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Original Research

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Protective effect of N-acetylcysteine against ethanol-induced gastric ulcer: A pharmacological assessment in mice

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ABSTRACT

Aim: Since there is an increasing need for gastric ulcer therapies with optimum benefit-risk profile. This study was conducted to investigate gastro-protective effects of N-acetylcysteine (NAC) against ethanol-induced gastric ulcer models in mice. **Materials and Methods:** A total of 41 mice were allocated into six groups consisted of 7 mice each. Groups 1 (normal control) and 2 (ulcer control) received distilled water at a dose of 10 ml/kg, groups 3, 4 and 5 were given NAC at doses 100, 300 and 500 mg/kg, respectively, and the 6th group received ranitidine (50 mg/kg). All drugs administered orally once daily for 7 days, on the 8th day absolute ethanol (7 ml/kg) was administrated orally to all mice to induce the acute ulcer except normal control group. Then 3 h after, all animals were sacrificed then consequently the stomachs were excised for examination. **Results:** NAC administration at the tested doses showed a dose-related potent gastro-protective effect with significant increase in curative ratio, PH of gastric juice and mucus content viscosity seen with the highest dose of NAC and it is comparable with that observed in ranitidine group. **Conclusion:** The present findings demonstrate that, oral NAC shows significant gastro-protective effects comparable to ranitidine confirmed by anti-secretory, cytoprotective, histological and biochemical data, but the molecular mechanisms behind such protection are complex.

KEY WORDS: Ethanol, gastric ulcer, gastroprotection, N-acetyle cystein

INTRODUCTION

N-acetylcysteine (NAC) is a derivative of thiol-containing amino acid that is a precursor for the intracellular antioxidant glutathione. It has potent antioxidant effects, NAC detoxifies reactive neutrophils and enhances the eradication of free radicals through either conjugation or reduction reactions [1], as well as suppression of cytokine expression and inhibition of nuclear factor kappa B [2]. Recently, there has been a growing interest in the therapeutic potential of NAC in a wide range of diseases where oxidative stress, cellular degeneration and inflammation are critical drivers in disease progression [3]. It is well-known that imbalance between antioxidants and oxidizing agents can create oxidative damages to cellular components; however, it has been hypothesized that high concentrations of antioxidants, such as glutathione and vitamins E and C could generate pro-oxidant effects, mainly through the reduction of transition metal ions and the involvement of Fenton reaction [4,5]. Several studies demonstrated that lipid peroxidation and protein oxidation are the major outcome of reactive oxygen species (ROS) generation [6], which plays a significant role in gastric ulceration. In accordance, thiol containing compounds like NAC reduce gastric mucosal injury that is caused by various ulcerogenic factors [7]. Gastric ulcer disease is characterized by the presence of sores in the gastric linings, which may occur due to imbalance between aggressive and gastro-protective factors [8]. Oxidative stress and lipid peroxidation play a crucial role in the etiology and development of gastric ulceration [9]. On the other hand, inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin [IL-6] and IL-10) and over expression and translocation of the nuclear factor-κB (NF-KB) subunits play additive roles in the acute phase of inflammation as well as in maintenance of gastric ulcers [10]. Globally, gastrointestinal problems have now become a common disorder, affecting 8-10% of the population, and increasingly many research resources are dedicated to address these problems [11]. Acid suppression therapy and eradication of *Helicobacter pylori* infection contributed to a reduction in the prevalence of gastric ulcer disease [12]; however, there is an increasing need for gastric ulcer therapies with optimum benefit-risk profile. Although the antioxidant anti-inflammatory properties of NAC have been established in other conditions, the gastro-protective effects of NAC as a potential treatment for gastric ulcer have not been previously studied [13]. This study aims to investigate gastro-protective effects of NAC against ethanol-induced gastric ulcer models in mice.

MATERIALS AND METHODS

Materials

NAC was purchased from America Medic and Science - AMS, USA. Absolute ethanol was purchased from BDH chemicals, Ltd., Poole, England. Ranitidine was obtained from SDI Company, Samarra, Iraq. Diethyl Ether was purchased from May and Baker, England. 42 male albino mice (27-32 g) were included throughout the study. The mice were purchased from College of science, University of Basra, housed in the animal house of College of Pharmacy, University of Basra, Iraq. This study was conducted in College of Pharmacy, University of Basra from May to August 2014. The animals were housed in polypropylene cages with three mice per cage under a 12-h light-dark cycle (7 a.m.-7 p.m., lights on), and maintained conventionally during the study with regulated air temperature (15-21°C), relative humidity (40-70%) and ventilation (air volume change 20 times/h). The animals were allowed to acclimatize for at least 1 week prior to the experiment. They had free access to standard diet and water *ad libitum*. Animal experiments were in accordance with National Institute of Health guidelines and approved by the Animal Ethics Committee of College of Pharmacy, University of Basra.

Experimental Design and Animal Treatment Protocol

The animals were allocated into six groups consisted of 7 mice each: Groups 1 (normal control) and 2 (ulcer control) received distilled water at a dose of 10 ml/kg, groups 3, 4 and 5 were given NAC at doses 100, 300 and 500 mg/kg, respectively, and the 6th group received ranitidine (50 mg/kg), as a reference drug for treatment of gastric ulcer. The doses of NAC were selected based on previously published reports suggesting that NAC was not toxic at these doses [14]. In addition, NAC does not show any signs of toxicity at doses even greater than those administered in this study [15]. All drugs dissolved in distilled water and administered orally once daily for 7 days using gastric gavage needle before induction of gastric ulceration. On the 8th day and after fasting for 22 h (but had free access to water), all mice received daily regular doses of drugs and vehicle 1 h before the induction of gastric ulcer. Absolute ethanol induced ulcer was performed according to the method described by Palacios-Espinosa *et al*. with slight modification [16], where absolute ethanol (7 ml/kg) was administrated orally to all mice to induce the acute ulcer except normal control group. Then, 3 h after ethanol administration, all animals were sacrificed by inhalation of high dose of diethyl ether, the abdomen opened and ligatures placed around the esophagus and pylorus then consequently the stomach was excised for examination.

Evaluation of Gastric Ulcer Area (UA) and Gastro -protection

The UA was measured using digital pictures under a dissection microscope, with the aid of the software UTHSCSA Image Tool 3.0 program to measure damage area [17,18]. The sum of total UA (mm^2) was divided by the number of animals to obtain the mean UA for each group. Meanwhile, the percent of gastro-protection was calculated according to the following equation [19]:

$$\text{Curative ratio} = ([\text{UA control} - \text{UA treated}] \times 100)/\text{UA control}$$

Histological Assessment

For histopathological examination, stomach was fixed in 10% formalin solution for 4 days, sectioned, and then embedded in paraffin. Histological sections were cut at 4-5 μm and then stained with routine hematoxylin and eosin [20], for examination under a light dissection microscope by a histopathologist for analysis of necrotic lesions, congestion, edema, hemorrhage, surface epithelium disruption and infiltration of epithelium.

Determination of Stomach Acidity and Mucus Content

The stomach of each mouse was cut along the greater curvature. Hydrogen ion concentrations of stomach content were measured using digital pH meter [16]. The gastric mucosa of all mice were gently scraped using a flat piece of glass slide and the mucus obtained was weighed using electronic weighing balance [21].

Statistical Analysis

Data were expressed as mean ± standard deviation; the values were first analyzed using one-way analysis of variance (ANOVA). Bonferroni's *post hoc* analysis was then performed to compare treated groups. Values with $P < 0.05$ were considered significantly different. Analysis was performed using GraphPad Prism software for Windows (version 5.0, GraphPad Software, Inc., San Diego, CA).

RESULTS

Photographic Investigation under Dissection Microscope

Gastric mucosa shows that absolute ethanol induced significant oxidative injury on the surface epithelial cells; the lesions produced were deep, inflamed with massive hemorrhage and edema in ulcer control group while in normal group no macroscopic or microscopic lesions were observed, as shown in Figure 1a and b. The mice pre-treated with different doses of NAC [Figure 1c-e] and ranitidine [Figure 1f] had considerably reduced areas of gastric lesions compared with ulcer control group depend on dose used with greatest gastro-protection observed in groups treated with the highest dose of NAC (500 mg/kg) and ranitidine.

Histopathological Investigation

Histopathological studies further confirmed that pretreatment with NAC inhibits the absolute ethanol induced gastric ulcer, necrotic lesions, congestion, edema, hemorrhage and extensive

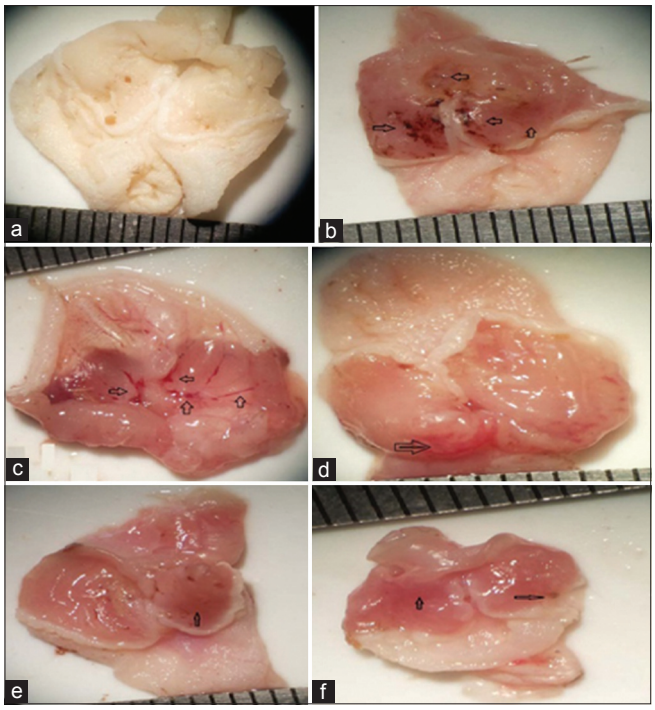

Figure 1: Photographs of gastric mucosa in controls and treated groups. Black arrows indicate ulcer areas. a Normal control group; b ulcer control group; (c) N-acetylcysteine (NAC) 100 mg/kg + absolute ethanol 7 mL/kg; (d) NAC 300 mg/kg + absolute ethanol 7 mL/kg; (e) NAC 500 mg/kg + absolute ethanol 7 mL/kg; (f) Ranitidine 50 mg/kg +absolute ethanol 7 mL/kg

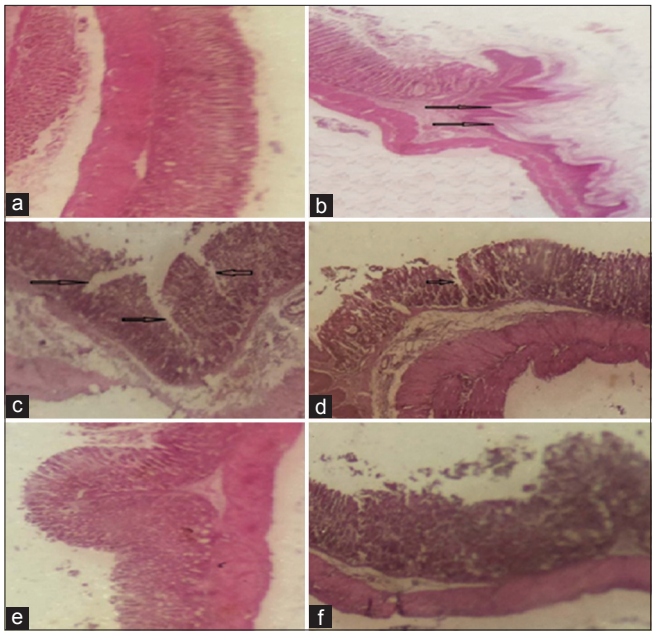

Figure 2: Photomicrographs of mice gastric mucosa stained with hematoxylin and eosin (×100). Ethanol induced damage to the gastric mucosa is indicated by the black arrows. (a) Normal control; (b) ulcer control; (c) NAC 100 mg/kg + absolute ethanol 7 mL/kg; (d) NAC 300 mg/kg + absolute ethanol 7 mL/kg; (e) NAC 500 mg/kg + absolute ethanol 7 mL/kg; (f) Ranitidine 50 mg/kg + absolute ethanol 7 mL/kg

disruption of the surface epithelium in gastric mucosa as summarized in Figure 2a-f

Ulcer Index and Curative Ratio

Orally administered absolute ethanol induced severe gastric mucosal lesion (lesion area = 24.5 ± 0.85); NAC at the tested doses (100, 300 and 500 mg/kg), showed a dose-related significant protective effect against gastric lesions, with significant reduction in the ulcer size achieved with the highest dose of NAC, and it is comparable with that observed in ranitidine group [Figure 3]. Figure 4 demonstrates both doses of NAC (300 and 500 mg/kg) exhibited marked increase in curative

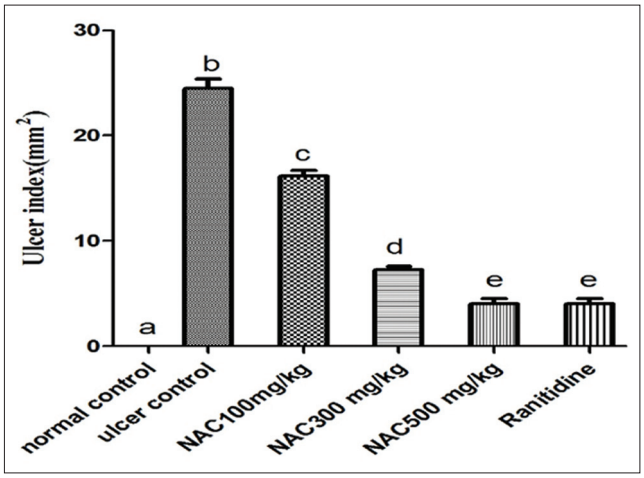

Figure 3: Gastric ulcer index of absolute ethanol induced acute gastric lesions in mice ($n = 7$), ANOVA: Values with different letters a, b, c, d and e, $P < 0.05$ versus normal control, ulcer control, N-acetylcysteine (NAC) 100 mg/kg, NAC 300 mg/kg and NAC 500 mg/kg respectively

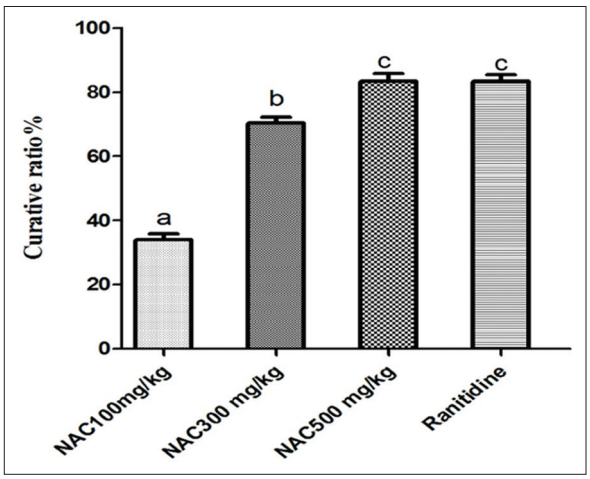

Figure 4: Curative ratio of different doses of NAC and ranitidine on gastric ulcer in mice ($n = 7$), ANOVA: Values with different letters a, b, c, $P < 0.05$ versus normal control, ulcer control, N-acetylcysteine (NAC) 100 mg/kg, NAC 300 mg/kg and NAC 500 mg/kg respectively

ratio, compared with that observed with ulcer control group; such increment were relatively comparable to that observed in ranitidine (as reference drug) treated group.

Biochemical Investigation

In comparison with ulcer control group as shown in Figures 5 and 6, there was a significant increase in pH of gastric juice and mucus content viscosity of the gastric layer in NAC pretreated group and ranitidine treated group, where NAC administration antagonizes mucus depletion by ethanol and raised the pH of gastric juice significantly. Nevertheless, NAC, administered in low dose (100 mg/kg), was found to be ineffective in preventing the depletion of mucus content, compared with other groups and comparable with that measured in ulcer control group.

DISCUSSION

The present study shows significant gastro-protective effects of oral NAC administration in ulcer induced by absolute ethanol. Aggressive factors such as acid-pepsin secretion, oxygen free radical production, lipid peroxidation, and matrix metalloproteinases play a crucial role in the pathophysiology of gastric ulceration [9,22]. Therapeutic management of peptic ulcer depends on modulation of such factors, and on this basis, several classes of drugs had been introduced, like proton pump inhibitors and histamine receptor blockers as antiulcer agent [23]. Aggressive factors involved in the pathogenesis of gastric ulceration are induced by several kinds of stress, including ethanol, NSAIDs or *H. pylori* [24]. In this study, we used a model of absolute ethanol-induced gastric ulceration for the evaluation of the activity of NAC as an antiulcer compound. Absolute ethanol affects the gastric mucosa within a few minutes of contact by different mechanisms, including gastric mucus depletion, decrease in mucosal blood flow and direct mucosal damage [25]. NAC (500 mg/kg) produces a significant gastro-protection, similar to that observed in ranitidine (reference drug), indicating the role of NAC protective mechanisms in providing reduced glutathione (GSH) as an endogenous antioxidant to protect the gastric mucosa through its function as a scavenger of free radical, which protect thiol groups from oxidation [3,26]. In the presence of NAC, the ROS production and consequently oxidative stress were significantly decreased, as reported in many *in vitro* and *in vivo* studies. This occurs directly or indirectly by restoring glutathione content [27]. Moreover, the significant inhibitory effect of NAC on ethanol-induced gastric injury can be explained by different mechanisms. NAC is a -SH containing compound with multiple regulatory and protective roles. They are involved in the defense against different chemicals that induce tissue injury, since both non protein and protein thiols can scavenge free radicals and affect enzyme activity, cell viability and membrane stability; such protective mechanism was reported in the stomach and liver injury [28]. Evidences from a variety of pathological and toxicological studies, such as ischemia-reperfusion injury, chemically induced oxidative injury, radiation damage, aging and degenerative disease, indicate that GSH is a major component of physiological systems protecting against oxidative and free radical mediated cell injury [29]. Many *in vitro* and *in vivo* studies support this mechanism. In an *in vitro* model by Mann *et al.* to assess gastro-protective effect of NAC, they found the healing of mucosal lesions that induced by HCl was promoted and hastened by low-dose of NAC [30]. Furthermore, in an *in vivo* study of gastric ulcer induced by HCl in the rat, depletion of glutathione with subcutaneous administration of diethylmaleate impaired gastric mucosal healing [31]. These results are supported by the findings of various previous studies. Intra peritoneal administration of GSH protected gastric mucosa against stress induced mucosal ulcerations [32]. In addition, oral administration of NAC has been found to protect the gastric mucosa against ethanol-induced gastric injury [33]. While oral NAC administration protected against ulcer [34], other study showed that intra-peritoneal administration of NAC increased the amount of gastric mucosal lesions after ethanol application secondary to reduced gastric mucosal blood flow [35]. Furthermore, NAC given intra-peritoneally in very high dose (1200 mg/kg) increased gastric injury [33]. The gastro-protective effect of oral NAC administration in the present study, which lowers mean ulcer size, may be due to the action against myeloperoxidase (MPO)/H_2O_2/Cl^- system. This fact was supported by a study done by Antwerpen *et al.*; they indicated that NAC and its lysinate salt were the most efficient thiol-containing molecules in scavenging of hypochlorous acid, which was produced by MPO, and appeared as efficient inhibitors of MPO/H_2O_2/Cl^- system, probably because of their relatively small size [36]. In addition, oxidative stress plays a critical role in enhancing inflammatory response. This leads to progression and deterioration of inflammatory conditions, where free radical may be implicated in the activation or modulation of a number of signaling pathways like NF-KB, and cytokines release, which promotes serious cellular injuries, and contributes to several inflammatory processes and human diseases [37,38]. NAC attenuates and reduces the inflammation through inhibiting variety of cytokines such as TNF-α, interferon-γ, IL-8, and IL-6, and/or to restore the cellular redox-status, and regulates the activity of redox sensitive cell signaling pathways, such as NF-KB that control pro-inflammatory genes, indicating its protective action against cytokines-induced organ damage [13,39]. In light of these issues, studies gave evidence that both anti-oxidant and anti-inflammatory activities of NAC are the major properties that actively participate in gastro-protective activity. Since ulcer formation is directly related to factors such as gastric juice acidity and mucus production, assessment of such factors gave an expression of ulcer status. Interestingly, our study showed that oral NAC administration significantly increased gastric juice pH level with concomitant increase in mucus content viscosity specifically in high dose of NAC. These results are contradictory to the assumption that NAC have mucolytic properties and should reduce mucus viscosity by breaking disulfide bonds between mucin polymers [40]. However, our finding came in tune with study done in estrous mares by Witte *et al.*, they conclude that NAC treatment seems to induce mucus production and viscosity in endometrium of mares [41]. While most of the previous literature data focus on therapeutic potential of NAC on acute lung injury, acute kidney injury, hemorrhagic shock, hepatic

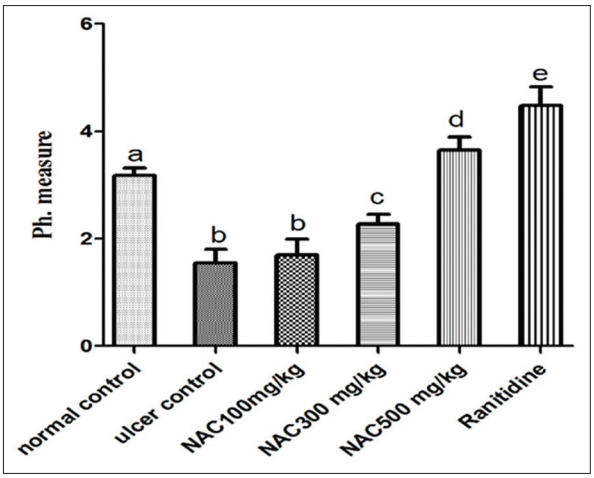

Figure 5: pH values in mice with acute gastric ulcer induced by absolute ethanol ($n = 7$), ANOVA: values with different letters a, b, c, d and e, $P < 0.05$ versus normal control, ulcer control, N-acetylcysteine (NAC) 100 mg/kg, NAC300 mg/kg and NAC500 mg/kg respectively

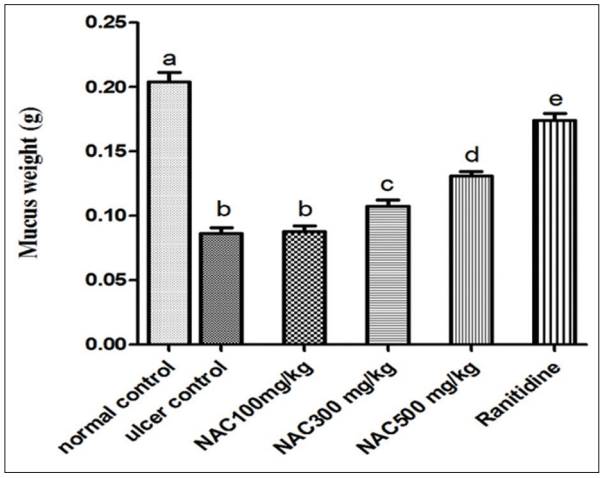

Figure 6: Mucus content in stomach of absolute ethanol induced acute gastric lesions in treated groups in mice ($n = 7$), ANOVA: values with different letters a, b, c, d and e, $P < 0.05$ versus normal control, ulcer control, N-acetylcysteine (NAC) 100 mg/kg, NAC300 mg/kg and NAC500 mg/kg respectively

damage after acetaminophen overdose, cardiovascular disease, diabetes, carcinogenesis and neuropsychiatric disorders [3,39], effect of NAC on gastric mucosa is an important matter. In conclusion, oral NAC administration shows significant gastro-protective effects comparable to ranitidine in absolute ethanol-induced gastric ulcer models confirmed by anti-secretory, cytoprotective, histological and biochemical data but the molecular mechanisms behind such protection are complex and still unclear. Indeed, further pharmacological and toxicological studies are required to delineate such mechanism(s).

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Anti-plasmodial activity of ethanolic extract of root and stem back of *Cassia sieberiana* DC on mice

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ABSTRACT

Aim: This study assessed within 4 days of suppressive test *in vivo* antimalarial activity of Ethanolic extract of root and stem bark of *Cassia sieberiana* DC against chloroquine sensitive strain of *Plasmodium berghei* NK65 in mice. **Methodology:** Two sets, each of five groups of four mice per each group were used. The groups of animals were administered with 100, 200, and 300 mg extract/kg body weight respectively, while positive control group were administered with 5 mg chloroquine/kg body weight and the negative control, were administered with 5 m1 distilled water/kg body weight. Oral acute toxicity was evaluated using up and down procedure. **Result:** Both the root and stem bark extract of *C. sieberiana* showed antimalarial property for suppressive tests. Chemo suppression of the root extract exerted significant ($P < 0.05$) dose-dependent reduction in the level of parasiteamia of 30.7%, 52.7%, and 55.8%. And from stem extract 17.6%, 38.0%, and 63.9% were recorded on mice when compared with 96.0% suppressive rate obtained from weight of chloroquine. The phytochemical screening of the plants root and stem bark extract revealed the presence of alkaloids, anthraquinones, flavonoids, triterpenoids, tannins, cardiac glycosides, saponins, reducing sugars and carbohydrates. The oral median lethal dose was determined to be >3000 mg/kg body weight. **Conclusion:** The acute toxicity results of this study showed that the plant parts used are assumed to be safe and has anti-plasmodial activity that can be explored for the management of malaria.

KEY WORDS: Anti-malaria, phytochemicals, suppressive test

INTRODUCTION

Cassia sieberiana (D.C.) belongs to the Family; Caesalpiniaceae Leguminosae, common name; African laburnum also called "Malga" in Hausa language, is a common plant in Saharan and sub-Saharan Africa. It is a savannah tree found in the dry areas of the forest and thickets [1]. It is often part of remedies in African veterinary and human therapies [2-4] particularly in north eastern and north western Nigeria where it is used for treatment of malaria, inflammatory conditions, rheumatism, jaundice, diarrhea, deworming, and aphrodisiacs [5]. The herb is usually available, affordable and acceptable to most of the consumers [6].

One of the major public health problems and greatest health challenges faced by 40% of the world's population is the malaria disease, which is caused by *Plasmodium* species, and transmitted by the bite of female Anopheles mosquito. The disease has caused much suffering and premature death in the poorer region of tropics and subtropics [7]. It has been estimated that 1.2 billion population are at high risk of transmission (≥1 case per 1000 population), and half of these population live in the African region; of which 80% cases are strenuous in 13 countries, and over half in Nigeria, Congo, Tanzania, Kenya, and Ethiopia. A quarter of all malaria cases in Africa, is presented in Nigeria where disease transmission occurs all year round in the southern part of the country and seasonal in the north [8]. At present, the still leading Africa's health problem is malaria, this occurs due to drug resistance to most anti-malarial drugs, insecticide resistance, war and civil disturbances, climatic changes, environmental changes, population increase and travel [8].

Therefore, the search for new anti-malarial compounds, either synthetic or natural is important for the killing of either the vector or parasite [9]. The use of plant-derived drugs for the treatment of malaria has a long and successful tradition [10,11]. For example, in the 1950's quinine was isolated from Cinchona and artemisinin from quinghaosu [12]. This illustrates the potential value of investigating traditionally used anti-malarial plants for

developing pharmaceutical anti-malarial drugs [13]. Unfortunately, chloroquine resistance now occurs throughout the whole world [14].

Certainly, native plants play an important role in the treatment of many diseases and 80% of people worldwide have been estimated to use herbal remedies [15]. However, information obtained on efficiency and safety of herbal use is few, despite the fact that motives of traditional practices could lead to novel strategies in malaria control. Different types of African plants used by traditional healers to treat and cure malaria symptoms have already been tested [16]. The increased number of drug-resistant malaria parasites, such as chloroquine-resistant malaria parasite has made the development of novel antimalarials urgent, and the high cost of malaria treatment has left the poor masses of Nigeria heavily reliant on traditional practitioners and medicinal plants for the treatment of the disease. For this study, the investigations on rodent malaria models for the antimalarial activity of *C. sieberiana* was carried out to verify the use experientially of the root and stem plant parts as ingredients in the traditional remedies for the treatment of malaria in aboriginal Northern Nigeria. With this view, the present study was executed to provide scientific evidence of its efficacy and continuous use in ethno therapeutic management of malaria.

METHODOLOGY

This study evaluates the in vivo antiplasmodial effect of ethanolic extract of *C. sieberiana* on mice. Stem bark and root of *C. sieberiana* were collected from Dundaye village of Usmanu Danfodiyo University, Sokoto State, Nigeria. The plant was identified and botanically authenticated at the herbarium unit, Department of Pharmacognosy Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen (pcg/udus/caes/0010) was prepared and deposited for future reference at the Pharmacognosy herbarium unit, Usmanu Danfodiyo University Sokoto. The root and stem samples were cleaned, air-dried and pounded into a fine powder using mortar and pestle. The powder was then stored in a dry air-tight container. Extraction was carried using soxhlet apparatus. The soxhlet method of extraction [17] was employed in extracting the plant material using ethanol. The extraction was carried out by placing 400 g of each of the powdered plant part in the upper chamber in a trimble and 700 ml of the solvent in the bottom of the flask. After the plant was extracted, the vapor was condensed to extract the sample to dryness using steam evaporator to obtain the crude extract of the plant. The dried ethanol crude extracts were weighed and stored in the refrigerator at 4°C in airtight plastic container until use for this study. The extracts were weighed and dissolved in distilled water for preparation of appropriate doses on each day of the experiment.

Phytochemicals Screening

A combination of several methods was used to identify the phytochemicals of the root and stem part. Standard screening tests using conventional protocol, procedure and reagents were used for detecting the major secondary metabolites present in the plant [18].

Experimental Animal

Sixty two mice of both sexes of Swiss Albino mice having body weight of 16-33 g, were obtained from Laboratory Animal Housing Unit of National Veterinary Research Institute (NVRI), VOM, Jos. They were kept at the Animal Housing Unit of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria. The consent of the ethical committee was obtained from the veterinary research and ethical committee on the use of Laboratory animal for research purposes of Usmanu Danfodiyo University Sokoto, Nigeria. The animals were housed in standard cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions in accordance with the National Institute of Health guideline for the care and use of Laboratory animals [18].

Rodent Parasite Used

The rodent parasite *Plasmodium berghei* NK 65 inoculated in four donor mice was sourced from the Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Research and Development (NIPRD), Idu Abuja, Nigeria, and were kept at the Animal housing unit of the Department of Phamarcology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria. Prior to commencing the study, four of the infected mice (donor mice) were kept and observed to reproduce disease symptoms similar to malarial human infection [19]. The donor mice (parasitaemia of about 20-30%) were anesthetized with chloroform, and their infected blood were collected via cardiac puncture with a pre-heparinized sterile syringe and needle. To avoid variability in parasitemia, the blood collected was pooled together. The collected blood was then diluted with 0.9% physiological saline, and the dilution was made based on the parasitemia of the donor mice and the red blood cell (RBC) count of averagely healthy mice (4.5×10^9 RBC/ml) [20] in such a way that 0.2 ml of blood contains 1×10^7 infected erythrocytes. Each mouse was administered intra-peritoneally with 0.2 ml of this diluted blood, which contains 1×10^7 *P. berghei* infected erythrocytes. The parasites were kept alive by continuous intra-peritoneal passage in mice every four days, and the re-infected mice were used for the study [21].

Acute Toxicity Studies

Acute toxicity of *C. sieberiana* ethanolic root and stem bark extract was carried out using up and down procedure to test the oral acute toxicity on two groups of five female mice which were selected at random and marked to permit individual identification. To conduct the limit test, food (excluding water) was withheld from the mice for 3 h. The fasted body weight of each animal was determined, and the dose was calculated according to the body weight. Water extract of crude extract of the leaves and fruits was administered orally in concentrations of 2000 mg/kg, body weights dose to five groups of animals (each consisting of one animal) using a stomach tube one after the other at the grace observation period of 24 h, 48 h and up to 14 days in a single oral dose while the control group received

distilled water only. After the substance has been administered, food was withheld for a further 3-4 h and observation of toxic symptoms was made and recorded systematically at 1, 2, 4 and 6 h after administration. Finally, the number of death and survivors was noted after 24 h, 48 h and up to 14 days for each animal. The dosed mice were observed for the first 1, 2, 4 and 6 h after administration and intermittently for the next 24 h for signs of toxicity. The second mouse was dosed at 24 h interval until all five mice were dosed. The control group received distilled water only. And all five mice were observed thereafter for the next fourteen days for any delayed toxic effect. Observation of toxic symptoms was made and recorded systematically at 1, 2, 4 and 6 h after administration. Finally, the number of death and survivors was noted after 24 h, 48 h and up to 14 days for each animal. The toxicological effect was assessed on the basis of toxicity sign: paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, tremor, off-feed, depression coma and mortality, which were expressed as lethal doze 50 (LD_{50}) [22].

Anti-plasmodial Analysis

The Peter's 4-day suppressive test against Chloroquine sensitive *P. berghei* (NK 65) infection in mice was employed [23]. Twenty albino mice of both sexes were inoculated. They were randomly grouped with the same number of male and female and four mice in each group. The animal were then administered extract daily for 4 consecutive days. On day 1 (D_0); heparinized blood was prepared from the donor mouse as explained earlier, treatment started 2 h after the mice were infected with the parasite. Group 1 that served as control was orally administered with 1 ml/kg body weight of normal saline, Groups 2, 3 and 4 were orally administered with 100, 200 and 300 mg extract/kg body weight daily respectively, while group 5 was administered with 5 mg chloroquine/kg body weight orally [23]. On Day 2-4 (D_1-D_3); the mice were treated again with the same doses and through the same route as in D_0 at interval of 24 h, 48 h and 72 h post-infection [24]. On the 5th day (D_4), blood was collected from the tail of each mouse and spread on a microscope slide to make a thin film. The blood films were fixed with methanol for 1 min stained with 3% Giemsa solution for 30 min and examined microscopically [25]. Eight fields containing 250 erythrocyte and the number of parasitized erythrocytes were recorded while the suppression of parasitemia was expressed as percent for each dose, by comparing the parasitemia in the control group with the treated one. The mice were observed for signs of toxicity after treatment for the first 4 (critical) h, then over a period of 24 h, thereafter daily for 7 days. Mortality occurring at a particular dose will indicate either to continue administration of subsequent higher dose or to estimate the LD_{50} by comparing the mortality to a fixed LD_{50} cut-off values provided in the guideline [26].

Data Analysis

Results were expressed as mean ± standard error of the mean. The data obtained were entered and analyzed using Analyze-it version 2.22 Excel 12+ statistical package. Chi-square test at a 95% confidence level was used to compare the result and values of $P < 0.05$ were considered as statistically significance.

Table 1: Antiplasmodial suppressive test of ethanolic root extract of *C. sieberiana* (suppressive test on *P. berghei*)

Treatment group/ extract concentration	Mean parasitaemia counts	% Inhibition
100 mg/kg	3.7±0.08	30.7
200 mg/kg	2.6±0.07	52.7
300 mg/kg	2.4±0.04	55.8
Chloroquine (5 mg/kg) (Standard)	0.24±0.04	96.0
Normal saline (5 ml/kg) (control)	5.7±0.32	0.00

C. sieberiana: Cassia sieberiana, P. berghei: Plasmodium berghei,

$$\text{Standard error of mean} = \frac{\text{Standard deviation}}{\sqrt{n}}$$

Table 2: Antiplasmodial suppressive test of ethanolic stem extract of *C. sieberiana* (suppressive test on *P. berghei*)

Treatment group/extract concentration	Mean parasitaemia counts	% Inhibition
100 mg/kg	4.4±0.12	17.6
200 mg/kg	3.3±0.05	38.0
300 mg/kg	1.93±0.05	63.9
Chloroquine (5 mg/kg) Standard	0.24±0.04	96.0
Normal saline 5 ml/kg (control)	5.7±0.32	0.00

C. sieberiana: Cassia sieberiana, P. berghei: Plasmodium berghei,

$$\text{Standard error of mean} = \frac{\text{Standard deviation}}{\sqrt{n}}$$

RESULTS

The results obtained showed significant decrease in parasitaemia of *P. berghei* infected mice treated with the ethanolic root and bark extract of *C. sieberiana* [Tables 1 and 2]. This significant suppression of parasitaemia observed was dose dependent ($P < 0.05$). The crude root extract (300 mg/kg) caused 55.8% suppression and the crude stem extract (300 mg/kg) caused 63.9% suppression in parasitaemia of *P. berghei* infected mice while chloroquine a standard antimalarial drug (5 mg/kg) exerted 96% suppression.

The oral median LD_{50} of the extract was estimated to be ≥2000 mg/kg in mice. There were no remarkable behavioral changes in the extract administered mice (reaction to food supply, contact and noise), though activity was reduced in some of the extract administered groups within the first 4 h. And no mortality occurred within the observation period of 14 days. However, behavioral signs of toxicity were observed in mice given 2000 mg stem extract/kg body weight which include; paw licking, salivation, stretching and reduced activity. There was however no mortality at all the dose levels used. And the oral median LD_{50} was estimated to be ≥2000 mg extract/kg body weight [Tables 3 and 4].

The result from the phytochemical analysis of ethanolic root bark extract of *C. sieberiana* showed; high amount of Carbohydrates using Molisch's test, high amount of Reducing Sugars using Fehling's test, high amount of Tanins using

Table 3: Acute toxicity study of ethanolic root extract of *C. sieberiana*

Group	Number of animal	Dose/kg body weight	Volume of plant extract (ml)	Behavioral sign/changes
1	1	2000 mg/kg	2.0	None
2	1	2000 mg/kg	2.5	R. activity
3	1	2000 mg/kg	3.0	R. activity
4	1	2000 mg/kg	1.9	None
5	1	2000 mg/kg	2.0	None
6	1	Distilled water	5	None

C. sieberiana: Cassia sieberiana

Table 4: Acute toxicity study of ethanolic root extract of *C. sieberiana*

Group	Number of animal	Dose/kg body weight	Volume of plant extract (ml)	Behavioral sign/changes
1	1	3000 mg/kg	2.0	None
2	1	3000 mg/kg	3.0	Paw licking, stretching
3	1	3000 mg/kg	2.6	R. activity
4	1	3000 mg/kg	2.1	R. activity
5	1	3000 mg/kg	3.0	R. activity
6	1	Distilled water	5	None

C. sieberiana: Cassia sieberiana, R. activity: Reduced activity

Ferric chloride test, Lead Actate test and Bromine water test, high amount of Flavonoids using Sulfuric acid test and Ferric chloride test, high amount of Saponins using Frothing test, high amount of Steroids and Triterpenoid using Liebermann–Buchard test and Salkowaski test, and also high amount of Cardiac Glycosides using Keller-Kiliani's test, using Borntrager's test Anthraquinones was in moderate amount while using Dragendorff's test and Wagner's test Alkaloids scored low [Table 5]. The ethanolic stem bark extract of *C. sieberiana* indicated; high amount of flavonoids in Sulfuric acid test and Ferric chloride test, high amount high amount of reducing sugars in Fehling's test, high amount of carbohydrates in Molisch's test, high amount of cardiac glycosides in Keller-Kiliani's test, high amount of tannins in Ferric chloride test, Lead Actate test and Bromine water test, in Salkowaski's test, Steroids and Triterpenoids were high but in Liebermann–Buchards test they were both low, there was moderate amount of Antraquinones in Borntrager's test and Saponins and Alkaloids were low using Frothing's test, Dragendorff's test and Wagner's, respectively [Table 6].

Table 5: Phytochemical analysis of ethanolic root extract of *C. sieberiana*

Component	Tests	Scoring
Alkaloids	Dragendorff's	+
	Wagner	+
Steroids and	Liebermann-Buchard	+++
triterpenoids	Salkowaski	+++
Saponins	Frothing	+++
Tannins	Ferric chloride	+++
	Lead actate	+++
	Bromine water	+++
Cardiac glycosides	Keller-Kiliani's	+++
Anthraquinones	Borntrager's	++
Flavonoids	Sulphuric acid	+++
	Ferric chloride	+++
Reducing sugars	Fehling's	+++
Carbohydrates	Molisch's	+++

C. sieberiana: Cassia sieberiana, +: Low concentration, +++: High

Table 6: Phytochemical analysis of ethanolic stem extract of *C. sieberiana*

Component	Tests	Scoring
Alkaloids	Dragendorff's	+
	Wagner	+
Steroids and	Liebermann-Buchard	+
triterpenoids	Salkowaski	+++
Saponins	Frothing	+
Tannins	Ferric chloride	+++
	Lead actate	+++
	Bromine water	+++
Cardiac glycosides	Keller-Kiliani's	+++
Anthraquinones	Borntrager's	++
Flavonoids	Sulphuric acid	+++
	Ferric chloride	+++
	Sodium hydroxide	+++
Reducing sugars	Fehling's	+++
Carbohydrates	Molisch's	+++

-: Not detected, +: Low concentration, ++: Moderate, +++: High, *C. sieberiana: Cassia sieberiana*

DISCUSSION

The four-day suppressive test which is a standard test commonly used for antimalarial screening and determination of percentage inhibition of parasitaemia in laboratory animal as used in this study indicated a mean group parasitaemia level of less than or equal to 96% of the mock-treated control animals given a suggestion that the test material is active in standard screening studies [23]. It has been reported that when a standard antimalarial drug is used on mice infected with *P. berghei*, it suppresses parasitaemia to non-detectable levels [27]. The observed antimalarial activity is consistent with the traditional use of the plant as an herbal medication against the disease in Nigeria. The extracts exerted significantly repository effect in mice treated with 100, 200 and 300 mg/kg body weight respectively [Tables 3 and 4]. This effect was however lower in groups that received low dose. This effect may be due to short duration of action of the extract occasioned by rapid metabolism, and so parasite clearance could not be total. It may also be explained by the fact that not all anti-malarials are completely active against *P. berghei* model [28]. The rodent model of malaria that was employed in this study for prediction of efficacy of anti-malarial effect of *C. sieberiana* root and stem bark extract, was also used for conventional antimalarial agents such as chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives [29]. *P. berghei* are used in the prediction of treatment outcomes. Hence it was an appropriate parasite for the study. Since this parasite was sensitive to chloroquine, this drug was used as the standard drug in this study. The choice of 4 weeks old male mice for the study was done to avoid the effect of anemia in the old mice and the effect of possible physiological changes associated with ageing and gender may induce on the treatment outcome [30]. An *in vivo* model was employed for this study because it takes into

account possible prodrugs effect and possible involvement of the immune system in eradication of infection [20]. Certain compounds form the basis of the pharmacologic effects of such plants [31,32]. Presence of alkaloids ranks among the most efficient and therapeutically significant plant compounds, moreover pure alkaloids and their synthetic derivatives are used as basic medicinal agents e.g. morphine is an analgesic, quinine is antiplasmodial, colchicines is used for gout, reserpine is a tranquilizer, vincristine and vinblastine have antitumor effects [31], terpenes, anthraquinones, and flavonoids screened plants has been implicated in antiplasmodial activity [33,34]. Earlier studies [35], reported the oxidant generation potential of *Acacia nilotica* extract, based on the ability of the extract to increase conversion of reduced glutathione to oxidized glutathione. Increased oxidation has also been shown to create an intracellular environment that is unfavorable to plasmodial growth [36,37]. The mechanism of action of artemisinin, which depends on oxidant action for its potent antimalarial activity, validates this [35]. However, lack of oxidizing action in some plants does not rule out anti plasmodial activity since they may be active through other biochemical mechanisms. The oral median LD of ≥3000 mg/kg body weight obtained for the Ethanolic root and stem bark extract of *C. sieberiana* DC is 31 times greater than the minimum effective dose of 100 mg/kg. Earlier reports have shown that if the median LD of a test substance is three times more than the minimum effective dose, the substance is considered a good candidate for further studies. It was also reported that oral administration is about 100 times less toxic than the intra-peritonial [38]. These findings suggested that the extract could be safe, and this partly explain the safe use of the plant by the local people who have been using it in traditional management of malaria in Nigeria.

CONCLUSIONS

It is evident based on these findings that *C. sieberiana* possess potent anti-plasmodial effect justifying its folkloric usage in the management of anti-malarial. However, the active principle(s) are yet to be identified, and there is a need for the identification. In view of this fact, attempts are being made to carry out anti-plasmodial curative test, prophylactic test as well as guided fractionation of the root and stem ethanolic extract to isolate the active compounds and also to test for the cytotoxicity of the extract.

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Original Research

ScopeMed

Hepatoprotective and Pancreatoprotective Properties of the Ethanolic Extract of Nigerian Propolis

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ABSTRACT

Objective: Increased oxidative stress is associated with the progression of diabetic mellitus. In the present study, we investigated the effects of the ethanolic extract of Nigerian propolis (N. propolis) on markers of oxidative stress, histology of the liver and pancreas and glycaemia in alloxan-induced diabetic rats. **Materials and Methods:** Alloxan-induced hyperglycemic Wistar rats were treated with either metformin (150 mg/kg/d) or N. propolis (200 mg/kg/d and 300 mg/kg/d) for 28 days. At the end of the treatment period, the rats were sacrificed; blood was collected for biochemical analysis while their pancreases and liver were excised and processed for histological studies. **Results:** Serum oxidative stress markers and blood glucose concentration were compared between the treated and control rats. In contrast to the non-treated diabetic rats, blood glucose concentration were not significantly different between treated rats and control ($P < 0.05$) at 28 days of treatment with N. propolis and metformin. Serum malondialdehyde levels was reduced while superoxide dismutase levels were elevated in the N. propolis group; these levels were converse in the diabetic group, these differences are statistically significant ($P<0.05$) when compared with the control. Histologically, there was improvement in the treated group compared to the untreated group. **Conclusion:** These findings suggest that the N. propolis confers protection against hyperglycemia-induced oxidative stress in both liver and pancreas of adult Wistar rats.

KEY WORDS: Diabetes, liver, metformin, Nigerian propolis, oxidative stress, pancreas

INTRODUCTION

Diabetic mellitus (DM) has long been defined as a heterogeneous group of metabolic disorders characterized by glucose intolerance and fasting hyperglycemia [1]. Type 1 DM accounts for approximately 10% of all cases and it is essentially immune-mediated with Type 2 DM afflicting about 6% of adult population, and it is essentially a result of insulin resistance and impaired β-cell function [2]. Besides fasting hyperglycemia, several organs develop complications in diabetes. Key organs in this respect include the kidney (nephropathy), eye (retinopathy), brain and peripheral nerves (neuropathy), liver (glycogen storage disease and steatohepatitis), blood vessels (atherosclerosis and microangiopathy) [3-5].

In long-standing DM, the morphology and function of the liver are disturbed. Liver biopsy findings in Type 1 diabetics with hepatomegaly are comparable to hepatic findings in Mauriac's syndrome and include marked glycogen deposition in hepatocytes [6]. Clark and Diehl [7] reported that in Type 2 diabetes, impaired insulin action usually result in non-alcoholic fatty liver disease, including steatosis and steatohepatitis.

There is ample evidence of an important role of oxidative and glycooxidative stress in the pathogenesis of diabetic complications [8]. The exact contribution of antioxidant enzyme to oxidative stress in diabetes is not fully understood. Learning and memory impairment has also been associated with oxidative stress in streptozotocin-induced diabetes rats [9].

Propolis is a natural product derived from plant resins and collected by honeybees (workers) to be used as glue and as draught-extruder for beehives [10]. Honey another product derived from honeybees was almost the only source of sugar available to the ancients and was valued for its medicinal benefits. It has been reported that propolis contain at least 200 compounds with more than 100 being present in any given sample [11]. These include fatty and phenolic acids and esters, substituted phenolic esters, bioflavonoids (flavones, flavanones, flavonols and others), terpenes, β-steroids, aromatic aldehydes and alcohols, and derivatives of sesquiterpenes, naphthalene and stilbenes [12-14].

The main types of flavonoids that have been reported include rutin (an antihypertensive agent), quercetin (a potent anti-diabetic material), galangin [15] and caffeic acid phenethyl

ester [16]. These entire constituents give propolis the ability to perform many functions. Propolis is reported to have hepatoprotective [17], antioxidant [18], antimicrobial [19], anti-inflammatory [19] and anticancer [20] properties.

Several factors is responsible for the variability in the propolis chemical components which includes: Unpredictability of growing plant species around the beehive, condition of the climate, beekeeper actions and nature of the soil [21]. Propolis has been reported to be geographically sensitive and each area has its own peculiar constituent [10]. Gómez-Caravaca *et al.* [22] reported that despite geographical differences, most propolis sample contain 50% resin, 30% wax, 10% essential oils, 5% pollen and 5% of other organic compounds in their chemical composition. Several studies have been carried out to examine the effects of propolis from different geographical regions on experimentally induced diabetes especially from the Arab world, Brazil and Croatia, but little or no information is available on the anti-diabetic properties of Nigerian propolis (N. propolis).

Currently, botanical drugs are being screened for their efficacy, safety and dosage in the management of DM because they are cheap and readily available. In this work, we report the blood glucose responses of hyperglycemic rats to a regimen of N. propolis. Besides this, histology of the liver and pancreas, activities of hepatic alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) and the antioxidant superoxide dismutase (SOD) and malondialdehyde (MDA) were reported.

MATERIALS AND METHODS

Animals

A total of thirty adult male Wistar rats were bred at the animal holdings of the Department. Animals weighed between 190 and 230 g and were 8 weeks old at the start of the experiment. They were exposed to 12 h light, 12-h dark cycle at 22-24°C. All animals were maintained on a pelletized growers feed (Flour Mill Ltd., Ibadan, Nigeria). Rat pellets and water were given freely. Study was performed in accordance with the ethical guidelines stipulated by the Ethical Committee of the College of the institution. These guidelines were in accordance with the internationally accepted principles for laboratory animal use and care.

Collection and Extraction of Propolis

N. propolis was purchased from the Federal University of Agriculture, Abeokuta, Ogun state in Nigeria. Raw propolis was obtained by scraping it off its hive frames. Ethanolic extract was prepared according to the method described by Ivan *et al.* [23].

2.3 Induction and assessment of DM

To induce hyperglycemia, 25 animals were fasted overnight. Diabetes was induced by single intra-peritoneal (ip) injection of alloxan monohydrate (100 mg/kg) (Sigma, St. Louis, USA) in sterile normal saline (0.9%). Five Control animals were injected intra-peritoneally with citrate buffer alone at a single dose of 1.2 ml/kg. All animals were allowed free access to feed and water after alloxan injection, and they were left undisturbed for a minimum of 72 h for hyperglycemia to develop [24]. Animals were identified as diabetic on the basis of blood glucose levels (higher than 230 mg/dL) in tail blood using a one touch ultra mini Glucometer (LifeScan Inc., Milpitas, USA) 3 days after alloxan treatment. Weekly record of blood glucose level was taken afterwards.

Administration of Drugs

A total of 25 adult Wistar rats (twenty surviving diabetic rats and five normal rats) were randomly assigned into one of the following treatment groups of five animals each: Control, diabetic, diabetic + N. propolis (200 mg/kg, p.o.), diabetic + N. propolis (300 mg/kg, p.o.) and diabetic + metformin. Metformin (Merck, Germany) was given orally at 150 mg/kg. Administration of both drugs lasted for 28 days and was carried out at 09:00-10:00 daily.

Blood Glucose, Feed Intake and Body Weight (BW)

A One Touch Ultra Mini Glucometer (LifeScan Inc., Milpitas, USA) was used to estimate the blood glucose of treated and control animals. Blood was obtained from the dorsal vein of the animals. Blood glucose was estimated at day 0 and at 1, 3, 5, and 7 h after the first dose of the extract. Thereafter, measurement was done twice a week for 4 weeks. Feed intake was monitored on a daily basis during the experimental period (data not show). BW of the animals was also recorded twice a week.

Termination of Treatment

All animals were fasted and killed under diethyl ether anesthesia 24 h after the last treatment day. Blood was collected into heparinized tubes and centrifuged at 3000 r/m for 20 min in a desktop centrifuge model 90-1 (Jiangsu Zhangji Instruments Co., China). Plasma was stored at −20°C until analyzed. Laparotomy was performed on each animal; the liver and pancreas were excised, rinsed in normal saline and fixed in Bouin's fluid.

Histological Studies

The livers and pancreases fixed in Bouin's fluid were processed and stained with hematoxylin and eosin for histological studies. Photomicrographs were taken with a JVC color video digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK).

Assessment of Liver Function

Biochemical analysis of the serum enzymes for AST and ALT was by the method of Reitman and Frankel [25].

Markers of Oxidative Stress Assessment

Serum level of MDA and SOD were assayed by the method of Ohkawa *et al.* [26] and Misra and Fridovich [27] respectively.

Determination of Serum Lipids

Triglycerides (TG) and total cholesterol (CHOL) were determined by enzymatic methods according to Diniz *et al.* [28] using commercial diagnostic kits (Randox, UK).

Statistical Analysis

Data obtained were presented as mean ± standard error of mean and analyzed for statistical significance using one-way analysis of variance, followed by Waller-Duncan *post-hoc* test. $P < 0.05$ was considered statistically significant.

RESULTS

To evaluate the effect of the ethanolic extract of N. Propolis on alloxan-induced diabetic rats, several biochemical estimations were carried out in all experimental animals for the estimation of plasma glucose, serum cholesterol, serum TG, liver function tests and oxidative stress markers. The histology of the liver and pancreas was also compared. The following pharmacological effects were observed:

Effect on Hyperglycemia

The mean blood glucose level of normal control rats fed on a normal diet was almost invariable throughout the experimental study. On the contrary, the blood glucose level of diabetic untreated rats was significantly increased ($P < 0.05$) when compared with the normal control group. When alloxan-induced diabetic rats were treated with metformin (150 mg/kg) and N. propolis at doses of 200 and 300 mg/kg, lowering in blood glucose was observed from the second week onwards when compared to the diabetic control group [Figure 1].

Effect on BW

Table 1 shows the initial and final BW of animals in all groups after the 28 day experimental period. BW of animals in the diabetic group decreased after 28 days while there was significant ($P < 0.05$) increase in the BW of animals in other groups.

Effect on lipid profile

Level of serum TG

Increase in level of serum TG was observed in the diabetic group when compared to the non-diabetic control group. Decrease in serum triglyceride levels was observed between metformin and extract treated groups when compared to the diabetic untreated group [Table 2].

Level of CHOL

Table 2 also shows that serum cholesterol levels of untreated diabetic rats were significantly higher than those in normal control group. Treatment with metformin and N. propolis lowered the level of serum cholesterol with maximum effect seen in the 300 mg/kg N. propolis group.

Effect on Serum ALT and AST

Figure 2a and b shows the serum levels of AST and ALT in all groups. Serum levels of ALT and AST were up regulated significantly ($P < 0.05$) in the diabetic untreated group

Table 1: Effect of ethanolic extract of N. propolis on BW

Group	A	B	C	D	E
Initial BW (g)	210±6.3	220±8.3	197±3.9	213±13.2	215±3.8
Final mass BW (g)	257±10.6*	198±9.7	225±6.5*	242±5.6*	244±6.5*

BW: Body weight, *$P<0.05$ significantly different from diabetic control group, A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg N. propolis), E: Diabetic treated (300 mg/kg N. propolis), N. propolis: Nigerian propolis

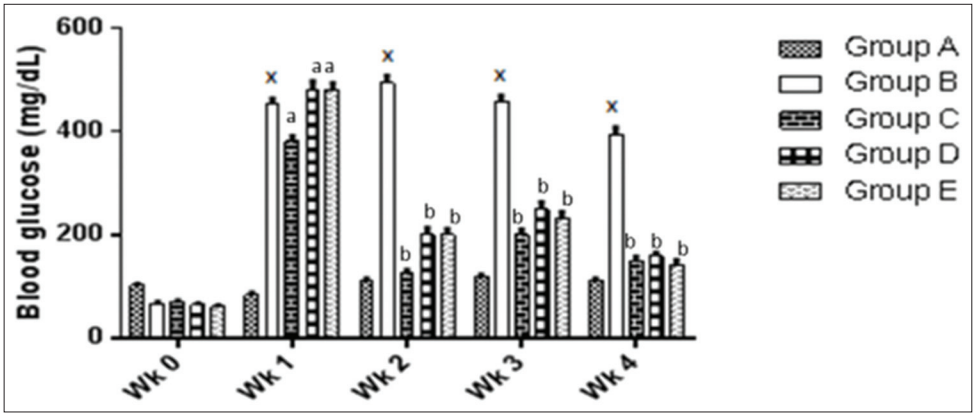

Figure 1: Showing and comparing weekly changes in the blood glucose level of the treated groups compared to the diabetic control group. *$P < 0.05$ significantly different from non-diabetic control group, [a]$P < 0.05$ significantly different from normal control group, [b]$P < 0.05$ significantly different from diabetic untreated group. A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg Nigerian propolis [N. propolis]), E: Diabetic treated (300 mg/kg N. propolis)

Table 2: Effect of ethanolic extract of N. propolis on lipid profile

Group	A	B	C	D	E
CHOL (mg/dL)	41.56±3.45*	105.46±6.06	42.13±1.47*	56.78±1.01*	36.12±3.13*
TG (mg/dL)	103.96±9.34*	185.34±7.11	180.71±8.68	183.88±13.01	147.16±9.96

*$P<0.05$ significantly different from diabetic control group, A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg N. propolis), E: Diabetic treated (300 mg/kg N. propolis). CHOL: Total cholesterol, TG: Triglyceride

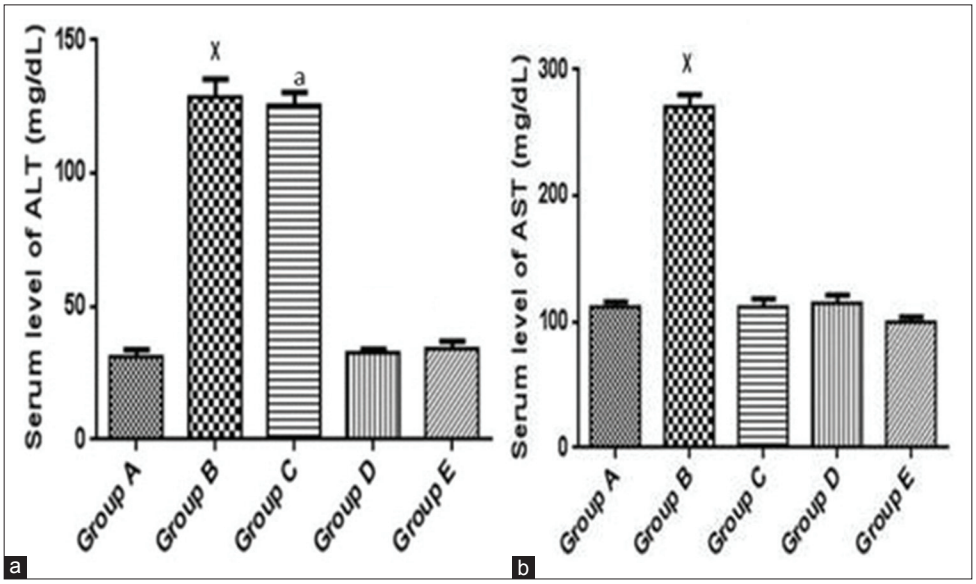

Figure 2: (a and b) Showing and comparing serum level of alanine aminotransferase and aspartate aminotransferase between treated groups and the diabetic untreated group. *$P < 0.05$ significantly different from normal control group, [a]$P < 0.05$ significantly different from normal control group. A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg Nigerian propolis [N. propolis]), E: Diabetic treated (300 mg/kg N. propolis)

when compared to the normal control group. Administration of metformin and N. propolis significantly decreased the elevated level of AST when compared to the normal control group. Metformin had no significant effect on the elevated level of ALT while N. propolis administration significantly brings down the level of ALT when compared to the normal control group.

Effect on oxidative stress

Serum MDA levels and SOD activities were assayed for. Alloxan-induced diabetes significantly lowered SOD levels in the diabetic group when compared to normal control group. After administration of metformin and N. propolis, SOD level was not significantly different ($P < 0.05$) between the normal control and the metformin treated group [Figure 3]; the administration of N. propolis raised the SOD level higher than the control [Figure 3]. Similarly, MDA levels were higher in the untreated diabetic group, which was significantly different ($P < 0.05$) when compared with the normal control. Administration of N. propolis and metformin significantly ($P < 0.05$) lowered the MDA levels when compared to the normal control group [Figure 4].

Histology

Hepatic histology

Figure 5a-e shows histology of the livers in control and treated groups at 28 days. In these groups, hepatic histology was comparable to the control, except in untreated diabetic rats, where hepatic sinusoids had become occluded, central veins were congested and hepatocytes appeared swollen.

Pancreatic histology

The section of rat pancreas from normal control group reveals normal pancreatic acini and islet cells, a similar result was found in the group treated with N. propolis and metformin. Alloxan-diabetic rats demonstrate degenerative and lytic changes in the islet of Langerhans of the pancreas as seen in Figure 6a-e.

DISCUSSION

In this study, we examined the possible antioxidants effects of N. propolis on diabetic rats. The noted significant decrease in blood glucose level in the N. propolis treated groups [Figure 1]

suggests that long-term administration/intake of this extract may have hypoglycemic effect. The reduction in the blood glucose level may be due to the presence of certain bioactive components and the protective effects that the antioxidant components of N. propolis may have on pancreatic β-cells which could enhance their production of insulin and more importantly, the possibility that propolis may enhance cellular response to insulin. In the propolis treated groups, despite the initial hyperglycemia, the antioxidants present in propolis were able to prevent oxidative stress effect of hyperglycemia on the liver and pancreas, this finding is in line with the reports of Al-Hariri *et al.* [29] who reported the efficacy of Arabian propolis in hyperglycemia. Hypoglycemic agents exact their effects via direct or indirect mechanisms in diabetes rats [30]. N. propolis acted as a direct hypoglycemic agent, by producing hypoglycemic effects when administered to alloxan-treated rats due to the severe destructive effect of alloxan on the β-cells of the pancreas [24]. N. Propolis

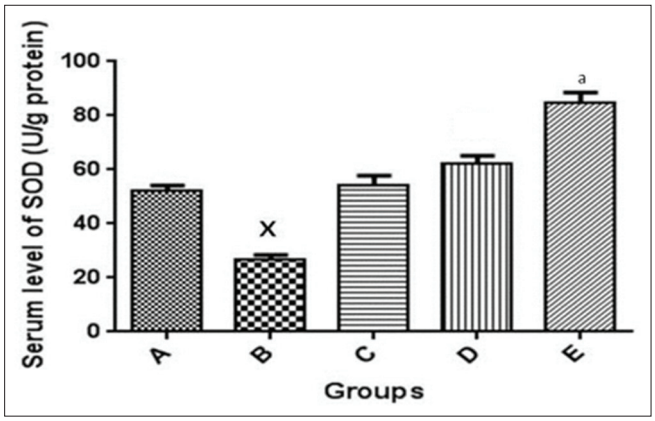

Figure 3: Serum level of superoxide dismutase in the control and experimental groups. *P < 0.05 significantly different from normal control group, [a]P < 0.05 significantly different from normal control group, A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg Nigerian propolis [N. propolis]), E: Diabetic treated (300 mg/kg N. propolis)

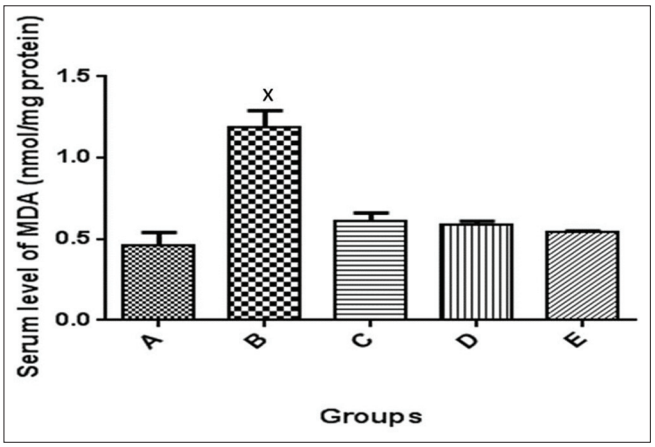

Figure 4: Serum level of malondialdehyde in the control and experimental groups. *P < 0.05 significantly different from the control and treatment groups. A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg Nigerian propolis [N. propolis]), E: Diabetic treated (300 mg/kg N. propolis)

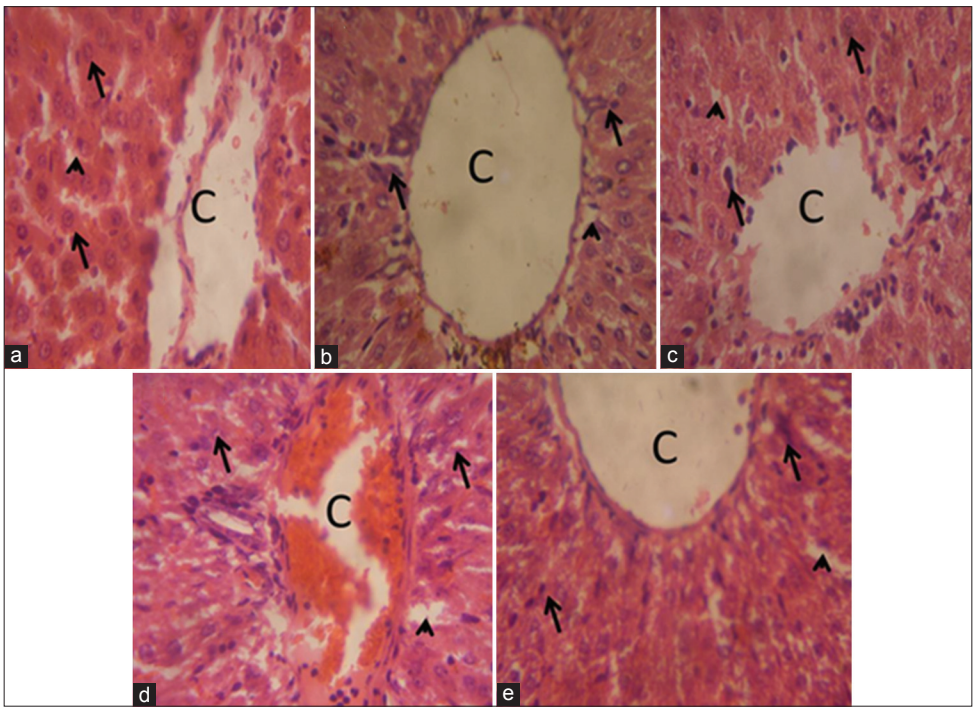

Figure 5: Liver of rats at 28 days of treatment, (a) Control group: The morphology of the liver is normal as indicated by intact central vein (c), sinusoids (arrowhead), and hepatocytes (arrows). (b) Diabetic group; Sinusoids have become largely occluded, perhaps due to swollen hepatocytes (arrows); central vein (c) is also congested. (c) Diabetic + metformin: The liver has normal morphology (c, central vein; arrow, hepatocyte; arrowheads, sinusoids). (d) Diabetic + 200 mg/kg of Nigerian propolis [N. propolis]: Hepatic morphology is comparable to control. (e) Diabetic + 300 mg/kg of N. propolis: The liver show normal morphology. Arrows indicate hepatocytes; arrowheads indicate sinusoids. H and E stain; ×400.

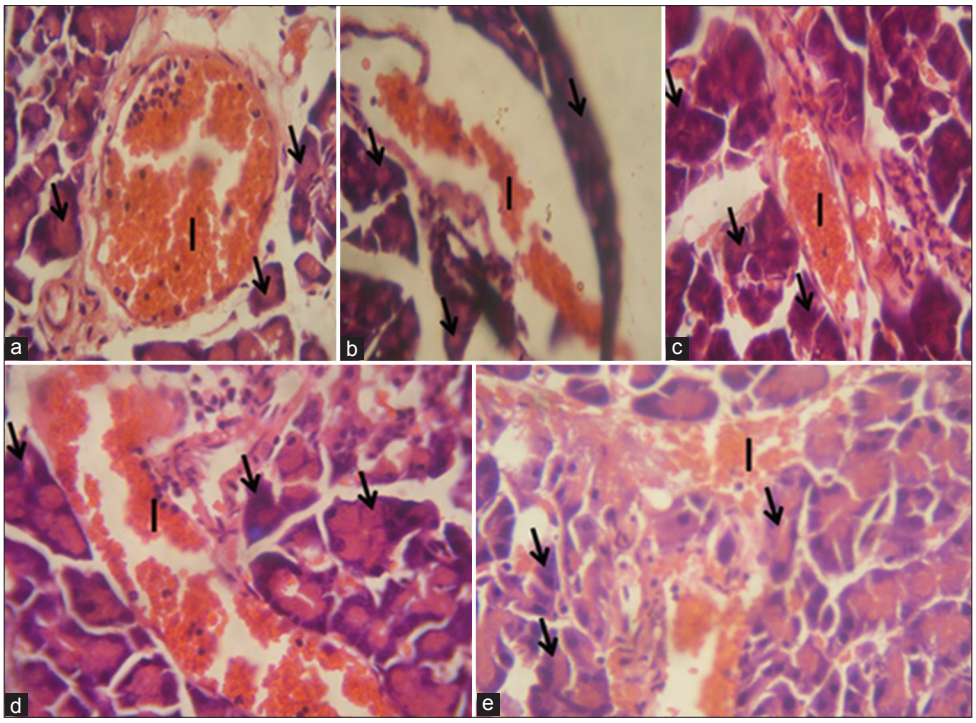

Figure 6: Pancreas of rats at 28 days of treatment. (a) Control group: The morphology of the pancreas is normal as indicated by intact islet of Langerhans cells (I) and acinar cells (arrows). (b) Diabetic group: There are degenerative and lytic changes in the islet of Langerhans of pancreas. (c) Diabetic + metformin: The pancreas has normal morphology (I show islet cells, arrow show acinar cells). (d) Diabetic + 200 mg/kg of Nigerian propolis; Pancreas morphology shows improvement comparable to control. (e) Diabetic + 300 mg/kg of Nigerian propolis. Pancreas morphology is comparable to control (H and E, 400×)

could also have acted indirectly by stimulating the few surviving β-cells to secrete more insulin rather than aiding the regeneration of necrotic β-cells of the pancreas. Observation from this study shows that N. Propolis exacts its activity by both direct and indirect mechanisms.

Lipid disorders assume a position of utmost importance in patients with diabetes, because of the high risk of microvascular disease in this condition but administration of N. propolis to the rats was able to reverse hyperlipidemia seen in the diabetic rats. Similar reports of Al-Hariri [29] and El-Sayed *et al.* [31] shows that treatment with propolis can reduce the TG and serum cholesterol level in diabetics. The hypolipidemic effects of N. propolis observed from this study is probably due to the hypoglycaemic potential of N. Propolis, which makes it possible to ameliorate lipid and lipoprotein disorders associated with diabetes.

The ethanolic extract of N. propolis possesses antioxidant components that ameliorated the oxidative stress induced damage associated with alloxan-induced diabetes. The bioflavonoids present in N. Propolis may have confers the antioxidant effect seen in this study as quecretin, a flavonoids present in N. Propolis have been reported to bring down hyperglycemia and oxidative stress in STZ-induced diabetes rats [32].

Increased levels of ALT and AST infiltrates and disturbs functioning of the hepatic cell membranes [33]. Administration of N. propolis ameliorated high levels of ALT and AST following induced-diabetes. The hepatoprotective activity of N. propolis was higher at 300 mg/kg than metformin.

A primary consideration in the assessment of the efficacy of a potential therapeutic agent for hepatic injury is its effect on liver histology. Histological sections of the liver in untreated diabetic rats show extensive occlusion of the sinusoids [Figure 5b]. Reports have revealed swelling of hepatocytes could arise from accumulation of glycogen in these cells - a condition referred to as hepatic glycogenosis of DM [34]. Treatment with the ethanolic extract of N. propolis improved hepatic injuries associated with induced-diabetes.

The pancreas is usually the main organ affected in diabetes with loss in both its exocrine and endocrine functions. This is mainly due to the close anatomical and functional links between the exocrine and endocrine pancreas [35]. Histologically, there was improvement in the islet and the acinar cells in the N. propolis treated group. This is different from what is seen in the untreated diabetic group. Pancreatic cells are usually lost because pancreatic β-cells are highly prone to oxidative stress and damage because they have low activity of antioxidant enzymes, which are the first line of defense against oxidative insult [27].

CONCLUSION

In conclusion, results from this study indicate that the ethanolic extract of N. propolis can ameliorate hyperglycemia, hypercholesterolemia, and hypertriglyceridemia as well as

protect the liver and pancreas against alloxan-induced diabetes. This significant protection of N. propolis may be due to the synergistic effect of the constituents of the extract. Further biochemical and pharmacological investigations would be required to know the comprehensive mechanism of action of the N. propolis which our laboratory is currently involve in.

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Original Research

ScopeMed

Antiproliferative activity and nitric oxide production of a methanolic extract of *Fraxinus micrantha* on Michigan Cancer Foundation-7 mammalian breast carcinoma cell line

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ABSTRACT

Aim: Methanolic extract of a *Fraxinus micrantha* (MeFM) was evaluated for antiproliferative activity *in vitro* using Michigan Cancer Foundation-7 (MCF-7) breast carcinoma cell line. This plant was selected and studied for naturally available bioactive compound as different synthetic drugs available for cancer treatment has certain limitations and side effects. **Materials and Methods:** The anti-proliferative activity of a methanolic extract from the aerial parts of *F. micrantha* was assessed on MCF-7 breast cancer cell line using 3(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide assay. Furthermore, to understand the mechanism of anti-proliferation, production of nitric oxide (NO) and DNA fragmentation was also determined on MCF-7 cells. Different phytoconstituents of the extract were determined qualitatively based on various biochemical assays. **Results:** The results demonstrated anti-proliferative activity of an MeFM in a concentration and time-dependent manner. The percentage viability determined was 31.24% at 125 μg/ml as compared to 80.46% in negative control group. An MeFM has also shown NO production in a concentration (0.2-125 μg/ml) and time-dependent manner (24-48 h). DNA fragmentation studies showed that a methanolic extract was causing DNA fragmentation thus inducing apoptosis in MCF-7 breast carcinoma cells. Biochemical analysis result showed the presence of flavonoids, polyphenols, and sterols in an MeFM. **Conclusion:** In conclusion, *F. micrantha* possesses potent anti-proliferative activity on the malignant MCF-7 cell line which is correlated with the production of NO and DNA fragmentation. Further studies are required to identify, isolate, and characterize the phytochemicals present in the methanolic extract that might have antiproliferative potential in the treatment of different cancer conditions.

KEY WORDS: Antiproliferation, DNA fragmentation, *Fraxinus micrantha*, nitric oxide, Michigan Cancer Foundation-7 breast cancer cell line

INTRODUCTION

Cancer is the disease characterized by uncontrolled and unregulated proliferation of cancer cells. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. By 2012, about 8.2 million died of cancers [1]. The conventional treatment of cancer includes chemotherapy causes various adverse and toxic side effects. These include damage to blood-forming cells in the bone marrow, hair follicles and cells lining mucous membrane, digestive tract, and reproductive system [2]. In view of this, there is a greater emphasis on search for novel and safer alternative. Plant are the attractive source as number of drugs such as sanguinarine, vinblastine, vincristine, teniposide, taxol, and camptothecin had being derived from these natural products [3]. In this continuing search for new anticancer compounds from plants, the present study evaluated the antiproliferative property of a methanolic extract of *Fraxinus micrantha* (MeFM) which had been previously reported for a number of medicinal properties.

The genus *Fraxinus* is in the olive family, *Oleaceae*. *F. micrantha* is one of the ashes found in Asia mainly in India and Nepal. In India, it is found in Himachal Pradesh and Uttar Pradesh region [4]. *F. micrantha* have been studied for its medicinal and economical value worldwide since ancient times. Local inhabitants of *Dharchula*, Himalayas use the inner bark infusion for the treatment of liver enlargement, jaundice, and other liver diseases [5]. The dried bark of *Fraxinus japonica* blume is

available in the market as an oriental medicine "*shinpi*" in Japan and has been used since olden times as diuretic, an antifebrile, an analgesic, and an anti-rheumatic [6]. *Fraxinus rhyncophylla* is traditional Chinese herb and has antidiarrheal properties [7].

Michigan Cancer Foundation-7 (MCF-7) cell line derived from the breast cancer cells was used as an *in vitro* model to study the anti-proliferative studies because the cell line has retained several ideal characteristics particular to the tumor epithelium [8].

MATERIALS AND METHODS

3(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-tetrazolium bromide (MTT), dimethylsulfoxide, Roswell Park Memorial Institute (RPMI) 1640, fetal bovine serum (FBS), streptomycin, penicillin G (Sigma-Aldrich Chemical Company Germany). Guanidium isothiocyanate reagent (EZ DNA KIT, Biological Industries, Israel, Beit Haemek Ltd.), Gene Ruler 1 kb DNA ladder (Fermentas) were used. Furthermore, Griess reagent was prepared of 1% sulphanilamide and 0.1% N-[naphthyl] ethylenediamine dihydrochloride in o-phosphoric acid (Sigma-Aldrich Chemical Company Germany).

Plant Material, Extraction and Preparation

The dried barks of *F. micrantha* were purchased from the local market in Delhi around February-March, 2012 and authenticated by a certified botanist. A voucher specimen (USBT#101-19012012FM) was submitted to the herbarium in the laboratory. The dried barks were grinded using an electric grinder and subjected to extraction (1:10) using methanol as solvent system by decoction method for 3-4 days. The solvent thus obtained was subjected to rota-evaporation for 24 h to get an MeFM. The fraction was reconstituted in complete RPMI (cRPMI - with 10% FBS and 1% antibiotic solution) medium and stock concentration of 2 mg/ml was made. The stock of an MeFM was filtered using 0.2 μ syringe filter before making different concentrations (0.2-125 μg/ml).

Anti-proliferative Study on MCF-7 Breast Carcinoma Cell Line

MCF-7 cells were plated at 5×10^5 cells/well in a 96-well plate in cRPMI (100 μl). 24 h after confluency (70-80%) and 48 h after more than 90% confluency, cells were treated with different concentrations of an MeFM (0.2-125 μg/ml). Each concentration was tested in triplicates (100 μl in each well). Untreated cells were used as negative control and taxol was used as a positive control. Morphological and quantitative changes were observed under inverted microscope at ×200 magnification and photographed.

MTT Assay

This assay was based on the cleavage of yellow tetrazolium dye MTT into soluble purple formazan, by succinate dehydrogenase in active mitochondria. Dead cells were unable to perform this reaction. In this assay, the amount of purple formazan generated is spectrophotometrically determined in a multi-well plate reader at 570 nm [9]. Cell viability was calculated by trypan blue dye exclusion method [10], and cells were plated in 96 well tissue culture plates at a final volume of 100 μl (in triplicates). Culture plates were incubated at 37°C and 5% CO_2 in a CO_2 incubator for 24 h. Afterward, different groups were given incubation with taxol (0.25 μM) and different concentrations (0.2-125 μg/ml) of an MeFM. After incubation, the supernatant was removed for nitric oxide (NO) analysis.

Griess Assay

The amount of NO formed was estimated from the accumulation of the stable NO metabolite, nitrite (NO_2^-) by Griess assay. In this assay, the culture supernatant (100 μl) from various treatment groups from the previous experimental set up and Griess reagent (100 μl) were mixed, and the absorbance was measured at 550 nm [11]. The amount of nitrite was calculated from a $NaNO_2$ standard curve (0-200 μM).

Detection of DNA Fragmentation

The MCF-7 cells were plated in 24-well plates (2×10^6 cells/well). The cells were treated with taxol and an MeFM at different concentration ranging from 0.2 μg/ml to 125 μg/ml for 24 h (in duplicates). After incubation cells were harvested using phosphate buffered saline (pH 7.4) and trypsin and centrifuged at 10,000 rpm for 10 min at room temperature. The pellet was reconstituted in 1 ml of EZ reagent. 1 ml of ethanol was added, and the contents were mixed thoroughly. After washing twice with ethanol, the pellet was air-dried and was dissolved in 8 mM sodium hydroxide. The samples (20 μl/well) were electrophoresed on 2.5% agarose gel along with molecular marker.

Biochemical Tests on an MeFM

Different biochemical tests such as Mayer's and Libermann Burchard's were performed for the qualitative detection of various phytoconstituents (alkaloids, flavonoids, polyphenols, sterols, and saponins) in an MeFM. Extract was treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids. Chloroform was added to 1 ml MeFM and then treated with few drops of acetic anhydride, boiled and cooled, followed by addition of concentrated sulfuric acid. Formation of brown ring at the junction indicates the presence of phytosterols. For the detection of saponins, an MeFM was diluted with distilled water to 20 ml, and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins. Flavonoids were detected using alkaline reagent test where an MeFM was treated with few drops of sodium hydroxide solution resulted in formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids. Addition of 3-4 drops of ferric chloride solution to an MeFM forms bluish black color indicating the presence of polyphenols [12].

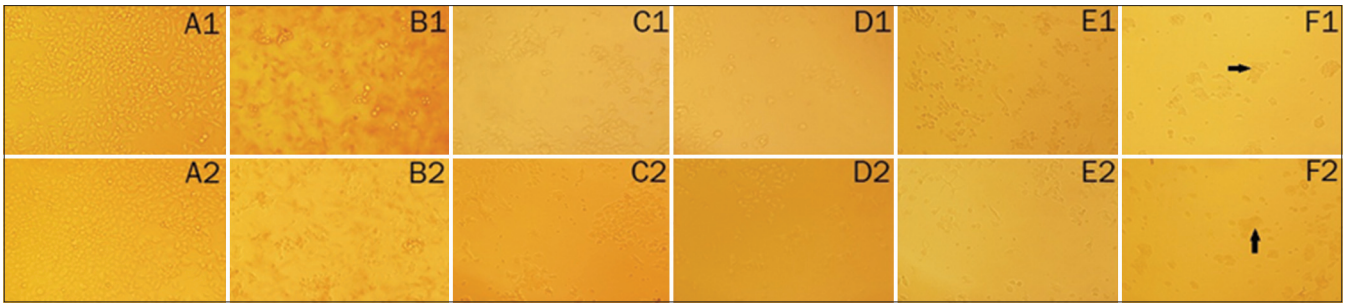

Figure 1: Comparison of Michigan Cancer Foundation-7 breast carcinoma cells at 24 h and 48 h - A1 and A2 only completed Roswell Park Memorial Institute (negative control), B1 and B2 taxol (positive control), C1 and C2 methanolic extract of a *Fraxinus micrantha* (MeFM) 0.2 µg/ml, D1 and D2 MeFM 0.9 µg/ml, E1 and E2 MeFM 31.25 µg/ml and F1 and F2 MeFM 125 µg/ml (arrow showing cell clumping)

Statistical Analyses

Statistical tests were performed using Graphpad Prism 4 (GraphPad software, Inc., CA/USA, 2011). Data were presented as mean±standard error. For single comparison, the significance of differences between means was determined by Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Morphological Examination of Anti-proliferative Activity of an MeFM against MCF-7 Human Breast Carcinoma Cell Line

The cells were treated with different concentration of an MeFM (0.2, 0.9, 31.25 and 125 μg/ml) for 24 h. After incubation morphological alterations were observed as compared to control groups. Untreated cells were elongated, expanding as monolayers forming interconnections between them on the well surface. Exposure of MCF-7 cells to concentrations of an MeFM as mentioned above at different time intervals (24 h, 48 h) resulted in retraction and rounding of cells. At 24 h incubation, the negative control group [Figure 1a1] showed 70% confluence with normal and healthy growing cells while cells debris were seen in positive control group [Figure 1b1]. At 0.2 μg/ml [Figure 1c1] and 0.9 μg/ml [Figure 1d1] the cells showed slow proliferation rate as they were less in number as compared to the negative control group. At high concentration of 31.25 μg/ml [Figure 1e1] and 125 μg/ml [Figure 1f1] the cells were seen in patches and clumps which is the sign of deteriorated morphology. At 48 h, the negative control cells [Figure 1a2] were fully confluent. The cells treated with taxol [Figure 1b2] showed dead cells and cell debris while cells incubated with an MeFM (0.2 μg/ml and 0.9 μg/ml) showed very less proliferative cells. An MeFM at high concentration (31.25 μg/ml [Figure 1e2] and 125 μg/ml [Figure 1f2]) showed complete inhibition of cell proliferation indicating strong anti-proliferative activity.

Evaluation of Cytotoxicity of an MeFM against MCF-7 Human Breast Carcinoma Cell Line

The relationship between concentration of an MeFM and its cytotoxic effect on MCF-7 cells was evaluated by MTT assay.

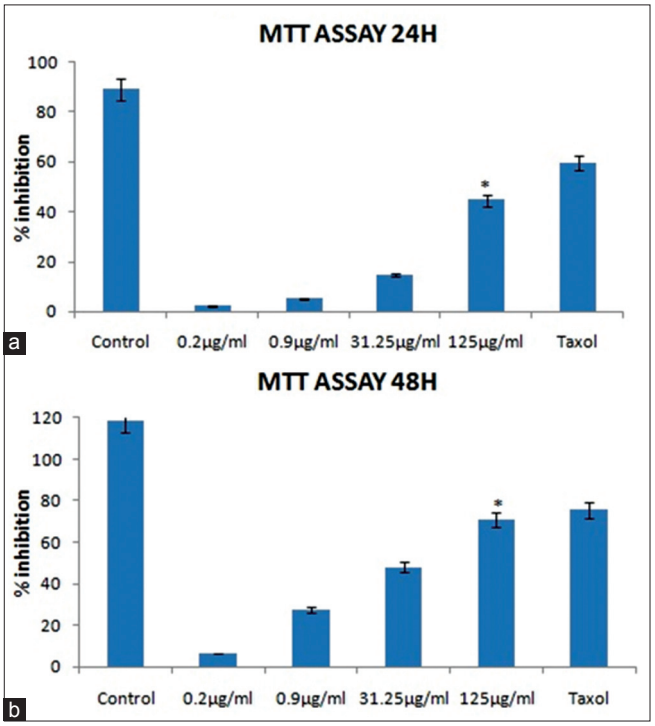

Figure 2: Effect of different concentrations of an methanolic extract of a *Fraxinus micrantha* (MeFM) (0.2-125 µg/ml) on cytotoxicity in Michigan Cancer Foundation-7 human breast cancer cell line at: (a) 24 h and (b) 48 h; *Antiproliferative potency of an MeFM was found to be significant, p<0.05 compared to untreated negative control group. Data are means±standard error for three independent experiments

The cells were treated with an MeFM at different concentrations (0.2, 0.9, 31.25 and 125 μg/ml) for 24 h [Figure 2a] and 48 h [Figure 2b]. The MeFM was found to be cytotoxic to MCF-7 cells in concentration and time-dependent manner. Similar results were observed when the taxol was used as a positive control. An MeFM at 31.25-125 μg/ml decreased the proliferation of MCF-7 cells by 15-45% at 24 h and by 82-89% at 48 h. Treatment with an MeFM significantly decreased the proliferation (31.24%) of MCF-7 carcinoma cells, $P < 0.05$ compared to negative control group (80.46%) which was untreated MCF-7 cells. The inhibitory concentration 50 value calculated from the line of best fit was 18.95 μg/ml.

NO Production by an MeFM against MCF-7 Human Breast Carcinoma Cell Line

The supernatant collected from different groups was subjected to NO assay by Griess method. The MeFM was found to be inducing NO production in a concentration and time-dependent manner [Figure 3]. With the increase in incubation time with an MeFM, the amount of NO produced was almost doubled (90 μM at 24 h and 187 μM at 48 h). Interestingly, the highest concentration of an MeFM induced NO production at a higher level than the positive control, taxol. The high production in NO after incubation with an MeFM was shown to be significant with $P < 0.05$ as compared to the positive control group treated with taxol.

DNA Fragmentation Analysis by an MeFM

DNA fragmentation of MCF-7 (2×10^6 cells/well) was detected on 2.5% agarose gel after exposing with control groups and concentrations of an MeFM ranging from 0.2 to 125 μg/ml for 24 h. At an MeFM concentration of 125 μg/ml, DNA smear was observed ranging up to 0.2 kb [Figure 4]. This smear (Lane 2) was obtained because of DNA fragmentation induced by an MeFM, which probably causing apoptosis. On the other hand, a sharp clear band was obtained in a negative control (Lane 6). A 1kb DNA ladder was used as a marker.

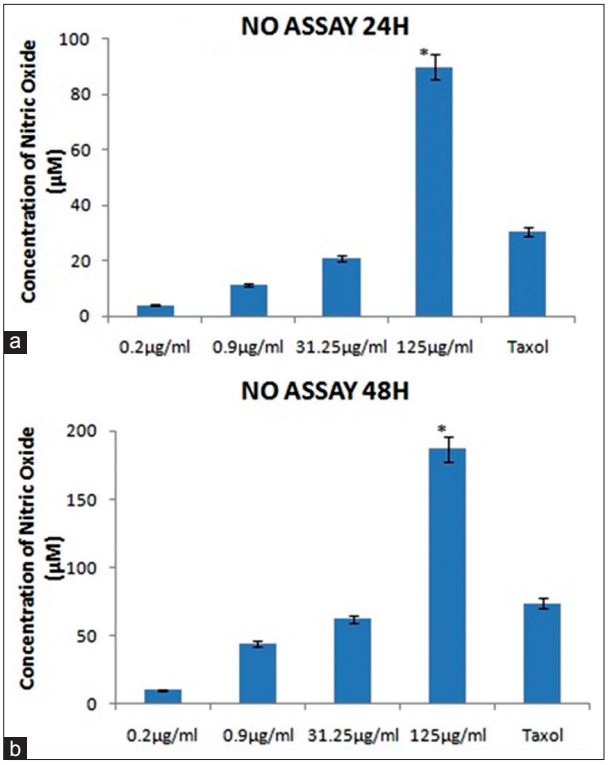

Figure 3: Effect of different concentrations of an methanolic extract of a *Fraxinus micrantha* (MeFM) (0.2-125 µg/ml) on nitric oxide (NO) production in Michigan Cancer Foundation-7 human breast cancer cell line at: (a) 24 h and (b) 48 h; *Induction of NO production was found to be significant, p<0.05 compared to positive control group. Data are means±standard error for three independent experiments

Biochemical Assays

Qualitative analysis showed the presence of flavonoids, polyphenols, and sterols while alkaloids and saponins were absent in an MeFM [Table 1].

DISCUSSION

Cancer is one of the invasive diseases caused by the uncontrolled proliferation and growth of otherwise normally growing cells. The drugs used in conventional chemotherapy causes various toxic and side effects such as depression of bone marrow leads to neutropenia, fall of hair follicles, damage to cell lining of mucous membrane, digestive tract, and reproductive system [2]. In view of this, there is an urgent need to search for novel and safer alternative molecules having anti-proliferative property. Natural resources especially plant like *F. micrantha* found in the Himalayan region of Indian subcontinent have been reported

Table 1: Result of phytochemical screening of bark of an MeFM

Phytoconstituents	Observation	Result
Alkaloid	No precipitate	-
Flavonoids	Colorless solution	+
Polyphenols	Black color	+
Sterols	Brown ring	+
Saponins	Unstable froth formation	-

+: Present, -: Absent, MeFM: Methanolic extract of a *Fraxinus micrantha*

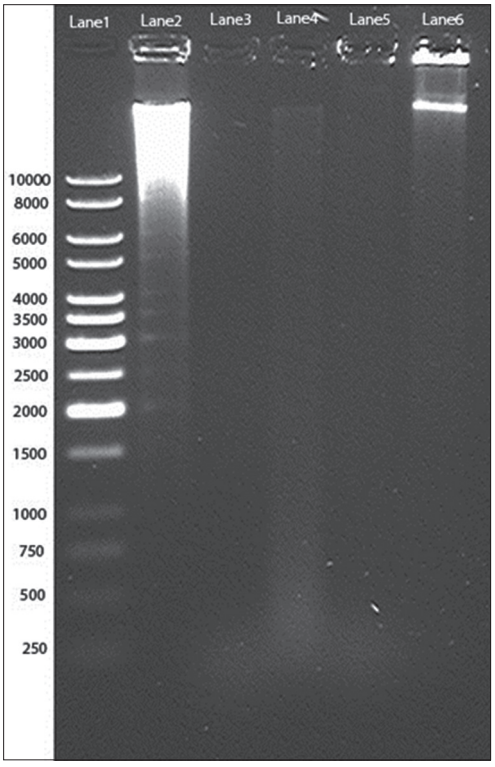

Figure 4: 2.5% agarose gel showing DNA fragmentation induced by an methanolic extract of a *Fraxinus micrantha* (Lane 1-1 kb marker, Lane 2-125 µg/ml, Lane 3-31.25 µg/ml, Lane 4-0.9 µg/ml, Lane 5-0.2 µg/ml, and Lane 6 - Negative control)

for various medicinal properties [13]. The present study for the first time reporting anti-proliferative property of an MeFM *in vitro* on MCF-7 cell line.

The MeFM demonstrated significant antiproliferative effect against MCF-7 human breast carcinoma cell line. MTT reduction assay showed concentration and time-dependent cytotoxicity of the MeFM. The morphological changes in MCF-7 breast cancer cells incubated with different concentrations of an MeFM were consistent with MTT assay. At the concentration of 31.25 μg/ml and 125 μg/ml striking morphological changes such as clumping of cells having round morphology, retraction and shrinking of cells were observed in inverted microscope in the same manner as MTT reduction assay showed strong cytotoxic action of an MeFM at these concentrations. The cytotoxicity of an MeFM at high concentration (125 μg/ml) used in the present study was almost comparable to cytotoxicity of taxol. This showed that the phytoconstituents present in the MeFM have strong anti-proliferation potential which might be useful in deriving the novel anticancer molecule in future.

NO is a short-lived free radical, endogenously acts as a signaling molecule in the body. Previous reports showed that an excessive and unregulated NO synthesis has been implicated to abrogation of tumorigenicity and metastasis of tumor cells [14]. NO is also known to inhibit cell proliferation and induces apoptosis in high concentrations [15]. Increased NO production has been reported in breast cancer cells treated with various apoptotic agents, such as tumor necrosis factor-α, phorbol esters, and peptide hormones [14,16]. This study demonstrated that on incubation of MCF-7 cells with different concentrations of an MeFM, NO was produced in concentration- and time-dependent manner. This phenomena might be possible due to enhancing effect of iNOS gene stimulated by an MeFM which may lead to enhanced production of NO [17] which might be responsible for damage of various cellular components (proteins, DNA, other organelles) and finally may result in cell death and apoptosis. This was consistent with our result of DNA damage in DNA fragmentation study after incubation with an MeFM. Similar DNA fragmentation results were reported in a study with positive control taxol [18].

Biochemical tests showed the presence of flavonoids, polyphenols, and sterols present in the MeFM, which might be involved in anti-proliferative activity. Flavonoids have been reported for potent cell growth inhibitory actions over a physiologically relevant concentration range [19].

In conclusion, the results of the present study demonstrated that the MeFM has potent antiproliferative activity which might be inducing the phenomena of programmed cell death or apoptosis. Further studies are required to identify, isolate, and characterize the active phytoconstituents from the extract in order to be used as a potential anticancer agent in the future for cancer chemoprevention.

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Original Research

ScopeMed

The effects of catechin isolated from green tea GMB-4 on NADPH and nitric oxide levels in endothelial cells exposed to high glucose

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ABSTRACT

Aim: This study aimed to investigate whether a catechin isolated from GMB-4 green tea is able to increase the reducing equivalent system and nitric oxide (NO) level in endothelial cells exposed to high glucose (HG) level. **Materials and Methods:** Endothelial cells were obtained from human umbilical vascular tissues. At confluent, human endothelial cells were divided into five groups, which included control (untreated), endothelial cells exposed to HG (30 mM), endothelial cells exposed to HG in the presence of green tea catechin (HG + C) at the following three doses: 0.03; 0.3; and 3 mg/ml. Analysis of $NADP^+$, NADPH, and NO levels were performed colorimetrically. **Results:** This decrease in NADPH was significantly ($P < 0.05$) attenuated by both the 0.3 and 3 mg/ml treatments of catechin. HG level significantly decreased NO compared with untreated cells. This increase in NO was significantly attenuated by the 0.3 mg/ml dose of the catechin. **Conclusion:** In conclusion, catechin isolated from GMB-4 green tea prohibits the decrease in NADPH and NO in endothelial cells induced by HG. Therefore this may provide a natural therapy for attenuating the endothelial dysfunction found in diabetes mellitus.

KEY WORDS: Endothelial dysfunction, green tea, high glucose level, oxidative status

INTRODUCTION

Reactive oxygen species (ROS) are causal factors for oxidative damage to various cells and induce organ dysfunction after high glucose (HG) level injury. However, they are also involved in cellular signal transduction pathway [1]. NADPH oxidase, a complex formed by Nox and other cytosolic subunits, whose only function is ROS production of molecular oxygen, has been extensively investigated in many cell types, including vascular endothelial cells [2,3]. The amount and site of ROS production as well as antioxidant defense determined its cellular's effect. Low levels of oxidative stress appear to be physiological and beneficial cellular signal in reparative angiogenesis, while excess amount of ROS contributes to endothelial cells injury and dysfunction [4-6]. Exposure of endothelial cells to HG induces ROS production in vitro [7,8]. NADPH oxidase have been reported to induce ROS production in diabetic vascular, which significantly contributes to endothelial dysfunction [9-11]. Excessive ROS production also induces a dysfunctional eNOS, call as eNOS uncoupling, caused by superoxide and nitric oxide (NO) production [9,12]. These product will reacts directly to form a more harmful molecule peroxynitrite ($ONOO^-$), thereby reducing NO bioavailability [12,13].

Previous studies were carried out to find specific agents to inhibit the NADPH oxidase activity. Peptides such as Gp91ds-tat and PR39 were shown to possess decoy p47phox binding sites, which prevent interaction between p47phox and NOX proteins, then suppress NADPH oxidase activation [14,15]. Many traditional plant products are in use due to their therapeutic potential, including garlic, ginger and green tea [16]. The Gambung Research Institute for Tea and Quinine has successfully developed a green tea with higher levels of catechin (14-16%) than other tea plant, known as GMB4 clones [17]. Previous studies showed that catechin from GMB4 clones act as insulin resistance inhibitor in visceral fats and adipose tissue [18], significantly decrease C/EBPα on the culture of visceral preadipocytes rat [19], and decreases eNOS expression, increases PI3K expression, and decreases p38 MAPK activity in rat

fed high lipid diet [20]. As far we know, there is no previous studies explore this catechin on endothelial cells. Therefore this study aimed to investigate whether green tea catechin able to modulates the level of NADPH as reducting equivalent and increase nitric oxide level in endothelial cells exposed to HG.

MATERIALS AND METHODS

Endothelial Cells Isolation and Culture

Human umbilical vein endothelial cells (HUVECs) were obtained from pregnant women at Gambiran Hospital, Kediri, East Java, Indonesia. The criteria for pregnant women are a healthy pregnancy (hemoglobin level ≥ 10 g/dl) accompanied by a section cesarean delivery (38 weeks of gestation). Immediately postpartum, 10 cm of the umbilical cord was placed in the buffer (100 ml Hank's Balance Salt Solution, gentamycine (GENTA, MERCK, Germany), sodium hydrogen carbonate, 4 ml red phenol, 2 ml 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid solution, and deionized water) and kept cold until endothelial cells were isolated. Endothelial cell isolation was performed no >12 h after delivery. For the cell isolation, umbilical veins were cleaned with alcohol to remove tissue debris and blood clots [21].

A cannula was then inserted (± 1.5 cm) at one end of the vein and secured tightly using suture. Using the cannula, veins were gently flushed with phosphate-buffered solution then closed off tightly at the distal to the cannula. Collagenase (5 mg/10 ml; 10 ml) was injected into the vein via 10 ml of syringe and incubated at 37°C approximately 8 min. The cell pellet was suspended in 4 ml 199 culture medium (SIGMA, M-5017, USA) supplemented with gentamycin, bicarbonate phenol red, 20% fetal bovine serum (GIBCO), and 20 ml newborn calf serum (SIGMA, N-4637, USA). This cell suspension was seeded into the culture wells that had been coated with gelatin. Cell were allowed to grow to confluency at 37°C 95%O_2 5% CO_2 [21].

Raw Extraction

A total of 25.1 g of green tea samples clones GMB - 4 is brewed with 500 mL of distilled water at temperature 90°C, then filtered with plastic tea strainer, which combined with cloth flannel and Buchner funnel. The process is repeated twice (using a sample that has been brewed in advance) to obtain a sample extract as much as 1.5 L.

Catechin Isolation

Extract samples were eluted into the column, the elution results then accommodated. After that, the column was eluted with 300 mL of 10% ethanol. The column was eluted with 10% ethanol 2.100 mL again, elution were done gradually with each elution of 100 mL, the elution were accommodated with a different container, then dried in a vacuum oven (fraction 10% ethanol). Subsequently, the column was eluted with 95% ethanol and 300 ml of elution results will accommodated. The column was eluted with 95% ethanol, elution was carried out gradually with each elution of 100 mL, and accommodated with containers different, then evaporate (95% ethanol fraction). The eluent that used for thin layer chromatography analysis fraction was 10% ethanol is ethyl acetate, while the eluent to 95% ethanol fraction was methanol:chloroform at ratio 1:9.

High-performance Liquid Chromatography (HPLC) analysis

Analysis of catechins by HPLC using standard methods and additional external standard. Eluent to catechins using HPLC analysis was made with aquabidest: acetonitrile: methanol: glacial acetic acid = 79.5:18:2:0.5; and for eliminating the dissolved gas is used ultrasonic bath. EGCG standard solution prepared as a comparison. Furthermore, analysis of catechins from ethanol fraction 10% done dissolving 1.3 mg of sample in 3 mL of eluent, while the fraction of ethanol 95 % made with dissolving 2.06 mg of the sample in 1 mL of eluent. Measurements were made using wavelength of 280 nm.

Endothelial Cells Treatment

Human umbilical vascular endothelial cells were cultured in 5-well dish (at 90% confluence) and grown in serum free medium for 4 h. After confluent, cells were exposed to three doses of the catechin (0.03 mg/ml, 0.3 mg/ml, 3 mg/ml) simultaneously with 30 mM glucose treatment. This catechin was isolated from GMB-4 green tea species. The negative control group of the HUVEC was not exposed to glucose (untreated group) and the positive control HUVEC was exposed to 30 mM glucose HG but not exposed to green tea catechins. We performed five replications each group.

Analysis of NO

NO levels were detected with a colorimetric method using NO Assay Kit KGE001 (R and D System, USA).

Analysis of NADPH

NADPH levels were assayed with a colorimetric method using NADPH Assay Kit ab65349. (Abcam,USA).

Ethics

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine Brawijaya University.

Statistical Analysis

Data are presented as mean ± standard deviation and differences between groups were analyzed using one-way ANOVA with SPSS 17.0 Statistical Package. The *post-hoc* test was used if the ANOVA was significant. $P < 0.05$ was considered as statistically significant.

RESULTS

Standard EGCG solution shows the presence of high peaks and large areas at a retention time was 5.153. Furthermore, the analysis of freeze dried extract, obtained seven major peaks, which indicate caffeine compound, C, EC, GC, EGC, EGCG and ECG. Fraction of 10 % ethanol shows four main peaks at retention time

of 1.663; 2.367; 2.950; and 4.890. This indicates that there are four components of catechins that the C, EC, GC, and EGC. Meanwhile, 95% ethanolic fraction found two main peaks at the time retention 5.167 and 9.82; which indicated EGCG and ECG.

Table 1 presents the levels of NADPH, NADP and NADPH/NADP$^+$ ratio in the cell culture media for each endothelial cell experimental group. The level of NADPH was significantly lower in the EC+HG group compared with the untreated control group ($P < 0.05$). Out of the 0.03 mg/ml, 0.3 mg/ml, 3 mg/ml doses of green tea catechin, only the two highest doses significantly prevented HG-induced decrease in NADPH level. There was significant no difference between the effects of these two highest doses ($P > 0.05$). In addition, the levels of NADP$^+$ and NADPH/NADP$^+$ were not significant differences between groups ($P > 0.05$).

Figure 1 presents the NO levels in the culture media from each endothelial cell experimental group. The NO levels were significantly lower in the HG group compared to the untreated group ($P < 0.05$). These decreased levels of NO in the HG group were significantly elevated by 0.3 mg/ml administration of green tea catechins. Indeed, administration of 0.3 mg/ml extract to the HG-treated endothelial cells reduced NO levels to those comparable to the untreated group.

DISCUSSION

In this study, the exposure of HG level in endothelial cells decreases the level of NADPH compared with the untreated control group ($P < 0.05$). This finding indicated that the HG level affects endothelial cells redox potential. Our finding confirming previous studies that hyperglycemia is associated with metabolic disturbances affecting cellular redox potential, particularly the NADPH/NADP$^+$ [22]. HG level exposure may induce metabolic insufficiency to support the increased metabolic demand for pentose phosphate pathway-generated NADPH [23]. There are 4 enzyme sources of cytosolic NADPH, 6-phosphogluconate dehydrogenase, malic enzyme and cytosolic NADP$^+$-dependent isocitrate dehydrogenase, and glucose 6-phosphate dehydrogenase (G6PD). G6PD regarded as the major enzyme to generate cytosolic NADPH [24,25]. Out of the 0.03 mg/ml, 0.3 mg/ml, 3 mg/ml doses of green tea catechin, only the two highest doses significantly prevented HG-induced decrease in NADPH level. There was no significant difference between the effects of these two highest doses ($P > 0.05$). Previous in silico study showed that gallated catechins, but not ungallated catechins, were NADP$^+$-competitive inhibitors of G6PD and other enzymes that employ NADP$^+$ as a coenzyme, such as IDH and G6PD [26]. In other side, catechins (and other flavonoids) isolated from green tea inhibit mammalian glucose transporters including GLUT1 located in the endothelial cells [27]. This effect was avoided by simultaneously treatment of cathecin and glucose. Furthermore, catechin were potent radical scavengers and provided protection against intracellular oxidative stress induced by hydrogen peroxide [28].

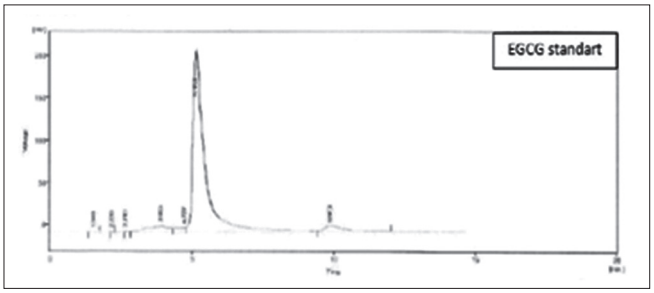

Figure 1: Level of nitric oxide endothelial cells induced by high glucose (HG). [a]$P < 0.05$ in comparison with control group; [b]$P < 0.05$ in comparison with HG group; NO: Nitric oxide, μM: Micromolar

The NO levels were significantly lower in the HG group compared to the untreated group ($P < 0.05$). These decreased levels of NO in the HG group were significantly elevated by 0.3 mg/ml administration of green tea catechins. This study confirming previous studies that treated with catechin increases the NO_2^-/NO_3^- production and not induces NO scavenging [29,30]. The mechanisms of NO production may be due to availability tetrahydrobiopterin as a co-factor for NO production. The presence of NADPH act as reducing equivalent for endogenous antioxidant defense. The integrity of endogenous antioxidant will inhibit oxidation of tetrahydrobiopterin, resulting in high NO bioavailability. Unfortunately, highest dose induces losing of NO bioavailability may be due to pro-oxidant effect of catechin. Catechin gallates has been found to produce H_2O_2 *in vitro* and in cell culture systems, where catechin reacts with dissolved oxygen in aqueous solution, and generates H_2O_2 [31,32].

CONCLUSION

Catechin isolated from GMB-4 green tea suppresses HG-induced reducing NADPH and NO, suggesting that this catechin may be a potential candidate for the treatment and prevention of diabetic vascular complications.

ACKNOWLEDGMENT

The author acknowledged to all technician in Laboratory of Physiology for supporting this study.

Table 1: Level of NADP and NADPH endothelial cells induced by HG

Level	Control	HG	HG+catechin		
			0.03 mg/ml	0.3 mg/ml	3 mg/ml
NADPH ($\times10^{-9}$ M)	15.47±1.58	5.16±4.40[a]	10.05±8.95	23.08±2.62[abc]	18.22±2.33[b]
NADP+ ($\times10^{-9}$ M)	13.60±2.89	8.91±1.78	13.53±8.66	20.44±8.06	21.68±1.99
NADPH/NADP$^+$	1.18±0.31	0.57±0.43	0.73±0.70	1.32±0.66	0.84±0.13

Values are presented as mean±SD, [a]$P<0.05$; in comparison with control group; [b]$P<0.05$; in comparison with HG group, [c]$P<0.05$; in comparison with first dose administered group, HG: high glucose, NADP: nicotinamide adenin dinucleotide phosphate, NADPH: reduced nicotinamide adenin dinucleotide phosphate; mg/ml: Miligram/mililiter; pmol: picomolar, SD: Standard deviation

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Original Research

GESDAV

Preliminary evaluation of hepatoprotective potential of the polyherbal formulation

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ABSTRACT

Aim: The aim of this study was to investigate the antioxidant and hepatoprotective effects of the polyherbal formulation (PHF)containing *Cajanus cajan* (L.)Millsp., *Lawsonia inermis* L. Linn, *Mimosa pudica* L., *Uraria picta* (Jacq.)DC. and *Operculina turpethum* (L.)Silva Manso on carbon tetrachloride (CCl_4)induced acute liver damage in albino rats. **Materials and Methods:** The groups of animals were administered with PHF at the doses 100, 200 and 400 mg/kg b.w. (per oral [p.o.])once in a day for 7 days and at day 6th and 7th the animals were administrated with Carbon tetrachloride (1.0 mL/kg b.w. 50% v/v with olive oil,; p.o.). The effect of PHF on serum glutamine pyruvate transaminase (SGPT), serum glutamine oxaloacetate transaminase, alkaline phosphatase (ALP)and total bilirubin were determined in CCl_4 - induced hepatotoxicity in rats. Further, the effects of PHF on glutathione (GSH), superoxide dismutase (SOD)level and lipid peroxidation (LPO)activity were also investigated. **Results:** The results demonstrated that PHF (400 mg/kg b.w.)significantly reduces the CCl_4 induced increase in level of serum SGPT, serum ALP and total bilirubin. PHF (400 mg/kg b.w.)prevents the depletion level of GSH and decrease in the activity of SOD in CCl_4 -induced liver injury in rats. In addition, PHF also showed a significant decrease in the LPO levels signifying the potent antioxidant activity. **Conclusion:** All our findings suggest that PHF could protect the liver cells from CCl_4 - induced liver damages and the mechanism may be through the anti-oxidative effect of PHF.

KEY WORDS: Antioxidant, carbon tetrachloride, hepatoprotective, a polyherbal formulation

INTRODUCTION

Liver regulates the various physiological processes of human body and plays a magnificent role in the metabolism of endogenous and exogenous agents. It has great capacity to detoxify toxic substances and synthesize useful metabolites. Indiscriminate use of certain therapeutic drugs such as paracetamol, anti-malarial drugs, anti-tubercular drugs, oral contraceptives, analgesics, antidepressants, anti-arrhythmic drugs and toxic substance (carbon tetrachloride [CCl_4], thioacetamide, aflatoxin)etc. are threatening the integrity of the liver. Uncontrolled consumption of alcohol, various infections and some autoimmune disorder are also facilitating hepatic damage [1]. These hepatotoxic agents are one of the leading causes for hepatitis, cirrhosis, liver cancer and at last death [2]. Overproduction of the reactive oxygen species (ROS)due to unnecessary exposure of toxic chemicals and depletion of antioxidant defense mechanisms attribute toward oxidative stress and culminating into severe hepatic injury [3].

A variety of medicinal plants are used for the treatment of the liver diseases in various systems of medicine [4-8]. Plants contain many phyto-constituents, but sometimes the individual phyto-constituent may not be enough to achieve the desired therapeutic effect. Therefore, the polyherbal formulation (PHF) was prepared in order to enhance the therapeutic effectiveness and improve the bioavailability. Besides synergism attenuates, reduction in undesirable side-effects is a key benefit of the formulation.

Operculina turpethum (L.)Silva Manso is a stout perennial climber which is reported to posses hepatoprotective, anticancer, antioxidant, antisecretory, ulcer protective, and antimicrobial activity [9].

Mimosa pudica L. is reported to possess wound healing, antimicrobial, analgesic, anti-inflammatory, anticonvulsant, antidiarrhoeal, antifertility, anti oxidant, an anti-hepatotoxic and anthelminthic activity [10].

Lawsonia inermis L. is much branched, deciduous, glabrous, sometime spinescent shrub. It possess hypoglycemic, nootropic, antimicrobial, antioxidant, cytotoxic and immunomodulatory activity [11].

Uraria picta (Jacq.)DC. is reported to have acaricidal [12]and anti-inflammatory [13]activity.

Cajanus cajan (L.)Millsp posses antimicrobial, hepatoprotective, hypocholesterolemic and analgesic activity [14].

In the present study, a PHF consisting *C. cajan* (L.)Millsp., *L. inermis* L., *M. pudica* L., *U. picta* (Jacq.)DC. *and O. turpethum* (L.)Silva Manso has been formulated and evaluated for hepatoprotective activity against CCl_4 induced liver toxicity in rats. All the plants of the formulation are taken from the Ayurvedic classical text "Chakradatta," where these are mentioned as ingredient of Ayurvedic formulation "Brihad Panchgavya Ghrit" indicated for jaundice [15].

MATERIALS AND METHODS

Materials

The plants materials *C. cajan* (Whole plant, 1 part), *O. turpenthum* (Root, 2 part), *M. pudica* (Root, 1 part), *U. picta* (Root, 1 part)and *L. inermis* (Leaves, ½ part)were collected from the Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh. The botanical authentication of the specimens was done by Dr. Anil Kumar Singh, Professor, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Science, Banaras Hindu University; Varanasi, India. For further reference the voucher specimens (APRL/HERB/12-13/112-116)of plant materials were deposited in Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh, India.

Preparation of Formulation

The shade dried plant material was coarsely powdered separately by using a mechanical grinder then mixed in a definite proportion. Extraction was performed with 98% methanol by cold maceration technique. After that extract was concentrated under vacuum evaporator and the dried extract was obtained. The dry polyherbal extract was suspended in 5% carboxymethyl cellulose solution before oral administration to animals.

Drug and Chemical

CCl_4 (Central drug house, New Delhi), Pentobarbitone (Ranbaxy India Pvt. Ltd., Mumbai, Maharastra, India), silymarin (Ranbaxy India Pvt. Ltd., Mumbai, Maharastra, India), NADH (Sisco Research Laboratories Pvt. Ltd., Mumbai, Maharastra, India)and NBT (Sisco Research Laboratories Pvt. Ltd., Mumbai, Maharastra, India)were used for the experimental purpose. All the reagents used were of analytical grade. Biochemical estimation kits (Span Diagnostic Surat, Gujarat, India)were used for serum glutamine pyruvate transaminase (SGPT), serum glutamine oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin estimation.

Preliminary Phytochemical Analysis (Qualitative Analysis)

PHF was subjected to preliminary phytochemical screening for the detection of the presence of various phytoconstituents like alkaloid, tannin, saponin, phenolics etc [16].

Hepatoprotective Activity of PHF

Animals

Adult Charles foster albino rats (140 ± 20 g)of either sex were procured from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University (Registration No-542/AB/CPCSEA); Varanasi. The animals were kept in a temperature-controlled room (22 ± 2°C)with humidity (55 ± 10%)and 12 h light and 12 h dark cycle. The animals were provided with standard pelleted feed (Amrit Pvt., Ltd., Pune, Maharastra, India)and fresh water *ad libitum*. Rats were kept at standard laboratory environment for at least 1 week before the experiment. The study has been approved by the Institutional Animal Ethical Committee (Dean/13-14/CAEC/318).

Acute toxicity study

The acute oral toxicity study was performed as per the OECD guideline 425 (2008)[17]. The 18 h fasted rats were divided into 3 groups, each group consisting of 3 animals. The PHF was given in various doses (1000, 2000 and 4000 mg/kg of b.w.)per oral (p.o.). The signs and symptoms of toxicity or any abnormalities coupled with administration of PHF were observed at every 30 min for the first 2 h, then at every one hour for the next two hours. Observation was continued for next 14 days, once a day. Any behavioral changes such as convulsion, salivation, dizziness, diarrhea, sleep and coma were noticed. Any death within 14 days was also noted.

CCl_4 induced liver toxicity

Overnight fasted thirty-six adult rats were divided into six groups of six animals in each group. Group I and II received normal saline for seven days. Group III was treated with standard drug silymarin (100 mg/kg b.w.), Group IV, V and VI were treated with PHF 100, 200 and 400 mg/kg b.w. respectively for seven days. All the animals except the group I were treated with CCl_4 (1.0 mL/kg b.w. 50% v/v with olive oil,; p.o.)on 6th and 7^{th} day [18].

Estimation of biochemical markers

After 24 h of the last administered dose, the rats were anesthetized using ether and sacrificed by cervical dislocation. Blood sample was collected by cardiac puncture method in a heparinized 1.0 mL tuberculin syringe and serum was separated for the estimation of biochemical parameters. The biochemical parameters were properly estimated with standard procedure by the diagnostics kits [19].

Histopathological examination

From sacrificed animals, liver was taken out and cut into small pieces and separated and weighed accurately. The liver was washed with normal saline and was preserved in formalin solution (10%). After fixation, processing of livers was done with routine histopathologic procedure and embedded in paraffin wax. Several 4-5 μm sections of each liver blocks were stained with hematoxylin and eosin. The sections were examined under high-resolution microscope [18].

Assay of antioxidant activity

The level of lipid peroxidation (LPO)were estimated and expressed in terms of malondialdehyde (MDA)as per the method of Ohkawa *et al*. 1979 [20]The activity of superoxide dismutase (SOD)were estimated as per procedure of Kakkar *et al.* (1984)[21]based on reduction of NBT to blue colored formazan in presence of phenazine methasulfate. The levels of glutathione (GSH)were expressed as μmol/g of wet tissue after estimation as per method of Sedlak and Lindsay, 1968 [22].

Pentobarbitone induces seep test

The animals were divided into five groups with 6 animals in each group. All groups were given pentobarbitone (45 mg/kg b.w., i.p.) after 2 h of administration of CCl_4 (1.0 mL/kg b.w. 50% v/v with olive oil; p.o.). Group II was treated with standard drug silymarin (100 mg/kg body weight p.o.). Group III, IV and V receive PHF 100, 200 and 400 mg/kg b.w.; p.o. respectively. The latency and duration of the sleeping time was noted in the each group [23].

Stastical Analysis

All results are expressed as mean ± standard error of the mean (n = 6 in each group). Statistical comparison was done by one-way ANOVA, followed by the Tukey's multiple comparison tests using Graph Pad Prism Software Version 5.01 (Fay Avenue, La Jolla, CA, USA).

RESULTS

Preliminary Phytochemical Analysis

Preliminary phytochemical screening shows the presence of flavonoids, tannins, saponins, steroids in the methanol extract [Table 1].

Table 1: Preliminary phytochemical investigation

Chemical test	Methanol extract
Test for alkaloids	−
Test for carbohydrates	−
Test for flavonoids	+
Test for tannins (phenolic compound)	+
Test for saponins	+
Test for steroids	+
Test for protein	−

+: Present, −: Absent

Acute Toxicity Study

No mortality or any behavioral changes were observed at all the doses 1000, 2000 mg/kg b.w. and 4000 mg/kg b.w. in the acute toxicity study. Hence, the PHF was considered to be safe up to 4000 mg/kg b.w. Therefore, doses of 100, 200 and 400 mg/kg b.w. were selected for the pharmacological studies.

Effect of PHF on Serum Biochemical Levels

The animals treated with CCl_4 (1.0 mL/kg)showed significant ($P < 0.05$)increase in the level of serum enzymes SGPT, SGOT and ALP when compared to the normal control group. The animals treated with silymarin as well as PHF 400 mg/kg showed significant ($P < 0.05$)reduction in the level of SGPT, SGOT and ALP when compared to the CCl_4 treated group. PHF 400mg/kg showed significant ($P < 0.05$)reduction level of serum enzymes SGPT, SGOT and ALP when compared to the PHF 100 and PHF 200mg/kg [Figure 1a-c].

The animals treated with CCl_4 showed significant ($P < 0.05$) increase in the level of total bilirubin and direct bilirubin as compared to the normal control group. The animals treated with silymarin, as well as PHF 400 mg/kg, showed significant ($P < 0.05$)reduction in the level of total bilirubin and direct bilirubin as compared to the CCl_4 treated group. PHF in the dosages of 100 and 200 mg/kg p.o. showed insignificant reduction in the level of total bilirubin and direct bilirubin as compared to animal treated with silymarin. PHF in the dosage level of 400 mg/kg showed significant ($P < 0.05$)reduction level of total bilirubin and direct bilirubin as compared to the PHF 100 and PHF 200 mg/kg [Figure 1d and e].

In vivo Assay of Antioxidant Activity

Effect of PHF on LPO level

The animal treated with CCl_4 showed significant ($P < 0.05$) increase level of LPO as compared to the normal control group. Silymarin treated group, PHF 200 and PHF 400 mg/kg showed significant ($P < 0.05$)reduction in the level of LPO as compared to the CCl_4 treated group. At the dosage level of PHF 100 mg/kg, it showed insignificant ($P < 0.05$)reduction in the level of LPO as compared to the silymarin treated group [Figure 2a].

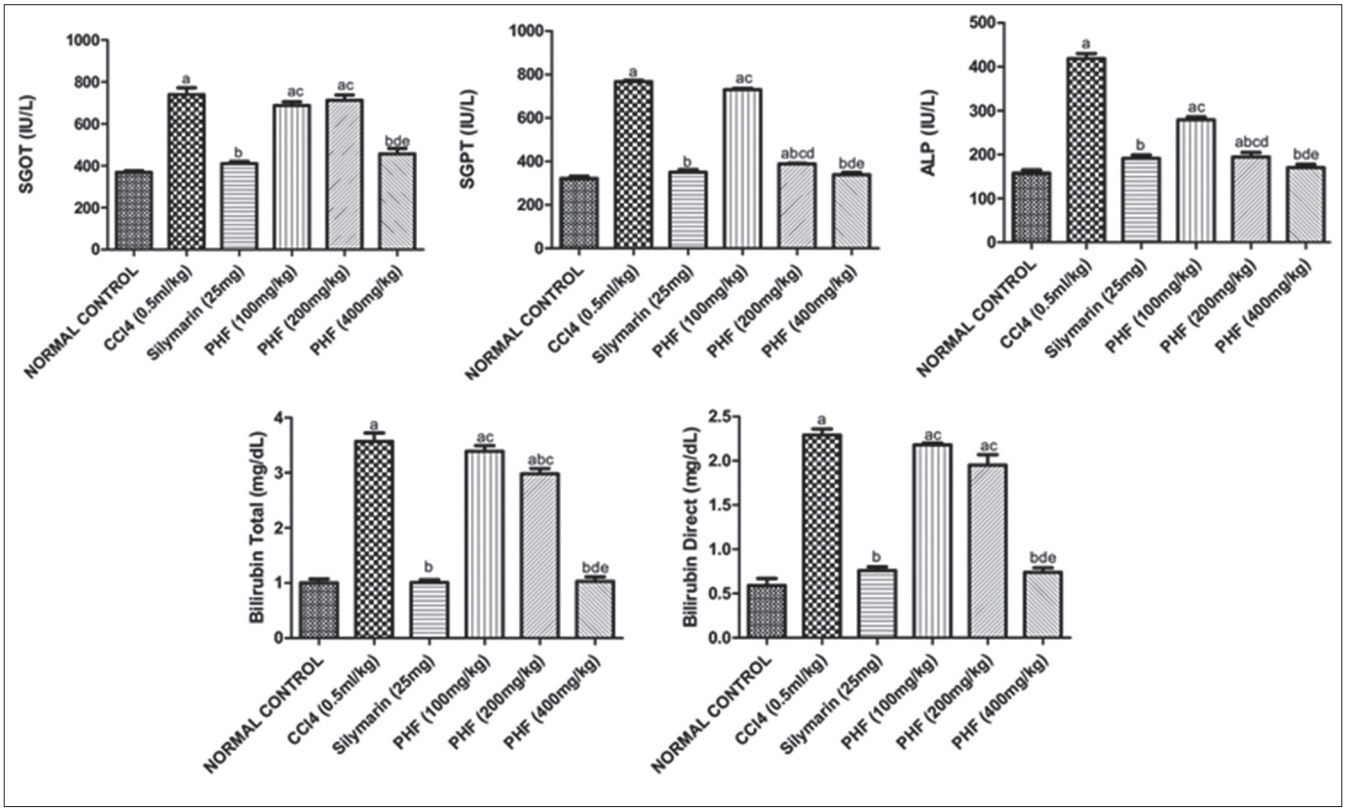

Figure 1: Effect of polyherbal formulation on biochemical parameters in carbon tetrachloride induced hepatic injury in rats

Effect of PHF on SOD level

The animal treated with the CCl_4 showed significant ($P < 0.05$) reduction in activity of SOD as compared to the normal control group. The standard drug silymarin and animals treated with test group PHF 100, PHF 200, PHF 400 mg/kg, p.o. showed significant ($P < 0.05$)increase in activity of SOD as compared to the animal treated with CCl_4 [Figure 2b].

Effect of PHF on GSH level

The animal treated with the CCl_4 showed significant ($P < 0.05$)decrease in the level of GSH as compared to the normal control group. The standard drug silymarin, as well as PHF, showed significantly ($P < 0.05$)increase in the level of GSH as compared to the CCl_4 treated group [Figure 2c].

Histopathological Result

Figures 3a and b shows the liver cells of rat in the normal control group. Liver cells were normal in shape and size with prominent nucleus. Cordlike arrangement of liver cells which was separated by sinusoids and central vein (CV)is clearly visible.

CCl_4 - treated animal's liver histopathology [Figure 3c and d] shows that the structural design of liver was totally damaged as compared to normal rats. Prominent cell vacuolation, pyknotic and degenerated nuclei along with damaged bile capillaries were observed. Cell lysis and aggregation of nuclei was also visible at some places. Wide spaces were formed at some sinusoids. These cellular changes were greatly reduced in CCl_4 with silymarin treated groups. A healthy population of hepatocytes interspersed with patches of mild necrosis was observed [Figure 3e and f].

In the liver cells of rats treated with PHF 400 and intoxicated with CCl_4 [Figure 3k and l], the nucleus were not very clear as in normal hepatocytes, but as compared to the CCl_4 treated group the number of hepatocytes with normal nucleus were much more. Cell vacuolation and pyknotic nuclei were observed to be low. Endothelium was disrupted at places and hepatic cells adjoining to intralobular vein shows atrophy. The overall architecture of the liver appears to be normal. The liver of PHF 100 [Figure 3g and h]and PHF 200 [Figure 3i and j]treated rats showed insignificant reversal of pathological alterations done by CCl_4.

Effect of PHF on Pentobarbitone Induced Sleeping Time

The animals treated with CCl_4 and pentobarbitone (45 mg/kg i.p.)showed a significant increase in sleeping time as compared to animals treated with only pentobarbitone. The animals treated with standard drug silymarin and test drugs PHF 200, 400 mg/kg showed significant ($P < 0.05$)reduction in the duration of sleep as compared to the CCl_4 treated group [Figure 4].

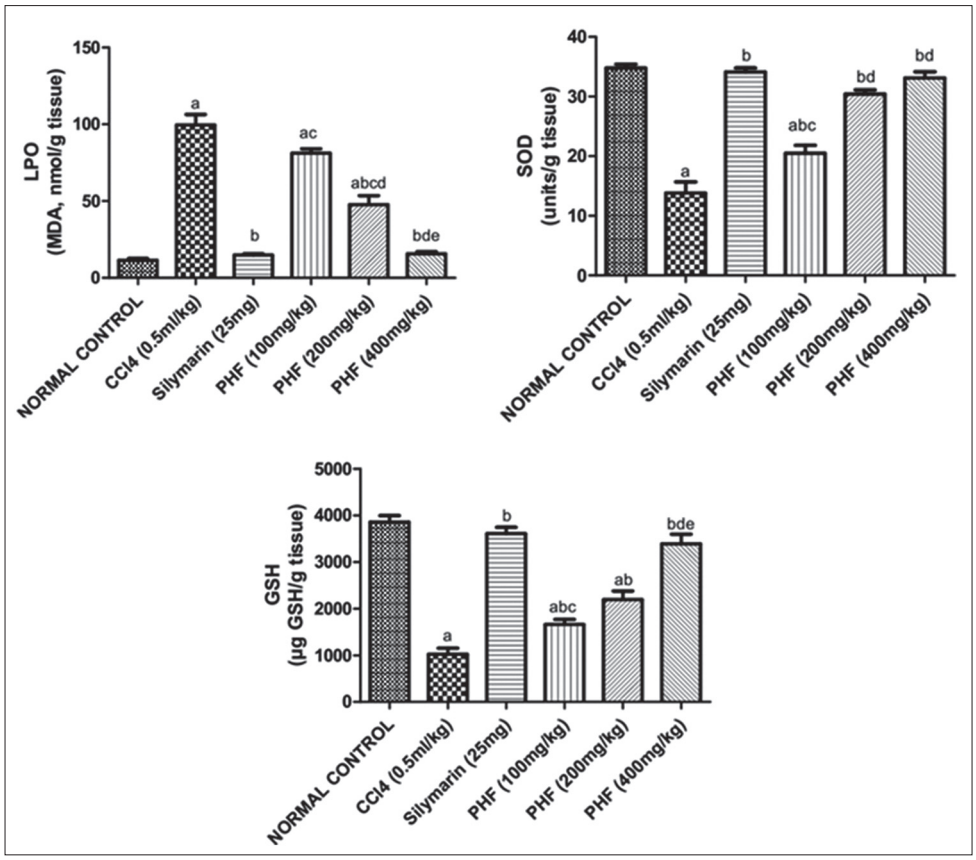

Figure 2: Effect of polyherbal formulation graded dose on the levels of lipid peroxidation, superoxide dismutase and glutathione in carbon tetrachloride induced hepatic injury in rats

DISCUSSION

In today's world liver diseases become a global health problem, lacking helpful curative approach. There are so many plants that are used as a hepatoprotective agent in traditional medicine systems [2,18,19,23]. It is, therefore, necessary to assess the scientific basis for the reported hepatoprotective activity of herbal drugs in the form of PHF. In this connection, a polyherbal formulation was prepared and evaluated against CCl_4 induced liver toxicity in rats.

It has been established that CCl_4 is metabolically activated by cytochrome P_{450} - dependent mono-oxygenases to form highly reactive free radical metabolites, tri-chloro-methyl free radical ($CCl_3^{\bullet}$)which later convert into more toxic tri-chloro-methyl-peroxyl radical (CCl_3OO^-)in presence of oxygen. The same is capable to produce disturbance in the transport function of the hepatocytes which leads to leakage of enzymes (SGOT and SGPT)from cells, hyper bilirubinaemia as well as rise in the level of serum ALP [24]. The result of the present study reveals that the pre-treatment with PHF antagonizes elevated enzyme parameters. The tendency of these enzymes to return towards the normal range in the PHF administered group was clearly indicating that the PHF 400 mg/kg challenge to protect liver tissue from CCl_4 injury. It was reported and accepted that serum levels of SGOT and SGPT return to normal with the healing of liver parenchyma and the regeneration of hepatocytes [25].

In the agreement with other reports, CCl_4 treated rat's recorded significant decrease in hepatic non enzymatic (GSH) and enzymatic (SOD)antioxidant markers [18]. Therefore, reduction in free radicals generation is a possible mechanism in the defense of the liver against different injuries. In this study dramatic increase in LPO after CCl_4 treatment suggested that natural antioxidant defense mechanism to scavenge excessive free radicals has been compromised [26]. Prophylactic treatment with PHF significantly inhibited the formation of MDA in the liver. These findings are in accordance with previous reports for other hepatoprotective agents [19].

In the present study, the PHF (400 mg/kg)showed a potential *in vivo* antioxidant activity as it elevates the reduced levels of liver cytosolic SOD and GSH. These antioxidant enzymes are involved in the reduction of ROS and peroxidase produced in the living organism thus play a vital role in the maintenance of a balance redox status. The restoration of the SOD towards a normal value indicates that the PHF can help in cellular defense mechanisms by preventing cell membrane oxidation. The PHF also restores some vital molecules such as NAD, Cytochrome, and GSH as indicated by increased peroxidase activity [27].

Although barbiturate sleeping time is not a direct measure of hepatic injury but it shows the status of the liver. It has been established that the phenobarbitone induced sleeping time is

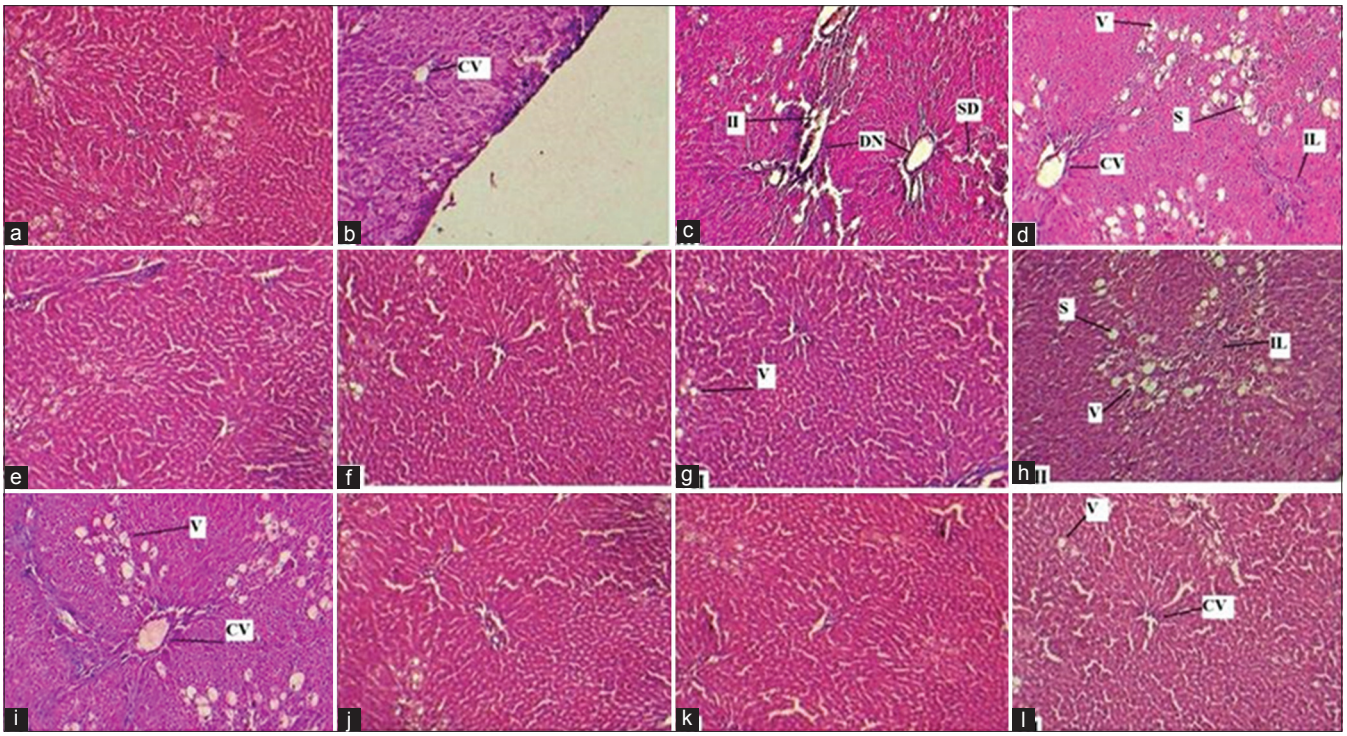

Figure 3: Photomicrographs of liver sections from: (a and b) Normal control group; (c and d) Carbon tetrachloride (CCl_4) (1:1 in olive oil) 1 mL/kg i.p., (e and f) silymarin (100 mg/kg) + CCl_4, (g and h) polyherbal formulation (PHF) 100 mg/kg + CCl_4, (i and j) PHF 200 mg/kg + CCl_4, (k and l) PHF 400 mg/kg + CCl_4, CV: Central vein, SD: Sinusoidal dilatation, V: Vacuolation, S: Steatosis, IL: Infiltration of lymphocytes, DN: Degenerated nuclei, II: Inflammatory infiltration

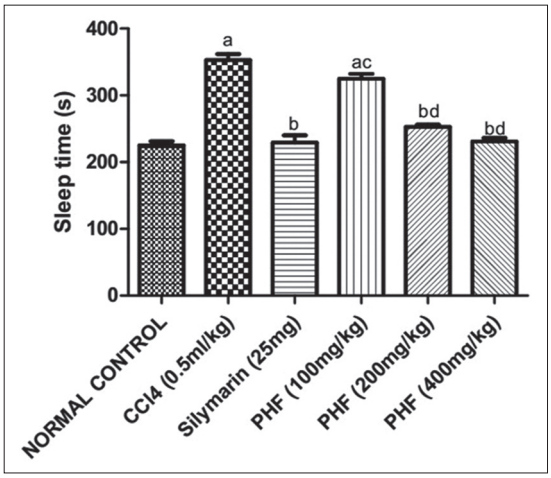

Figure 4: Effect of polyherbal formulation graded dose on the duration of barbiturate induced sleep in carbon tetrachloride induced hepatic injury in rats

a measure of hepatic metabolism since the barbiturates are metabolized primarily in the liver [28]. If there is any existing liver damage by CCl_4 intoxication, the amount of the hypnotic metabolized per unit time will be less, which results in prolonged sleeping time after the given dose of barbiturates [23]. In this study, there is a considerable reduction and restoration of Pentobarbitone sodium-induced sleeping time after PHF administration i.e. PHF was effective in regulating the level of microsomal drug metabolizing enzymes in the liver. This outcome supported the use of this formulation in the chronic hepatic diseases.

The experiential hepatoprotective activity of the PHF may be on account of the presence of polyphenolic compounds found in the preliminary phytochemical screening [Table 1] as these compounds are reported to have the free radical scavenging ability, which stabilizes lipid oxidation. Flavonoids correspond to a group of polyphenolic compounds, which exhibits a wide range of biological activities primarily due to their antioxidant property, probably due to their free radical scavenging activity and ability to reduce free radical formation [29]. Some studies suggest a correlation between phenolic content and hepatoprotective activities [30]. Saponins present in the PHF are also effective against hepatotoxins [25]. Report also indicates that some steroids may be responsible for hepatoprotective effect [31].

The biochemical findings were also confirmed by histopathological observation. Histopathological studies under light microscope confirm the curative efficacy of PHF against CCl_4 induced liver damage. Vacuolated hepatocytes along with fatty deposition, necrosis and degenerative changes were observed in CCl_4 treated rats. In this experimental group infiltration of inflammatory cells in the CV was also evident. This could be due to the formation of highly reactive free radicals because of oxidative stress caused by CCl_4. These set of changes have also been reported by other researchers following CCl_4 treatment [32].

This severity of toxic effects was much less when seen in sections treated with PHF and was comparable to silymarin. Simultaneous treatment of PHF with CCl_4 exhibits less damages to the hepatic cells as compared to the rats treated with CCl_4 alone. Intralobular veins were found to damaged but to a lesser extent. Endothelium is disrupted at places. Hepatic cells are adjoining to Intralobular vein show atrophy. Pyknotic nucleus and cell vacuolation are observed to be low. The correlation between liver biomarkers and histopathological changes suggested that they could be used for early detection of acute liver damage. Reduction of biochemical and histological damages exerted by PHF confirms its hepatoprotective potential.

Results indicated improvement in metabolic activity and cellular stability. On the whole hepatoprotective effect of PHF is most likely due to antioxidant action. This data provide a primary base for the use of these five plants in the form of an herbal preparation for the treatments of hepatic disorders.

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Original Research

Improvement of sperm density in neem-oil induced infertile male albino rats by *Ipomoea digitata* Linn

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ABSTRACT

Aim: Investigation has been carried out to validate folkloric claim of the potential of *Ipomoea digitata* (ID) based on reproductive health status in experimentally induced male albino rats. **Materials and Methods:** Emulsified neem oil fed albino rats were orally administered root powder of ID suspended in water for the doses of 250 and 500 mg/kg body weight for 40 days. Change in organ weight, sperm density and motility, serum hormonal levels and histomorphological changes were evaluated. **Results:** Significant increase in the sperm density and the sperm motility ($P < 0.01$) along with increase in the testis, and epididymes weight in neem-oil induced infertile rats treated with ID at both dose levels. This effect is vis-à-vis to serum hormonal levels. Presence of β-sitosterol in the root of ID likely to enhance the process of spermatogenesis as it is evident from histomorphological studies. **Conclusion:** Results of the present investigation reveal that ID is a good candidate for the management of male infertility.

KEY WORDS: *Ipomoea digitata*, male infertility, oligozoospermia, sperm density, testosterone

INTRODUCTION

Ipomoea digitata Linn. (ID) is a well-known medicinal plant used in Ayurveda for its health promoting effects. ID, a member of family Convolvulaceae is known as Ksheera-vidaari, Ksheervalli, Payasvini, Swaadukandaa, Ikshukandaa, Gajavaajipriyaa, Kandapalaasha, Bhuumikuushmaanda in Sanskrit; Bilai-khand, Bidarikand in Hindi; Bhui-kohala, Pattana in Marathi and Gujarati; Bhumikumra, Bhuikumra in Bengali; Matta-paltiga, Nelagummudu in Telugu; Phalmodika, Nelli-kumbala in Tamil; Mothalkanta, Palmodikka in Malyalam and Milky Yam in English. Its synonyms are *Ipomoea paniculata* (R.Br.); *Convolvus paniculata* (Linn.); *Batatas paniculata* (Choisy) and *Ipomoea mauritiana* (Jacq.). Ayurveda described it in Bhav-Prakash Nighantu as, [1].

विदारी स्वादुकन्दा च सा तु क्रोष्ट्रीसिता स्मृता
इक्षुगन्धा क्षीरवल्ली क्षीरशुक्ला पयस्विनी १८०
विदारी मधुरा स्निग्धा बृंहणी स्तन्यशुक्रदा १८१

Root is a tonic, alternative, aphrodisiac, demulcent, galactagogue, mucilaginous and has a bitter taste [2]. Flour of raw rhizome of this plant is given in enlargement of liver and spleen, also for menorrhagia, debility and fat accumulation [3]. The tuberous root of ID bend with other plants part used in spermatorrhea. Aphrodisiac activity of tuberous root of ID was documented earlier [4]. Tuberous root contains a resin (similar to Jalap resin), sugar, principally starch and β-sitosterol. Carbohydrates, glycosides, proteins and amino acids, saponins, alkaloids, flavonoids, phytosterol, gum and mucilage are present in the aqueous extract of tuberous root [5]. Primary and secondary metabolites except saponins in root powder of ID were also reported [6].

Low sperm density and motility are major causes of male infertility. Common male infertility factors include azoospermia (no sperm cells are produced except obstructive azoospermia), asthenozoospermia (decreased motility of sperm) and oligozoospermia (few sperm cells are produced) [7]. A number of researchers focused this issue for establishing the appropriate reason for declination in sperm density and seminal volume worldwide in last five decades [8,9]. Sperm produce controlled concentration of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and nitric oxide, which are needed for fertilization. However high concentrations of these free radicals can directly damage sperm cells and ultimately decline sperm density [10-12]. High levels of ROS in semen

have been correlated with reduced sperm motility and damage to sperm nuclear DNA [13].

Medicinal plants play an important role in the development of potent therapeutic agents. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine [14]. Inadequate information is available about the ID in the literature regarding use of its root to elevate sperm density in experimental animals. Therefore, in the present study an indigenous medicinal plant, ID was investigated for its use in increasing sperm density.

MATERIALS AND METHODS

Plant Material and Authentication

In the present study, ID was collected from Satpura ranges and authenticated at the Department of Botany, SSVPS College, Dhule (Maharashtra). Filtered neem oil was bought from the local market.

Animals

Healthy, sexually mature male and female white albino rats (*Rattus norvegicus*) weighing between 240 and 260 g were selected. They were kept in a well-ventilated animal house and fed with commercial rat feed purchased from Prashant traders, Pune (Maharashtra) India. They were allowed unrestricted access to clean tap water *ad libitum*.

Preparation of Root Powder

Tuberous root of ID [Figure 1] was sliced and dried under shed. The dried pieces were then pulverized using an electric blender. The powdered material stocked in a plastic container.

Selection of Animals, Grouping and Treatment

Sexually mature two female rats and one male albino rat were placed in one cage. Presence of vaginal plug was taken as the day one of pregnancy. The females allowed to delivered pups and pups were counted and observed their growth. Such male rat is identified and considered fertile. Thus they were selected for this experimentation. Twenty-four proved fertile male rats were divided into four groups. Each group contains six rats. Group I (control group) animals, received 5 ml of the "vehicle" (distilled water). Group II animals, received emulsified neem oil (ENO) (ENO 0.5 ml + 4.5 ml Distilled water) for 15 days. Group III and Group IV animals, received ENO for 15 days and a day gap were treated with ID root powder suspended in water twice daily at the dose of 250 mg/kg and 500 mg/kg body weight respectively for 40 days using feeding needle. Experimental rats were allowed free access to rat feed and tap water *ad libitum*. All the animal experimentation was carried out under the guidelines of Institutional Animal Ethical Committee (IAEC) of CPCSEA, India. (No: IAEC/08/CPCSEA/MJ/2010).

Figure 1: Sliced *Ipomoea digitata* root

Experimentation

The day after their daily doses for 15 and 55 days for Groups II and III to IV, the blood was collected from the retro-orbital plexus after treatment. After clotting of blood, the serum was collected and stored at 8°C in refrigerator. Experimental rats were dissected. The epididymes were separated from the testes by blunt dissection. They were weighed separately. The epididymes were cut open longitudinally and with gentle pressure on the serosa, a drop of semen was expressed on a pre-warmed slide (37°C). A drop of 2.9% sodium citrate buffer was added to the expressed semen drop and cover-slip was applied to evaluate motility under ×40 of microscope. Semen examinations were done using methods described by Zemjanis [15]. Following separation of the epididymes, the testicles were fixed in 10% formaldehyde saline in labeled bottles and processed routinely for histological examinations. This was later observed using Olympus (model-41) research microscope at ×40, ×100 and photomicrographs were taken. The serum testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined on the VIDAS instrument using the enzyme linked fluorescent assay kits of BioMerieux (France) according to the methods of Wide, Bardin and Butt [16-18].

Statistical Analysis

The treated groups were compared to control by One-way ANOVA with Dunnett's post-test was performed using GraphPad Prism Demo version 4.00 for Windows, GraphPad Software, San Diego California USA, was used. Differences were considered significantly when $P < 0.05$.

RESULTS

Treatment with ENO to male albino rats (Group-II) for 15 days, a significant reduction was seen in almost all the parameters *viz*. testes and epididymes weight [Figure 2], sperm density and motility, serum levels of testosterone, FSH and LH compared with control group rats. Previous studies on acute toxicity of ID allowed us to take the dose levels of 250 and 500 mg/kg body weight (b.w) [5,19].

Treatment with ID (Group III and IV) at both doses to male albino rats for 40 days results in the significant increase in all the parameters viz. testes and epididymes weight [Figure 2]; sperm density and sperm motility; serum levels of testosterone, FSH and LH compared with control group rats. Change (%) was calculated and shown in [Table 1].

Testis of albino rat treated with ENO shows arrest of spermatogenesis, derangement of germinal epithelial along with blood clotting [Figure 3]. Testis of albino rat treated with ID shows spermatozoa and sperm bundle illustrates restoration of the process of spermatogenesis [Figure 4].

DISCUSSION

Long term anti-fertility effect of neem oil was noted in rats [20]. Our findings in neem oil treated group evaluate the reduction in sperm density and motility was resulted due to arrest of spermatogenesis. Decrease in sperm volume, sperm motility and serum testosterone level after administration of *Azadirachta indica* stem bark extract was also observed in rat [21]. Histopathological studies showed changes like disruption to spermatogenesis in some seminiferous tubules included derangement of the first layer of spermatogonial cells and necrotic spermatocytes of male rats treated with commercial neem (*A. indica Juss*) extract for 25 days [22]. These results are in accordance with our findings [Figure 3]. Albino rats treated with ENO for 15 days subsequently with ID showed high degree of integrity of Leydig cells with seminiferous tubules having spermatogonia, primary and secondary spermatocytes, Sertoli cells along with nucleus and bunches of maturing late spermatids [Figure 4]. Spermatozoa and sperm bundles in the lumen of seminiferous tubules are the evident of the restoration of the process of spermatogenesis. It has been postulated, however, that reproductive organ weight and function such as testes, epididymes and seminal vesicles, are closely regulated by androgens [23]. Improvement in body weight is generally attributed to steroid genesis and is a biological indicator for effectiveness of the herbal drugs in improving the genesis of

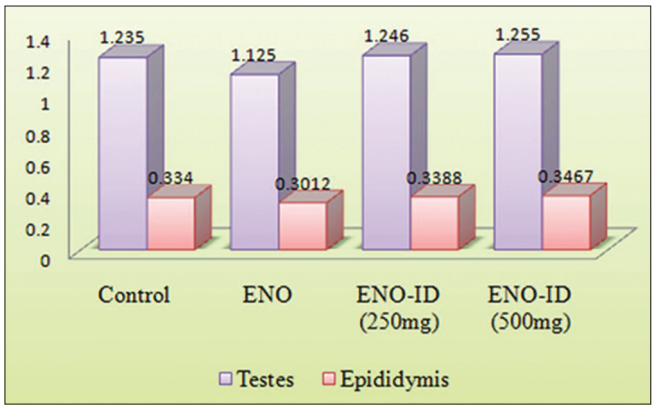

Figure 2: Regain in reproductive organs weight (g) of male albino rats. X-axis represents weight and Y-axis represents treatment groups

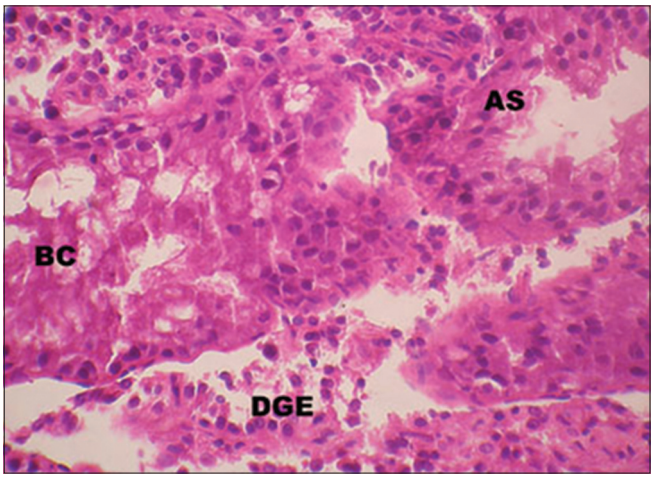

Figure 3: Photomicrograph of testis treated with ENO shows arrest of spermatogenesis (AS), derangement of germinal epithelium (DGE) with blood clotting (BC). Five microns tissue sections stained with hematoxylin and eosin

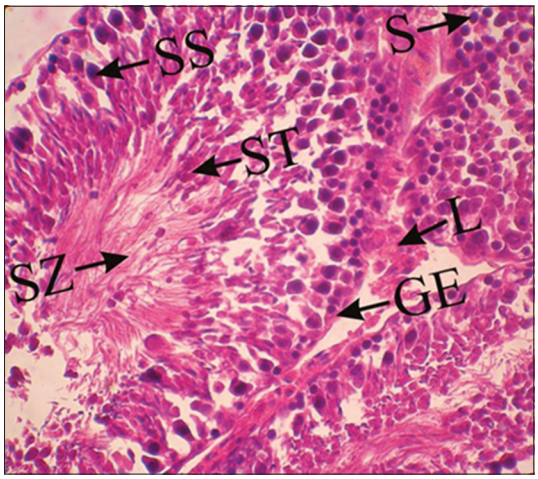

Figure 4: Photomicrograph of testis treated with ID shows restoration of the process of spermatogenesis. Tissue sections stained with hematoxylin and eosin. S: Spermatogonia; SS: Secondary spermatocyte; ST: Spermatid; SZ: Spermatozoa; GE: Germinal Epithelium and L: Leydig cells.

Table 1: Regain in sperm density, motility and serum hormonal levels of male albino rats

Parameter	Control	ENO	ENO-ID (250 mg)	ENO-ID (500 mg)
Sperm density (million/ml)	45.45±1.69	8.71±1.80[a] (80.82)↓	45.95±1.68[b] (1.10)↑	46.65±1.71[a] (2.64)↑
Sperm motility (%)	31.50±1.04	5.66±0.81[a] (82.00)↓	34.42±0.49[a] (9.26)↑	35.00±0.57[a] (11.11)↑
Testosterone (ng/ml)	3.35±0.28	2.34±0.23[a] (30.19)↓	3.53±0.19[b] (5.27)↑	3.59±0.18[a] (7.06)↑
LH (mIU/ml)	3.38±0.37	2.26±0.32[a] (33.13)↓	3.55±0.37[a] (5.02)↑	3.59±0.36[a] (8.21)↑
FSH (mIU/ml)	7.72±0.172	6.54±0.341[a] (15.24)↓	7.97±0.162[b] (3.27)↑	8.00±0.164[b] (3.62)↑

Values (Mean±SD), [a]significant at $P<0.01$, [b]significant at $P<0.05$, ENO: Emulsified neem-oil, Figures in bracket indicate change (%), ↓: Indicate reduction and ↑: Indicate rise, SD: Standard deviation, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, ID: *Ipomoea digitata*

steroidal hormones [24]. It was assumed that ID possesses progenitors of testosterone biosynthesis because of the presence of β-sitosterol in the roots, which was confirmed in our laboratory using high performance thin layer chromatography. It is evident from our findings that rats treated with ID at both doses attributed increase in sperm density and testosterone which established a reciprocal relation.

Numerous studies demonstrates that ID possesses moderate antioxidant potential which may be due to the presence of phenolic compounds, coumarins, flavonoids and steroids [6,25,26]. Overproduction of ROS can lead to a state of oxidative stress that compromises sperm function [27]. ROS are neutralized by an elaborate antioxidant system consisting of enzymes such as catalase, superoxide dismutase and glutathione peroxidase/reductase, and numerous non-enzymatic antioxidants such as vitamin C, vitamin E, vitamin A, pyruvate, glutathione, taurine and hypotaurine. The male and female genital tracts are rich in both enzymatic and non-enzymatic antioxidants [28,29]. We have a strong assumption that the phytochemicals present in the said plant are capable of synthesizes the ROS scavengers in infertile male albino rats particularly to repair the oxidative damage of spermatozoon characteristics. Rats treated with ID at the dose of 500 mg/kg b.w. exhibits more penile erections lead to establishment of physiological and biochemical mechanisms remain uncertain. In nutshell, ID has spermatogenic activity along with anabolic effect in experimental rats.

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Original Research

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Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae)

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ABSTRACT

Objective: The aim was to determine the chemical constituents and antimicrobial activity of the hexane leaf extract of *Anisopus mannii* against a wide range of human pathogenic microorganisms. **Methods:** The chemical constituents of the hexane leaf extract was determined using gas chromatography-mass spectrometry (GC-MS) analysis; and the antimicrobial activity was evaluated on "standard strains," clinical susceptible and resistant bacterial and fungal isolates using the disc diffusion and broth microdilution methods. **Results:** GC-MS analysis of the hexane leaf extract revealed 32 compounds, representing 73.8% of the identified components. The major compounds were hexadecanoic acid, ethyl ester (34%), oxirane, hexadecyl- (11%) and 9, 12, 15-octadecatrienoic acid, ethyl ester, (Z, Z, Z) (9.6%). Results from the antimicrobial activity demonstrated higher inhibition zones against *Bacillus cereus* (29 mm), followed by *Streptococcus pyogenes* (28 mm). Other notable inhibitions were observed with *Enterococcus faecalis* (27 mm), *Proteus vulgaris* (26 mm) and MRSA (25 mm). The MIC values ranged from 0.625 mg/mL to 1.25 mg/mL while the MBC/MFC values ranged from 2.5 mg/mL to 5.0 mg/mL. **Conclusion:** These results support the traditional use of the plant and demonstrate the huge potential of *A. mannii* as a source of antimicrobial compounds.

KEY WORDS: *Anisopus mannii*, antimicrobial activity, hexane extract, gas chromatography-mass spectrometry

INTRODUCTION

Infectious diseases are the primary cause of death, representing about 50% of all the human deaths in tropical countries [1]. The is due to decreasing efficacy of the antimicrobial chemotherapy because of the emergence of drug-resistant pathogens especially in developing countries where poverty and ignorance are high among the populace and basic health facilities are inadequate [2]. The range of these emerging infectious diseases such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Vancomycin resistant enterococci* (VRE) and the prevalence of drugs resistant *Pseudomonas aeruginosa* are threats to public health around the world [1]. This situation calls for an urgent need to identify new or complementary antimicrobial agents from natural sources to combat the resistant pathogens [3]. This screening of plant extracts or plant products for bioactive agents have shown potentials for new compounds or templates for new antibiotics [4,5]. It is

desirable to evaluate our indigenous herbal plants as sources of bioactive compounds for drug discovery.

Anisopus mannii N.E. Br. (family Asclepiadaceae), is a perennial herb currently used in the traditional medicinal preparations of Northern Nigeria. Different parts of the plant are used as a remedy for diabetes [6], pile, diarrhea and other infectious diseases (Personal communication). The plant is locally known as "kashe zaki" in Hausa language. Previously, the proximate composition, mineral elements and anti-nutritional factors of *A. mannii* were reported [7]. The phytochemical and antimicrobial screening of the stem aqueous extract [8], the analgesic and anti-inflammatory studies of methanol leaf extract [9] as well as the isolation of chemical constituents such as 1, 7-naphthyridine alkaloid- named anisopusin, 5-hydroxy-lup-20(29)-en-3-yl eicosanoate, 6-gingerdione, 6-dehydrogingerdione and ferulic acid from acetone extract of the stem bark have been reported [10]. However, no previous phytochemical or biological studies on the non-polar constituents of *A. mannii* have been reported. Thus, the need to evaluate the lipophilic components is imperative to validate the use of the plant as traditional phytomedicine. It is unarguable that bioactive constituents of medicinal plants are widely distributed between polar and non-polar regions [11,12]. Because *A. mannii* leaf is the commonly used recipe for infectious disease treatment in Northern Nigeria; we investigated the chemical composition using gas chromatography-mass spectrometry (GC-MS) and antimicrobial effects of hexane extract on some standard, clinical susceptible and resistant strains of human pathogenic microorganisms with had not been reported hitherto.

MATERIALS AND METHODS

Plant Material

The leaves of *A. mannii* were collected in February, 2011 in Zaria, Kaduna State, Nigeria. It was taxonomically authenticated by Umar Shehu Galla of the Herbarium Unit, Department of Biological Sciences Ahmadu Bello University Zaria, Nigeria. A specimen (Voucher No. 217) was deposited there. The leaves were air-dried for 2 weeks and pulverized to powder using pestle and mortar.

Extraction

The pulverized plant sample (120 g) was extracted with hexane (750 ml) by simple percolation on a shaker (Labcon, South Africa) for 24 h. The extract was filtered using Whatman filter paper No. 2, and concentrated on a rotary evaporator (Büchi Rota vapor R-124) at 40°C to give 12.5 g of the crude hexane extract.

GC-MS

The GC-MS analyses was carried out on an Agilent Technologies (6890 Series) GC coupled with a (5973 Series) Mass Selective Detector. It was equipped with an Agilent HP-5MS capillary column (0.25 μm film thickness) with dimensions 30 m (length) $\times$ 0.25 μ I.D). The sample ionization energy of 70eV for GC-MS detection was used. Helium was used as the carrier gas at a pressure of 60 kPa, with the oven temperature programming at 100°C (for 2 min) to 280°C (for 30 min) at a ramping rate of 4°C per min. A 2.0 μl diluted sample was manually injected while the injection temperature was 280°C with a split ratio of 1:50. The system software was driven by Agilent Chemstation software. The relative percentage of each component was calculated by comparing its average peak to the total areas. The identification of the various compounds was carried out by comparison of their mass spectra with those of authentic samples or those obtained from isolated pure compounds in our laboratory. The NIST/NBS 2005 mass spectral database of the GC-MS system was also used to identify some compounds whose structures were confirmed by published data [13].

Test Organisms

The standard strains of organisms such as: *Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418, *Salmonella typhi* ATCC 9184, *Pseudomonas aeruginosa* NCTC 6750 and clinical isolates including MRSA, VRE, *Bacillus cereus*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Candida tropicalis*, *Candida stellatoidea*, *Candida krusei* and *Candida albicans* used in this study were obtained from the Department of Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika. The isolates were purified on nutrient agar (OXOID) plates and characterized using standard microbiological and biochemical procedures as previously described [14,15].

Determination of Antibacterial and Antifungal Activities

The disc diffusion method was used [16]. Stock solution (100 mg/mL) of the hexane extract was prepared using methanol. Disc (6 mm diameter) were prepared using Whatman filter paper and sterilized by autoclaving. The blank sterile discs were placed on the inoculated Mueller Hinton Agar (OXOID) surface and impregnated with 15 μL of stock solutions (300 μg/dics). The plates were incubated at 37°C for 24 h. Standard antibiotic discs were used as positive control: Sparfloxacin (Himedica Laboratories, India) (100 μg/dics) for bacteria and Fluconazole (Saga Laboratories, India) (30 μg/dics) for fungal species. All tests were performed in duplicate, and the antimicrobial activity was expressed as the mean diameter of inhibition zones (mm) produced by the extracts.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was carried out using micro broth dilution in accordance with National Committee for Clinical Laboratory Standards [17]. Serial dilution of sample extract was prepared between 0.1 mg/ml and 6.50 mg/ml concentration. The tests tubes were inoculated with the suspension of the standardized inocula and incubated at 37°C for 24 h. MICs were recorded as the lowest concentration of the extract showing no visible growth of the broth.

Determination of Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC)

MBC/MFC were determined by aseptically inoculating aliquots of culture from MIC tubes that showed no growth, on sterile nutrient agar plates and incubating at 37°C for 48 h. MBC/MFC were recorded as the lowest concentration of the extract showing no bacterial growth.

RESULTS

The studies of the active principles in the hexane leaf extract of *A. mannii* by GC-MS analysis showed the presence of 32 compounds. The active principles with their retention time (RT) and percent relative composition are presented in Table 1. The results of the antimicrobial activity of the hexane leaf extract are presented in Tables 2 and 3.

DISCUSSION

The use of *A. mannii* leaf extracts in the traditional treatment of some infectious diseases had prompted us to investigate the antimicrobial activity and subsequently, the chemical composition of the extract was investigated using GC-MS as a first step towards understanding the nature of bioactive principles. In traditional medicine, *A. mannii* recipes are prepared as aqueous concoction or taken in powdered form with milk (Personal communication) for different remedies. Plant extracts contain both polar and non-polar components. Thus the phytochemical analysis of non-polar portion is desirable to validate the use as effective phytomedicine against the microbial infections.

Because hexane is non-polar, it was used to extract lipophilic components that have been determined to be largely fatty acid methyl esters (FAME) with hydrocarbons and terpenes as traces. Terpenoids volatile components of essential oil were not detected; they are obtainable from fresh plant samples using a Clevenger hydro distillation technique. Thus, the chemical composition of *A. mannii* hexane leaf extract is an aggregate mixture of fat soluble compounds of different structural motifs driven by lipophilicity of the solvent. These compounds vary in content or composition from plant to plant due to environmental or genetic factors [18].

The hexane extract demonstrated good *in vitro* antimicrobial profile especially against MRSA, *S. faecalis*, *S. pyogenes*, *B. cereus* and *P. aeruginosa* which are known to cause infections that are extremely difficult to treat due to multiple drugs resistance. Furthermore, the extract showed potent activity against both Gram-positive and Gram-negative bacteria, as well as different species of fungi. These findings could indicate that the extract had broad-spectrum antimicrobial effects against a wide range of infectious agents that could be the basis for the folkloric use of the plant. However, it is noteworthy that the test extract showed better antimicrobial activity on *B. cereus* (29 mm), *S. pyogenes* (28 mm), *E. faecalis* (27 mm) and MRSA (25 mm). Previous antibacterial studies on the stem-bark aqueous extract of *A. mannii* showed higher MIC values of 50 mg/ml (indicating low activity) for *S. aureus*, *S. pyogenes* and *P. aeruginosa* [8] when compared to our findings in which the MIC of the hexane extract for *S. aureus* and *P. aeruginosa* was 1.25 mg/ml with *S. pyogenes* having 0.625 mg/ml [Table 3]. This indicates the potency of the lipophilic hexane extract of *A. mannii* due to the compounds identified.

FAME have been studied in relation to antibacterial activity and the structural properties, including carbon chain length, unsaturation, esterification and functional groups have enormous influences on the activity [19]. Hexadecanoic acid, ethyl ester (34%), oxirane, hexadecyl (11%) and 9, 12,

Table 1: Chemical composition of hexane extract of *A. mannii* leaf

Compounds	[a]RT	(%) composition
Tetradecanoic acid, ethyl ester	14.8	1.5
Ethyl 9-hexadecenoate	16.8	1.5
Hexadecanoic acid, ethyl ester	16.9	34.0
Eicosane	17.0	1.3
Phytol	18.0	2.0
9, 12, 15-octadecatrienoic acid, ethyl ester, (Z, Z, Z)-	18.5	9.6
Oxirane, hexadecyl-	18.9	11.0
Heptadecane, 9-octyl	20.4	3.3
3-Hexadecyne	20.5	1.0
Ethyl tetracosanoate	23.7	2.6

[a]Compounds' retention times (min) as eluted from DB-5MS column, Compounds identified (%)=67.8, Compounds identified as traces (<1.00%) 6.0, Total Compounds identified (%)=73.8, RT: Retention time, *A. mannii: Anisopus mannii*

Table 2: Antimicrobial activity of hexane extract of *A. mannii* leaf

Zone of inhibition (mm)			
Test organisms	Hexane extract	Sparfloxacin	Fluconazole
MRSA	25	6	-
VRE	6	6	-
S. aureus NCTC 6571	24	39	-
E. faecalis	27	42	-
S. pyogenes	28	41	-
B. cereus	29	47	-
E. coli NCTC 10418	6	35	-
S. typhi ATCC 9184	6	31	-
P. vulgaris	26	34	-
P. aeruginosa NCTC 6750	22	6	-
C. albicans	22	-	37
C. tropicalis	20	-	32
C. stellatoidea	6	-	40
C. krusei	24	-	36

MRSA: methicillin resistant *Staphylococcus aureus*, VRE: vancomycin resistant enterococci, *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, E. faecalis: Enterococcus faecalis, B. cereus: Bacillus cereus, S. typhi: Salmonella typhi, S. pyogenes: Streptococcus pyogenes, P. vulgaris: Proteus vulgaris, P. aeruginosa: Pseudomonas aeruginosa, C. tropicalis: Candida tropicalis, C. stellatoidea: Candida stellatoidea, C. krusei: Candida krusei, C. albicans: Candida albicans, A. mannii: Anisopus mannii*

Table 3: Results of MIC and MBC/MFC of hexane extract of *A. mannii* leaf

Test organisms	MIC (mg/ml)	MBC/MFC (mg/ml)
MRSA	1.25	5.0
VRE	NT	NT
S. aureus NCTC 6571	1.25	5.0
E. faecalis	1.25	2.5
S. pyogenes	0.625	2.5
B. cereus	0.625	2.5
E. coli NCTC 10418	NT	NT
S. typhi ATCC 9184	NT	NT
P. vulgaris	1.25	5.0
P. aeruginosa NCTC 6750	1.25	5.0
C. albicans	1.25	5.0
C. tropicalis	1.25	5.0
C. stellatoidea	NT	NT
C. krusei	1.25	5.0

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration, MFC: Minimum fungicidal concentration; NT: Not tested, *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, E. faecalis: Enterococcus faecalis, B. cereus: Bacillus cereus, S. typhi: Salmonella typhi, S. pyogenes: Streptococcus pyogenes, P. vulgaris: Proteus vulgaris, P. aeruginosa: Pseudomonas aeruginosa, C. tropicalis: Candida tropicalis, C. stellatoidea: Candida stellatoidea, C. krusei: Candida krusei, C. albicans: Candida albicans, A. mannii: Anisopus mannii*

15-octadecatrienoic acid, ethyl ester, (Z, Z, Z) (9.6%) are the major constituents identified in the hexane leaf extract. Some of the minor constituents identified are heptadecane, 9-octyl (3.3%), ethyl tetracosanoate (2.6%) and phytol (2.0%). The 32 compounds identified represent 73.8% of the extract. Among the detected major phytochemicals, hexadecanoic acid ethyl ester (palmitic acid ethyl ester) is a neutral lipid soluble form of palmitic acid. Interestingly, the antimicrobial activity of this compound has been reported and subsequently proposed to be the main bioactive antimicrobial agent identified in the ethyl acetate extract of marine *Burkholderia cepacia* [20]. Furthermore, the other major compound detected in the extract, oxirane tetradecyl has been identified as one of the bioactive antimicrobial compounds of the red algae *Laurencia brandenii* and *Senecio pedunculatus* [21,22]. In another study, the leaf extract of *Andrographis peniculata* demonstrated strong antimicrobial, antioxidant and anticancer activities, which were attributed, in part, to 9, 12, 15-octadecatrienoic acid, ethyl ester, (Z, Z, Z) [23]. However, the antimicrobial activity could probably be accentuated by synergy effects of the different chemical components of the extracts [24].

CONCLUSION

This is the first report on the chemical composition and antimicrobial activity of hexane extract of *A. mannii* leaf. The findings validate the folkloric use of the plant against infectious agents and identified some chemical entities such as hexadecanoic acid ethyl ester, oxirane, tetradecyl and 9, 12, 15-octadecatrienoic acid, ethyl ester, (Z, Z, Z), which have acted perhaps synergistically for the enhanced antimicrobial activity.

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Original Research

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Single session of integrated "silver yoga" program improves cardiovascular parameters in senior citizens

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ABSTRACT

Aim and Objective: This pilot study was carried out to determine cardiovascular effects of a single session of an integrated "silver yoga" program in senior citizens of Serene Pelican Township, Pondicherry. **Materials and Methods:** Heart rate (HR) and blood pressure (BP) measurements were recorded in 124 senior citizens (75 female, 49 male) with mean age of 67.19 ± 10.61 year who attended an integrated "Silver Yoga" program at Centre for Yoga Therapy, Education and Research from August to October 2014. Participants practiced the protocol that was specially designed for senior citizens, keeping in mind their health status and physical limitations. This included simple warm-ups (jathis), breath body movement coordination practices (kriyas), static stretching postures (asanas), breathing techniques (pranayamas), relaxation and simple chanting. Non-invasive BP apparatus was used to record the HR, systolic (SP) and diastolic pressure (DP) before and after the 60 min sessions. Pulse pressure (PP), mean pressure (MP), rate-pressure product (RPP) and double product (DoP) indices were derived from the recorded parameters. Student's paired t-test was used to compare data that passed normality testing by Kolmogorov–Smirnov Test and Wilcoxon matched-pairs signed-ranks test for those that did not. $P < 0.05$ were accepted as indicating significant differences for pre-post comparisons. **Results:** All parameters witnessed a reduction following the single session. This was statistically more significant ($P < 0.0001$) in HR, RPP and DoP while it was also significant ($P < 0.01$ and $P < 0.05$) in SP and PP, respectively. The decrease in MP just missed significance ($P = 0.054$) while it was not significant in DP. **Conclusion:** There is a healthy reduction in HR, BP and derived cardiovascular indices following a single yoga session in geriatric subjects. These changes may be attributed to enhanced harmony of cardiac autonomic function as a result of coordinated breath-body work and mind-body relaxation due to an integrated "Silver Yoga" program.

KEY WORDS: Cardiovascular, psycho-somatic harmony, senior citizen, yoga

INTRODUCTION

Ageing is a natural process characterized by declining physical performance, slower speed of reaction, inadequate working of various systems with poor motor and sensory conduction. There is a progressive and generalized impairment of homeostasis resulting in declining ability to respond to external or internal stresses and increased risk of diseases [1]. The loss of adaptive response to stress increases risk of many age-related, degenerative disorders [2]. Dr. Dean Ornish, the renowned American physician and bestselling author who has shown that a yogic lifestyle can reverse heart disease, says, "Yoga is a system of perfect tools for achieving union as well as healing" [3]. Dr. Ramamurthy, the eminent neurosurgeon, has observed that yoga practice reorients the functional hierarchy of the entire nervous system while benefiting cardiovascular, respiratory, digestive, and endocrine systems, in addition to bringing about many positive biochemical changes.

The practice of yoga has been shown to have preventive, curative as well as rehabilitative potential that can be explained on the basis of modulation of autonomic functions, stress reduction,

improvement in physiological functions and enhanced quality of life [4-6]. It has become quite apparent that yoga is a relatively low-risk, high-yield approach to improving overall health and wellbeing [7].

Yoga is qualitatively different from other modes of physical activity as it has a unique combination of isometric muscular contractions, stretching exercises, relaxation techniques, and breathing exercises. A recent report on the acute effects of one session of hatha yoga practice on blood pressure (BP) and other cardiovascular responses in healthy volunteers has showed that systolic (SP), mean (MP), and diastolic (DP) BP increased significantly during the yoga practice [8]. However, they did not compare pre-post effects of the entire session but focused only on the individual techniques used during the session. Very few studies have focused on immediate effects of a single yoga session, and these include one that investigated the effectiveness of a single 90-min hatha yoga class concluding that it significantly reduced perceived stress [9].

The only major report on effects of a single session of yoga, was a retrospective review of clinical data of 1896 patients done by us at Centre for Yoga Therapy, Education and Research (CYTER) in 2013 [10]. We found significant reductions in all the studied cardiovascular parameters following the yoga session. However, the magnitude of reductions differed in different groups of patients depending on pre-existing medical conditions as well as the specific yoga therapy protocol.

With the above in mind, this pilot study was done to determine cardiovascular effects of a single, 60-min, integrated "Silver Yoga" session in senior citizens of Serene Pelican Township attending regular sessions at CYTER.

MATERIALS AND METHODS

This pilot study was conducted at the CYTER functioning in Mahatma Gandhi Medical College and Research Institute of the Sri Balaji Vidyapeeth University, Puducherry, India. These sessions were carried out in the CYTER Yoga hall between 11 am and 12 noon on Thursdays in a quiet environment, with a comfortable temperature and subdued lighting. The participants had been advised to finish their breakfast at least 2 h earlier and come after emptying bowel and bladder.

Heart rate (HR) and BP measurements were recorded in 124 senior citizens (75 female, 49 male) with mean age of 67.19 ± 10.61 y who were attending an integrated "Silver Yoga" program at CYTER from August to October 2014. Participants practiced the protocol that was specially designed for senior citizens, keeping in mind their health status and physical limitations. This included simple warm-ups (jathis), breath body movement coordination practices (kriyas), static stretching postures (asanas), breathing techniques (pranayamas), relaxation and simple chanting. The complete protocol is given in Table 1.

Table 1: Components of an integrated 'Silver Yoga' program

Jathis and kriyas (loosening techniques)
Standing asanas
Veera asana 1
Tada asana
Ardhakati and kati chakra asana
Ardha utkat asana
Sitting asanas
Vakra asana
Paschimottana/purvottana asana
Chatuspada kriya/vyagraha pranayama
Nava kriya
Face prone asanas
Bhujanga asana/bhujangini mudra
Makara asana
Supine asanas
Pawanamukta series
Pada uttana series
Sethu kriya
Pranayamas
Chandra nadi
Pranava
Bhramari
Mudras
Brahma mudra
Relaxation
Savitri pranayama in shava asana
Marmanasthanam/kaya kriya
Chakra awareness sequence

Non-invasive BP apparatus was used to record HR, systolic pressure (SP) and diastolic pressure (DP) readings before and after the 60 min session. To ensure objectivity, all recordings were performed using non-invasive automatic BP monitor (Omron HEM 7203, Kyoto, Japan) that uses oscillometric method with an instrumental accuracy of ± 5% for HR and ± 3 mm Hg for BP. The pre-session recordings were taken after 5 min of quiet comfortable sitting while post-session recordings were taken at the end of the session. Pulse pressure (PP), mean pressure (MP), rate-pressure product (RPP) and double product (DoP) indices were derived from the recorded parameters.

Data were assessed for normality using GraphPad InStat version 3.06 for Windows 95, (GraphPad Software, San Diego California USA, www.graphpad.com). Student's paired t test was used to compare data that passed normality testing by Kolmogorov-Smirnov Test and Wilcoxon matched-pairs signed-ranks test for those that didn't. $P < 0.05$ were accepted as indicating significant differences for pre-post comparisons.

RESULTS

The results are given in Table 2. All cardiovascular parameters and derived indices witnessed a reduction following a single session of "Silver Yoga." This was statistically very significant ($P < 0.0001$) in HR, RPP and DoP while it was also significant ($P < 0.01$ and $P < 0.05$) in SP and PP, respectively. The decrease in MP just missed significance ($P = 0.054$) while it was insignificant in DP.

DISCUSSION

There is a healthy reduction in HR, BP and derived cardiovascular indices following a single yoga therapy session. This implies a healthier autonomic regulation of the heart that may be

Table 2: HR, SP, DP, PP, MP, RPP and DoP before (B) and after (A) a single session of Silver Yoga

	B	A	*P* value
HR (bpm)	77.74±11.99	73.92±12.12	<0.0001
SP (mmHg)	131.93±13.63	128.97±14.34	0.0047
DP (mmHg)	71.63±11.83	70.94±11.52	0.4059
PP (mmHg)	60.30±12.48	58.03±13.00	0.0372
MP (mmHg)	91.73±10.98	90.28±10.93	0.0541
RPP (units)	103.20±22.51	95.60±20.57	<0.0001
DoP (units)	71.95±16.99	67.17±15.71	<0.0001

HR: Heart rate, SP: Systolic pressure, DP: Diastolic pressure, PP: Pulse pressure, MP: Mean pressure, RPP: Rate-pressure product, DoP: Double product, Student's paired *t*-test was used to compare data that passed normality testing by Kolmogorov-Smirnov test (SP, DP, PP, MP, RPP) and Wilcoxon matched-pairs signed-ranks test for those that didn't (HR and DoP). $P<0.05$ were accepted as indicating significant differences for pre-post comparisons

attributed to either an overall increase of parasympathetic tone and/or a reduction in sympathetic tone. As the RPP and DoP are indirect indicators of myocardial O_2 consumption and load on the heart, their reductions signify a lowering of strain on the heart that is beneficial for the senior citizens [11,12]. RPP also provides a simple measure of HR variability (HRV) in hypertensive patients and is a surrogate marker in situations where HRV analysis is not available [13]. Hence, reduction in RPP in our subjects is an indirect evidence of better cardiac autonomic modulation in them. The reduction of adrenergic tone coupled with normalization of parasympathetic tone is potentially very useful in this population as increased adrenergic tone has been implicated in precipitation of heart attacks. The elderly often have cardiac disorders with higher risk of heart attacks and strokes. Hence, such a program has potential benefits in prevention, management, and rehabilitation of such conditions.

One of the few earlier studies on acute effects of a single session of hatha yoga showed that SP, MP and DP increased significantly during the yoga practice [8]. Elevation in BP due to yoga practice was associated with increases in cardiac output (CO) and HR. This is similar to those changes observed in isometric exercise. However that study continuously measured HR, SP, DP, MP, stroke volume and CO. On the other hand we are evaluating the pre-post effects and hence are commenting on overall effects of the whole integrated practice session rather than the individual techniques. As suggested in our earlier report the conscious self-effort made in asana practice may be understood as the *spanda* (tension) component, whereas the relaxation of effort (*Prayatna shaithilya*) may be understood as the *nishpanda* (relaxation) component [10]. Hence, it is essential to physiologically evaluate the cardiovascular changes occurring not only during the actual performance of an asana, but maybe more importantly during and after period of recovery following it. We are supported in this assertion by a previous report by Telles *et al*. that concluded that a combination of stimulating and relaxing techniques reduced physiological arousal better than the mere practice of relaxation techniques alone [14]. They also pointed out that though the practical performance of yoga techniques seem to be stimulatory in nature, their physiological effects are, in fact, more relaxatory. This is again corroborated by another report that shavasana relaxation is enhanced with the addition of savitri pranayama thus decreasing O_2 consumption by 26% [15].

In another study, we compared cardiovascular changes immediately after performance of different asanas and during the recovery phase [16]. In that study, there was a temporary rise of HR following dhanurasana due to relative difficulty of the posture. However cardiovascular recovery was found to be greater after performance of asanas when compared to merely relaxing in shavasana (a supine relaxation posture). This implies a healthier cardiac autonomic de-activation response when effort precedes relaxation.

We have also previously also studied the immediate effects of uninostril breathing techniques in a geriatric population and reported that exclusive left nostril breathing (chandranadi pranayama) resulted in a decrease of cardiovascular parameters with a slowing down of the reaction time [17]. This is one of the techniques used in the present study and hence may have also contributed toward these positive cardiovascular relaxatory changes.

As the integrated "Silver Yoga" program was devised with the needs of the seniors in mind, it consisted of more breath-body work, breathing awareness and yogic relaxation. This may be the main factor behind the cardiac autonomic balance in our subjects irrespective of their initial condition. Yoga is defined as the state of balance (samatvam yoga uchyate - Bhagavad Gita) and the restoration of physical, mental, emotional and spiritual balance may be the prime factor behind the changes seen in our study.

Our findings are in agreement with a previous suggestion that yoga appears to modulate stress response systems by reducing perceived stress and anxiety, which in turn, decreases physiological arousal with decreases in HR and BP and respiration [18]. They also reiterate the results of our earlier retrospective study on 1896 patients where we found significant reductions in all the studied cardiovascular parameters following a single yoga session [10].

CONCLUSION

There is a healthy reduction in HR, BP and derived cardiovascular indices following a single yoga session in geriatric subjects. These changes may be attributed to enhanced harmony of cardiac autonomic function as a result of coordinated breath-body work and mind-body relaxation due to an integrated "Silver Yoga" program. We recommend that such an integrated Yoga program should be part of the heath care facilities for the elderly as it can enhance their quality of life and improve their overall health status. Our study is limited by the fact that it only addressed immediate effects of a single session. Therefore, further studies on the effects of short and long-term training may deepen our understanding of the intrinsic mechanisms by which such positive changes are occurring. This would help strengthen our conclusion about the cardiovascular benefits of Yoga in a geriatric population.

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Original Research

ScopeMed

Use of household ingredients as complementary medicines for perceived hypoglycemic benefit among Sri Lankan diabetic patients; a cross-sectional survey

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ABSTRACT

Background: Biologic based therapies are frequently used as complementary medicines in diabetes. The aim of this study was to identify the commonly used herbal remedies and their preparations in Sri Lankan patients with Type 2 diabetes. **Methods:** This is a descriptive, cross-sectional study on 220 diabetic patients using herbal remedies for perceived glycemic benefit. **Results:** All the patients used their regular conventional medications together with herbal remedies. The most commonly used medication was metformin (91.4%). Ivy gourd (*Coccinia grandis*) was the most commonly used herbal remedy (32%), followed by crepe ginger (*Costus speciosus*) (25%) and bitter gourd (*Momordica charantia*) (20%). Herbal remedies used less frequently were finger millet (*Eleusine corocana*) (5%), anguna leaves (*Wattakaka volubilis*) (5%), goat weed (*Scoparia dulcis*) (4%), *Salacia reticulata* (4%), fenugreek (*Trigonella foenum-graecum*) (3%) and tree turmeric (*Coscinium fenestratum*) (0.5%). None of the patients used commercially available over-the-counter herbal products. The common preparations were salads (72.8%), curries (12.8%), herbal tea (6%), and herbal porridges (6%). **Conclusion:** The practice of using household ingredients as complementary medicines is common in Sri Lanka. Few herbal remedies and their methods of preparation have limited evidence for efficacy. In view of the frequent use by diabetic patients each needs to be documented for reference and scientifically explored about their hypoglycemic potential.

KEY WORDS: Bitter gourd, crepe ginger, complementary medicine, ivy gourd, Type 2 diabetes

INTRODUCTION

The concept of combining dietary constituents to manage illnesses is ingrained in the history. At present, there is a booming market for the complementary and alternative medicines (CAM) globally. Five categories of CAM have been identified; biological based therapies such as herbal and dietary supplement; alternative medical systems like acupuncture and Ayurveda; energy therapies like Reiki; manipulative and body-based systems like chiropractic or massage; and mind-body interventions like Yoga [1]. Of these, the herbal remedies are the most abundantly used form of therapy. Global prevalence for the use of CAM in diabetes varies from 30% to 57% [1,2]. Diabetic patients are 1.6 times more likely than non-diabetics to use a CAM for a host of reasons [3].

In a previous study, we observed the prevalence of CAM use in diabetic patients in Sri Lanka to be 76% [4]. Recent estimates show that over 80% of people living in developing countries still depend on CAM for treatment of health conditions [5]. Similarly even in the absence of good supporting evidence, developed countries like the United States also has recorded an increase of 380% in the use of herbal remedies. [6] In the western world most of the herbal remedies used like Asian Ginseng, St John's wart and garlic are available in the form of proprietary products at supermarkets and pharmacies. In contrast in the developing Asian countries non-proprietary raw herbal products sourced locally are used with minimal processing. Most often they are common household vegetables perceived to process hypoglycemic properties [4]. While commercial preparations may have safety related information together with the product [7], the Physician needs to be knowledgeable about the locally sourced products.

Over 400 plants and compounds have so far been evaluated for use in Type 2 diabetic patients and over 1200 have been claimed to be remedies for the same illness [8]. Although the perceived adverse events of herbal remedies are low, they are not entirely devoid of adverse events [7]. Some are known to cause interactions with conventional medicines. For example,

Tanners cassia (*Cassia auriculata*) used in the Asian region as an herbal remedy for diabetes is known to cause elevated levels of Carbamazepine [9].

Since patients believe herbal products to be devoid of adverse events most do not inform their regular physician of their use [7].

While it will be exhaustive for physicians to be informed about all the existing herbal products used in diabetes, each country or region should have on record a list of common preparations used by their patients for reference.

The aim of this study was to identify the commonly used herbal remedies and their preparations in patients with Type 2 diabetes, attending a tertiary care diabetes clinic in Sri Lanka.

METHODS

A cross-sectional survey was performed between April and August 2014 at the diabetes clinic of Teaching Hospital Peradeniya Sri Lanka. This diabetes clinic caters to 2200 registered patients with diabetes from multi-ethnic backgrounds. From a previous study [4] we knew the prevalence of herbal remedy use to be around 76% among our diabetic patients.

Inclusion criteria included Type 2 diabetes for at least 6 months and use of at least one type of herbal remedy in addition to conventional medications. Potential subjects were briefed regarding the study during a regular clinic day and were asked to report back during their next clinic visit if they were willing to participate. Out of 234 patients briefed 220 patients agreed to participate. Informed verbal consent was obtained and formally recorded from those who consented.

An interviewer-administered questionnaire was used to collect data. The questionnaire contained questions on the 3 domains of demographic information, diabetes related information and herbal remedy use. Demographic data included age, gender, level of education, occupation, income, the diabetes related information included duration of diabetes, presence of complications and current anti-diabetic medication. CAM related information included the type of CAM, source of the product and the method of preparation. The participants were asked to describe their method of preparing the herbal product and the details recorded. Excluding minor variations the methods were then re-categorized during post coding of the data to a limited number of preparations. To ensure validity of these methods they were read out to local housewives and cross checked with local recipes.

A two-stage sampling technique was used and consisted of an initial pilot-test being administered to 25 patients to establish face validity and identify any questions that needed re-phrasing. The questionnaire was then administered to the selected 220 participants.

Ethical approval for the study was obtained from the Ethics Review Committee (ERC) of the Faculty of Medicine, University of Peradeniya.

RESULTS

Demographic Characteristics

The study included 220 patients with 47 (21.4%) males and 173 (78.6%) females. The mean age was 60.6 years. The majority (93.6%) was Buddhist with the Hindus and Muslims making up the remaining 6.7%. Only 12.7% had completed secondary education, and 4.5% had completed tertiary education. Only 38 (17.2%) patients were actively employed, and 149 (67.7%) were unemployed. The mean duration of diabetes in the participants was 9.79 years. The socio-demographic profile of the participants is given in Table 1.

Background Hypoglycemic Therapy

All the participants were on one or more oral hypoglycemic agents (OHG). The majority (68.3%) was on 2 OHGs. Metformin was used by 91.4% of the herbal remedy users and sulfonylureas by 64%. Insulin was used by 15.5%. None of the patients had reduced or stopped taking conventional medicines while using herbal therapies.

Types and Preparations of Herbal Remedies

Ivy gourd (*Coccinia grandis*) was the most commonly used herbal remedy (32%), followed by crepe ginger (*Costus speciosus*) (25%) and bitter gourd (*Momordica charantia*) (20%). Herbal remedies used less frequently were finger millet (*Eleusine corocana*) (5%), anguna leaves (*Wattakaka volubilis*) (5%), goat weed (*Scoparia dulcis*) (4%), *Selacia reticulata* (4%),

Table 1: Demographic and clinical characteristics of the diabetic respondents

	CAM users *n*=220 (%)
Gender	
Male	47 (21.4)
Female	173 (78.6)
Religion	
Buddhist	206 (93.6)
Christian	03 (1.3)
Muslim	09 (4.0)
Hindu	02 (1.0)
Education	
Primary education	124 (56.4)
Secondary education	58 (26.3)
Completed secondary	28 (12.7)
Grad/Post graduate	10 (4.5)
Occupation	
Student	1 (0.5)
Unemployed	149 (67.72)
Retired	32 (14.54)
Employed	38 (17.27)
Individual income (Rs)	
No income	158 (71.8)
<10000	07 (3.2)
10000-20000	30 (13.6)
20000-30000	03 (1.3)
30000-40000	06 (2.7)
>40000	2 (1)

CAM: Complementary and alternative medicines

fenugreek (*Trigonella foenum-graecum*), (3%) and tree turmeric (*Coscinium fenestratum*) (0.5%). Cinnamon (*Cinnamomum zeylanicum*) and garlic was used by less than 1% of instances. None of the patients used over-the-counter (OTC) proprietary herbal medications.

All of the participants sourced the remedies locally, with most being grown in their gardens or bought from local groceries. The preparation of the herbal products was done as part of the general household cooking and consumed as a constituent of a daily meal. In most instances (48-78%) individual remedies were consumed on a weekly basis. The common preparations were salads (72.8%), curries (12.8%), herbal tea (6%) and herbal porridges (6%). This largely depended on the part of the plant that was consumed.

In cases of ivy gourd, crepe ginger and *W. volubilis* the leaves were consumed, in case of bitter gourd the fruit, in cases of fenugreek and finger millet the seeds and finally in cases of *S. reticulata* and tree turmeric parts of the trunk were consumed. The leaves were generally consumed as a leafy raw or tempered salad or herbal porridge, the seeds as porridge or boiled extracts, parts of trunk as boiled extracts and the fruit in many number of ways ranging from curries to blended juices. The frequency of use and the preparations of the commonly used herbal products are tabulated in Table 2.

Table 2: Frequency of use of CAM and the preparations of each herb/vegetable

Biological based (Herbal therapy)	Frequency	Percentage	Part of plant used	Common types of preparation (%)
Ivy gourd (*C. grandis*)	160	32	Leaves	1. Fresh salad (70.62) 2. Tempered salad (24.4) 3. Herbal porridge (16.8)
Bitter gourd (*M. charantia*)	103	20	Fruit	1. Curry (67.9) 2. Herbal tea (15.5) 3. Fresh salad (13.6)
Crepe ginger (*C. speciosus*)	127	25	Leaves	1. Fresh salad (77.1) 2. Tempered salad (30.7) 3. Curry (7.1)
Finger millet (*E. coracana*)	13	5	Seeds	1. Porridge (60) 2. Other (40)
W. volubilis	11	5	Leaves	Herbal tea (100)
Goat weed (*S. dulcis*)	9	4	Leaves	Porridge (100)
S. reticulata	9	4	Trunk	Herbal tea (100)
Fenugreek (*T. foenum-graecum*)	6	3	Seeds	Herbal tea (100)

C. grandis: Coccinia grandis, M. charantia: Momordica charantia, C. speciosus: Costus speciosus, E. coracana: Eleusine coracana, W. volubilis: Wattakaka volubilis, S. dulcis: Scoparia dulcis, S. reticulate: Selacia reticulate, T. foenum-graecum: Trigonella foenum-graecum, CAM: Complementary and alternative medicines

The salads were made by shredding the leaves, mixing it with grated raw coconut and adding spices. The herbal porridge was made by first shredding the leaves, grinding to make it into a pulp then adding coconut extract and water and cooking it over a slow fire. Finger millet was also used as porridge by grinding the seeds into a paste and later adding coconut extract. In most instances fenugreek seeds, *S. reticulata* and tree turmeric had been left to soak overnight in a jar of water and the water drunk in the morning as herbal tea.

DISCUSSION

We studied the patterns of herbal remedy use on 220 diabetic individuals attending a tertiary care diabetes clinic at Teaching Hospital Peradeniya in Sri Lanka. In a previous study we observed that the use of herbal remedies among this population was high [4]. During the current study, we observed that almost all patients used common household herbs and spices as complementary medications in contrast to the Western practice of using commercially available herbal products. During the current study, participants were specifically probed to ensure that the herbal use was meant as a complementary medication as opposed to everyday use as a vegetable or spice. Although many studies exist that explored socio-demographic correlates of CAM use [2,10], none have documented in detail the patterns of herbal use.

The use of common household vegetables and spices as herbal remedies in diabetes has not been reported in Sri Lanka before. However, their use as CAMs has been reported elsewhere. In India, Modak *et al.* [11] reported the use of several herbs, which included bitter gourd and garlic. Al-Saeedi *et al.* reported the use of fenugreek among the diabetic patients in Mecca [12]. Similarly, herbs or biologic based therapies were used in 80% of CAM users in Malaysia with the majority using bitter gourd [1]. The high prevalence of herbal use in the Asian region is probably due to their low cost, easy accessibility and perceived safety. A common traditional know-how seems to exist with regard to selection and preparation of these substances in the Asian region. This is probably the result of common ancient healing systems that linked these countries through cultural and religious links [1].

In contrast, the western countries use commercially available herbal products as CAMs in diabetes. Cinnamon and Asian Ginseng are widely used for diabetes in USA and Canada [13]. In western Sidney Australia, multivitamins, cinnamon, co-enzyme Q and garlic were used as CAMs [2].

In the current study, only 12.7 and 4.5% of the participants had completed secondary and tertiary education respectively. A large proportion (67%) was unemployed. This may have influenced the pattern of CAM use observed by us as opposed to the use of OTC products.

All the participants of the current study used the herbal preparations alongside with their conventional medications. This raises the likelihood of interactions between conventional

medications and herbal therapies [7]. Little is known on such interactions, and there is no formal reporting of such events. There is reported reluctance on the patient's part in informing about herbal remedies to their respective physicians. One study in the UK found that a vast majority did not inform their physicians regarding herbal use [14].

There are no reported cases of interactions with any of the medications used in our study.

However, in view of the high prevalence of use, attending physicians should have a basic knowledge of the types of remedies used and their possible glycaemic and adverse responses. This task is made considerably difficult by the non-uniformity of the products and preparations from different regions of the world.

There are many studies that explored the efficacy of the herbal remedies used by the participants of our study [15]. Ivy gourd, the most commonly used herb in the current study, has been used in clinical trials in the Asian region and has shown promise in reducing hyperglycemia [16-18]. Munasinghe *et al*. [18] used ivy gourd as a traditional salad in exactly the same context as used by the participants of the present study and demonstrated a significant reduction of post prandial blood glucose in a set of Sri Lankan patients. In 2 other trials different preparations alien to its natural use was mentioned. Use of crepe ginger leaves for hypoglycemic effect was reported by us in a previous study [4]. In this study we demonstrated a significant association between the use of crepe ginger and the incidence of hypoglycemia in diabetic patients. The preparation was done in the same way as in the present study. It is interesting to note that the use of crepe ginger leaves in diabetes is unique to Sri Lanka. Use of various parts of the bitter gourd plant in treating diabetes is common in Asia [1]. Bitter gourd too has been studied in many short-term clinical trials of varying designs and has shown mixed results in reducing hyperglycemia [19-23]. However the use of bitter gourd as curry, salad or tea has not been reported. Bitter gourd seems to have been widely studied albeit having mixed outcomes. The effects of ivy gourd and crepe ginger has a much smaller pool of evidence and needs to be studied in more organized well designed studies to explore their hypoglycemic activity.

In our study, finger millet, anguna leaves (*W. volubilis*), *S. reticulata*, fenugreek, and goat weed were used by few people only. Fenugreek has been studied for its hypoglycemic effect, and there have been favorable results of its hypoglycemic efficacy [24-26]. *S. reticulata* has evidence for inhibition of intestinal alpha glucosidase and may have clinical significance in reducing post prandial glucose values[27]. Goat weed (*S. dulcis*) has been studied widely regarding its intestinal alpha glucosidase inhibitory activity in rat models but clinical trials are lacking [28]. Finger millet use and glycemic response in diabetes had previously been studied, but its use as an herbal remedy was not reported [29].

Unfortunately, there is very little published information on individual methods of preparation of these products. These traditional remedies are often subjected to scientific testing in preparations that are wholly outside their natural use. Often, different parts of the plant, complex methods of extractions and extensive processing occur, which may alter their naturally occurring hypoglycemic effects.

With regard to fenugreek and bitter gourd the method of preparation seems to play a key role in its demonstrated hypoglycemic potential. Kassaian *et al*. [26] demonstrated patients who were given fenugreek powder in water had a significant reduction in FPG compared to those who received the same dose in yoghurt. Similarly, patients receiving blended raw bitter gourd juice showed significant reductions in blood glucose value compared to other methods of preparation [22,23]. At present, the use of ivy gourd and crepe ginger leaves as salads and the use of blended bitter gourd is supported by limited evidence [4,18,23].

It is important as an initial measure to document the traditional methods of use of herbal remedies so that these methods could be tested scientifically in future studies. We reiterate that whenever these substances are subjected to scientific vigor, that they at least initially get tested within their limits of natural use.

Strengths and Limitations

The major strength of this study is the detailed reporting of the type of herbal remedies used by a group of Type 2 diabetic patients in Sri Lanka. As limitations, we identify the inclusion of patients only from the state sector and a relatively small sample size of 220.

CONCLUSION

A variety of household ingredients is commonly used as CAMs by diabetic patients. Because of their non-proprietary nature and individual variations in dosing, the perceived effects and adverse effects are difficult to establish. The safety and efficacy of these products are not backed by sound evidence. Considering the high prevalence of use, efficacy and safety information on the common types of herbal products need to be freely available to attending physicians to better understand and manage their patients.

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Original Research

ScopeMed

Combination treatment of lycopene and hesperidin protect experimentally induced ulcer in laboratory rats

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ABSTRACT

Aim: Lycopene, a carotenoid and hesperidin, a flavonoid are naturally occurring in vegetables and fruits. Synergistic effect of a combination of carotenoid and flavonoid has been reported due to its antioxidant activity. Therefore, the present study was aimed to evaluate the protective effect of this combination on pylorus ligation induced ulcers in rats. **Materials and Methods:** Thirty Wistar albino rats were divided into five groups ($n = 6$). Rats were fasted for 24 h before pylorus ligation. After 24 h of fasting the rats were treated with hesperidin (100 mg/kg) and lycopene (2 mg/kg) and their combination 1h prior to surgery. After an hour under ether anesthesia pylorus ligation was performed, after 5 h the animals were sacrificed, stomach was dissected, and gastric contents were collected and measured. Total acidity and pH of gastric content was estimated. Ulcer index was calculated, and *macroscopic* examination of the stomach was carried out. **Results:** The sham operated rats showed a significant increase in pH, volume of gastric content and total acidity and ulcer index. The rats pretreated with lycopene and hesperidin showed significant improvement in the ulcer conditions. However, rats treated with a combination of lycopene and hesperidin showed more significant restoration of gastric function as compared to sham operated rats. Moreover, a significant difference was also noted in rats treated with a combination as compared to lycopene and hesperidin treatment alone. **Conclusion:** Thus experimentally the combination was seen to treat ulcers by anti-secretory, neutralizing, cytoprotective and mainly due to its antioxidant property.

KEY WORDS: Hesperidin, lycopene, pylorus ligation, ulcer index

INTRODUCTION

Peptic ulcer has been recognized as the most important problem in developing countries. Even though the etiology of ulcers is still debated, it is accepted that ulcers are caused due to imbalances between the mucosal defensive and aggressive factors [1]. The incidence of ulcer has shown a significant increase in the past decade. According to the recent survey it shows that peptic ulcer disease remains a relatively common condition worldwide, with annual incidence ranging from 0.10% to 0.19% diagnosed by physician and 0.03-0.17% diagnosed during hospitalization [2]. Traditional treatment options available are mucoprotectives, antacids, alginates, motility stimulants, and acid suppressants and anti-reflux surgery is done in severe cases [3]. In spite of this, the rational therapy of ulcers is elusive and research advances in search for more potent drugs. Since ulcer is a disease that widely affects the population daily and if not treated can be fatal; studying it and finding a solution for the same is the need of the hour. It has become a matter of grave concern and hence the world shifts towards the safe and efficacious herbal drugs.

The present drug lycopene is a carotenoid and is a constituent of many fruits and vegetables [4]. It has been scientifically reported as an antioxidant [5], hepatoprotective [6], anti-inflammatory [7], anti-diabetic [5], anti-proliferative [8] and anti-cataract [9]. Hesperidin is a bioflavanone glycoside found abundantly in citrus fruits and reported to possess antioxidant [10], anticonvulsive [11], hypoglycemic [12], sedative [13] and anti-cancer [14] activity. Moreover, several studies show the synergistic antioxidant activities of carotenoids and bioflavonoids [15,16]. The synergistic effects of the two components have a concatenate effect together, but may have only a fraction of efficacy when assessed individually.

Therefore, the aim of the present was designed to study the combination treatment of hesperidin and lycopene for antiulcer activity in laboratory rats.

MATERIALS AND METHODS

Animals

Thirty Wistar albino rats of either sex weighing 200-250 g were obtained from National Institute of Biosciences, Pune. The animals were acclimatized at standard laboratory conditions maintained at temperature 23±2°C with relative humidity 55±10% and 12 h light and dark cycles. Animals had free access

to water and laboratory feed. The animals were deprived of food 24 h before the study. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC).

Drugs and Chemicals

Hesperidin was obtained as a gift sample from NANS Products (Mumbai, India) and lycopene from Zedip Formulations (Ahemdabad, India). All other chemicals and reagents were purchased from local supplier of Pune.

Experimental Design

Wistar albino rats were divided into five groups ($n = 6$). The animals were fasted for 24 h with water ad libitum before pylorus ligation [17]. To prevent cannibalism and coprophagy they were housed singly in cages with raised bottoms of wide wire mesh. After 24 h of fasting the rats were treated with hesperidin and lycopene 1 h prior to surgery. Group I rats received a vehicle (2% gum acacia solution) at a dose of 10 ml/kg. Group II and Group III rats were treated with hesperidin and lycopene at a dose of a 100 mg/kg and 2 mg/kg; respectively. Group IV were treated with a combined dose of lycopene (2 mg/kg) and hesperidin (100 mg/kg). Group V rats were administered with a standard drug omeprazole at a dose of 10 mg/kg. Then after an hour under ether anesthesia one inch midline abdominal incision was given below the xiphoid process. The pylorus was then carefully lifted and ligated without damaging its blood supply. The stomach was replaced, and the abdominal wall was sutured. After 5 h of ligation, the animals were sacrificed, and the stomach was dissected out. The contents of the stomach were drained by cutting it through the greater curvature. The gastric contents were then centrifuged at 3000 rpm for 10 min and the supernatant was collected and measured.

Estimation of Gastric pH

A volume of 1 ml of the gastric juice was diluted with 1 ml of distilled water and the pH of the solution was measured using a pH meter (EQUIPTRONICS - EQ-614) [18].

Estimation of Total Acidity

An aliquot of 1 ml of gastric juice diluted with 1 ml distilled water was taken in a conical flask and titrated against 0.01N NaOH with phenolphthalein as an indicator till a permanent pink color is obtained [19]. The volume of NaOH was then noted. The total acidity expressed as mEq/L was then calculated by the following formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{N} \times 100\text{mEq/L}}{0.1}$$

Ulcer Index

The stomach mounted was then scored from 0 to 3 on the basis of severity as normal colored stomach (0); red coloration (0.5); spot ulcer (1); hemorrhagic streak (1.5); deep ulcers (2) and perforations (3). Mean ulcer score for each animal will be expressed as ulcer index [20].

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

Where, U_I = Ulcer index; U_N = Average number of ulcers per animal; U_S = Average number of severity score;

U_P = Percentage of animals with ulcer.

Statistical Analysis

All the data were expressed as Mean ± standard error of the mean. The statistical analysis was done by one-way ANOVA, followed by Tukey's test. $P < 0.05$ was considered as minim level of significance.

RESULT

Effect of Combination Treatment of Lycopene and Hesperidin on Gastric Content, Total Acidity and pH

As shown in Table 1, a significant reduction in gastric content and total acidity as well as increase in gastric pH was observed in rats treated with lycopene ($P < 0.05$, $P < 0.001$ and $P < 0.01$; respectively) and hesperidin ($P < 0.01$, $P < 0.001$ and $P < 0.001$; respectively) as compared to control rats. Further, the rats treated with the combination of hesperidin and lycopene showed more significant results ($P < 0.001$, $P < 0.001$ and $P < 0.001$) compared to control rats. Moreover, significant reduction in gastric content and total acidity as well as increase in pH was observed in combination treated rats when compared to lycopene treated rats ($P < 0.01$, $P < 0.001$ and $P < 0.001$; respectively), but as compared to hesperidin treatment it shows the significant improvement only in gastric content and total acidity ($P < 0.05$ and $P < 0.01$; respectively). No significant difference was found in combination treated rats as compared to omeprazole treatment.

Effect of Combination Treatment of Lycopene and Hesperidin on Ulcer Index

Rats treated with individual doses of lycopene and hesperidin significantly decreased the ulcer index (2.21 and 2.00 vs. 3.917;

Table 1: Effect of combination treatment of lycopene and hesperidin on gastric content, total acidity and pH

Group	Gastric contents (ml)	Total acidity (mEq/L)	pH
Control	9.46±0.33	78.36±0.77	2.46±0.15
Lycopene (2 mg/kg)	7.02±0.81*	67.18±1.13$^{@}$	4.18±0.27$^{\#}$
Hesperidin (100 mg/kg)	6.42±0.45$^{\#}$	59.83±1.19$^{@}$	5.40±0.40$^{@}$
Lycopene (2 mg/kg)+ Hesperidin (100 mg/kg)	4.36±0.20$^{@,a,\$}$	51.17±2.57$^{@,b,\wedge}$	6.23±0.31$^{@,b}$
Omeprazole (10 mg/kg)	3.27±0.32$^{@}$	47.33±1.89$^{@,\$}$	5.91±0.26$^{@}$

*$P<0.05$, $^{\#}P<0.01$, $^{@}P<0.001$ versus control, $^{a}P<0.01$, $^{b}P<0.001$ versus Lycopene (2 mg/kg), $^{\$}P<0.05$, $^{\wedge}P<0.01$ versus Hesperidin (100 mg/kg)

$P < 0.05$) as compared to control rats. Rats treated combination of lycopene and hesperidin showed more significant reduction in ulcer index compared to control rats ($P < 0.01$) [Figure 1].

Effect of Combination Treatment of Lycopene and Hesperidin on Macroscopic Changes

As shown in Figure 2, marked deep ulcers with perforations were observed in the stomach of control rats (A). However, lycopene (B) and hesperidin (C) treatment at individual doses reduced the severity of the ulcers with mild red coloration and spot ulcers. The perforations and hemorrhagic streaks were not observed in the rats treated with the combined dose of lycopene and hesperidin (D). The omeprazole treated rats were also found to ameliorate these macrophagic changes (E).

DISCUSSION

Peptic ulcer is considered as one of the modern age epidemics which causes a high rate of morbidity particularly in non-industrialized nations. As reported previously, it is mainly caused due to the imbalance between aggressive and defensive factors [1]. The deleterious effects of smoking and the excessive use of non-steroidal anti-inflammatory drugs inducing ulcers have also been reported. Stress is another factor that triggers the formation of ulcers [21]. The current treatment of ulcers is the "Triple therapy." This makes the use of a proton pump inhibitor or H2 antagonist (reduce gastric acid secretion) combined with either two different antibiotics or an antibiotic and a bismuth salicylate [3]. However, these drugs produce several side effects like gynecomastia [22], thrombocytopenia [23], nephrotoxicity and hepatotoxicity [24]. To overcome these side effects and provide a safe and efficacious therapy, a paradigm shift to natural herbal remedies is necessary, and the combination of carotenoids and bioflavonoids is one of such approaches.

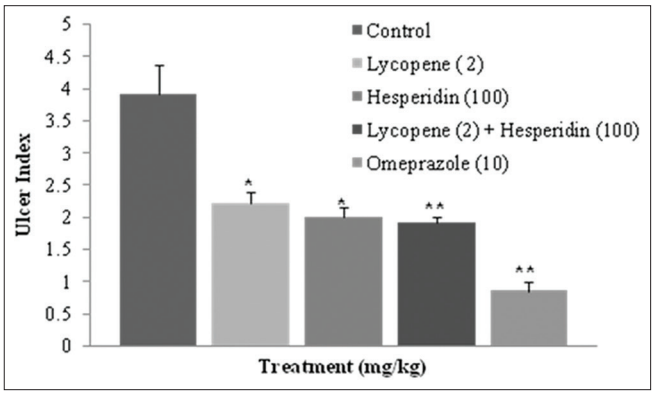

Figure 1: Effect of combination treatment of lycopene and hesperidin on Ulcer index. *$P < 0.05$ and **$P < 0.01$ versus control

In the present study, the pylorus ligation model was used. Pylorus ligation induced ulcer model is mainly used to check the effect of drugs on gastric secretions. Pylorus is the end portion of the stomach after which the duodenum of the small intestine begins. Due to the ligation of the pylorus, there is formation of stress conditions in the membrane. Stress generated can impair the ion transport system thus disturbs the H^+/K^+ ATPase pump and hence results in the production of excessive hydrochloric acid that leads to the development of ulcers. Further, stress may also cause inactivation of prostaglandin synthetase leading to decreased biosynthesis of prostaglandin and thereby reduced the thickness of the protective gastric mucosa [25].

The generation of reactive oxygen species (ROS) is another major reaction of stress induced ulcers [25]. Lycopene and hesperidin are powerful antioxidants, scavenge these ROS and prevent lipid peroxidation of the membrane thereby preventing ulcers [16]. In the present study, we have observed the individual dose of lycopene (2 mg/kg) and hesperidin (100 mg/kg) significantly decreases the gastric secretions and total acidity

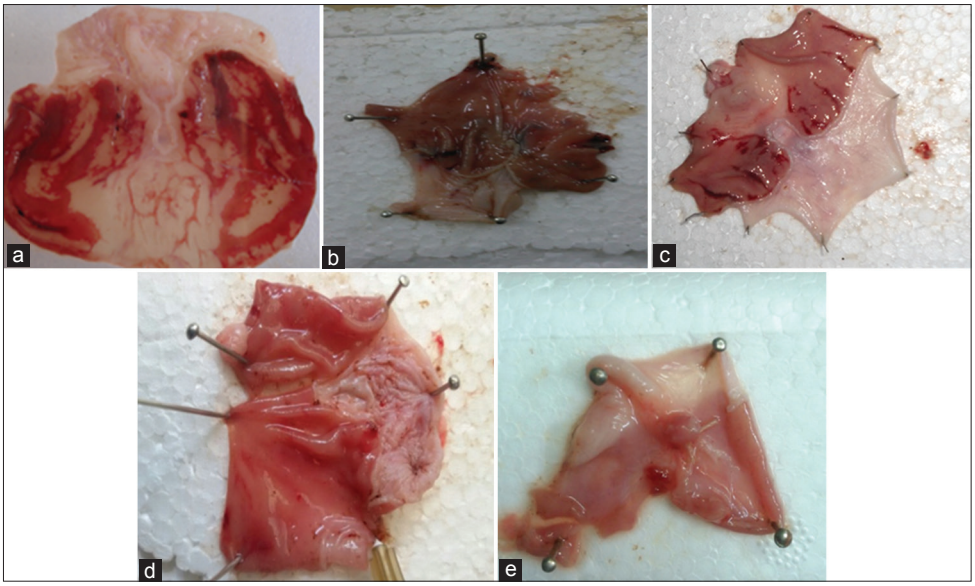

Figure 2: Effect of combination treatment of lycopene and hesperidin on macroscopic changes in the pylorus ligation in rats, a: Control; b: lycopene (2 mg/kg); c: Hesperidin (100 mg/kg); d: hesperidin (100 mg/kg) + lycopene (2 mg/kg) and e: omeprazole (10 mg/kg)

as well as increases gastric pH due to the restoration of the normal gastric acid conditions. Liang *et al*., reported the possible mechanism for the antioxidant synergism of flavonoids and carotenoids due to their interaction on the basis of their water/lipid partition coefficient as well as fast electron transfer reaction from (iso)flavonoid or anion to carotenoid radical cation [26,27]. This electron transfer is facilitated by the flavonoids. In the agreement of the previous reports, we observed more significant improvement in lycopene and hesperidin combination treated rats when compared to control rats, as well as rats treated with lycopene and hesperidin alone. The individual effects of both the phytochemicals are effective, but they are proven to have a synergistic effect as antioxidants, and hence they potentiate the anti-ulcer effect when combined [15].

Thus, the present study suggests interplay between anti-secretory, cytoprotective, neutralizing and the antioxidant properties of a combination of lycopene and hesperidin.

CONCLUSION

Therefore, in conclusion, our results showed synergism in anti-ulcer activity of lycopene and hesperidin. A pharmacologically and analytically well standardized extracts of lycopene and hesperidin together can be cheap and very effective herbal alternative for the treatment of ulcers.

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Original Article

Medicinal plants with potential anti-arthritic activity

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ABSTRACT

Ethno Pharmacological Relevance: Traditional medicinal plants are practiced worldwide for treatment of arthritis especially in developing countries where resources are meager. This review presents the plants profiles inhabiting throughout the world regarding their traditional usage by various tribes/ethnic groups for treatment of arthritis. Materials and Methods: Bibliographic investigation was carried out by analyzing classical text books and peer reviewed papers, consulting worldwide accepted scientific databases from the last six decades. Plants/their parts/extracts/polyherbal formulations, toxicity studies for arthritis have been included in the review article. The profiles presented also include information about the scientific name, family, dose, methodology along with mechanism of action and toxicity profile. Research status of 20 potential plant species has been discussed. Further, geographical distribution of research, plants distribution according to families has been given in graphical form. Results: 485 plant species belonging to 100 families, traditionally used in arthritis are used. Among 100 plant families, malvaceae constitute 16, leguminasae 7, fabaceae 13, euphorbiaceae 7, compositae 20, araceae 7, solanaceae 12, liliaceae 9, apocynaceae, lauraceae, and rubiaceae 10, and remaining in lesser proportion. It was observed in our study that majority of researches are carried mainly in developing countries like India, China, Korea and Nigeria. Conclusion: This review clearly indicates that list of medicinal plants presented in this review might be useful to researchers as well as practioners. This review can be useful for preliminary screening of potential anti-arthritis plants. Further toxicity profile given in the review can be useful for the researchers for finding the safe dose.

KEY WORDS: Arthritis, plant, polyherbal, traditional uses

INTRODUCTION

Immune system of our body plays a crucial role, as an overactive immune system may lead to certain fatal disease because of various hypersensitive or allergic reactions which may cause numerous derangements; loss of normal capacity to differentiate self from non-self resulting in immune reactions against our own's cells and tissues called autoimmune diseases. Certain common autoimmune diseases like myasthenia gravis, serum sickness, pernicious anemia, reactive arthritis etc., are the severe issues for medical and pharmaceutical community because of unknown etiology [1]. According to WHO, 0.3-1% of the world population is affected from rheumatoid arthritis (RA) and among them females are three times more prone to the disease as compared to males [2]. RA is a chronic, inflammatory, and systemic autoimmune disease [3]. The primary symptoms of RA include pain, swelling, and destruction of cartilage and bone as a result of which permanent disability occur. Although the exact etiology is unknown but several hypotheses said that it is triggered by the combination of genetic predisposition and exposure to environmental factors like viruses [4]. The exact pathophysiology is still unknown but release of certain free radicals such as nitrous oxide and superoxide radicals generated as by-products of cellular metabolism. The release of such free radicals may induce the production of interleukins (IL) and tumor necrosis factor (TNF-α) from T-cells which ultimately influence the production of growth factors, cytokines and adhesive molecules on immune cells as such factors may cause tissue destruction and inflammation [5]. Pathological changes in RA are hyperplasia of synovial membrane, infiltration of inflammatory cells and neovascularization, which results into cartilage erosion and articular destruction [3].

The goal of treatment for rheumatoid arthitic patients is to eliminate symptoms, slow disease progression, and optimize quality-of-life [6]. Therefore, before starting the treatment of RA certain goals must be kept in mind such as relief of analgesia, reduction of inflammation, protection of

articular structure, maintenance of function, and control of systemic involvement [5]. Presently for the treatment of RA, strategies have changed from traditionally used non-steroidal anti-inflammatory drugs (NSAIDs) or disease modifying antirheumatic drugs (DMARDs) to novel biological agents, like TNF monoclonal antibody. Clinically, the treatment of RA includes five strategies. The foremost approach is the use of NSAIDs followed by mild doses of glucocorticoids to minimize the signs of inflammation as well as progression of disease. In chronic patients, the use of DMARDs such as methotrexate, sulfasalazine, gold salts or D-pencillamine can be included in the treatment. In certain cases, TNF-α neutralizing agents like infliximab, etanercept etc; IL-1 neutralizing agents like anakinra; and the drugs which interfere with T-cell activation such as abatacept can also be included in treatment of chronic cases. Finally, immunosuppressive and cytotoxic drugs such as cyclosporine, azathioprine, and cyclophosphamide are used for the treatment of chronic patients [5,7,8]. The above-mentioned therapeutic agents reduce the inflammation and joint destruction but their long-term risks are still unknown. However, long-term risks of drugs includes gastrointestinal ulcers, cardiovascular complications, hematologic toxicity, nephrotoxicity, pulmonary toxicity, myelosuppression, hepatic fibrosis, stomatitis, cirrhosis, diarrhea, immune reactions, and local injection-site reactions. Moreover, higher costs and side effects which include high risks of infections and melagnancies reguires continous monitoring [1].

Herbal Therapy for the Treatment of Arthritis

Herbal medicines are used for the treatment of various ailments from ancient times and it is not an exaggeration to say that the use of the herbal drugs is as old as mankind [9]. Herbal medicines are synthesized from the therapeutic experience of generation of practicing physicians of ancient system of medicine for more than hundreds of years [10]. Nowadays, researcher shows a great interest in those medicinal agents that are derived from plants because the currently available drugs are either have certain side effects or are highly expensive [11]. Nature has blessed us with enormous wealth of herbal plants which are widely distributed all over the world as a source of therapeutic agents for the prevention and cure of various diseases [12]. According to WHO, world's 80% population uses herbal medicines for their primary health care needs. Herbal medicines will act as parcels of human society to combat disease from the dawn of civilization [13]. The medicinally important parts of these herbal plants are chemical constituents that produce a desired physiological action on the body [14].

Since ancient time India uses herbal medicines in the officially alternative systems of health such as Ayurveda, Unani, Sidha, Homeopathy, and Naturopathy [15]. In India, there are more than 2500 plants species which are currently used as herbal medicaments. For than 3000 years, the herbal medicines are used either directly as folk medication or indirectly in the preparation of recent pharmaceuticals [16]. Thus, from the knowledge of traditional plants, one might be able to discover new effective and cheaper drugs [17]. In this review article, we have tried to cover all the ayurvedic strategies that are followed for the treatment of RA without any possible side effects. The future treatment of RA should provide more effective relief [5].

MATERIALS AND METHODS

In this review, bibliographic investigation was carried out during July 2011-December 2013, by refering various text books and certain review papers and research papers, consulting globally accepted databases from last some decades. The data were gathered from various databases i.e. Science Direct, PubMed, and Google and the information is compiled by reviewing more than 250 research and review articles. The data which are relevant would be considered. The botanical correct names and families were mentioned after verification from published literature and databases.

The method of scrutining the data for this review article includes those plants: (i) Which are native to India and other countries such as America, Africa or Europe, (ii) used in traditional systems and in various polyherbal preparations, (iii) with reported anti-arthritic activity, (iv) appropriate dosage, (v) mechanism of action, (vi) safety profile, and (vii) models used. Plants/their parts/extracts used traditionally in acute rheumatic attacks, chronic analgesia, and chronic rheumatism have been considered as anti-arthritic agents. Further, detailed information on research status of 20 plant species has been explained.

Polyherbal Formulations for Arthritis

Analgesics and NSAIDs are helpful in reducing pain and inflammation in either acute or chronic RA patients [18]. Although the treatment of RA is available but due to potential adverse effects or irreversible organ damage the new approaches are developed for maintaining the balance between these potential risks and acknowledged benefits [19]. Currently for the treatment of RA safer and more potent medicaments are developed from oriental sources. Large number of herbal extracts and products such as polyherbal formulations are prepared to reduce such side effects and increase the benefits [18].

Rheum off Gold is a poyherbal formulation that is commonly recommended by Ayurvedic medical practitioners for the treatment of arthritis. The anti-arthritic activity was confirmed on complete Freund's adjuvant (CFA) induced arthritis model in wistar rats and it was observed that significant reduction in arthritis index, paw thickness and inflammatory markers such as C-reactive protein, serum rheumatoid factor and erythrocyte sedimentation rate (ESR) when compared with dexamethasone. Thus, the formulation possesses a potential anti-arthritic activity [20].

A Unani polyherbal formulation was evaluated for its anti-arthritic activity in rats. The anti-arthritic efficacy of Manjoon Suranjan was evaluated using formaldehyde and CFA induced arthritis models. The data obtained suggested the anti-arthritic activity of the formulation [21].

Evaluation of Sudard as a potent anti-arthritic polyherbal formulation was studied using formaldehyde and adjuvant induced arthritis models in wistar rats. The formulation at the doses of 150 mg/kg and 300 mg/kg p.o. proves to have an anti-inflammatory and anti-arthrtic activity [22].

Anti-arthritic potential of Tongbiling (TBL-II) which was prepared by some modification in Chinese herbal formulation TBL. The anti-arthritic efficacy of formulation was studied using the collagen induced arthritis model in wistar rats and it was revealed that at the doses of 100 and 300 mg/kg p.o. the levels of IL-1β and TNF-α was significantly reduced. Thus it was concluded that the formulation have an anti-arthritic potential [23].

Chinese herbal formula HLXL was used in the treatment from last hundred years for the treatmnent of inflammation and arthritis. Moreover, after certain modifications in HLXL herbal formulation it was evaluated for its anti-arthritic property using CFA model in rats. It was concluded that the polyherbal formulation shows an anti-arthritic activity through significant inhibition of paw edema and levels of TNF-α and IL-β [24].

The therapeutic effect of Ganghwaljetongyeum on RA in rabbit knee synovial membrane was evaluated. It was observed that there would be significant inhibition of proliferation of HG-82 cells which shows that the polyherbal formulation have an anti-arthritic activity. Moreover, there was significant reduction in TNF-α, IL-10 and NO species [18]. Various polyherbal formulations are described in Table 1.

RESULTS

About more than 350 articles were reviewed. More than 20 articles were studied for searching the traditional use of plants in arthritis [Table 2]. Around 108 articles were referred for citing the proved anti-inflammatory and anti-arthritic activities of plants along with mechanism of action, acute toxicity profile, and doses [Table 3].

The detailed information on research status of following 20 plant species was gathered from multiple references.

Alstonia scholaris Linn. (AS)(Family-Apocynaceae)

AS is commonly known as saptaparni or devil's tree, widely distributed in dried forests of India as Western Himalayas, Western Ghats, and in the Southern region. AS is a medium to large tree about 40 m high with a somewhat tessellated corky grey to grey-white bark [25]. Traditionally, bark of AS is used in the treatment of rheumatism, malarial fevers, abdominal disorders, leprosy, asthma, bronchitis, pruritis, and chronic ulcers [12]. Milky juice is mixed with oil and was applied in rheumatic pains. The chief alkaloids present in AS are echitamine, tubotaiwine, akaummicine, echitamidine, picrinine, and strictamine. AS flowers also contains amino acids, carbohydrates, phenol, tannins, cardiac glycosides, saponins, flavanoids, steroids, fixed oil, and fats [26]. The plant showsimmune-stimulatory, hepato-protective [27], anti-cancer [28], anti-plasmodial [29], and anti-hypertensive [30] activities. Extract of AS possess an anti-diabetic, anti-hyperlipidemic [31], anti-bacterial [32], anti-inflammatory, analgesic [33], antioxidant [27], immunostimulant [34], anti-cancer [35], anti-asthmatic [36], hepatoprotective [37], and anti-anxiety activity [12,25,38]. The ethanolic extract of AS leavesat doses of 100 and 200 mg/kg confirmed anti-arthritic activity in male wistar rats. The anti-arthritic activity was mainly by reducing the total leukocyte migration as well as lymphocytes and monocytes/macrophages migration. It can be concluded that AS shows an anti-arthritic activity on male wistar rats [39].

Aristolochia bractaeta Lam. (AB)(Family-Aristolochiaceae)

AB commonly known as worm killer or kidamari is a shrub found in Deccan Gujarat, western and southern India, Bihar, Sindh, and Bengal [16]. Traditional use of AB was found in gonorrhea, syphilis, inflammation, ulcer, amenorrhea, skin disease, dermatitis, leprosy, jaundice, and helminthiasis [16]. The major chemical constituents of the AB are alkaloids, triterpenoids, steroids, flavonoids, saponins, carbohydrates, proteins, and cardiac glycosides [40,41]. The studies of extract have shown anti-pyretic [42], anti-allergic [43], anti-inflammatory, anti-arthritic [1], anti-ulcer [44], anti-fungal [45], anti-microbial [46], antioxidant [47], wound healing [48], anti-implantation, and abortificient activities [49]. The petroleum ether, methanol, and chloroform extract of whole plant of AB possess comparable anti-arthritic activity at doses of 100, 200, and 400 mg/kg body weight. AB revealed anti-arthritic activity by maintaining the synovial membrane and vascular permeability thus inhibiting cytokines and leukotriene infiltration. In conclusion, AB possesses an anti-arthritic effect on wistar albino rats of either sex [1].

Boerhaavia diffusa Linn. (BD)(Family-Nyctagineae)

BD is found all over India especially during rain. Two varieties of BD are explored, one with white flowers called "shwethpurna" and other flowers called "raktapurna." The medicinally important part is root (MateriaMedica, 1982). BD is traditionally significant due to their laxative, diuretic, expectorant, diaphoretic, and emetic properties [50]. A paste made up of roots together with *Colchicum*, *Solanum nigrum*, Tamarind stone, Stag's horn and dried ginger, all in equal parts, are used in rheumatic and gouty painful joints. Root is used as powder in drachm doses or decoction or infusion for the treatment of inflammatory disorders like arthritis. Chakradatta used it in the treatment of chronic alcoholism and various other ailments i.e. phthisis, insominia, and rheumatism [51]. The air-dried plant was found to contain large quantities of potassium nitrate and also contains an alkaloid, panarnavine, present in very small quantity of 0.01%. Recent investigations reported that BD possess an antistress, adaptogenic [52], antioxidant [53], immunosuppressive [54], anti-carcinogenic [55], hepatoprotective [56,57], diuretic [58], anti-diabetic [59], anti-viral [60], and anti-inflammatory

Table 1: Polyherbal formulations

Product name	Ingredients	Botanical name	Quantity	Manufactured by
Rumalaya forte - Tablet	Shallaki	*Boswellia serrata*	240 mg	Himalaya Global Holdings Ltd.
	Camphor	*Cammiphora wightii*	200 mg	
	Rasna	*Alpiniaga galangal*	70 mg	
	Yashtimadhu	*Glycyrrhiza glabra*	70 mg	
	Gokshura	*Tribulus terrestris*	60 mg	
	Guduchi	*Tinospora cordifolia*	60 mg	
	Nirgundi	*Vitex negundi*	60 mg	
	Sunthi	*Zinger officinalis*	60 mg	
Rumalaya - Liniment	Bakuchi	*Psoralea caryofolia*	35 mg	Himalaya Global Holdings Ltd.
	Maricha	*Piper nigrum*	35 mg	
	Karpura	*Cinnamomum camphor*	90 mg	
	Pudina	*Mentha arvensis*	40 mg	
	Ajamoda	*Carum capticum*	35 mg	
	Tila	*Sasamum indicum*	365 mg	
	Gandhapura	*Gaultheria fragrantissima*	350 mg	
	Sarala	*Pinus longifolia*	50 mg	
Artha cure - Oil	Clovos	*Syzgium aromaticum*	50 mg	Be Sure Health Care (P) Ltd.
	Mithazahar	*Aconitum ferox*	25 mg	
	Kupilu	*Strychnos nux vomica*	25 mg	
	Garlic	*Allium sativum*	50 mg	
	Akasbel	*Cuscuta reflexa*	50 mg	
	Jatiphalam	*Myristica fragrans*	50 mg	
Arthcure - Capsule	Khorpad	*Aloe vera*	50 mg	Be Sure Health Care (P) Ltd.
	Hiranya-tuttha	*Colchicum leuteum*	50 mg	
	Nisoth	*Operculina terpethum*	50 mg	
	Shonpat	*Crotalaria juncea*	50 mg	
	Pippali	*Piper longum*	50 mg	
	Jatiphalam.	*Myristica fragrans*	50 mg	
	Clovos	*Syzgium aromaticum*	50 mg	
	Sonth	*Zingiber officinale*	50 mg	
	Asphalt	*Black bitumen*	25 mg	
	Mahayogaraja guggulu	*Commipora mukul*	25 mg	
	Mithzahar	*Aconitum ferox*	25 mg	
	Kupila	*Strychnos nux vomica*	25 mg	
	Ashvagandha	*Withania somnifera*	50 mg	
	Shatavari	*Asparagus racemosus*	50 mg	
	Garlic	*Allium sativum*	50 mg	
	Akasbel	*Ciscuta refluxa*	50 mg	
Rheumartho gold - Capsule	Suranjan kadwi	*Colchicum luteum*	60 mg	Baidyanath
	Asgandh	*Withania somnifera*	60 mg	
	Shodhit kuchla	*Strychnos nux vomica*	50 mg	
	Salai guggul	*Boswellia serrata*	215 mg	
	Maharasnadi qwath	*Ghanna sativa*	64 mg	
	Abrak bhama	*Biotite calx*	5 mg	
	Harsingar	*Nyctanthes arbor-tristis*	30 mg	
	Swarnamakshik bhasma	*Calx of copper pyrites*	5 mg	
	Yograj guggulu	*Commiphora mukul*	30 mg	
	Swarn bhasma	*Ipomoea digitata*	0.6 mg	
	Loha bhasma	*Calx of corat*	5 mg	
Ortho joint oil	Vishagarbha taila	*Ricinus communis*	2.5ml	SBS Biotech Ltd.
	Mahamasha taila	*Vign unguiculata*	2.5 ml	
	Dalchini taila	*Cinnamomum zeylanicum*	0.5 ml	
	Gandhapuro	*Gaultheria fragrantissima*	2.0 ml	
	Camphor	*Cinnamomum camphor*	100 mg	
	Sat pudina	*Menthe arvensis*	2.5 mg	
	Narayan taila	*Wthania somnifera*	2.5 ml	
Rheuma off gold	Mahayogaraja guggulu	*Commiphora mukul*	72 mg	Virgo UAP Pharma (P) Ltd. [20]
	Maharasnadi kwath	*Suvarna bhasma*	112 mg	
	Suvarna bhasma	*Strychnos nux vomica*	1.6 mg	
	Suddha kuchala	*Boswellia serrata*	9.6 mg	
	Shallaki		4.8 mg	
Majoon suranjan	Kalaparni	*Ipomea turpethum*	445 mg	Qarshi herbal products [21]
	Pathya	*Terminalia cheluba*	223 mg	
	Hiranya-tuttha	*Colchicum luteum*	223 mg	
	Kakadani	*Capparis spinosa*	44.5 mg	
	Kustumbari	*Coriandrum sativum*	44.5 mg	

Contd...

Table 1: Polyherbal formulations

Product name	Ingredients	Botanical name	Quantity	Manufactured by
	Fish baries	*Rosa damascus*	44.5 mg	
	Lancaster rose	*Plumbago zelanicum*	44.5 mg	
	Chitra	*Zingiber officinalis*	44.5 mg	
	Sonth	*Aloe barbadensis*	44.5 mg	
	Khorpad	*Apium graveolens*	33 mg	
	Ajmoda	*Convulvulus scammony*	33 mg	
	Sakmunia	*Sepia latimanus*	33 mg	
	Cuttle fish bone	*Foeniculum vulgare*	33 mg	
	Fennel	*Lawsonia inermis*	33 mg	
	Mendhi	*Piper nigrum*	33 mg	
	Black pepper	*Sodium chloride*	33 mg	
	Table salt	*Zataria multiflora*	33 mg	
	Satar	*Ricinus communis*	33 mg	
	Eranda		0.668 mg	
	Saccharum base			
	Preservatives			
Huo Luo Xiao Ling Dan (HLXL)	Ruxiang	*Boswellia carterii*	15 g	[24]
	Qianghuo	*Notopterygium incisum*	12 g	
	Danggui	*Angelica sinensis*	12 g	
	Chishao	*Paeonia lactiflora*	12 g	
	Gancao	*Glycyrrhiza uralensis*	12 g	
	Yanhusuo	*Corydalis yanhusuo*	12 g	
	Danshen	*Salvia miltiorrhiza*	12 g	
	Chuanxiong	*Ligusticum chuanxiong*	12 g	
	Qinjiao	*Gentiana macrophylla*	12 g	
	Guizhi	*Cinnamomum cassia*	15 g	
	Duhuo	*Angelica pubescens*	12 g	
Ganghwaljetongyeum (GHJTY)	Angelicae koreanae	*Angelica koreanum*	06 mg	[18]
	Atractylodis rhizoma	*Atractylodes chinensis*	06 mg	
	Manchurian spikenard	*Aralia continentalis*	04 mg	
	Paeonia radix rubra	*Paeonia obovata*	04 mg	
	Stephaniae tetrandrae	*Sinomenium acutum*	04 mg	
	Clematidis radix	*Clematis mandshurica*	04 mg	
	Giant angelica	*Angelica gigas*	04 mg	
	Hoelen	*Poria cocos*	04 mg	
	Alismatis rhizoma	*Alisma orientale*	04 mg	
	Akebiae caulis	*Akebia quinata*	04 mg	
	Tangerine	*Citrus unshiu*	04 mg	
	Chaenomelis fructus	*Chaenomeles sinensis*	04 mg	
	Phellodendri cortex	*Phellodendron amurense*	03 mg	
	Glycyrrhizae radix	*Glycyrrhiza uralensis*	02 mg	
	Juncus medulla	*Juncus effuses*	04 mg	
	Gleditsiae spina	*Gleditsia sinensis*	04 mg	
	Lonicerae caulis	*Lonicera japonica*	04 mg	
	Taraxaci herba	*Taraxacum platycarpum*	04 mg	
Sudard	Guggulu	*Commiphora mukul*	100 mg	Anglo French Drugs and Industries
	Rasna	*Pluchea lanceolata*	50 mg	Ltd, Bangalore, India [22]
	Gandha prasarini	*Paederia foetida*	50 mg	
	Nirgundi	*Vitex negundo*	50 mg	
	Ginger	*Zingiber officinalis*	50 mg	
	Eranda mula	*Ricinus communis*	50 mg	
	Chandra sura	*Lepidium sativum*	30 mg	
	Suranjan	*Colchicum luteum*	30 mg	
	Dwipantra wacha	*Smilax glabra*	30 mg	
	Kupilu	*Strychnous nuxvomica*	10 mg	
	Shilajatu	*Mineral pitch*	50 mg	
TBL-II	*Cinnamomi cassiae*	*Cinnamomi cassiae*	15 g	Zhong-Yue Herbal Pharmaceutical
	Paeoniae alba radix	*Paeoniae alba*	30 g	Union Company in China [23]
	Radix aconiti lateralis	*Aconiti lateralis*	09 g	
	Achyranthes bidentata	*Achyranthes bidentata*	09 g	
	Celastrus orbiculatus	*Celastrus orbiculatus*	18 g	
	Millettia reticulata	*Millettia reticulata* Benth	06 g	

TBL: Tongbiling

activities [61,62]. The petroleum ether extract of roots at dose 1000 mg/kg has been evaluated as anti-arthritic using CFA model and showed 81.5% response as compared to indomethacin [63].

Table 2: Traditionally used anti-arthritic plants

Botanical name	Family	Common name	Part used	Dosage form	References
Abrus precatorius Linn.	Papilionaceae	Indian liquorice, chirmiti, gunchi	L	Oil	[278]
Acacia catechu Willd.	Fabaceae	Mimosa catechu	R	Extract	[279]
Acalypha indica Linn.	Euphorbiaceae	Kuppu, Arittmanjarie	L	Juice	[19]
Acanthus illicifolius Linn.	Acanthaceae	Sea holly, Moranna harikusa	L	Extract	[278]
Achillea millefolium Linn.	Compositae	Rojmari, bloodwort, arrow-root	H	Extract	[280]
Achyranthus aspera Linn.	Amaranthaceae	Chirchitta, aghada, prickly chaff-flower	R	Infusion	[281]
Acampe wightiana Lindl.	Orchidaceae	Marabale	Wh	Extract	[278]
Aconitum ferox Wall.	Ranunlaceae	Mithazahar, visha	R	Liniment, paste	[51]
Aconitum napellus Linn.	Ranunlaceae	Monk' hood	R, L	Liniment	[51]
Aconitum palmatum Don.	Ranunlaceae	Bikhma	R	Paste	[51]
Acorus calamus Linn.	Aroideae	Bach, vacha	R	Powder	[51]
Actaea racemosa Linn.	Ranunculaceae	Black cohosh	R, Rh	Extract	[51]
Actaea spicata Linn.	Ranunculaceae	Baneberry, grapewort	R	Powder	[278]
Adansonia digitata Linn.	Malvaceae	Gorakh amli	L	Poultices	[19]
Adenthera pavonina Linn.	Leguminosae	Kuchandana	L, B	Decoction	[51]
Adhatoda vasika Nees.	Acanthaceae	Adosa, adarushah	L	Poultices	[51]
Aegel marmolosa Corr.	Rutaceae	Stone apple, bael	F	Juice	[14]
Aesculus indica Colebr.	Sapindaceae	Bankhor, pankar	F	Oil	[278]
Agave americana Linn.	Amaryllidaceae	American aloe, kantal, bilatipat	L	Paste	[278]
Aghati grandiflora Desv.	Leguminosae	Hathia, agastya	R	Paste	[51]
Agropyron repens Beauv.	Graminae	Couch G, quilch	Rh	Extract	[278]
Ailanthus excels Roxb.	Simaroubaceae	Indian tree of heaven	L	Oil, extract	[279]
Alangium lam*arckii* Thwaites.	Cornaceae	Akola, shoedhanam	R, B	Oil	[51]
Allium cepa Linn.	Liliaceae	Onion, palandu	Bu	Paste	[19]
Allium sativum Linn.	Liliaceae	Garlic, lasun	S	Oil	[51]
Alocasia indica Schott.	Aroideae	Mankanda, alooka	T	Hot T	[51]
Alpinia galangal Willd.	Scitaminaceae	Sugandhavacha	Rh	Paste	[51]
Alstonia scholaris R.Br.	Apocynaceae	Datyuni, saptaparna	Mj	Juice	[39]
Althaea rosea Cav.	Malvaceae	Hollyhock, round dock	Fl	Oil	[278]
Ammannia baccifera Linn.	Lythraceae	Dadmari, agni-garva	L	Blisters	[2]
Amorphophallus campanulatus Roxb.	Aracea	Zamikand, kandula kandvardhana	S	Oil	[278]
Anacylus pyrethrum DC.	Compositae	Akarkaro	R	Infusion	[51]
Andropogon citrates DC.	Gramineae	Bhushtrina,true lemon grass	L	Oil, liniment	[51]
Androphogon iwarancusa Roxb.	Gramineae	Lamjak	R	Paste	[51]
Andropogon martini DC.	Gramineae	Grass of nemaur	G	Oil	[51]
Andropogon nardus Linn.	Graminae	lemon grass	St	Oil	[282]
Anemone obtusiloba Don.	Ranunculaceae	Padar, rattanjog	R	Oil	[51]
Anisomeles malabarica Linn.	Labiatae	Alamoola	L	Oil, decoction	[19]
Aphanamixis polystachya Blatter.	Meliaceae	Harinhara, janavallabha	S	Oil, liniment	[278]
Apium graveolens Linn.	Umbellifeae	Ajmoda, celery	R	Decoction	[280]
Aquilaria agallocha Roxb.	Thymelaeaceae	Aloe-wood, garu	W	Decoction	[51]
Arctium lappa Linn.	Compositae	Garden celery	L	Infusion	[280]
Argyreia speciosa Sweet.	Convulvulaceae	Elephant creeper	R	Powder	[19]
Aristolochia bracteata Linn.	Aristolochiaceae	Birthworts, pipevines	Wh	Extract	[1]
Aristolochia serpentaria Linn.	Aristolochiaceae	Virginian snake root	R	Infusion	[282]
Artanema sesamoides Benth.	Scrophularineae	Kokilaksha	R	Decoction	[51]
Artemisa absinthium Linn.	Compositae	Indhana, worm-wood	H	Juice	[51]
Asparagus filicinus Ham.	Liliaceae	Allipalli, sansarpal	R	Extract	[278]
Asparagus officinalis Linn.	Liliaceae	Marchuba	R	Powder	[51]
Asparagus racemosus Willd.	Liliaceae	Shatavari	R	Oil	[51]
Asystasia coromandeliana Nees.	Acanthaceae	Lavana-valli	Wh	Juice	[51]
Asystacia gangetica T. Anders.	Acanthaceae	Avokombily, puruk	Wh	Juice	[278]
Atalantia monophylla DC.	Rutaceae	Wild-lime, atavi-jambira	Be	Oil	[51]
Atropa belladonna Linn.	Solanaceae	Black cherry, sagangur	R, L	Extract	[278]
Atylosia barbata Baker.	Leguminosae	Mashaparni	R	Extraction	[51]
Azadirachta indica A. Juss.	Meliaceae	Bakayan, Indian lilac, balnimb	L	Decoction	[278]
Azima tetracantha Lam.	Salvadroraceae	Kundali	L, R	Decoction	[19]
Bacopa monnieri Penell.	Plantaginaceae	Brahmi	Wh	Extract	[283]
Balsamodendron mukul Hook.	Burseraceae	Guggula, salaitree	Gm	Paste	[51]
Balsamodendron playfairii Hook.	Burseaceae	Meena-herma	Gm	Paste	[51]
Barleria courtallica Nees.	Acanthaceae	Wahiti, artagala	R	Decoction	[278]
Barleria cristata Linn.	Acanthaceae	Jhinti, tadrelu	R	Decoction	[51]
Baliospermum montanum Muell.	Euphorbiaceae	Dantimul, hakum, anukheti	S	Oil	[278]
Bassia butyracea Roxb.	Sapotaceae	Phulwara butter	K	Fat	[51]
Bassia latifolia Roxb.	Sapotaceae	Madhuka, jangli moha	B	Decoction	[51]
Bassia longifolia Linn.	Sapotaceae	Madhuka, mohua	S	Oil	[51]

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Table 2: Contd...

Botanical name	Family	Common name	Part used	Dosage form	References
Bassia malabarica Bedd.	Sapotaceae	Illuppi	F, S	Oil	[51]
Barosma crenulata Hook.	Rutaceae	Bucchu, buku	L	Powder	[282]
Bauhinia racemosa Lam.	Fabaceae	Bidi leaf tree, kachnal	B	Extract	[14]
Bauhinia tomentosa Linn.	Fabaceae	Yellow bell orchid	L	Infusion	[13]
Berberis asiatica Roxb.	Berberidaceae	Kilmora	St	Decoction	[51]
Berberis petiolaris Wall.	Berberidaceae	Chachar, ambar	R	Decoction	[278]
Berberis vulgaris Linn.	Berberidaceae	True barberry	St	Decoction	[51]
Bidens pilosa Linn.	Compositae	Black jack, phutium	Sh	Young shoots	[278]
Blumea balsamifera DC.	Compositae	Nagal camphor, kakaronda	L	Fumigation	[278]
Blumea ripens DC.	Asteraceae	Red stink wood	S	Oil	[279]
Bula alba Linn.	Cupuliferae	White birch bark	L	Extraction	[51]
Boerhaavia diffusa Linn.	Nyctagineae	Punarnava, thikri	R	Paste	[63]
Boucerosia aucheriana Dcne.	Asclepiadaceae	Charungli, chungi pamanke	St, Wh	Juice	[278]
Borassus flabellifer Linn.	Arecaceae	Toody palm, sugar palm	F	Juice	[14]
Boswellia glabra Roxb.	Burseraceae	Kapithaparni, lobhan	Gm	Gum	[51]
Boswellia serrata Triana.	Burseraceae	Salai gugul	Gm, Rs	Gum	[76]
Brassica campestris Linn.	Cruciferae	Wild turnip, bangasarson	S	Oil	[278]
Brassica integrifolia West.	Cruciferae	Rai, Indian mustard, rajika	S	Oil	[278]
Brassica juncea Coss	Cruciferae	Rai, rajika	S	Oil	[51]
Brassica nepus Linn.	Cruciferae	Van dai, onuma	Wh	Extract	[278]
Brassica nigra Linn. & Koch.	Cruciferae	Kalori, sarshapah	S	Oil	[51]
Brassica oleracea Linn.	Cruciferae	Cabbage, karamkalla	L	Extract	[278]
Bridelia retusa Spreng.	Euphorbiaceae	Gaya, kajja, assana	B	Oil	[278]
Bryonia epigoea Rottl.	Cucurbitaceae	Rakas-gaddah, mahamula	R	Powder	[51]
Buxus sempervirens Linn.	Euphorbiaceae	Papari	L	Extraction	[51]
Caccinia glauca Savi.	Borageneae	Goazaban	L	Extraction	[51]
Cadaba indica Lamk	Capparidaceae	Indian cadaba	L	Decoction	[19]
Caesalpinia bonduc Roxb.	Caesalpiniaceae	Katkaranj, latakaranja	S	Oil	[51]
Callicarpa macrophylla Vahl.	Verbenaceae	Sumali	R	Decoction	[51]
Calophyllum apelatum Wild.	Guttiferae	Cherupinnai, sarapuna	S	Oil	[278]
Calophyllum inophyllum Linn.	Guttiferae	Surpan, punnaga	K	Oil	[19]
Calotropis gigantean R.Br.	Asclepiadaceae	Gigantic, arka	R	Powder (mucilage)	[51]
Calotropis procera R.Br.	Asclepiadaceae	Madar	R-B	Extract	[282]
Cammphora officinarum Bauh.	Lauraceae	Camphor, kapur	C	Liniments	[51]
Canangium odoratum Baill.	Annonaceae	Kadapanyan, maladi	Fl	Oil	[278]
Canarium bengalense Roxb.	Burseraceae	Bisjang, dhuna, goguldhop	L, B	Extract	[278]
Canarium commune Linn.	Burseraceae	Java almond, jangali badam	T	Ointment	[278]
Canarium strictum Roxb.	Burseraceae	Black damer	Rs	Ointment	[51]
Cannabis sativa Linn.	Urticaceae	Ganja, charas	S	Oil	[98]
Canella alba Murry.	Canellaceae	Jamaica	B	Oil	[282]
Capparis aphylla Roth.	Capparideae	Caper plant, karira	R-B	Powder, infusion	[51]
Capparis deciduas Edgew	Capparideae	Chayruka	L	Extract	[278]
Capparis heyneana Wall.	Capparideae	Chayruka	L	Decoction	[51]
Capparis spinosa Linn.	Capparideae	Kabra, kakadani	L	Decoction	[51]
Capsicum annum Linn.	Solanaceae	Lal mirchi, spanish pepper	F	Tincture	[51]
Cardiospermum halicacabum Linn.	Sapindaceae	Balloon vine, winter cherry	R, L	Decoction	[284]
Carissa carandas Linn.	Apocynaceae	Karamardaka	S	Extract	[279]
Carissa spinarum Linn.	Apocynaceae	Karaunda, garna	R	Extract	[278]
Carthamus tinctorius Linn.	Compositae	Wild saffron, kamalottara	Fl	Hot infusion	[51]
Cassia fistula Linn.	Caesalpiniceae	Sonhali, nripadruma	B, L	Paste	[19]
Cassia sophera Linn.	Caesalpiniceae	Bas-ki-kasunda	L	Infusion	[51]
Cassia tora Linn.	Fabaceae	Charota, taga	L	Infusion	[279]
Cadreia toona Roxb.	Meliaceae	Toona, khusing	B	Infusion	[51]
Cedrus deodara Lou Don.	Coniferae	Deodar, kilan, geyar	W	Oil	[278]
Cedrus libani Barrel	Coniferae	Deodar, devadaru	Gm	Gum	[51]
Celastrus paniculata Willd.	Calastraceae	Malakanguni, vanhiruchi	S	Decoction	[19]
Celosia argentia Linn.	Amaranthaceae	Paanai keerai	L	Decoction	[13]
Centella asiatica Urban.	Mackinlayaceae	Gotu kola	St	Extract	[285]
Cephaelis ipecacuanha A.Rich.	Rubiaceae	Poaya	R	Extract	[282]
Chenopodium album Linn.	Chenopodiaceae	Goosefoot, fathen	Wh	Extract	[279]
Chloroxylon swietenia DC	Meliaceae	Bheria, girya, yellow wood	L	Oil	[278]
Cicuta virosa Linn.	Apiaceae	Cowbane, water hemlock	Wh	Poultice	[282]
Cimicifuga racemosa Ellicot.	Ranunculaceae	Balck snake root, bugbane	R	Extract	[282]
Cinchona calisaya Hook.	Rubiaceae	Peruvian bark	B	Infusion	[51]
Cinnamomum camphora Nees.	Lauraceae	Camphor laurel	W	Oil	[282]
Cinnamomum cassia Blume.	Lauraceae	Dalchini, gudatvak	L	Oil	[51]

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Table 2: Contd...

Botanical name	Family	Common name	Part used	Dosage form	References
Cinnamomum tamala Fr.Nees.	Lauraceae	Cassia lignea, tejpat	L	Extract	[278]
Cinnamomum macrocarpum Hook.	Lauraceae	Dalchini, tejpatra	R, B,L	Oil	[109]
Cinnamomum parthenoxylon DC.	Lauraceae	Kaaway, kayogadis	F	Oil	[278]
Cissus quadrangularis Linn.	Vitaceae	Devil's backbone	Wh	Extract	[279]
Cistus creticus Linn.	Cistaceae	Ladano	L	Oil	[282]
Citrullus colocynthis Schrad.	Cucurbitaceae	Indrayan, colocynth	R	Powder	[19]
Citrus aurantium Linn.	Rutaceae	Narengi, sweet orange	Fl	Liniment	[51]
Citrus bergamia Ris.	Rutaceae	Jambha, nimbu	F	Juice	[51]
Citrus limonum Sp.Risso.	Rutaceae	Jambira, limpaka	F	Juice	[51]
Cleome brachycarpa Linn.	Capparidaceae	Panwar, kasturi	Wh	Extract	[278]
Cleome gynandra Linn.	Capparaceae	African cabbage, spiderwisp	Wh	Extract	[286]
Cleome rutidosperma DC.	Cleomaceae	Fringed spider flower	Wh	Decoction	[14]
Clerodendron colebrookianum Walp.	Lamiaceae	Glowery bower	Rh	Extract	[279]
Clerodendron inerme Gaertn.	Verbenaceae	Garden quinine, binjoam	R	Liniment	[51]
Clerodendron phlomides L.F.	Verbenaceae	Agnimantha, jaya	L	Paste	[14]
Clerodendron serratum Spreng.	Verbenaceae	Barangi, baleya, angaravalli	R	Decoction	[278]
Clerodendron siphonanthus R.Br.	Verbenaceae	Bharangi, arnah, chingari	W	Rs	[278]
Clitoria ternatea Linn.	Verbenaceae	Butterfly-pea	Wh	Extract	[279]
Cocculus cordifolius Miers.	Menispermaceae	Heart-leaved, gulancha	St, L, R	Infusion	[51]
Cocculus hirsutus Diels.	Menispermaceae	Broom creeper, chireta	R	Infusion	[278]
Cocculus villosus DC.	Menispermaceae	Jaliamni, faridbel	R	Decoction	[19]
Cochlearia armoracia Linn.	Cruciferae	Horse-radish	R	Condiment	[282]
Colchicum autumnale Linn.	Melanthaceae	Wild saffron	S	Extract	[282]
Colchicum luteum Baker.	Liliaceae	Golden collyrium, hiranya-tuttha	R	Extract	[51]
Coldenia procumbens Linn.	Borageineae	Tripungkee	L	Extract	[51]
Coptis teeta Wall.	Ranunculaceae	Gold thread, mishamitita	R	Paste	[51]
Corallocarpus epigeous Rottl & Willd.	Cucurbitaceae	Akasgaddah, karwinai, lufa	R	Decoction	[19]
Coriandrum sativum Linn.	Umbelliferae	Coriander, kustumbari	F, L	Oil	[120]
Costus speciosus Sm.	Scitaminaceae	Kemuka, kushtha, padmapatra	R	Extract	[287]
Cotula anthemoides Linn.	Compositae	Babuna	R	Infusion	[51]
Crataeva nurvala Linn.	Capparidaceae	Bhatavarna, biiana	L	Juice	[278]
Crataeva religosa Hook& Forst.	Capparidaceae	Three leaved creeper, pashuganda	L	Juice	[51]
Crinum asiaticum Linn.	Amaryllidaceae	Poison bulb, chindar	Bu	Roasted Bu	[51]
Crinum latifolium Linn.	Amaryllidaceae	Chakrangi, dadhyani	Bu	Roasted Bu	[278]
Crocus sativus Linn.	Irideae	Saffron, bhavarakta	Sg	Tincture, infusion	[51]
Crotalaria prostrate Rottler.	Fabaceae	Prostate rattlepod	Wh	Extract	[279]
Croton oblongifolus Rox.	Euphorbiaceae	Chucka, bhutamkusam	B	Infusion	[51]
Croton tiglium Linn.	Euphorbiaceae	Jamalgota, naepala	S	Liniment	[51]
Curcuma longa Linn.	Scitaminaceae	Turmeric, haldi, varnavat	Rh	Powder	[130]
Cymbopogon citrates Stapl.	Graminae	Melissa grass, gandhatrina	G	Oil	[278]
Cymbopogon jwarancusa Schult.	Graminae	Ghatyari, amrinala, izkhir	G	Oil	[278]
Cymbopogon schoenanthus Spreng.	Graminae	Geranium grass, bhutika	G	Oil	[278]
Cynodon dactylon Pers.	Graminae	Bahama grass, amari, bhargavi	Wh, Rh	Extract	[278]
Daemia extensa R.Br.	Asclepiadeae	Utranajutuka, phala-kantak	L	Juice	[19]
Dalbergia lanceolaria Linn.	Fabaceae	Bithua, takoli	B	Oil	[278]
Daphne mezereum Linn.	Thymelaceae	Mezereon	B	Extract	[282]
Datisca cannabina Linn.	Datiscaceae	Akalbar, bhangjala drnkhari	R	Decoction	[278]
Datura alba Nees.	Solanaceae	Thornapple, tattur	L	Juice	[51]
Datura metel Linn.	Solanaceae	Downy datura, dushtura	L	Paste	[278]
Datura stramonium Linn.	Solanaceae	Apple of peru, tattur, devika	L	Infusion	[278]
Delonix elata Gamble Fl.	Fabaceae	Vayni, tiger bean	Wh	Extract	[288]
Delphinium cenudatum Wall.	Ranunlaceae	Vishalakarni, jadwar	R	Decoction	[51]
Delphinium consolida Linn.	Ranunculaceae	Larkspur	S	Oil	[282]
Delphinium staphisagri Linn.	Ranunculaceae	Spach	S	Oil	[282]
Derris uliginosa Benth.	Papilionaceae	Panlata, worm killer	B	Decoction	[51]
Dichrostachys cinera W.&A.	Fabaceae	Kheri, vertuli, bahuvaraka	R	Extract	[278]
Diospyros candollena Wight.	Ebasnaceae	Nila-variksha	B	Decoction	[51]
Diospyros paniculata Dalz.	Ebanaceae	Tinduka, karinthuvari	B	Powder	[51]
Dipterocarpus alatus Roxb.	Dipterocarpaceae	Gurjan, battisal, kanyin	B	Extract	[278]
Dipterocarpus indicus Bedd.	Dipterocarpaceae	Ennei	Rs	Rs	[51]
Dodonaea viscose Linn.	Sapindaceae	Aliar, sanatta, Dhasera	L	Poultice	[51]
Dolichos falcatus Klein.	Papilionaceae	Kattamara	S	Decoction	[51]
Dysoxylum malabaricum Bedd.	Meliaceae	Agaru, kana-mulla	W	Decoction	[51]
Eclipta prostrate Linn.	Asteraceae	Bhringaraj	R, L	Juice, decoction	[14]
Elaecarpus obolongus Gaertn	Tiliaceae	Malankara	F	Oil	[51]
Elaeocarpus serratus Linn.	Tiliaceae	Julpai, olang-karai	L	Extract	[51]

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Table 2: Contd...

Botanical name	Family	Common name	Part used	Dosage form	References
Elaeis guineensis Jacq.	Palmae	African oil palm	Sr	Oil	[278]
Elaeocarpus tuberculatus Roxb.	Tiliaceae	Rudraksha, rutthraksham	B	Decoction	[51]
Elephantopus scaber Linn.	Asteraceae	Elephant foot, tutup bumi	L	Oil	[279]
Emblica officinalis Gaertn.	Euphorbiaceae	Amla	F	Juice	[14]
*Ephedra gerardiana*Wall.	Gnetaceae	Amsania, budshur	St, R	Decoction	[278]
Ephedra vulgaris Rich.	Ephedraceae	Khanda, ma-hung	Be	Decoction	[289]
Erythrina stricta Roxb.	Papilionaceae	Mura, murukku	B	Powder	[51]
Eucalyptus globlus Labill.	Myrtaceae	Blue gum tree	B, L	Oil	[282]
Eugenia operculata Roxb.	Myrtaceae	Rai-Jaman, piaman	F	Oil	[51]
Eupatorium perfoliatum Linn.	Asteraceae	Boneset, crosswort	L	Extract	[282]
Euphorbia antiquorum Linn.	Euphorbiaceae	Triangular spurge, Tidhara, vajratundi	Br	Gum, milky juice	[290]
Euphorbia helioscopia Linn.	Euphorbiaceae	Hirruseeah, gandabhuti	Br	Juice	[51]
Euphorbia neriifolia Linn.	Euphorbiaceae	Snoohi, common milk hedge	Br	Juice	[51]
Euphorbia nivulia Ham.	Euphorbiaceae	Katathohar, vajri	L	Juice	[278]
Euphorbia tirucalli Linn.	Euphorbiaceae	Milk bush, sehund	Wh	Milky juice	[278]
Euryale ferox Salisb & Roxb.	Nymphaeaceae	Makhana, foxnut, machana	L	Extract	[278]
Erythrina stricta Roxb.	Fabaceae	Indian coral tree	B	Decoction	[279]
Excoecaria acerifolia Didrichs.	Euphorbiaceae	Basing	Wh	Juice	[51]
Fagopyrum escuentum Moench.	Polygonaceae	Buckweat	R	Extract	[279]
Farsetia aegyptiaca Turr.	Cruciferae	Mulei, faridbuti	F, L	Extract	[51]
Farsetia hamiltonii Royle.	Cruciferae	Farid-buti	F, L	Extract	[51]
Farseaia jacquemontii Hk.F. & T.	Cruciferae	Mulei	F, L	Extract	[51]
Feaula asafoetida Linn.	Umbelliferae	Hing, bhutnasan	R	Oil	[51]
Ferula galbanifulua Bioss.	Umbelliferae	Gandhabiroza, galbanum	R	Oil	[51]
Ferula narthex Boiss.	Umbelliferae	Hingra, bhutari, devil's dung	L	Infusion	[278]
Ficus bengalensis Linn.	Urticaceae	Banyan tree, sriksha	S, F	Juice	[291]
Ficus religosa Linn.	Urticaceae	Pippala, peepul tree	B	Decoction, oil	[51]
Ficus retusa Linn.	Urticaceae	Nandruk, pilala, kamrup	L, B	Poultice	[278]
Flacourtia sepiara Roxb.	Cyperzceae	Kondai, kingaro	L, R	Infusion	[51]
Fraxinus excelsior Linn.	Oleaceae	European ash	L	Exudates	[282]
Garcinia pictorial Roxb.	Guttiferae	Mysore gamboges tree, tamal	Rs	Powder	[51]
Gaultheria fragrantissima Wall.	Eriaceae	Indian wintergreen gandapuro	L	Oil	[51]
Gelsemium nitidum Michaux.	Loganiaceae	Wild yellow jessamine	R	Extract	[282]
Gendarussa vulgaris Nees.	Acanthaceae	Nili-nargandi, kala-bashimb	L	Infusion	[51]
Gentian lutea Linn.	Gentianaceae	Yellow gentian	R	Powder	[282]
Geodorum densiflorum Lam.	Orchidaceae	Shepherd's crook orchid	Rh	Extract	[279]
Geranium maculatum Linn.	Geraniaceae	Alum-root	Rh	Oil, liniment	[282]
Gmelina asiatica Linn.	Verbenaceae	Badhara, vikarini	R	Extract	[19]
Gossypium arboretum Linn.	Malvaceae	Tree cotton	S	Oil	[279]
Gossypium barbadense Linn.	Malvaceae	Sea island cotton	S	Cotton	[282]
Gossypium haceum Linn.	Malvaceae	Levant cotton	L	Oil	[279]
Gossypium indicum Linn.	Malvaceae	Indian cotton plant, anagnika	S	Oil, liniment	[51]
Grangia maderaspatana Poir.	Compositae	Mukhatari, afsantin	R	Decoction	[278]
Grewia asiatica Linn.	Tiliaceae	Palsa, dharmana	B	Infusion	[51]
Grewia tenax Fiori.	Tiliaceae	Gowali, kakarundah	L, F	Oil	[278]
Guaiacum offcinale Linn.	Zygophyllaceae	Lignum vita	St	Rs	[282]
Guizojia abyysynica Cass.	Compositae	Nigers, kala-til	S, F	Oil	[51]
Gynandropsis gyuandra Marill.	Capparidaceae	Churota, hulhul, ajagandha	L	Extract	[278]
Gynocardia odorata R.Br.	Flacourtiaceae	Chaulmugra, biringmogra	S	Oil	[51]
Hedeoma pulegioides Persoon.	Labiatae	Ameican pennyroyal	L	Infusion	[282]
Heliotropium indicum Linn.	Boraginaceae	Hattasura, siriari bhurundi	R, L	Plasters	[278]
Hemidesmus indicus R.Br.	Asclepiadaceae	Sugandhi, indian sarsaparilla	R-B	Infusion	[292]
Herpestis monniera H.B.K.	Scrophularineae	Brahmi, thyme-leaved	L	Juice	[51]
Hibiscus tillaceus Linn.	Malvaceae	Cork wood, pola	R	Embrocation	[51]
Hiptage benghalensis Linn.	Malpighiaceae	Hutimukta, kampti	L	Juice	[278]
Hiptage madablota Gaertn.	Malpighiaceae	Madhabi, madavilata	L	Extract	[51]
Holarrhena antidysenterica Wall.	Apocynaceae	Kurchi, kutaja, kewar	B	Lep	[51]
Hedera helix Linn.	Araliaceae	Barren ivy, mandia bind wood	Be	Infusion	[278]
Holoptelea integrifolia Planch.	Urticaceae	Papri, vavala	B	Juice	[51]
Humulus lupulus Linn.	Cannabineae	Hop	Wh	Infusion	[282]
Hydnocarpus wightiana Blume.	Flacourtiaceae	Jangli almond, tuvaraka, chaulmoogra	S	Oil	[51]
Hydrocotyle asiatica Linn.	Umbelliferae	Brahmi, Indian penny-wort	Wh	Juice extract	[51]
Hygrophila spinosa T.Anders	Acanthaceae	Kolistha, gokhula-kanta	R	Decoction	[51]
Hyssopus officinalis Linn.	Labiatae	Zupha	L	Infusion, syrup	[51]
Illicium verum Hook.	Magnoliaceae	Star anise, anasphal	F	Oil	[51]
Indigofera oblongifolia Forsk.	Papilionaceae	Jhilla, mridupatraka	R	Decoction	[278]

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Table 2: Contd...

Botanical name	Family	Common name	Part used	Dosage form	References
Indigofera paucifolia Delile.	Papilionaceae	Kuttukkar-chammathi	St	Decoction	[51]
Indigofera trifoliate Linn.	Papilionaceae	Vekhario, malmandi	S	Confection	[51]
Inula helenium Hook.	Compositae	Rasan	L	Oil	[51]
Ipomoea eriocarpa Br.	Convolvulaceae	Nakhari, pulichevidu	Wh	Oil	[51]
Ipomoea hispida Roem & Schult.	Convulvulaceae	Bhanwar, harankhuri	Wh	Oil	[278]
Ipomoea pescaprae Purga.	Convolvulaceae	Goat's foot creeper, chagalanghri	R, L	Decoction	[51]
Ipomoea reniformis Chois.	Convolvulaceae	Mushakani, mooshakarni	Wh	Decoction	[51]
Ipomoea turpethum Br.	Convolvulaceae	Indian jalap, kalaparni	R-B	Powder	[51]
Jasminum grandiflorum Linn.	Oleaceae	Spanish jasmine, chambeli	R	Oil	[278]
Jatropha curcas Linn.	Euphorbiaceae	Jangli-erandi, angula-leaved physic nut	S	Oil	[293]
Jatropha glandulifera Roxb.	Euphorbiaceae	Nikumba, lal-bhranda	S	Oil	[51]
Juglans regia Linn.	Juglandaceae	Akhor, darga, walnut tree	B	Decoction	[280]
Juneperus communis Linn.	Coniferae	Juniper berry, hapusha	Be	Powder	[51]
Justica ecbolium Linn.	Acanthaceae	Odoojati	Wh	Extract	[51]
Justica gendaruusa Burm.	Acanthaceae	Nilinargandi, kapika, bhutakeshi	L	Decoction	[294]
Justica procumbens Linn.	Acanthaceae	Carmeutine couchee	H	Infusion	[51]
Koelpinia linearis Pallas.	Asteraceae	Koelpinia	Wh	Extract	[279]
Lantana aculeate Linn.	Verbenaceae	Wild Sage, ghaneri	Wh	Decoction	[278]
Launaea pinnatifida Cass.	Compositae	Pathri, almirao	L	Juice	[51]
Lavandula stoechas Linn.	Labiatae	Arabian lavender, dharu	Fl	Formentation	[51]
Lawsonia alba Linn.	Lythraceae	Heena, mendhi, mehndi	L	Paste	[295]
Leea indicum Merr.	Vitaceae	Bandicoot berry	Wh	Extract	[279]
Leonotis nepetaefolia R.Br.	Labiatae	Hejurchei, matijer	L	Decoction	[278]
Leucas aspera Spreng.	Labiatae	Chotahalkusa, tamba,	L	Juice	[296]
Lipidium crassifolium Hung.	Cruciferae	Hairy cress	S	Extract	[278]
Lipidium sativum Linn.	Cruciferae	Cress, chandrasura halim, chansaur	S	Paste	[51]
Leucas linifolia Spreng.	Labiatae	Dronapushpi, hulkussa	L, Fl	Infusion	[51]
Linum usitatissimum Linn.	Linaceae	Lins, uma, tisi	S	Poultice	[297]
Litsea chinensis Lam.	Lauraceae	Garur, adhavara, chamana	Be	Oil	[278]
Litsea sebifera Pers.	Lauraceae	Garbijaur, menda, medasak	B	Powder, paste	[51]
Lolium temulentum Linn.	Graminae	Darnel	S	Powder	[282]
Lycopodium clavatum Linn.	Lycopodiaceae	Clubmoss spores, wolf claw	Sp	Tincture	[51]
Lygodium flexuosum Linn.	Polypodiaceae	Vallipanna, kalazha	R	Oil	[51]
Machilus macrantha Nees.	Lauraceae	Kolamavu	B	Extract	[51]
Marrubium vulgare Linn.	Labiatae	White hore-hound, farasiyun	H	Infusion	[51]
Matricaria chamomillla Linn.	Compositae	Babunphul, camomile	Fl	Oil	[51]
Melaleuca leucadendron Linn.	Myrtaceae	Cajuput tree, kayaputi	L	Oil	[51]
Melaleuca minor Smith.	Myrtaceae	Kaya-puti, cajuput	L	Oil	[282]
Melia azadirachta Linn.	Meliaceae	Ravipriya, neem, nimb	B	Decoction	[51]
Melia azedaracha Linn.	Meliaceae	Mahanimba, persian lilac, bakayan	S	Oil	[51]
Menthe piperita Linn.	Labiatae	Peppermint, gamathi phudina	L	Oil	[51]
Menyanthes trifoliate Linn.	Gentianaceae	Bogbean, water shamrock	R	Extract	[278]
Merremia tridentate Hallier.	Convulvulaceae	Prasarini	Wh	Extract	[298]
Mesua ferrea Linn.	Guttiferae	Cobra's saffron, nagkesara	S	Embrocation	[51]
Michella champaca Linn.	Magnoliaceae	Golden champa, champaka	Fl	Oil	[51]
Mimosa pudica Linn.	Fabaceae	Humble plant, lajjavati, kandiri	Wh	Extract	[278]
Mollugo cerviana Ser.	Ficoidaceae	Taph-jhad, phanya, grishmasundara	R	Oil	[51]
Momordica chirantia Linn.	Cucurbitaceae	Bitter gourd, karavella, karela	F	Juice	[51]
Momordica cochinchinensis Spreng.	Cucurbitaceae	Gangerua, kakrol, krindana	R	Decoction	[278]
Moniera cuneifolia Michx.	Scrophulariaceae	Bama, brahmi, svetchammi	L	Juice	[278]
Monarda punctate Linn.	Labiatae	Horse-mint	L	Oil	[282]
Morinda citrifolia Linn.	Rubiaceae	Indian mulberry, barraal	L	Juice	[278]
Moringa oleifera Lam.	Moringaceae	Horse-radish, sobhanjana	S	Oil	[51]
Mucuna gigantean DC.	Papilionaceae	Kakuvalli	B	Powder	[51]
Mukia maderaspatana Linn.	Cucurbitaceae	Madras pea pumpkin, agumaki	L	Decoction	[13]
Murraya exotica Linn.	Rutaceae	Honey bush, ekangi, kamini	Fl, L	Infusion	[51]
Murray koenigii Linn.	Rutaceae	Bristly bryoni	L	Powder	[13]
Myristica fragrans Houtt.	Myristaceae	Nutmeg, jati-phalam., jaiphal	S	Oil	[19]
Myristica malabarica Lamk.	Myristaceae	Malabar nutmeg, malati, kamuk	S	Embrocation	[51]
Myropyrum similacifolium Blume.	Oleaceae	Chatura-mallikei	L	Extract	[51]
Myrtus caryophyllus Linn.	Myrtaceae	Cloves, lavangaha, laung	F	Oil	[51]
Myrtus communis Linn.	Myrtaceae	Myrtle, murad	L	Oil	[51]
Naregamia alata W.& A.	Meliaceae	Goanese ipecacuanha, amlavalli	Wh	Extract	[278]
Nicotiana tabacum Linn.	Solanaceae	Tobacco, tambaku, tamrakuta	L	Decoction	[51]
Nycthanthes arbor-tristis Linn.	Oleaceae	Night jasmine, siharu, parijata	L	Infusion	[51]
Ocimum gratissimum Linn.	Labiatae	Shrubby basil, ramtulasi	Wh	Fumigations	[19]

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Table 2: Contd...

Botanical name	Family	Common name	Part used	Dosage form	References
Ocimum sanctum Linn.	Lam.iaceae	Basil	L	Decoction	[14]
Odina wodier Roxb.	Anacardiaceae	Jingini, ajashringi, jingan	L	Paste	[51]
Oldenlandia heynei Hk.	Rubiaceae	Nonganam-pillu	L	Extract	[51]
Olea cuspidate Wall.	Oleaceae	Kahu, zaitum	R	Ashes	[278]
Onosoma bracteatum Wall.	Boraginaceae	Goazaban, kazabun	Wh	Decoction	[51]
Onosoma echoides Linn.	Boraginaceae	Ratanjot, laljari, koame	Fl	Oil	[51]
Origanum majorana Linn.	Labiatea	Wild marjoram, sathra	Wh	Oil	[51]
Origanum vulgare Linn.	Labiatae	Sathra	Wh	Oil	[51]
Oroxylum indicum Vent.	Bignoniaceae	Prathusimbhi, miringa, snapatha	B	Powder	[51]
Osmunda regalis Linn.	Osmundaceae	Royal fern, osmonde	Wh	Extract	[51]
Paederia feotida Linn.	Rubiaceae	Prasarini, gandhali, Chinnese flower plant	L	Juice	[51]
Pandanus odoratissimus Willd.	Pandanaceae	Ketaki, fragrant screwpine	F	Oil	[51]
Pandanus tectorius Soland.	Pandanaceae	Umbrella tree, keora, ketgi	Bt	Oil	[278]
Panicum italicum Linn.	Gramineae	Italian millet, kanku	S	Extract	[51]
Papaver dubium Linn.	Papaveraceae	Pale-red poopy	R	Cooked	[278]
Papaver somniferum Linn.	Papaveraceae	Opium poppy, khas khas	S	Liniment	[51]
Pavetta indica Linn.	Rubiaceae	Papat, Indian pellet kankra	W	Infusion	[278]
Pavonia odorata Willd.	Malvaceae	Kalavala, hribera, sugandhabala	R, Wh	Extract	[278]
Pedalium murex Linn.	Pedaliaceae	Faribduti, gaja daunstree	L	Powder	[51]
Peganum harmala Linn.	Rutaceae	Foreign henna, harmal, kaladana	L	Decoction	[278]
Peucedanum graveolens Benth.	Umbelliferae	Dill, misroya, soya	S, R	Paste	[19]
Pergularis daemia Linn.	Apocynaceae	Utaran, akasan	L	Oil	[279]
Pergularis extensa N.E.	Asclepiadaceae	Sadowani, karial, vishanika	L	Juice	[278]
Phaseolus Roxb.urghii Linn.	Papilionaceae	Black gram, masha	P	Poultice, oil	[51]
Physalis alkekenji Linn.	Solanaceae	Strawberry tomato, rajaputrika	Sbe	Strawberries	[51]
Pinus australis Michaux.	Coniferae	Broom pine, yellow pine	Rs	Liniments	[282]
Pinus balsamea Linn.	Coniferae	American silver fir	St	Oil	[282]
Pinus gerardiana Wall.	Coniferae	Gunobar, neozapine edible pine	S	Confection	[51]
Pinus picea Du Roi.	Coniferae	Norway spruce	St	Extract	[282]
Piper longum Linn.	Piperaceae	Pippali, long-papper	F, R	Oil	[172]
Pisonia aculeate Linn.	Nictaginaceae	Baghachura, kuruindu	B, L	Extract	[51]
Pittosporum floribundum W.& A.	Pittosporaceae	Vchkali, tibilti	B	Decocotion	[51]
Pittosporum napaulense Rehdre.	Pittosporaceae	Yekadi, phurke, vehkali	B	Oil	[278]
Plantago ispagula Forsk.	Plantaginaceae	Ispaghula, snigdhajeera	S	Poultice	[51]
Plantago major Linn.	Plantaginaceae	Barhang, ripple grass, luhuriya	Wh	Extract	[278]
Plantago ovate Forsk.	Plantaginaceae	Shlakshnajira, bartang	S	Poultice	[278]
Plumbago rosea Linn.	lumbaginaceae	Chitraka, rose-colored lead-wort	R	Liniment	[51]
Plumbago zeylanica Linn.	Plumbaginaceae	Ceylon leadwort, chitra	R	Powder	[19]
Plumieria acuminate Poir.	Apocynaceae	Gulchin, kshira	L	Juice	[51]
Plumieria acutifolia Poir.	Apocynaceae	Frangipani, gulachin,chameli	R-B	Extract	[278]
Podphyllum peltatum Linn.	Berberidae	May-apple, mandrake	Rh	Extract	[282]
Poinciana elata Linn.	Papilionaceae	Vayni	Wh	Extract	[51]
Pongamia glabra Vent.	Papilionaceae	Karanja, Indian beech	L	Decoction	[19]
Polygala snega Linn.	Polyganaceae	Rattle snake root	Rs	Extract	[282]
Portulaca oleracea Linn.	Portulacaceae	Pigweed, pursley	L	Juice, poultice	[13]
Premna Hacea Roxb.	Verbenaceae	Bharangi, bhargi	R	Juice	[51]
Premna integrifolia Linn.	Verbenaceae	Arni, agni-mantha	R	Decoction	[299]
Prinsepia utilis Royle.	Rosaceae	Vhekal	O	Oil	[51]
Prosopis spicigera Linn.	Fabaceae	Chhikura, jhand, bhadra	B	Extract	[278]
Prunus persica stokes bot.	Rosaceae	Peach tree, aru	F	Oil	[278]
Prunus triflora Roxb.	Rosaceae	Aruwa, gadharu	F	Oil	[278]
Pseudarthria viscid W.&A.	Papilionaceae	Sanaparni, neermali	Wh	Extract	[51]
Psidium guyava Linn.	Myrtaceae	Safedsafari, amrud, guava tree	L	Oil	[278]
Psoralea corylifolia Linn.	Papilionaceae	Babachi, avalguja, kamboji	S	Maceration	[278]
Ptychotis ajowan DC.	Umbelliferae	Yavanika, bishop's weed, ajowan	F	Oil	[51]
Pueraria tuberose DC.	Fabaceae	Bilaikand, pona, saloha	R	Crushed	[278]
Pyrethrum indicum DC.	Compositae	Mitha akalakara	R	Paste, confection	[51]
Pyrus malus Linn.	Rosaceae	Sebhaphala, crab apple	F	Juice	[51]
Randia dumetorum Lamk.	Rubiaceae	Madana, emetic nut, mainphal	B	Paste	[19]
Ranunculus avensis Linn.	Ranunculaceae	Corn crow foot, devil's claws	Wh	Extract	[51]
Ranunculus muricatus Linn.	Ranunculaceae	Chambul	Wh	Extract	[278]
Ranunculus trichophyllus Linn.	Ranunculaceae	Water crowfoot	Wh	Extract	[278]
Rhamnus catharticus Linn.	Rhamnaceae	Buckthorn	Rbe	Juice	[282]
Rhazya stricta Dcne.	Apocynaceae	Sunwar, wena, sehar	R, St, L	Infusion	[51]
Rhodendron campanulatum D.Don.	Eriaceae	Cherallu, gaggar, surngar	L	Extract	[51]
Rhododendron javanicum Benn.	Eriaceae	Kechung	L	Extract	[51]

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Table 2: Contd...

Botanical name	Family	Common name	Part used	Dosage form	References
Ribes nigrum Linn.	Saxifragaceae	Currants, nabar	C	Currants	[51]
Ricinus communis Linn.	Euphorbiaceae	Castor oil plant, eranda, endi	S	Oil, poultice	[19]
Ruta graveolens Linn.	Rutaceae	Satap, garden rue, pismarum	L	Tincture	[207]
Rubia cordifolia Linn.	Rubiaceae	Madar, manjit, khuri	R	Decoction	[278]
Rourea santaloides W. & A.	Conoraceae	Vardara, wakeri	R	Tonic	[51]
Rosa alba Linn.	Rosaceae	Gulseoti, gulab bahupatrika	Fl	Oil	[278]
Saccolabium pappilosum Lindl.	Orchidaceae	Nakuli, rasna	Rs	Resins	[51]
Salacia oblonga Wall.	Celastraceae	Ponkoranti	R-B	Extract	[51]
Salacia reticulate Wight.	Celastraceae	Ekanayakam, koranti	R-B	Extract	[300]
Salix alba Linn.	Salicaceae	Huntingdon willow, bushan	B	Decoction	[301]
Salvadora oleoides Dcne.	Salvadoraceae	Kabber, mithidiar, jhal	S	Oil	[51]
Salvadora persica Linn.	Salvadoraceae	Pilu, tooth brush tree, chhota-pilu	Fl	Oil	[51]
Samadera indica Gaertn.	Simaroubaceae	Kathai, nibam, daraput	F	Oil	[278]
Sambucus canadensis Linn.	Adoxaceae	American elder	Fl	Oil	[282]
Sambucus nigra Linn.	Adoxaceae	Bore tree	Fl	Oil	[282]
Sansevieria urghiana Roxb and Schult.	Hemodoraceae	Muruva, murahri, katukapel	R	Extract	[51]
Santalum rubrum Linn.	Sapindaceae	Arishta, indian filbert, ritha	R, L	Extract, juice	[51]
Sarcocephalus missionis Wall.	Rubiaceae	Jalamdasa, nirvanji	B	Decoction, powder	[51]
Sassafras officinale Nees.	Laurineae	Sassafras	R	Oil	[51]
Saussurea lappa Clarke.	Compositae	Puskara, costus, kushta, kut	R	Infusion	[217]
Schleichera trijuga Willd.	Sapindaceae	Ceylon oak, gausam	S	Oil	[278]
Schoenocaulon officinale A.Gray.	Melanthaceae	Sabadilla	F, S	Ointment	[51]
Scindapsus officinalis Schitt	Araceae	Poriabel, gajapipal, shreyasi	F	Juice	[282]
Semecarpus anacardium Linn.	Anacardiaceae	Marking-nut tree, bhallataka, bhela	F	Juice	[302]
Sesamum indicum Linn.	Pedaliaceae	Gingelly, bariktel	S	Oil	[278]
Sesbania aegyptiaca Pers.	Papilionaceae	Jayantika, jetrasin	L	Poultice	[51]
Sesbania grandiflora Pers.	Papilionaceae	Agasta, hatiya	R	Paste	[278]
Setaria italic Beauv.	Graminae	Foxtail millet, kangu	Gr	Parching	[51]
Shorea robusta Gaertn.	Dipterocarpaceae	Sal tree, sakhu asvakarna,	B, Rs	Paste	[51]
Sida acuta Burm.	Malvaceae	Bariaca, bala, pranijivika	L	Oil	[19]
Sida cordifolia Linn.	Malvaceae	Bariar, batyalaka, simak	R, S	Oil	[51]
Sida rhombifolia Linn.	Malvaceae	Sahadeva, kharenti	R	Oil	[218]
Siegesbeckia orientalis Linn.	Compositae	Katampam, kau-kan	Wh	Tincture	[51]
Skimmia laureola Sieb.	Rutaceae	Ner	Wh	Extract	[279]
Smilax china Linn.	Liliaceae	Dwipautra, china root, chobchini	R	Decoction	[19]
Smilax lanceafolia Roxb.	Liliaceae	Bari-chobchini	R	Juice	[51]
Smilax officinalis Kunth.	Smilaceae	Jamaica sarsaparilla	R	Powder, extract	[282]
Smilax zeylaniea Linn.	Liliaceae	Chobchini, ramdatun	R	Paste	[278]
Smithia conferta Sm.	Papilionaceae	Smithia	Wh	Extract	[278]
Solanum dulcamara Linn.	Solanaceae	Kakmachi, bitter-sweet, rubabarik	Be	Decoction	[51]
Solanum nigrum Linn.	Solanaceae	Makoi, kambei, kamuni	L	Poultice	[51]
Solanum xanthocarpum Schrad & Wendll.	Solanaceae	Kantakari, warumba, bhutkatya	Wh, Be	Juice	[51]
Spilanthes acmella Murr.	Compositae	Pokormul, akarkara	L	Decoction	[278]
Spondis pinnate Kurz.	Anacardiaceae	Amarah, Indian hog plum, ambra	L	Juice	[278]
Stachytarpheta indica Vahl.	Verbenaceae	Aaron's rod	L	Juice	[278]
Strychnos bourdilloni Trees.	Loganiaceae	Nirmali, clearing nut tree	R	Decoction	[278]
Strychnos cinnamomifolia Thw.Enum.	Loganiaceae	Etakirindiwel, welbeli	R	Decoction	[278]
Strychnos nux-vomica Linn.	Loganiaceae	Kupilu, poison-nut, kagphala	S	Powder	[303]
Strychnos potatorum Linn.	Loganiaceae	Clearing-nut tree	S	Powder	[303]
Teucrium polium Linn.	Labiatae	Cat thyme, poley	L	Infusion	[280]
Teramus labialis Spreng.	Combretaceae	Masha-parui, mashani	B	Decoction	[51]
Terminalia belerica Roxb.	Combretaceae	Vibhitaki, bhaira	K	Oil	[51]
Terminalia chebula Retz.	Combretaceae	Pathya, myrobalan, Indian gall-nut	F	Powder	[19]
Tinospora cordifolia Miers.	Menispermaceae	Ambarvel, gharol, gulwel	R, St	Starch	[278]
Tinospora malabarica Miers.	Menispermaceae	Gurch, giloe, padmagaluncha	L, St	Extract	[278]
Thevetia nerifolia Juss.	Apocynaceae	Yellow oleander, pilakanir, ashvaha	S	Oil	[278]
Thymus vulgaris Linn.	Labiatae	Garden thyme	Wh	Oil	[282]
Toddalia aculeate Lamk.	Rutaceae	Kanchana, jangli-kali-mirch, limri	F, R	Oil	[51]
Toddalia asiatica Lam.	Rutaceae	Dahan, lopez root, forest pepper	F, R	Liniment	[278]
Toddalia bilocularis W. & A.	Rutaceae	Krishna-aguru, devadarom	W	Oil	[51]
Toluifera pereirae Baill.	Fabaceae	Peru balsam	B	Balsam	[282]
Trewia nudiflora Linn.	Eiphorbiaceae	Pindara, pitali, sivani	R	Decoction	[51]
Tribulus terrestris Linn.	Zygophyllaceae	Small caltrops, gokshura, chota-gokhura	F	Decoction	[19]
Trichosanthes palmate Roxb.	Cucurbetaceae	Indrayan, mahakala kaundal	F	Juice	[278]
Trigonella foenum-gaeceum Linn.	Papilionaceae	Methi, medhika	S	Confection	[247]
Tylophora asthmatica W. & A.	Acslepiadaceae	Jangli-pikvan, antamul	L	Powder, decoction	[51]

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Table 2: Contd...

Botanical name	Family	Common name	Part used	Dosage form	References
Unona narum Dun.	Anonaceae	Gunamanijhad, unaminigida	L	Extract	[51]
Uraria lagopoides DC.	Papilionaceae	Pitvan, prasniparni Dowla	Wh	Decoction	[51]
Urena lobata Linn.	Malvaceae	Latloti, kunjia, tapkote	R	Extract	[51]
Urgenia indica Kunth.	Liliaceae	Indian squill, jangli-piyaz, phaphor	Bu	Tincture, powder	[51]
Urtica dioica Linn.	Urticaceae	Common nettle, scaddie	L	Decoction, powder	[278]
Vanda roxburghii Br.	Orchidaceae	Rasna-nai, vandaka	R	Extract	[51]
Vanda tessellate Hook.	Orchidaceae	Rasna, Perasara	R	Decoction	[278]
Valeriana officinalis Linn.	Valerianaceae	Capon's tail, setwall	R	Oil	[278]
Vateria indica Linn.	Dipterocarpaceae	White damimer tree, kahruba, ajakarna	S	Oil	[51]
Vepris bilocularis Engler.	Rutaceae	Vepris	W	Oil	[278]
Veratrum viride Solander.	Melanthaceae	Indian poke	Rh	Extract	[282]
Verbascum thapsus Linn.	Scrophulariaceae	Feltwort, blanket-leaf, rag paper	L	Paste	[278]
Verbena officinalis Linn.	Verbenaceae	Frog foot, karaita, tears of juno	L	Paste	[278]
Vernonia anthelmintica Willd.	Compositae	Somaraja, bakchi, purple fleabane	L, R	Extract	[51]
Viola tricolor Linn.	Violaceae	Flame flower, gardengate	Wh	Infusion	[278]
Vitex negundo Linn.	Verbenaceae	Nirgundi, mewri, sawbhalu	L	Poultice	[258]
Vitex trifolia Linn.	Verbenaceae	Jalanirgundi, nichindi, surasa	L	Infusion	[51]
Vitis pallida W.&A.	Vitaceae	Chunnampuvalli	L	Juice	[51]
Vitis vinifera Linn.	Vitaceae	Grapes, draksha, kishmish	F	Resins	[51]
Withania somnifera Dunal.	Solanaceae	Ashvagandha, winter cherry	R	Oil, Taila	[304]
Xylia dolabriformis Benth.	Papilionaceae	Schmsapa, irula	B	Decoction	[51]
Zingiber officinale Roscoe.	Taminaceae	Nagaram, ginger sonth	Rh	Infusion	[280]
Zizyphus jujube Mill.	Rhamnaceae	Badari, baer	R-B	Juice	[19]

L: Leaves, R: Roots, H: Herb, Wh: Whole plant, Rh: Rhizome, B: Bark, F: Fruit, Bu: Bulb, S: Seed, T: Tubers, Fl: Flowers, Mj: Milky juice, G: Grass, St: Stems, W: Wood, Be: Berries, Gm: Gum, K: Kernel, Sh: Shoot, C: Camphor, Rs: Resin, Br: Branches, Sg: Stigmas, Sp: Spores, Bt: Bracts, Sbe: Strawberries, Rbe: Ripeberries, Gr: Grain, C: Currants, O: Oil, P: Pulse, Sr: Sarocarp, Nt: Nuts, Al pt: Aerial parts, Ug pt: Underground parts-Ug pt

Boswellia serrata Roxb. (BS)(Family-Burseraceae)

BS is a deciduous middle-sized tree, grown in tropical parts of Asia and Africa [64]. Boswellic acid is the first terpenoids isolated from oleo gum resins. The oleo gum resin of BS is used in various Unani and Ayurvedic preparations. Folkloric uses of BS are in the treatment of bronchitis, rheumatism, asthma, cough, intestinal problems, syphilitic, jaundice, dysentery, and pulmonary diseases. It acts as both internal and external stimulant, expectorant, diuretic, and stomachic [51,64]. Boswellia is a traditional natural remedy that has been used for thousands of years to treat swelling and inflamation in Ayurvedic medicine and traditional Chinese medicine. In 2003, medical researchers conducted a randomized blind placebo controlled trial of BS on 30 patients suffering from osteoarthritis of the knee. The data showed an increased range of motion and less swelling in their knees from arthritis than before they began the treatment. The essential oil of BS predominantly comprised monoterpenoids, of which β-pineneis the major constituent. Other monoterpenoids includes β-pinene, cis-verbenol, trans-pinocarveol, borneol, myrcene, verbenone, limonene, and p-cymene, while α-copaene was the only sesquiterpene identified [65,66]. BS possess an anti-inflammatory [67], analgesics [68], immunomodulatory [69], anticancer [70,71,72], hepatoprotective, hypolipidemic [73], antiasthmatic [74], osteoarthritis, and hypoglycemic activities [75]. The n-hexane extract of gum resins of BS in combination with methanolic extract of rhizomes of *Glycyrrhiza glabra* (GY) exhibited anti-arthritic activity at doses of 50 or 100 mg/kg in male wistar rats. The anti-arthritic activity is mainly by decreasing the activity of membrane marker enzymes such as alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and by the prevention of leucocytes migration in the inflamed area. In conclusion, BS possesses a significant anti-arthritic activity on male albino wistar rats [76].

Caesalpinia sappan Linn. (CP)(Family-Leguminosae)

CP commonly known as sappanwood, bakam or patang, is a native of South India, Madhya Pradesh, Orissa, West Bengal, Malaya, and Sri Lanka. The tree spreads to a height of 10 m and is cultivated for its large, ornamental penicals of yellow flowers. A very strong barrier is formed by the branches when they are interlaced [11]. The heartwood of the CP is traditionally used for the treatment of ulcers, leprosy, rheumatism, skin disease, diarrhea, dysentery, epilepsy, convulsions, diabetes, odontopathy, stomatopathy, and leucorrhea. The heartwood of the CP is bitter, astringent, sweet, acrid, refrigerant, constipating, sedative, and hemostatic. In Yunani system, the decoction of wood was useful in rheumatism [77,78]. CP is reported to have an anti-anaphylactic [79], anti-coagulant [80], anti-bacterial [81-83], anti-fungal [83], anti-inflammatory [84], anti-tumor [85-87], anti-viral [88,89], immunostimulant [87], and semen coagulating activities [86]. CP also causes the inhibition of phosphodiesterase [90] and stimulation of glutamate pyruvate transaminase [91] and tyrosinase enzymes [92]. The ethanolic extract at doses 1.2, 2.4, and 3.6 g/kg of CP wood showed anti-arthritic activity on wistar rats by declining the levels of IL-1β, IL-6, TNF-α, and prostaglandin E2 (PGE2) in serum. The study concluded that CP possesses an anti-arthritic activity on rats [93].

Cannabis sativum Linn. (CT)(Family-Urticaceae)

CT, a pistillate plant, is a native of Persia, Western and central Asia, and is now largely cultivated all over India.

Table 3: Plants with reported anti-arthritic activity

Botanical name	Family	Common name	Part	Extract	Dose (p.o.)	Acute toxicity (p.o.)	Model	Mode of action	Reference
Acyranthus aspera Linn.	Amaranthaceae	Devil's horsewhip	S, R	Alcohol	15 mg/100 g	Safe upto 8 g/kg	FIA	Inhibition of secondary lesions	[281,305]
Achyranthes aspera Linn.	Amaranthaceae	Apamarga	R	Ethanol	100-200 mg/kg	Safe upto 8 g/kg	CFA	Prevented the recruitment of leukocytes	[217,305]
Aconitum vilmorinianum Kom.	Ranunculaceae	Huang Cao Wu	R	Ethanol	10-100 mg/kg	D.N.A	CFA	Improvement of join allodynia, swelling, hyperaemia and vascular permeability	[306]
Ajuga bracteosa Wall.	Labiatae	Ground pine	Wh	Ethanol	5, 10, 20 mg/kg	LD_{50}>5 g/kg	TIA FOIA CFA	COX-1 and COX-2 inhibition	[307,308]
Ajuga decumbens Thunberg.	Lamiaceae	Bugle weed	Wh	70% ethanol	30, 50, 150 mg/kg	D.N.A	CFA	Regulates the balance between bone resorption and bone formation	[309]
Alstonia boonei De Wild.	Apocynaceae	Cheese wood, pattern wood	St, B	Methanol	50, 100, 200, 400 mg/kg	D.N.A	FIA CFA	Inhibition of both the early and late phases of pain stimulus.	[310]
Alstonia scholaris Linn. R.Br.	Apocynaceae	Dita bark, devil tree	L	Ethanol	100, 200, 400 mg/kg	≥2 g/kg	CFA	Reduction of total leukocyte migration as well as lymphocytes and monocytes/ macrophages migration	[27]
Ammania bracifera Linn.	Lythraceae	Acrid weed, tooth cup	L	Aqueous alcoholic	250, 500 mg/kg	≥5000 mg/kg	CFA	Decrease the ESR and WBC count	[2]
Aristolochia bracteata Lam.	Aristolochiaceae	Kidamari	Wh	Pet ether, chloroform, methanol	100, 200, 400 mg/kg	Safe upto 4 g/kg	CFA	Maintenance of synovial membrane and vascular permeability, thereby inhibiting cytokines and leukotriene infiltration	[43]
Argyreia speciosa Sweet.	Convulvulaceae	Elephant creeper	R	Ethanol	50-100 mg/kg	≥3000 mg/kg	CFA	Prevented the recruitment of leukocytes	[217,311]
Arisaema rhizomatum Fischer.	Aroideae	Jack in the pulpit	Rh	Methanol	130, 261, 522 mg/kg	Safe upto 40 g/kg	CIA	Inihibits arthritis deterioration the secretion of pro-inflammatory cytokines and RA factor	[3]
Arnebia euchroma Johnst.	Boraginaceae	Pink arnebia, demok	R	95% ethanol	2.5, 5, 10 mg/kg	D.N.A	CIA AIA	Suppressing the levels of TNF-α and IL-1β	[312]
Artocarpus tonkinensis A. Cheval.	Moraceae	Chay	L	Ethyl acetate	10-200 mg/kg	D.N.A	CIA	Apoptosis induction in activated T-cells	[6]
Asystasia dalzelliana Santapau.	Acanthaceae	Violet asystasia	L	Ethanol	200, 400, 800 mg/kg	≥2000 mg/kg	CFA	Decreasing synthesis/release of T-cell mediators	[4]
Baccharis genistelloides Linn.	Asteraceae	Carqueja	Ar pt	Aqueous	4.2 mg/kg	Safe upto 42 mg/kg	CIA	IL-1 induced production of progelatinase B and PGE2, and synovial fibroblast proliferation have been suppressed	[313]
Bacopa monniera Penell.	Scrophulariaceae	Herpestis monniera	Wh	Methanol	100 mg/kg	≥3000 mg/kg	CFA	Stabilizing action on lysosomal membranes	[283,314]
Barleria lupulina Lindl.	Acanthaceaea	Hophead	L	Methanol	300, 600 mg/kg	D.N.A	CFA CIA	Assisting cell mediated immune responses	[7]
Barleria prionitis Linn.	Acanthaceae	Katsareya, karunta	Wh	Hydro-alcoholic	12.5, 25, 50, 100 mg/kg	Safe upto 3000 mg/kg	AIA	Lowers the ESR level and have an immune-modulatory activity	[315]
Bauhinia variegate Linn.	Caesalpiniaceae	Kachnar, chingthrao	St	Ethanol	250 mg/kg	Safe upto 2000 mg/kg	CFA	Superoxide dismutase, catalase, glutathione peroxidase and lipid peroxide	[8,316]
Bergenia stracheyi Linn.	Saxifragaceae	Paashaanbhed	Rh	Pet ether and methanol	40 mg/kg	Safe upto 2000 mg/kg	AIA	Potential Th1/Th2 cytokine balancing activity	[317]
Boerhaavia diffusa Linn.	Nyctaginaceae	Punarnava	R	Pet ether	500-1000 mg/kg	≥1000 mg/kg	CFA	Inhibition of inflammatory 7 inhibitor	[63]
Boswellia carterii, Birdw.	Burseraceae	Olibanum	Rs	70% aqueous acetone	0.90 g/kg	Safe upto 0.90 g/kg	CFA	Decrease the formation of leukotriene LTB4 and reduce the infiltrarion of leucocytes	[318]

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Table 3: Contd...

Botanical name	Family	Common name	Part	Extract	Dose (p.o.)	Acute toxicity (p.o.)	Model	Mode of action	Reference
Boswellia serrata Triana.	Burseraceae	Salai gugul	Rs	n-hexane	50 mg/kg	Safe upto 50 mg/kg	CFA	Lysosomal membrane stability modulating effect, inhibiting leukocyte migration, controlling the production of auto antigens and anti-proteinase activity	[76]
Butea monosperma Linn.	Fabaceae	Palash, keshu, bastard Teak	Wh	Pet ether	100-200 mg/kg	Safe upto 2000 mg/kg	CFA	Improvement in levels of hemoglobin and RBC; levels of WBC, ESR were suppressed	[319,320]
Caesalpinia sappan Linn.	Leguminosae	Sapanwood, suou	W	Ethanol	2.5, 5, 10 ug/ml	Safe upto 5000 mg/kg	*In vitro*, cartilage/ chondrocyte protection	Inhibited the expression of pro-inflammatory cytokines IL-1ß and TNF-α	[321,322]
Caesalpinia sappan Linn.	Leguminosae	Sappanwood, suou	W	Ethanol	1.2, 2.4, 3.6 g/ kg	Safe upto 5000 mg/kg	CIA	Decreasing the levels of IL-1β, IL-6, TNF-α and PGE2 in serum and the expression of COX-2 and transcription factor NF-κB	[93,322]
Calotropis gigantean R.Br.	Asclepiadaceae	Milkweed	Al Pt	Petroleum ether	50 mg/kg	Safe upto 2000 mg/kg	CFA	Pro-inflammatory cytokines as well as anti-inflammatory cytokines are reduced	[323,324]
Calatropis procera R.Br.	Apocynaceae	Sodom apple	Al pt	Methanol	50-500 mg/kg	Safe upto 2000 mg/kg	CFA	Inhibit cellular influx and vascular permeability	[325,326]
Caltha palustris Linn.	Ranunculaceae	Kingcup, marsh marigold	Wh	Methanol	10 mg/kg	D.N.A	CIA	Decrease in the percentage and the absolute count of splenic T-regulatory cells ($CD4^+CD25^+FOXP3^+$)	[327]
Cannabis sativum Linn.	Cannabaceae	Ganja, indian hemp	L	Alcoholic	10, 25 mg/kg	D.N.A	CIA	Diminished CII-specific proliferation and IFN-g production	[98]
Capparis erythrocarpus Isert.	Capparaceae	Flamingo lily	R	Ethanol	100, 300 mg/kg	D.N.A	AIA	Inhibit the release of pre-inflammatory cytokines and immunosupressant action	[328]
Capparis spinosa Linn.	Capparaceae	Flinders rose	F	Hydroalcoholic	240-600 mg/kg	D.N.A	CFA	Conteract the effects of IL-1	[329]
Cardiospermum halicacabum Linn.	Spindaceae	Ballon plant	L	Ethanol	250-500 mg/kg	D.N.A	CFA	Reduction of RF and CRP levels in the serum	[284]
Cassia uniflora Mill.	Caesalpiniaceae	One leaf senna	L	Methanol, pet ether, ethyl acetate	50, 100 mg/kg	≥1000 mg/kg	CFA	Histamine and prostaglandin synthesis inhibition	[330]
Cayaponia tayuya Cogn.	Cucurbitaceae	Tayuya	R	Hydroalcoholic	1 mg/kg	D.N.A	AIA	Modifying the cell infiltration and the expression of both nitric oxide synthase-2 and COX-2. Decreases TNF-α & IL-1β production in lymphocytes	[331]
Celastrus aculeatus Merr.	Celastraceae	Gua shan fena	R, St	Ethanol	1-3 g/kg	LD_{50}=20.5 mg/kg	CFA	Down modulation of immunological and biochemical mediator	[332,333]
Centella asiatica Urban.	Mackinlayaceae	Brahmi booti	L	Methanol	0.5 ml		HRBC-MS	Inhibition of protein denaturation membrane stabilization and proteinase inhibitory	[285]
Cinnammomum zeylcanium Breyn.	Lauraceae	Dalchini	B	Aqueous	8 mg/kg	D.N.A	CFA	Inhibition of leukocyte emigration and prostaglandins	[109]
Cissampelos pareira Linn.	Menispermaceae	Abuta, barbasco, butua	R	50% aqueous ethanol	200-400 mg/kg	Safe upto 2000 mg/kg	CFA	Levels of acid phosphatase and N-acetyl glucosaminidase were reduced and hexose, sialic acid increased.	[334,335]
Chelidonium majus Linn.	Papaveraceae	Tetterwort	Al pt	Methanol	40/400 mg/kg	D.N.A	CIA	Lower the absolute number of CD4+T cells in spleen and lymph node, induce immunosuppressive response by lowering the CD4+T-cells and enhancing CD8+T-cells.	[336]

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Table 3: Contd...

Botanical name	Family	Common name	Part	Extract	Dose (p.o.)	Acute toxicity (p.o.)	Model	Mode of action	Reference
Clematis chinensis Osbeck.	Ranunculaceae	Wei Ling xian	R	Aqueous Methanol Acetone	100 mg/kg	D.N.A	LPS	Inhibited PGE2 production and COX-2 expression	[337]
Cleome gyandra L.	Cleomaceae	Shone cabbage	L	Ethanol	150 mg/kg	Safe upto 2000 mg/kg	CFA	Modifying the lysosomal membrane or by inhibiting the release of lysosomal enzymes	[286,338]
Coriandrum sativum Linn.	Apiaceae	Cilantro, dhania	S	Hydroalcoholic	8, 16, 32 mg/kg	Safe upto 2000 mg/kg	CFA FOIA	Inhibit the secretion of pro-inflammatory cytokines including TNF-α	[120,339]
Costus speciosus Sm.	Zingiberaceae	Keukand	Al pt	Methanol	400, 800 mg/kg	Safe upto 2000 mg/kg	CFA	Suppression of inflammatory mediators	[287,340]
Curcuma longa Linn.	Zingiberaceae	Turmeric	Rh	N-hexane	520 mg/kg	D.N.A	SCW	Activation of genes critical to articular inflammation	[130]
Curcuma zeodaria Rosc.	Zingiberaceae	White turmeric	R	Pet ether Chloroform	200, 400 mg/kg	Safe upto 5000 mg/kg	CFA	Decrease the latency time to explore	[341]
Delonix elata, Gambles.	Ceasalpinoideae	White gulmohar, waykaran	B	Pet ether Chloroform Hydroalcoholic	250 mg/kg	Safe upto 5000 mg/kg	CFA	Blocking the action of COX, LO and AT and thus preventing the generation of mediators	[288]
Dipsacus asperoides Linn.	Dipsacaceae	Japanese teasel root	R	Aqueous	50-100 mg/kg	D.N.A	CIA	Reduced the levels of anti-CII IgG2a antibody, PGE2, TNF-α, IL-1β and IL-6	[342]
Drynaria quercifolia L.	Polypodiaceae	Oak leaf fern	Rh	Aqueos	100-200 mg/kg	Safe upto 2000 mg/kg	CFA	Inhibition of ROS release	[342,259]
Elaecarpus sphaericus L.f.	Elaecarpaceae	Blue marble tree, Indian oil fruit	Wh	Ethanol	250 mg/kg	Safe upto 2500 mgkg	CFA	Immunosupressant action and inhibition of leokocytes migration in inflamed areas	[344]
Ephedra sinica Staph.	Ephedrceae	Ma Haung	H	Water	50 ul s.c.	D.N.A	CFA	mRNA expressions of TNF-α and IL-6 genes restored to normal levels	[289]
Euphorbia antiquorum Linn.	Euphorbiaceae	Antique spurge	Wh	Aqueous, ethanol	400 mg/kg	≥2 g/kg	CFA	Inhibition of the arachidonic metabolites and suppression of cell-mediated immunity	[290]
Ficus bengalensis Linn.	Moraceae	Banyan tree bargad	St B	Methanol	100, 200, 300 mg/kg	Safe upto 4 g/kg	CFA FIA AGIA	Inhibition of early phase of inflammation	[291]
Ginkgo biloba Linn.	Ginkgoaceae	Maidenhair tree	L	Methanol	2 mg/kg	D.N.A	CFA CACW	Inhibition of NO production from the macrophages that infiltrated to the inflamed site	[345]
Glycosmis pentaphylla Linn.	Rutaceae	Orange berry	B	Ethanol	400, 800 mg/kg	Safe upto 4 g/kg	CFA	significant improvement of the hematological parameters like RBC count, Hb level and the ESR	[346]
Glycyrrhiza glabra Linn.	Fabaceae	Liquorice mulethi	Rh	Methanol	150 mg/kg	Safe upto 5 g/kg	CFA	Lysosomal membrane stability modulating effect, inhibiting leukocyte migration, controlling the production of auto antigens and anti-proteinase activity	[76]
Hedera helix Linn.	Araliaceae	European ivy	L	Ethanol	2.5-7.5 ml/kg	LD_{50}=2.5 g/kg	FIA	Reduction inarthritic symptoms	[347,348]
Hemidesmus indicus R.Br.	Asclepdiaceae	Indian sarsaparilla	R	Hydroalcoholic	450 mg/kg	LD_{50}>2000 mg/kg	CFA	Inhibition of inflammation induced by caragenin, bradykinin and serotonin	[292]
Hippocratea excels H.B.K.	Hipocreataeceae	Mata piojo, cancerina	B	Ethanol	25, 50, 100 mg/kg	D.N.A	FIA CFA	Activity against both exudative and proliferative phases of inflammation	[349]
Hybanthus enneaspermus Muell.	Violaceae	Humpback flower	Wh	Aqueous Ethanol	500 mg/kg	Safe upto 5000 mg/kg	CFA	Inhibits the release of mediators like cytokines (IL-1β and TNF-α), GM-CSF, IFN and PGDF	[350]

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Table 3: Contd...

Botanical name	Family	Common name	Part	Extract	Dose (p.o.)	Acute toxicity (p.o.)	Model	Mode of action	Reference
Jatropha isabellei Mull.	Euphorbiaceae	Physic nut	Ug pt	Ethanol	100-300 mg/kg	Safe upto 300 mg/kg	MSUIA	Prevent the neutrophil infiltration	[293]
Justica gendarussa Linn.	Acanthaceae	Willow leaved justice	L	Ethanol	100 mg/kg	LD_{50}=1000 mg/kg	CFA CIA	Inhibition of migration of leukocytes	[294]
Lantana camara Linn.	Verbinaceae	Lava	L	Ethanol	5, 10, 20 mg.kg	D.N.A	TIA	Lipoxygenase and/or cyclooxygenase inhibition	[151]
Laportea bulbifera Weddell.	Urticaceae	Mukago-irakusa	R	Ethanol	20, 40, 60 mg/kg	D.N.A	CIA	Decrease in the production of IFN-α and IL-2, an increase of IL-10 and TGF-β	[351]
Lawsonia inermis Linn.	Lythraceae	Henna, mehandi	L	70% aqueous ethyl alcohol	200, 400 mg/kg	Safe upto 400 mg/kg	CFA FOIA	Decrease in both acute and chronic phase of inflammation due to suppression of inflammatory mediators	[295]
Leucas aspera Willd.	Lamiaceae	Thumbai	Al pt	N-hexane chloroform ethyl acetate ethanol	100, 200 mg/kg	Safe upto 2000 mg/kg	CFA	Increased levels of CRP, TNF-α and IL-2 were decline	[296]
Linum usitatissimum Linn.	Linaceae	Flax	S	Petroleum ether	1, 3 ml/kg	Safe upto 5000 mg/kg	CFA, FIA	Inhibitory effect on arachidonate metabolism	[297,352]
Lonicera japonica Thumb.	Caprifoliaceae	Japanese honey suckle	L	Methanol	1-2 mg/kg i.p.	Safe upto 5000 mg/kg	CACW	Suppress T-cell proliferation	[353,354]
Mallotus oppositifolium Mull.	Euphorbeaceae	Geisel	L	Methanol	100 mg/kg	≥6000 mg/kg	FIA	Anti-proliferative activity	[355,356]
Merremia emarginata Burm.	Convolvulaceae	Kupit-kupit	Wh	Ethanol	100, 200, 400 mg/kg	Safe upto 2000 mg/kg	CFA	Improves ESR and hemoglogin values and restores body weight	[357]
Merremia tridentate Hall.	Convulvulaceae	Mudiarkunthal, savulikodi, thrippan- pullu	Wh	Ethanol	100, 200 mg/kg	LD_{50}=400 mg/kg	CFA	Inhibition of second phase of inflammation and release of kinins and PG's	[298]
Operculina turpethum Linn.	Convolvulaceae	Turpeth	R	Ethanol	200,400, 600, 800 ug/ml	≥2000 mg/kg	IPDN	Inhibit the denaturation of proteins	[358,359]
Panax ginseng C.A.Meyer.	Araliaceae	Ginseng	R	Ethanol	10 mg/kg	D.N.A.	CIA	Suppressed TPA-induced acute inflammation	[360]
Phyllanthus amarus Schum.and Thomm.	Euphorbiaceae	Chanca piedra	Wh	Aqueous	100, 200, 400 mg/kg	Safe upto 2000 mg/kg	CFA	ALT and 1T levels were reduced	[165]
Physalis angulate Linn.	Solanaceae	Fisalia	L	Aqueous, ethanol, methanol	100-1000 ug/ml	Safe upto 5000 mg/kg	HRBC-MS	Inhibit the denaturation of proteins	[361,362]
Pinus maritime Roxb.	Pinaceae	Maritime pine	B	Hydroalcoholic	1%	Safe upto 4000 mg/kg	CIA	Inhibiting acute and chronic inflammatory lesions and production of NO	[363,364]
Piper betle Linn.	Piperaceae	Tambula	L	Hydroalcoholic	0.25, 0.5, 1, 2, 4 mg/kg	Safe upto 1000 mg/kg	CFA	Elevated levels of CD4+T cell specific IFN-c in splenocytes are reduced	[365]
Piper longum Linn.	Piperaceae	Pippali	F	Aqueous	200, 400 mg/kg	≥2500 mg/kg	CFA	inhibited the adherence of neutrophils to endothelial monolayer by inhibiting the TNF-α -induced expression of ICAM-1, VCAM-1 and E-selectin and also inhibits activation of NF-kB	[172,111]
Pisonia grandis R.Br.	Nyctaginaceae	Grand devil's-claws	L	Ethanol	300 mg/kg	Safe upto 2000 mg/kg	CFA	Release of mediators like cytokines, GM-CSF, interferons and PGDF are suppressed	[366]
Pistia stratiotes Linn.	Araceae	Water lettuce	L	Aqueous Ethanol	30, 100, 300 mg/kg	LD_{50}=850 mg/kg i.p.	AIA	Low levels of C-reactive proteins and ESR	[367,368]

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Table 3: Contd...

Botanical name	Family	Common name	Part	Extract	Dose (p.o.)	Acute toxicity (p.o.)	Model	Mode of action	Reference
Pleurotus sajorcaju Singer.	Pleurotaceae	Oyster mushroom	F	Aqueous Methanol	500, 1000 mg/kg	D.N.A.	AIA	Suppression of splenic lymphocytes	[369]
Premna serratifolia Linn.	Verbenaceae	Agnimantha	W	Ethanol	300 mg/kg	Safe upto 2000 mg/kg	CFA	Suppression of migration of leukocytes	[299]
Pseudocdrea kotschyi Schweinf.	Meliaceae	Hard cedar, Senegals basari	L	Aqueous	200, 400 mg/kg	Safe upto 2000 mg/kg	COIA	Reduction in inflammation due to mediators suppression	[370]
Punica granatum Linn.	Lythraceae	Pomegranate	F	Solid phase extraction	13.6-34 mg/kg	Safe upto 2000 mg/kg	CIA	Inhibition of spectrum of signal transduction pathway	[196,371]
Rhus verniciflua Stokes.	Anacardeaceae	Chinese lacquer tree	B	N-hexane	50 mg/kg	5 g/kg	CIA	Suppressive effects on inflammatory cytokines/chemokines and angiogenic factor in IL-1β-stimulated RA	[372]
Ruta graveolens Linn.	Rutaceae	Rue	Al pt	Aqueous	10 mg/kg	≥10 g/kg	CFA	Reduces cell influx, release of mediators, lipid peroxidation and oxidative stress	[207,373]
Salacia reticulate Wight.	Celastraceae	Khothala himbutu	L	Ethanol	25 ug dry powder/ml	2000 μg/ml	CIA	Inhibition of IL-1β - activated cell proliferation and regulation of mRNA expression	[300]
Salix nigra Linn.	Saliaceae	Black willow	B	Methanol	100 mg/kg	D.N.A.	CIA	Inhibition of pro inflammatory inhibitors	[301]
Saraca asoca Roxb.	Rubeacea	Sorrow less	B	Methanol	1-5 g/kg	LD_{50}=6.5 gm/kg	CFA	Antagonistic action against the pro-inflammatory cytokines and stabilizing effect on lysosomal membrane, reduction in release of acid hydrolase	[343,374]
Saussurea lappa Clarke.	Compositae	Kuth roots	R	Ethanol	50, 100, 200 mg/kg	Safe upto 2000 mg/kg	CFA	Inhibited TNF-release from LPS-stimulated murine macrophage cell line	[217,375]
Semecarpus anacardium Linn.	Anacardiaceae	Bhallatak	Nt	Nut milk extact	150 mg/kg	Safe upto 5 g/kg	CFA	Inhibition of cytokine production	[302,376]
Sida rhombifolia Linn.	Malvaceae	Cuban jute, jelly leaf	Al pt	Methanol, petroleum ether	30-100 mg/kg	Safe upto 5000 mg/kg	CFA	Generation of reactive oxygen species was suppressed	[218,377]
Sinomenium acutum Rehd.	Menispermaceae	Tudurafuji	R	Alcoholic	15, 50, 150 mg/kg i.p.	D.N.A.	CFA	Inhibition of lymphocyte proliferation and macrophage Function and reduction of the ESR	[378]
Smithia sensitive Smith.	Fabaceae	Odabirni	Wh	Methanol pet ether chloroform	10 ml/kg	Safe upto 2000 mg/kg	FIA	Inhibition in the hypotonicity	[379]
Sophora flavescens Aoton.	Fabaceae	Kurara worm killer	R	Ethanol	100 mg/kg	D.N.A.	AIA	Inhibition of COX-2 -catalyzed PGE2 and iNOS	[380]
Strobilanthus callosus Nees.	Acanthaceae	Marudona	R	Pet ether	200, 400, 800 mg/kg	Safe upto 2000 mg/kg	CFA	Reduce levels of lipid peroxides, glutathione peroxidase and catalase	[381,382]
Strychnus potatorum Linn.	Loganaceae	Clearing nut tree	S	Water	200 mg/kg	D.N.A.	CFA	Suppressive action on mediators of inflammation	[303]
Torilis japonica Houtt.	Apiaceae	Upright hedge parsley	F	Methanol	90, 270 mg/kg	Safe upto 5000 mg/kg	CIA	Inhibitory effects on immune cell trafficking. CD4 T-cells	[200]
Toxicodendron pubescens P. Mill.	Anacardiaceae	Atlantic poison oak	Wh	aqueous	10 mg/kg	Safe upto 2000 mg/kg	CFA	Immunosuppressant activity	[383,384]
Trewia polycarpa Benth.	Euphorbiaceae	Gambhari, prathinidhi	R	Ethanol	100 mg/kg	Safe upto 3.2 g/kg	CFA	Superoxide dismutase, glutathione peroxidase, ascorbic acid levels were increase while lipid peroxide content was decrease	[385]

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Table 3: Contd...

Botanical name	Family	Common name	Part	Extract	Dose (p.o.)	Acute toxicity (p.o.)	Model	Mode of action	Reference
Tridax procumbens Linn.	Asteraceae	Ghamra	L	Ethanol	300 mg/kg	≥2000 mg/kg	CFA	Suppression of migration of lukocytes	[386]
Trigonella foenum raecum Linn.	Fabeceae	Fenugreek	S	Mucilage	75 mg/kg	D.N.A.	CFA	Reduces cell influx, release of mediators, and oxidative stress	[247]
Urtica pilulifera Linn.	Urticaceae	Roman nettle	L	Methanol	1.33, 2.0 g/kg	Safe upto 2 g/kg	CFA	Suppress the activation of NF-kB	[387]
Vernonia cinerea Less.	Asteraceae	Bitterleaf ndole	Fl	Ethanol	25-100 mg/kg	Safe upto 5000 mg/kg	CFA	Membrane stability-modulating effect	[388,389]
Vitex negundo Linn.	Verbenaceae	Nirgundi, sindhuvara	L	Ethanol	1 ml/100 g	LD_{50}>2000 mg/kg	CFA	Immunosuppressive activity	[258,390]
Withania somnifera Dunal.	Solanaceae	Indian winter cherry	R	Hydralcoholic	500-1000 mg/kg	LD_{50}=1750 mg/kg	UIA	Inhibiting the release of inflammatory mediators	[14]
Xanthium srtuarium Linn.	Compositae	Cocklebur, burdock datura	L	Ethanol	200, 400 mg/kg	Safe upto 2000 mg/kg	CFA	Inhibiting the release of inflammatory mediators, lowers the elevated levels of NO, urinary hydroxyproline and neutrophil infiltration	[277]
Yucca schidigera Roezl.	Liliaceae	Spanish dagger	B	Hydro-alcohol	300-400 mg/kg	D.N.A.	APA	Inhibition of NFkB activation	[391]

CIA: Collagen induced arthritis, CFA: Complete freund arthritis, SCW: Streptoccol cell wall induced arthritis, HRBC-MS: HRBC membrane stabilization, FIA: Formalin induced arthritis, TIA: Turpentine oil induced arthritis, IPDN: Inhibition of protein denaturation, APA: Anti-protozoal activity, MSUIA: MSU induced arthritis, CACW: *Candida ablicans* cell wall, FOIA: Formaldehyde induced arthritis, COIA: Croton oil induced arthritis, ICAM-I: Intercellular adhesion molecule-1, VCAM-I: Vascular cell adhesion molecule-1, D.N.A.: Data not available, ESR: Erythrocyte sedimentation rate, WBC: White blood cell, RA: Rheumatoid arthritis, TNF-α: Tumor necrosis factor, IL: Interleukins, RBC: Red blood cell, PGE2: Prostaglandin E2, GM-CSF: Granulocyte-macrophage colony-stimulating factor, PGDF: Platelet- derived growth factor, TGF-β: Transforming growth factor beta, TPA: Tissue-type plasminogen activator, ALT: Alanine aminotransferase, IFN: Interferon, iNOS: Inducible nitric oxide synthase, NF: Nuclear factor

Dried flowering or fruiting tops are medicinally important. CT possesses traditional significance in infections of eye, local inflammation, neuralgia, acute mania, whooping cough, asthma, and to relieve pain in dysmenorrhea and menorrhagia. Oil extracted from seeds is used in rheumatism. The chief chemical constituent is a resin volatile oil composed of canabene, canabene hydride, canabinon, and canabin; which consist of cannabinol, pseudo-cannabinol, cannabinin, and several terpenes [51,94]. Around more than 166 research papers confirm that cannabis and related therapies will be helpful in relieving the pain associated with arthritis. Moreover, cannabinoid component of cannabis shown to possess anti-arthritic activity. It has been claimed to use as anxiolytic, antidepressant [95,96] in schizophrenia [97] and RA. The active moiety of CT i.e. cannabidiol at a dose of 10 and 25 mg/kg, orally, administered in collagen-induced arthritic ratssignificantly decreases the arthritic score and inhibits the release of inflammatory mediators. Thus, it was concluded that the cannabidiol have an anti-arthritic activity by possessing anti-inflammatory and immunosuppressive action [98].

Cinnammomum zeylicanium Blume. (CZ)(Family-Lauraceae)

CZ a topical evergreen tree grows to a height of 7-10 m in its mild state and has deeply veined ovate leaves that are dark green underneath. It is commonly known as cinnamon or Ceylon cinnamon. CZ is cultivated in Sri Lanka, Mayanmar, and Southern Coastal strips of India. Treatment of vaginitis, rheumatism, neuralgia, wounds, toothache, diabetes, inflammation of eyes, impotence, and leucorrhea is its traditional uses. CZ was also used to treat abdominal pain associated with diarrhea, dysmenorrhea, and amenorrhea. The active constituents of the CZ are cinnamaldehyde and eugenol. The other constituents are cmphene, sibinene, myrcene, fenchone, nerol, bornyl acetate, cinnamyl acetate, and geranial [99]. The CZ is reported to have an analgesic, anti-pyretic [100], anti-fungal [101], anti-inflammatory, anti-microbial [102,103], insecticidal [104], anti-diabetic [105,106], and antioxidant activities [107,108]. The polyphenolic extract of the CZ bark at a dose of 8 mg/kg revealed anti-arthriticpotential in male wistar rats in CFA model by improving the body weight and the level of serum C-reactive proteins when compared with control group. Thus, anti-arthritic activity was mediated through inhibition of leukocyte emigration and prostaglandin synthesis [109].

Coriander sativum Linn. (CS)(Family-Umbelliferae)

CS is a herbaceous plant distributed all over India and used for its seeds, fruits and leaves. Traditionally, plant is used as stimulant, carminative, stomachic, diuretic, tonic, and aphrodisiac. Oil is very useful for rheumatism in a dose of 1-4 minim on sugar or in emulsion. Coriander oil which contains linalool/coriandrol, geraniol, and boborneol, extracted from its fruit, is volatile and essential [51,110]. Externally seeda can be used as a lotion or have been bruised and used as a poultice for the treatment of arthritis. Cineole, one of the 11 components of the essential oils, and linoleic acid, present in coriander, possess antirheumatic and anti-arthritic properties [111]. CS possesses an antibacterial [112,113], anti-spasmodic [114], antioxidant [115-117], anticarcinogenic [118], and hypolipidemic activities [119]. The hydroalcoholic extract of seeds at doses of 8, 16, and 32 mg/kg showed reduction in paw swelling induced by formaldehyde and CFA methods in male wistar rats by inhibiting the pro inflammatory cytokines and TNF-α. In conclusion, the extract of CS shows a potent anti-arthritic activity on rats [120].

Curcuma longa Linn. (CL)(Family-Scitaminaceae)

CL is a perennial herb that measures up to 1 m high with a short stem, distributed throughout tropical and subtropical regions of the world, and is widely cultivated in Asian countries, mainly in India and China [121]. There are two varieties of CL one with rich-colored oval rhizomes and other with softer, larger, lighter-colored long rhizomes which are edible. Turmeric paste mixed with lime and saltpeter can be used externally in rheumatism. The major chemical constituents are curcumin, methylcurcumin, demethoxy curcumin, sodium curcuminate, and Ar-turmerone. Traditionally, CL is used in wound healing, helminthic infections, fevers, skin eruption, conjunctivitis, cough, parasitic infections, and liver diseases [51,121]. Later on, it was investigated the effect of herbomineral formulation (comination of turmeric, ashwagandha, sallai guggul, and jasad bhasma based on Ayurveda medicinal system) on 90 patients suffering from arthritis. It was observed that there was significant reduction in disability and pain. The plant is reported to be highly valued as anti-inflammatory [122,123], antiprotozoal [124,125], nematocidal [126], antibacterial [127], anti-tumor [128], and hepatoprotective [129]. The anti-arthritic activity was shown by essential oils of rhizomes of CL with streptococcal cell wall induced arthritis. It can be concluded that the turmeric essential oil possess an anti-inflammatory as well as anti-arthritic activities [130].

GY (Family-Fabaceae)

GY commonly known as mulethi is a herb/shrub of 2 m height mainly found in subtropical or temperate areas. The underground growth of stem is up to 2 m and is highly branched consisting short taproot with number of rhizomes. GY is commercially grown in Spain, Sicily and England. In India, it is mainly cultivated in Punjab and Sub Himalayan tracts [51]. The plant is reported to be traditionally used in anemia, gout, asthma, epilepsy, fever, cough, skin disease, rheumatism, paralysis, and hemorrhagic diseases. Roots in the form of infusion, decoction, extract or lozenge are useful as a demulcent in inflammatory affections [10,51]. The clinical trials reveal that glycyrrhizin has favorable effects on RA, when administered along adrenocorticotropic hormone or cortisone, in comparison, when administered alone. Hence, it was suggested that the main effect of liquorice is to potentiate rather than mimic endogenous steroids.The active chemical constituent is glycyrrhizin present in the form of potassium and calcium salts of glycyrrhizic acid. GY also contains sucrose,

glucose, resins, bitter principles, mannites, asparagines, and fat [131]. GY have shown anti-microbial, hypolipidaemic, antiviral, hypotensive, anti-ulcer, anti-diuretic, anti-inflammatory, anti-mutagenic, expectorant, hepatoprotective, antioxidant, and antipyretic activities [132-134]. The methanolic extracts of rhizomes of GY at a dose of 150 mg/kg possess anti-arthritic activity in male wistar rats by inhibiting the leukocyte migration and auto antigens production and exhibit anti-protinase activity. The study concluded that GY possess a significant anti-arthritic activity [76].

Lantana camara Linn. (LC)(Family-Verbinaceae)

LC popular as lava or red sage is a low erect or subscandent vigorous shrub with tetrangular stem, stout recurved pickles and comprises strong odour ofblack currents. LC is native to India and reaches to a height of 1-3 m [135]. Traditionally, LC is used in the treatment of sores, chicken pox, measles, fever, cold, rheumatism, asthma, ulcers, and high blood pressure [135]. In Asian countries like India, the decoction of leaves of the plant LC was used traditionally for the treatment of rheumatism. In Ghana, the infusions of whole plant are used against arthritis. *Nyctanthes arbor tristis* is used in Bangladesh for treatment of fever, bacterial infections, and rheumatism as well as other ailments [136]. The active constituents are flavones, isoflavones, antocyanins, coumarins, lignins, alkaloids, tannins, saponins, triterpinoids, catechins, and isocatechins [137]. LC is reported to have an antioxidant [138], anti-diabetic [139,140], anti-inflammatory [141], anti-motility [142], anti-fungal [143,144], anti-bacterial [145,146], anti-fertility [147], cytotoxic [148], larvicidal [149], and wound healing activities [17,150]. The ethanolic extract of leaves of LC at doses 5, 10 and 20 mg/kg proved to have anti-arthritic activity by inhibiting the lipoxygenase and cyclooxygenase [151].

Phyllanthus amarus Schum and Thomm. (PA)(Family-Euphorbiaceae)

PA is a 10-60 cm tall herb which grows in tropical and subtropical sandy regions. Its common name is chancapiedra. Traditionally, PA is used in jaundice, dropsy, diarrhea, dysentery, urino-gental disease, scabies, ulcer, and wounds. In addition, it is used as astringent, stomachic, diuretic, antiseptic, bitter, and febrifuge [51,152]. In the Hand Book of African Medicinal Plants it is reported that PA was traditionally use for its anti-inflammatory activity. Moreover, in Amazonia and Brazil, the whole plant was used for the treatment of various inflammatory disorders like arthritis. PA comprised of active constituents found in all parts of the plant aslignans, glycosides, flavonoids, alkaloids, ellagitannins, and phenylpropanoids [152]. Studies have proved that PA have anti-inflammatory [153], anti-microbial [154,155], anti-cancer [156], anti-fertility [157], hepatoprotective [158], anti-diabetic [159], anti-diarrheal [160], antioxidant [161], anti-oedemotgenic [162], diuretic [163] and chmoprotective [164] activity. The aqueous extract of whole plant at a dose of 100, 200, and 400 mg/kg shows anti-arthritic activity in male wistar rats. The extract at various doses reduced the levels of aspartate transaminase and alanine transaminase and thus maintains its anti-arthritic activity [165].

Piper longum Linn. (PL)(Family-Piperaceae)

PL is a slender, climbing, under shrub, creeping, and rooting below. The young shoots are downy, the leaves are 5-10 cm long; 5 cm wide; ovate; cordate with broad rounded lobes at the base; sub-acute and entire. PL is indigenous to North-Eastern and Southern India and Ceylon [51]. PL is used in cold cough, asthma, hoarseness, and snake bite since ancient times. In rheumatism, roasted aments are bitten up with honey and taken in a prescribed dose. In Java and Indonesia, the whole plant was applied topically, as it relieves muscular pains and inflammation [51,166]. Major constituents are piperine, piperlongumine, piperlonguminine, and methyl 3,4,5-trimehoxycinnamate. Others include resin, volatile oil, starch, fatty oil, and inorganic matter [167]. Medicinally, PL finds its importance as an anti-inflammatory [168], anti-amoebic [169], anti-asthmatic [170], hepato-protective, and immune-modulatory activities [171]. The aqueous extract of seeds of PL at two doses (200 and 400 mg/kg) shows a 46.32% inhibition in paw swelling in Freund's complete adjuvant induced arthritis in rats by inhibiting the adherence of neutrophils to endothelial monolayer by suppressing the TNF-α induced expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, E-selectin, and also inhibits the NF-κB. In conclusion, PL possess a significant anti-arthritic activity on male wistar rats [172].

Punica granatum Linn. (PG)(Family-Lythraceae)

PG is popular as pomegranate is a native of India, East Indies, Southern Asia, tropical Africa, California, and Arizona. PG grows tillan height of 12-16 feet with number of spiny branches and has long lifespan. Traditionally, PG is used in diarrhea, ulcers, and diabetes and also useful as antiparasitic agent and blood tonic [51,173]. In Iranian Traditional Medicinal system, the seeds and juice are considered as a tonic for the treatment of rheumatism. Pomegranate fruit consumption reduced composite disease activity index in RA patients, and this effect could be related to the anti-oxidative property of pomegranates. Dietary supplementation with pomegranates may be a useful complementary strategy to attenuate clinical symptoms in RA patients [174]. Some of the major chemical constituents present in the PG aregallic acid, anthocyanins, ellagitannins, flavones, flavonoids, antocyanidins, sterols, quercitin, rutin, and other fatty acids [173]. The plant is of high value due to its anti-inflammatory [175], anti-carcinogenic [176,177], antioxidant [178,179], hypotensive [180], hypolipidaemic [181], anti-artheroseclerotic [182], and anti-diabetic activities [183]. PG is also used in the treatment of myocardial ischemia [184], prostrate cancer [185,186], dental plaques [187], denture stomatitis [188], bacterial infections [189,190], erectile dysfunctions [191], male infertility [192], alzheimer's disease [193], and ischemic brain injury [194,195]. The fruits of PG show an anti-arthritic activity at doses of 13.6-34 mg/kg

by inhibiting the spectrum of signal transduction pathway in male wistar rats. Thus, it can be concluded that PG have potent anti-arthritic activity [196].

Ruta graveolens Linn. (RG)(Family-Rutaceae)

Rue is an herbaceous perennial plant, originally growing in the Mediterranean region [197]. RG is traditionally used as antiseptic, anthelminthic, antispasmodic, stimulant, abortificient, expectorant, and anti-rheumatic [51]. The major chemical constituents isolated from the RG are rutin, quercitin, rutacridone, rutacridone epoxide, graveoline, and gravacridonodiol [197]. RG is reported to have anti-inflammatory [198,199], analgesics [200], antiandrogenic [201,202], antihyperglycemic [203,204], antihyperlipidemic [205], anticancer activity [206], and anti-rheumatic properties. The polyphenolic fraction of aerial parts of RG at a dose of 10 mg/kg, b.w. showed an anti-arthritic activity in male wistar rats induced by CFA model. The polyphenolic fraction revealed its activity by inhibiting the prostaglandins synthesis, decreasing CRP level, ceruloplasmin, lipid peroxidation and release of other inflammatory mediators. In conclusion, RG possess anti-arthritic activity [207].

Saussurea lappa Clarke. (SL)(Family-Compositae)

SL herbs grow abundantly on the Himalayas and Valley of Kashmir. Roots contain odorous principle composed of a solid resin, salt of valeric acid and ash which contains manganese. SL is mainly useful in asthma, helminthiasis, fever, cough, skin disease, rheumatism, malaria, and leprosy. Roots in the form of infusion with little cardamoms are used in chronic rheumatism. Oil of the root composed of camphene, phellandrene, costene, aplotaxene, costol, and costic acid [51]. In the Southern part of Kashmir, Himalaya, and Punjab regions, the roots and root stalk are used for the treatment of rheumatism. In Unani system of medicine, it is useful in rheumatism [208]. The combination of *Cyperus rotundus*, *Tinospora cordifolia* and SL clinically proved to have an anti-arthritic activity through significant reduction of pain in double-blinded, comparative, parallel clinical trial design [209]. The SL extracts exhibited other biological activities including anti-diarrheal [210], antiulcerogenic [211,212], antibacterial [213], anticancer [214], anticonvusant [212], hepatoprotective [215], antiviral [216], anti-inflammatory, antioxidant [217], and anti-arthritic activities. The ethanolic extract of SL at dose levels of 50-400 mg/kg showed potent anti-arthritic activity. A sesquiterpene lactone "cynaropicrin" isolated from SL strongly inhibited TNF-α release from lipopolysaccharide (LPS) - stimulated murine macrophage cell line and dose-dependently suppressed the proliferation of lymphocytes stimulated. Another sesquiterpene lactone "dehydrocostus lactone" from SL suppressed LPS-induced nitric oxide production. The investigation concluded that the SL shows a significant anti-inflammatory and anti-arthritic activity [217].

Sida rhombifolia Linn. (SR)(Family-Malvaceae)

SR is a small erect under shrub having rough branches with stellate hairs commonly found in dry countries such as India and Ceylon [218]. Traditionally, the plant is used as nutritive, tonic and for the treatment of gonorrhea, piles, rheumatism, as diuretic, and aphrodisiac [51]. In Indonesia and Johore medicinal system, juice of whole plant pounded with little water is given indoses of ¼ seer for the treatment of rheumatism. β-phenethylamine, N-methyl-β-phenethylamine, S-(þ) N-β-methyl tryptophan methyl ester, vasicinol, vasicinone, vasicine, choline, hypaphorine methyl ester, hypaphorine, and betaine [219] have been isolated from the plant. The reported activities of plant include cytotoxic [220], antimicrobial [221], antibacterial [222], anti-inflammatory, antipyretic [223], and anti-arthritic. The aqueous and ethanol extract of aerial parts of the SR at doses 30 and 100 mg/kg reduced the paw edema induced by CFA method. Thus, it is concluded that the plant possess a potent anti-arthritic activity [218].

Terminalia chebula Retz. (TC)(Family-Combrataceae)

TC is a well-known traditional plant of Indian traditional medicinal system and the most frequently used herb in ayurveda. In tribal of Tamil Nadu in India, the TC is commonly known as Kadukkai and was used for treating various ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infections, and wound infections [51]. TC is a medium-sized deciduous tree of variable appearance with usually short cylindrical bole of 5-10 m length and 60-80 cm diameter. The phytoconstituents of TC are tannins, flavonoids, resins, fixed oil, fructose, amino acids, and sterols. Moreover, the active constituents of tannins include chebulic acid, ellagic acid, chebulagic acid, chebulinic acid, and gallic acid. TC was used in Thai traditional system as a carminative, expectorant, and antioxidant. A polyherbal formulation "Triphala" of TC, *Terminalia bellerica* and *Emblica officinalis* is commonly used in chronic constipation, detoxification, poor digestion and rejuvenator of the body [224]. TC possesses an anti-bacterial [225], anti-viral [226], anthemintic [227], anti-fungal [228], anti-ameobic [229], anti-neoplastic [230], anti-plasmodial [231], antioxidant [232], anti-diabetic [233] and anti-ulcerogenic [234] activity. The TC reported to have an immunomodulatory[229],radioprotective[235],cytoprotective[236], cardioprotective [237], and hepatoprotective [238] activity. Moreover, the hydroalcoholic extract of TC produces a significant inhibition of joint swelling in formaldehyde induced arthritis and CFA induced arthritis models. The anti-arthritic potential of the extract was due to significant reduction in the levels of TNF-α, IL-6, and IL-1β [239].

Trigonella foenum-graecum Linn. (TF)(Family-Papilionaceae)

TF, commonly known as Fenugreek, is an herbaceous plant which has found wide applications as a food, a food additive, and as a traditional medicine. Albuminoids, soluble carbohydrates, woody fibers, and ash are present in TF [240,241]. The plant has wide uses in the traditional medicine and reportedly used to treat diabetes, high cholesterol, wounds, inflammation, and gastrointestinal ailments. Several confections of TF like methi modaka, *Svalpamethimodaka* etc., are used in rheumatism [51]. Fenugreek seeds have high

content of mucilage, choline, and trigonelline. Studies of its extract have shownantihyperglycemic [242], estrogenic [243], antioxidant [244], anticancer [245], anti-inflammatory [246], and antirheumatic activities. The fenugreek mucilage obtained from seeds of the TF at dose 75 mg/kg possess an anti-arthritic activity anddecreased the elevated levels of SGOT, SGPT, CRP, nitrites, ESR, and white blood cell count. The TF may act by decreasing the oxidative stress, cell influx, and release of mediators associated with arthritis. In conclusion, TF showed anti-arthritic activity [247].

Vitex negundo Linn. (VN)(Family-Verbenaceae)

VN is referred to as five leaved chaste tree and a large aromatic shrub or sometimes a smaller slender tree with quadrangular, densely whitish tomentosebranchets. VN is originated in Southern India and Burma [51]. VN have its traditional use in rheumatism, headache, enlarged liver, syphilis, diarrhea, and cholera. Leaves along with garlic, rice and gul is a remedy for rheumatism. In Ayurvedic, Unani and Chinese medicine system the leaves extract of VN was used to treat the rheumatism and inflammation of joints. The Konkan community in Maharashtra used the plant for rheumatism [248]. The chief chemical constituents are nishindine, flavones, luteolin-7-glucoside, casticin, iridoid glycosides, vitamin C, β-sitisterol, and phthalic acid [249]. VN possess different pharmacological activities including anti-inflammatory, analgesic [250-253], anticonvulsant [254], antioxidant [250,255], insecticidal [256,257], and antirheumatic [249]. The active compound agnusideisolated from ethanolic extract of leaves administered at doses of 1.56 mg/10 ml, 3.12 mg/10 ml, 6.25 mg/10 ml and 1.25 mg/10 ml p.o. decreased the elevated levels of ESR, leukotriene B4, PGE2, cytokines, IL-17, TNF-α and interferon gamma. Hence, it can be concluded that the VN possess an anti-arthritic activity [258].

Xanthium strumarium Linn. (XS)(Family-Compositae)

XS commonly known as cochlebur, burweed or burdock datura is anindigenous of tropical parts of India. XS is an annual herb of 1m height with a short, stout, hairy stems, and commonly grows in waste places, roadsides and along river banks in warmer parts. Traditionally, it is used as laxative, anthelmintic, tonic, digestive, antipyretic and also improves appetite, voice, complexion, and memory. XS is also used to cure leukoderma, biliousness, poisonous bites of insects, epilepsy, salivation, and fever. The infusion of plant has been used in treatment of rheumatism in ayurvedic and Chinese medicine system. The active principle of aerial parts of XS are alkaloids; sesquiterpenes lactones such as xanthinin, xanthumin, xanthatin; sulphated glycoside such as xanthostrumarin, atractyloside, carboxyatractyloside; phytosterols, xanthanol, isoxanthanol, xanthosin, 4-oxo-bedfordia acid, hydroquinone, xanthanolides, and deacetylxanthumin [259]. However, recently investigated that XS possess an anti-bacterial [260], anti-tumor [261], anti-cancer [262], anti-tussive [263], anti-fungal [264,265], anti-inflammatory [266,267], vasorelaxant [268], hypoglycaemic [269], antimitotic [270], anti-malarial [271], anti-trypanosomal [272], diuretic [273], anti-allergic [274], and antioxidant activity [275,276]. Oral doses (200 and 400 mg/kg) ofethanolic extract of XS when administered exhibited anti-arthritic activity by inhibiting the release of inflammatory mediators. In conclusion, XS have a potent anti-arthritic activity [277].

DISCUSSION

Since Neanderthal times, the plants had been used for the prevention and cure of various ailments such as RA and other inflammatory diseases. Natural sources such as plants have been considered as the safest and valuable treatment for the disease. From the ethno botanical knowledge, we included the plants that are used in Indian traditional systems such as herbalism, folklore and shamanism. The review article includes more than 485 different plant species that are used for the prevention and cure of RA during last few decades. The botanical name of the plant, family, common name, part used, and various dosage forms studied are summarized in the Table 2. Around more than 100 families are included for 485 plants among them papilionaceae, fabaceae, euphorbiaceae, acanthaceae, compositae, ranunculaceae, malvaceae, rutaceae, liliaceae, labiatae, solanaceae, cruciferae, verbenaceae, lauraceae, and rubiaceae are in major proportion. As shown in Figure 1, around 485 plants have been mentioned in which 19 (4.4%) belongs to family papilonaceae, 17 (4%) to compositae and euphorbiaceae, 15 (3.5%) to rutaceae, 14 (3.3%) to vabenaceae, 13 (3%) to labiatae and fabaceae, 12 (2.7%) to malavaceae and crucuferae, 11 (2.5%) to solanaceae and acanthaceae, 10 (2.3%) to ranunculaceae and liliaceae, 9 (2.1%) to apocynaceae, lauraceae and rubiaceae, 8 (1.8%) to graminae, meliaceae, and umbelliferae, and remaining (48.2%) are categorized as others [Figure 1].

From our review, we have noticed that majority of researches were carried mainly in developing countries such as India, China, Korea, and Nigeria. But some developed countries like USA and Japan also continue their research on RA so as to increase the potential benefits [Figure 2].

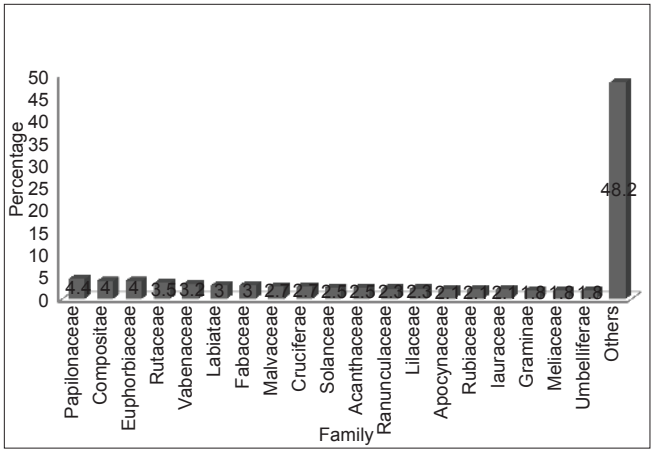

Figure 1: Plants in diverse families with % anti-arthritic activity

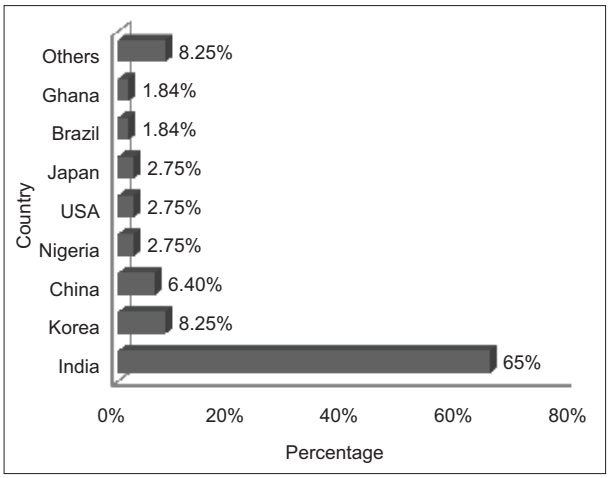

Figure 2: Geographical distribution of researches in the review

For the treatment of RA, various parts of plants are used such as leaves, roots, fruits, rhizomes, and seeds in distinguished dosage forms like extract, decoction, juice, infusion, paste, oil etc. The most potent anti-arthritic plants such as *Aconitum ferox*, *Balsamodendron mukul*, BD, *Boswellia serrata*, CS, CL, PL, *Ricinus communis*, *Plumbago zeylanica*, SL, SR, and *Strychnos nux vomica* have been elaborated in the review article. Among these listed plants, certain plants have been used in acute attack or in chronic pain or chronic rheumatism.

CONCLUSION

Traditional medicines used for the treatment of arthritis are used in various tribal/rural cultures worldwide. At present, investigation of anti-arthritic activity of traditional medicine has led to the development and studies of many herbal remedies employed for such purpose. The information that has been gathered from various sources is helpful in preserving folk indigenous knowledge as well as discovery of potential compounds having promising anti-arthritic activity. The information gathered from the data provides the information on toxicity profile and mechanism of action of tested extracts. Therefore, this review article has been prepared to provide the plants/their parts having specific traditional use in the treatment of arthritis upto year 2013. Moreover, this review has included latest data on new plant species/polyherbal formulations which are not covered in previews reviews on arthritis therapy as per our knowledge.

In conclusion, about 485 plant species mentioned in the list would have a promising anti-arthritic activity in humans. Information about the ethnic proof of the traditionally used anti-arthritic plants was cross-validated from various articles/reviews published in journals. Till know, no such review has analyzed which correlates the plant family, parts used, dosage form with anti-arthritic effects of the plants. Data mentioned in Table 2 show that papilonaceae family contains more plants with anti-arthritic activity whereas among parts, leaves have been maximally used in oil dosage form for the treatment of arthritis. Table 1 provides wealth of information indicates the beneficial effects of polyherbal formulations in the treatment of the arthritis. These includes Rumalaya forte, Rumalaya-liniment, arthacure, ortho joint oil, rheum off gold, Majoon suranjan, HLXL, GHJTY, Sudard, and TBL-II [18,20-24]. The data mentioned in Table 3 in addition provides the dose, toxicity profile, and models with mechanism of action for anti-arthritic activity.

The data discussed in this review might be quite useful in obtaining monographs on plants and recommendations on their use. In this review, we mainly deal with the safety profile, mechanism of action, and toxicity studies of plant extracts. The plant extracts and polyherbal formulations would be served as an alternate therapy for the treatment of arthritis with lesser side effects. Moreover, current knowledge can be helpful in materializing the commercial products, where the evidence can be quite limited.

Future Needs

Majority of traditionally used plants which have been mentioned in Table 2, have not been experimentally proved to have anti-arthritic activity. In addition, data in Table 3 show experimentally, the plants possess anti-arthritic activity only on animals but no clinical data are provided for proving the activity in humans. The data also lack information on exact activity of isolated compounds. However, the emphasis should be given in an area that needs further investigations as studied in animals needs to be translates to humans in order for a natural extract to be recommended for the treatment of arthritis. Therefore, further research of such less explored plants is still needed to determine their anti-arthritic activity.

Limitations

The data studied and prepared had been collected from the literature published in English language only and ignoring the studies published in other languages. The data mentioned in other languages, if had been included, will also be helpful in validating the current data. Further studies on isolated compounds of plants are not included, which otherwise, might be useful in scruitning the cause of anti-arthritic activity of plants.

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Review Article

Chemotherapeutic potential of cow urine: A review

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ABSTRACT

In the grim scenario where presently about 70% of pathogenic bacteria are resistant to at least one of the drugs for the treatment, cue is to be taken from traditional/indigenous medicine to tackle it urgently. The Indian traditional knowledge emanates from ayurveda, where *Bos indicus* is placed at a high pedestal for numerous uses of its various products. Urine is one of the products of a cow with many benefits and without toxicity.

Various studies have found good antimicrobial activity of cow's urine (CU) comparable with standard drugs such as ofloxacin, cefpodoxime, and gentamycin, against a vast number of pathogenic bacteria, more so against Gram-positive than negative bacteria. Interestingly antimicrobial activity has also been found against some resistant strains such as multidrug-resistant (MDR) *Escherichia coli* and *Klebsiella pneumoniae*. Antimicrobial action is enhanced still further by it being an immune-enhancer and bioenhancer of some antibiotic drugs. Antifungal activity was comparable to amphotericin B. CU also has anthelmintic and antineoplastic action. CU has, in addition, antioxidant properties, and it can prevent the damage to DNA caused by the environmental stress. In the management of infectious diseases, CU can be used alone or as an adjunctive to prevent the development of resistance and enhance the effect of standard antibiotics.

KEY WORDS: Antibiotic, antifungal, antineoplastic, bioenhancer, Bos indicus, immune-enhancer

INTRODUCTION

Infectious diseases remain a major threat to the public health despite tremendous progress in human medicine. Emergence of widespread drug resistance to the currently available antimicrobials is a matter of deep concern. A high percentage of nosocomial infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* or multidrug-resistant (MDR) Gram-negative bacteria. Each year in the United States, about 2 million people become infected with antibiotic resistant bacteria and at least 23,000 people die every year as a consequence of these infections. Many more people die from other conditions that are complicated by an antibiotic-resistant infection [1]. In 2012, there were about 450000 new cases of MDR tuberculosis. Extensively drug-resistant tuberculosis has been identified in 92 countries. Development of resistance to oral drug of choice fluoroquinolones, for urinary tract infections caused by *Escherichia coli* is very widespread, often sensitivity remains only for injectables [2]. Infections caused by resistant microorganisms often fail to respond to the standard treatment, resulting in prolonged illness, higher health care expenditures, and a greater risk of death. There is a dire need for the development of new antimicrobial agents with sensitivity intact against microorganisms [3,4]. The rational designing of novel drugs from traditional medicines to treat these difficult to treat infections offers a new prospect for the modern health-care system.

Ayurvedic texts (Sushruta Samhita, Ashtanga Sangrah and Bhav Prakash Nighantu) describe cow urine (CU) (gomutra) as an effective medicinal substance/secretion of animal origin with innumerable therapeutic uses. Cow (Kamadhenu) has been considered as a sacred animal in India. In Rigveda (10/15), CU is compared to nectar. In Susruta (45/221) and in Charak (sloka-100) several medicinal properties of CU have been mentioned such as weight loss, reversal of certain cardiac and renal diseases, indigestion, stomach ache, diarrhea, edema, jaundice, anemia, hemorrhoids and skin diseases including vitiligo. Gomutra is capable of removing all the imbalances in the body, thus maintaining the general health [5]. CU contains 95% water, 2.5% urea, minerals, 24 types of salts, hormones, and 2.5% enzymes. It also contains iron, calcium, phosphorus, carbonic acid, potash, nitrogen, ammonia, manganese, iron, sulfur, phosphates, potassium, urea, uric acid, amino acids, enzymes, cytokine and lactose [6].

CU is an effective antibacterial agent against a broad spectrum of Gram-negative and Gram-positive bacteria and also against some drug-resistant bacteria. It acts as a bio-enhancer of some antimicrobial drugs. It has antifungal, anthelmintic, antineoplastic action, is useful in hypersensitivity reactions and

in numerous other diseases including increasing the life-span of a person. Recent researches have shown that CU is an immune-enhancer also [7-9]. Therapeutic properties of CU have been validated by modern science also.

MECHANISM OF ACTION OF CU

Different fractions of CU possess antimicrobial activity due to the presence of certain components like volatile and nonvolatile ones [10-13]. Presence of urea, creatinine, swarn kshar (aurum hydroxide), carbolic acid, phenols, calcium, and manganese has strongly explained the antimicrobial and germicidal properties of CU [14-16]. Presence of amino acids and urinary peptides may enhance the bactericidal effect [17] by increasing the bacterial cell surface hydrophobicity. CU enhances the phagocytic activity of macrophages. Higher amounts of phenols in fresh CU than CU distillate (CUD) makes it more effective against microbes.

After photo-activation, few biogenic volatile inorganic and organic compounds such as CO_2, NH3, CH4, methanol, propanol and acetone, and some metabolic secondary nitrogenous products are also formed [18]. Photo-activated CU (PhCU) becomes highly acidic in comparison to fresh CU. An increase in bactericidal action may be due to a significant decrease in pH [12], presence of inorganic phosphorus, chloride and dimethylamine may also play an important role [19], along with increased formation of some reactive compounds like formaldehyde, sulfinol, ketones and some amines during photo-activation and long term storage [20]. CU prevents the development of antibacterial resistance by blocking the R-factor, a part of plasmid genome of bacteria [21].

CU contains phenolic acids (gallic, caffeic, ferulic, o-coumaric, cinnamic, and salicylic acids) which have antifungal characteristics [22].

Antioxidant property of uric acid and allantoin present in CU correlates with its anticancer effect. CU reduces apoptosis in lymphocytes and helps them to survive better [5]. This action may be due to the free radical scavenging activity of the urine components, and these components may prevent the process of aging [10]. It efficiently repairs the damaged DNA [5].

Daily consumption of CU improves immunity due to the presence of swarn kshar and fastens the wound healing process, which is due to allantoin [8]. CU enhances the immunocompetence by facilitating the synthesis of interleukin-1 and -2 [23,24], augments B - and T- lymphocyte blastogenesis, and IgA, IgM and IgG antibody titers [25].

Early morning first voided CU is more sterile and have more macro and micronutrients along with other enzyme/urea content could be more effective [26].

AS ANTIMICROBIAL AGENT

Antimicrobial activity of CU from both indigenous and hybrid breeds against *E. coli*, *Salmonella typhi*, *Proteus vulgaris*, *S. aureus*, *Bacillus cereus*, Staphylococcus *epidermidis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Pseudomonas fragi*, *Streptococcus agalactiae*, *Enterobacter aerogenes*, *Aeromonas hydrophila*, *Micrococcus luteus*, *Streptococcus pyogenes*, *Streptomyces aureofaciens*, *Lactobacillus acidophilus* and *Bacillus subtilis*, and *Leishmania donovani* has been observed in various studies. In these studies the antimicrobial activity of CU was found to be comparable with ofloxacin, ciprofloxacin, ampicillin, chloramphenicol, nalidixic acid, rifampicin, tetracycline, streptomycin, cefpodoxime and gentamycin in different studies [27-36].

Studies with Indigenous *Bos indicus* Breeds of Cow

Fresh CU (FCU), Sterile, PhCU and CUD from a healthy Geer cow, was used to assess the antibacterial effect against different strains of bacteria. Against *E. coli*, FCU had the bigger mean of inhibition zone (15 mm) than Sterile, PhCU, and CUD (~10 mm). FCU had good activity of 15, 16 and 20 mm of inhibition against *E. coli*, *B. subtilis*, and *S. typhi*, respectively. Other forms of CU showed activity against *E. coli*, *S. typhi*, *P. vulgaris*, *S. aureus* and *B. subtilis* [27].

Rana and De [28] observed a greater activity against Gram-positive than Gram-negative bacteria with CU obtained from Geer cow. The minimum inhibitory concentration (MIC) in all the four tested Gram-positive bacteria was 134 mg/ml. Among Gram-negative organisms, *P. aeruginosa* was more sensitive (MIC 134 mg/ml) than *P. vulgaris* (MIC 268 mg/ml). Mean zone of inhibition (mm)± standard error of the mean against *B. subtilis* was found to be 18.67±1.15, which was less than 27 for Gentamycin 10 mcg and cefpodoxime 10 mcg. Activity (18.67±1.15) against *B. cereus* and was similar to that of cefpodoxime (19) but less than with gentamycin (26). Activity (16) against *S. aureus* and *S. epidermidis* was <25 for Gentamycin and ~23 with Cefpodoxime. No inhibition against *P. aeruginosa* was observed with Cefpodoxime while CU had an inhibition of 19.33 ± 1.15 mm and Gentamycin 35 mm. Against *P. vulgaris* inhibition was comparable between CU (16 ± 1.73), gentamycin (21) and cefpodoxime (20). There was comparable inhibition of *P. vulagris* by CU (16 ± 1.73), gentamycin (21) and cefpodoxime (20). Against *K. pneumoniae*, inhibition observed with CU (15.67 ± 0.57) was less than gentamycin (34) and cefpodoxime (20).

Comparatively FCU obtained from Gujarati Geer cow was found to have more antimicrobial activity than its distillate (Table 1).

Similar findings were reported by Jarald *et al.* [29]. Mean zone of inhibition (mm), using Sahiwal CUD, was found to be 19.2 for *S. aureus*, 20.2 for *P. fragi*, 18.8 for *E. coli*, 23 for *B. subtilis*, 19 for *S. agalactiae* and 17 for *P. vulgaris*. There was a progressive decrease in optical density (indicator of antimicrobial activity and was measured by spectrophotometer at 600 nm) over 5 h when FCU was added to the respective inoculums [29].

The antibacterial efficacy (as mean zone of inhibition in mm) of CU Concentrate (CUC) obtained from Karnataka breed, Amrit

Table 1: Antimicrobial activity of CU, CUD (Gujrati Geer cow) in comparison to standard drug Ofloxacin [10]

	E. coli	*S. epidermidis*	*S. aureus*	*K. pneumoniae*	*P. vulgaris*	*B. subtilis*
FCU	23	22	24	25	23	24
CUD	20	20	18	20	20	21
Ofloxacin	30	28	25	28	28	32

E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, P. vulgaris: Proteus vulgaris, B. subtilis: Bacillus subtilis, S. epidermidis: Staphylococcus epidermidis, FCU: Fresh cow urine, CUD: Cow urine distillate, CU: Cow urine

mahal was comparable with Streptomycin on *B subtilis* (16:18), *S. aureus* (16:19), *E. coli* (14:18) and *E. aerogenes* (15:18) using Disc diffusion method [30].

In an *in vitro* study, 30 μL of PhCU of Hariana breed was found to be comparable in efficacy to Tetracycline (30 μg mL). Antimicrobial activity (mean zone of inhibition in mm) of PhCU and Tetracycline, respectively against *B. cereus* was 17 and 22, *S. aureus* was 18 and 21, *S. typhimurium* was 21 and 22, A. *hydrophila* was 22 and 24, *E. aerugenes* was 13 and 18 and *M. luteus* was 15 and 17 [31]. Similar results were found in another study with PhCU of Hariana breed against these bacteria [32].

Studies where breed of cow is not mentioned

In an *in-vitro* test, activity of FCU was comparable to Streptomycin. Similar mean zone of inhibition (mm) was seen against gram positive organisms *E. coli* (16:16:13), *K. pneumonia* (15:17:12) and *P. aeruginosa* (17:19:15) with FCU and Streptomycin and lesser with PhCU (by keeping urine in sunlight in sealed glass bottles for 72 h), respectively. Comparatively lesser antibacterial activity against gram negative organisms *S. aureus* (18:26:17), coagulase negative *Staphylococci* (18:29:15), *B. subtilis* (20:29:15), and *S. pyogenes* (20:26:14) was seen for FCU than streptomycin, and still lesser than with PhCU [33]. No antibacterial activity was seen for CUD, which is contradictory to some previous reports [34].

Vats *et al*. [35] studied the synergistic antimicrobial effect of PhCU and herbs against bacterial and fungal strains. PhCU and *Azadirachta indica* combination showed a remarkable synergistic antimicrobial activity against *Candida tropicalis*, *Candida glabrata*, *P. aeruginosa*, and *S. aureofaciens*. PhCU and *Terminalia chebula* showed maximum activity against *S. auereofaciens* (45 mm), and *P. aeruginosa* (zone of inhibition of 40 mm). *Piper nigrum*, *T. chebula* and PhCU in combination were most effective against *C. glabrata* (35 mm) and *C. tropicalis* (45) mm.

Upadhyay *et al*. [18] found in *in-vitro* tests that PhCU has better bactericidal activity against *S. aureus*, *B. cereus*, *L. acidophilus*, *M. luteus*, *K. pneumonia*, *S. pneumonia* and *E. coli*, when compared with Tetracycline, Ampicillin and Ciprofloxacin. PhCU showed MIC value of 0.25 μl/ml against *S. aureus*, *B. cereus*, *L. acidophilus* and *M. luteus*, while it was found to be 0.125 μl/ml against *E. coli*, which was less than that for antibiotics. A combination of CU with Neem (*A. indica*) oil and Bavchi (*Psoralea coryfolia*) oil showed a synergistic effect (MIC 0.125-0.25 μl/ml), which was less than that for antibiotics. Neem oil and CU showed 33-35 mm inhibition zones against *B. cereus*, *L. acidophilus*, *M. luteus*, *K. pneumoniae* and *S. pneumonia*.

Sathasivam *et al*. reported the antibacterial activity of CUD (5, 10 and 15 μl) against the *B. subtilis*, *P. aeruginosa*, *K. pneumoniae* and *S. typhi*. Antibacterial activity (mean zone of inhibition in mm) was observed against *B. subtilis* (7.6 ± 0.04, 8.6 ± 0.17, 8.8 ± 0.17, respectively) *P. aeruginosa* (12.6 ± 0.04, 13.6 ± 0.17, 15.4 ± 1.23, respectively) and *S. typhi* (12 ± 1.23, 13.6 ± 0.17, 15.4 ± 1.23, respectively). This antibacterial activity was more than the positive control of ampicillin (30 mg/disc), which was 7.1 ± 0.01 mm against *B. subtilis*, 11.2 ± 0.01 mm against *P. aeruginosa* and 9.6 ± 0.02 mm of inhibition zone against *S. typhi*. Antibacterial activity against *K. pneumonia* was 11 ± 0.14 mm with 15 μl of CUD, which was more than the activity (9.5 ± 0.05 mm) with Ampicillin [34].

Yadav *et al*. reported the antimicrobial property of a herbal formulation containing CU, *Dalbergia sissoo*, and *Datura stramonium*. The antimicrobial activity of CU alone was also found to be significant ($P > 0.001$). It was found that CU extract showed the highest inhibition in gram-positive *S. aureus* (CI, 213%) and comparable activity in *S. pneumoiae* (95%) compared to chloramphenicol (30 μg), nalidixic acid (10 μg), rifampicin (30 μg), and ampicillin (10 μg). In gram-negative bacteria all antibiotics were inactive, except chloramphenicol (30 μg), while CU extract showed significant ($P < 0.05$) activity (35% and 37%, respectively) against *E. coli* and *K. pneumonia* as compared to Chloramphenicol [36].

CU has anti-*Leishmania donovani* effect (Kala-azar) in an *in-vitro* study [37]. This fact can be further validated by more intensive studies.

PREVENTION OF ANTIBIOTIC RESISTANCE

Pathogenic bacteria are remarkably resilient and have developed several ways to resist antimicrobial drugs. Due to increasing use and rampant misuse of existing antibiotics in human and veterinary medicine, and also in agriculture, threat from antimicrobial resistance is increasing. Resistant strains like Penicillin- and Methicillin- resistant *S. aureus*, vancomycin resistant *Enterococcus*, and ciprofloxacin resistance *P. aeruginosa* are an ever increasing global threat. After photoactivation and purification, CU has been found to be effective against certain drug resistant bacterial strains [38]. CU extract of *A. indica* showed better MIC values than the organic fractions for MDR *E. coli* (12.68 mm) and *K. pneumonia* (9 mm). CU extracts of *A. indica* showed >8.66 mm zone of inhibition for MDR *S. aureus*, *P. aeruginosa* and *P. vulgaris* [39].

FUNGICIDE AND BIOFUNGICIDE

Fungicidal effect against *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus*, *Malassezia*, *C. tropicalis* and *C. glabrata* has been observed in various studies. CU was highly stable and capable in inhibiting the growth of *Malassezia* fungi (90-95%) responsible for causing dandruff for a longer time (4-5 days), than rice water (due to *B. cereus* growth in rice water) which was stably capable of inhibiting 85-90% of the growth for 3-4 days. Neem leaves extract and Lemon Juice extract were comparatively less effective in this study [40].

15% CU was most active against *Aspergillus*, *Rhizophus* and the percentage of inhibition obtained with it was 85% [41]. 5% CUC showed maximum antifungal activity against *A. niger* (93%), followed by *A. oryzae* (92.67%) and *A. flavus* (83%) [30]. CUD showed better antifungal activity against *A. fumigatus* and *C. albicans* with mean zone of inhibition of 13 and 11 mm than PhCU [27]. More fungal growth suppression (as mm in diameter) was observed with CUD in *A. niger* (8 ± 0.14, 11.3 ± 1.2 and 12.6 ± 0.04, respectively) than *A. flavus* (7.3 ± 0.25, 10 ± 0.26 and 11 ± 1.2, respectively) with the use of 5, 10 and 15 μl of CUD [34].

In vitro antifungal activity (in mm) of Geer CU against *A. flavus* (17.33 ± 0.57) was in between 50 μg of amphotericin B (15) and 10 μg of clotrimazole (24) and against *C. albicans*, activity was similar with CU (18.67 ± 1.15) and amphotericin B (19), but less than clotrimazole (30) [28]. Antifungal activity of Geer CU is better than the others where source of CU is not mentioned.

In an *in vitro* study, it was found that the urine samples of outdoor feeding cow (OCU) was more effective and inhibited growth of fungi more strongly as compared to indoor feeding CU (ICU). This inhibition was concentration dependent. No growth of *Penicillium notatum*, *Trichoderma viridae*, and *Alternaria solanii* was observed with 10% OCU and with 20% ICU and that of *Claviceps purpurea*, *Rhizopus oligosporius*, *C. albicans* and *A. candidus*, no growth was observed with 20% of OCU only [42].

ANTISEPTIC

Sanganal *et al*. observed the enhanced wound healing activity of CU in Wistar albino rats [43]. On 4th day, the external application of CU showed significant and progressive increase in wound healing in rats compared to different concentrations of CU and 1% w/w nitrofurazone ointment locally. Similar findings were also observed by Maheshwary *et al*. [44].

ANTHELMINTIC ACTIVITY

CUC was found to be more effective than piperazine citrate as anthelmintic agent at both 1% and 5% concentrations. For anthelmintic activity, adult Indian earthworm *Pheretima posthuma* was studied due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Paralysis of earthworm occurred in 53 and 48 min with 1% piperazine and CUC, respectively and 16 and 13 min with 5% piperazine and CUC, respectively. Time taken for the death of earthworms decreased from 72 min with 1% piperazine to 60 min with 1% CUC, respectively. It further decreased from 28 min with 5% piperazine to 18 min with 5% CUC, respectively [30].

Different compositions of Panchgavya (five products of cow namely milk, curd, ghee, urine and dung) alone and combination of Panchgavya and ethanolic extract of *Bauhinia variegata* Linn (10%, 50%, 75% in Panchgavya) were found to have excellent anthelmintic activity against adult Indian earthworm (*P. posthuma)* when we compared to control Piperazine (50 and 100 mg/ml). In combination, the anthelmintic activity was synergistic and with increasing doses, time (in minutes) of onset of paralysis and death in earthworm decreased [45].

BIOENHANCER

A 'bioenhancer'/'biopotentiator' is an agent capable of enhancing the bioavailability and efficacy of a drug with which it is co-administered, without any pharmacological activity of its own at the therapeutic dose used. In ayurveda, this concept is known as 'yogvahi' and is used to increase the effect of medicines by increasing the oral bioavailability (especially of medicines with poor oral bioavailability), decreasing their dose and adverse effects, and were used to circumvent the parentral routes of drug administration. We can develop more such useful and economically viable drug combinations, by integrating the knowledge of time tested ayurveda with modern methods of research [8]. CU is the only agent of animal origin which acts as bioenhancer of antimicrobial, antifungal, and anticancer agents [30]. The indigenous CU contains '*Rasayana*' tatva, which is responsible for modulation of the immune system and also act as a bioenhancer [21].

CUD is more effective bioenhancer than CU [30,46]. CUD enhances the transport of antibiotics like rifampicin, tetracycline and ampicillin across the gut wall by 2-7 folds [47]. It also enhances the potency of taxol against MCF-7 cell lines [48]. It enhances the bioavailability of rifampicin by 80 fold in 0.05 microgm/ml concentrations, ampicillin by 11.6 fold in 0.05 μ g/ml concentrations and clotrimazole by 5 fold in 0.88 μ g/ml concentration [49]. The activity of rifampicin increases by about 5-7 folds against *E. coli* and 3-11 folds against Gram-positive bacteria, when used along with CU [50]. Potency of paclitaxel has been observed to increase against MCF-7, a human breast cancer cell line in *in-vitro* assays [49]. The bioenhancing ability is by facilitating the absorption of drugs across the cell membrane. The CU has been granted US Patents for its medicinal properties, particularly as a bioenhancer along with antibiotics, antifungal and anticancer drugs (6896907, 6410059).

CUD alone caused more inhibition of Gram-positive bacteria. Inhibition caused by streptomycin (1 mg/ml) alone was higher (31-34 mm) than that of CUD alone (19-22 mm). With the

combination of *Pinguicula longifolia*, CUD and streptomycin together, *S. aureus* was inhibited to a more extent (38 mm) followed by *P. aeruginosa* (37 mm) and an equal inhibition was exhibited by *B. subtilis* and *E. coli* (36 mm) [51]. *S. aureus* and *P. aeuroginosa* have been recognized as most common bacteria, which have developed resistance against several antibiotics and is a major hospital borne pathogen, which is particularly dangerous to immunocompromised patients. This study is of importance in this scenario.

This bioenhancing activity of CU has been aptly and widely used in various ayurvedic formulations. It is one of the constituents of Hingwadh ghrita, Lashunadh ghrita, Sidhartak ghrita for psychiatric illness used in abdominal tumors and in other formulations like Mandurvatak, Darvi ghrita, and Punnarvamandur. CU is used as yogvahi along with Hareetakyadi yog, Swarnkshiryad yog, Swarnmakshik bhasma and Gvakshyadi churana. These preparations are commercially available in the Indian market. Ghritas are available as semisolid preparations while bhasms, yogs, and churans are in the powder form.

ANTICANCER PROPERTIES

CU has antioxidant properties and is a free radical scavenger, and thus it neutralizes the oxidative stress. Scientists proved that the pesticides even at very low doses cause apoptosis of lymphocytes and tissues through fragmentation of DNA while CU helps the lymphocytes to survive by inhibiting their apoptosis and by repairing the damaged DNA and is, therefore, effective as anti-cancer therapy [52,53].

Chemopreventive potential of CU was observed in a study, which was conducted on 70 Swiss albino mice for 16 weeks. Papillomas were induced by 7, 12 dimethyl benzanthracene and later promoted by repeated application of croton oil. In mice treated with CU, the incidence of tumor (papillomas), tumor yield, and its burden was statistically less than the untreated group [54].

Jain *et al*. studied the effect of CU therapy on various types of cancers in Mandsaur area. The severity of symptoms (pain, inflammation, burning sensation, difficulty in swallowing, and irritation) decreased from day 1 to day 8 with CU therapy. Percent of patients with severe symptoms decreased from 82.16 to 7.9 on day 8, patients with moderate symptoms increased from 15.8 to 55.3 and with mild symptoms, patients increased from 1.58 to 36.34. The severity of symptoms decreased further with continued CU therapy [15].

Dutta *et al*. reported the anti-clastogenic and anti-genotoxic effect of redistilled CUD (RCUD) in human peripheral lymphocytes, which have been challenged with manganese dioxide (MnO_2) and hexavalent chromium (Cr+6). Protection in number of chromosomal aberrations and frequency of micronuclei were more prominent when these cells were pretreated with CU than simultaneous treatment with CU [55].

IMMUNOSTIMULANT

The use of herbs and minerals (like chavanprash and panchgavya) for improving the overall resistance of the body against common infections and pathogens has been a guiding principal of Ayurveda. Ancient books on Ayurveda state that consuming CU daily increases the resistance to diseases by up to 104%. CU enhances the humoral, and cell-mediated immune response in mice [5]. CUD was found to augment B- and T-lymphocyte blastogenesis; IgG, IgA and IgM antibody titers in mice. It has been observed that CU also increases the phagocytic activity of macrophages and is thus helpful in the prevention and control of bacterial infections. The level of both interleukins -1 and - 2 in mice was increased by 30.9% and 11.0%, respectively, and in rats these levels were increased significantly by 14.75% and 33.6%, respectively [52]. CUD has been reported to be a potent and safe immunomodulator, which increases both humoral, and cell-mediated immunity in mice.

Cell-mediated immune response was evaluated on various parameters in a study by Verma *et al*. using CU for 30 days. There was a 55% increase in phagocytic index, and a significant increase (16%) in neutrophil adhesion on regular use of whole freeze dried CU. CU has the potential to boost the immune functions by increasing the white blood cells counts and subsequently reducing the red blood cells count to a certain extent [56].

Traditional uses of CU

Some of the traditional uses of CU are in fever, where CU along with pepper, curd and ghee is used; in leprosy, CU is used along with dhruhardi and in deformities associated with leprosy, it is used with Nimbuchal, whereas in chronic leprosy, CU is used along with leaves of Vasaka and kanar, and bark of kuraila and neem [11]. Local traditional healers in Mandsaur prescribe CU for worm infestations, to develop immunity and to avoid aging. They suggest 10-25 ml of CU to be taken on an empty stomach for the same [15].

CONCLUSIONS

On analyzing the effect of different preparations of CU, FCU had better activity than CUD [27-32]. Activity of FCU and CUD from indigenous cows was similar to streptomycin and tetracycline. Ayurveda also mentions that FCU of indigenous cows' is the best.

More well-planned studies in human subjects are required to fully assess its potential as an effective antimicrobial agent as most of the studies quoted are *in vitro* studies. Comparative studies between CU obtained from indigenous breeds and of inbred strains may be undertaken, as ayurveda was written when inbred strains of cows were not present. Future development of newer drugs can involve CU in its repository.

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Review Article

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Caffeic acid phenethyl ester as a remedial agent for reproductive functions and oxidative stress-based pathologies of gonads

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ABSTRACT

In recent years, the studies on the roles of caffeic acid phenethyl ester (CAPE) in several disease models and cell cultures are tremendously growing. It is such a great molecule that was used by ancient times to ameliorate some diseases and nowadays, it is used by modern medicine to test the effectiveness. In this mini-review article, the protection capability of CAPE, as a liposoluble antioxidant and a potent nuclear factor kappa B inhibitor, on oxidative and non-oxidative ovary, and testis damages has been summarized. In view of our laboratory findings/experience and those reported in the hitherto literature, we suggest that CAPE possesses protective effects for pathologies of the reproductive organs induced by untoward effects of harmful molecules such as free oxygen radicals, pesticides, methotrexate, and MK-801 (dizocilpine).

KEY WORDS: Caffeic acid phenethyl ester, gonads, ovary, oxidative damage, protection, testis

INTRODUCTION

Caffeic acid phenethyl ester (CAPE) [Figure 1] is one of the most active compounds found in propolis. Propolis has been known to be used many ethnic and/or cultural groups as folkloric medicine for hundreds and thousands of years. CAPE is known as a potent antioxidant substance that inhibits the production of xanthine/xanthine oxidase (XO) and free oxygen radicals in human neutrophils [1,2]. Besides its antioxidant and potent Nuclear Factor kappa B (NFκB) inhibitory activities, CAPE has been reported to show some other effects, i.e., vasorelaxant, anti-inflammatory, anticarcinogenic, and immunomodulatory as well. CAPE, as a liposoluble antioxidant and it is used in a number of inflammatory and infectious diseases as traditional medicine. As an antioxidant, CAPE can be used in ischemic/reperfusion injuries or other types of organ damages due to oxidative stress [3-5]. Cells mainly have two defense mechanisms against oxidative damage; one of them pertains to antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GSH-Px), GSH reductase (GSH-Red), superoxide dismutase (SOD), and the other one being antioxidant defense compounds such as GSH, vitamin C, and vitamin E.

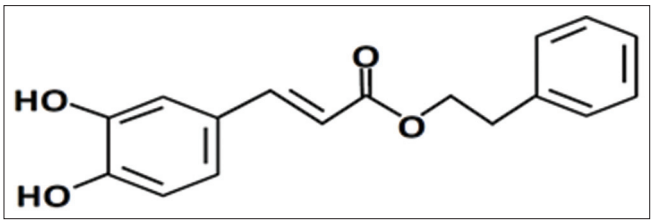

Figure 1: The chemical structure of caffeic acid phenethyl ester

This study aimed to underscore the effects of CAPE on the oxidative and non-oxidative ovary and testicular damages induced by several factors such as free oxygen radicals, pesticides, methotrexate, and MK-801 which have untoward effects on the organs.

Ipsilateral and Contralateral Ischemia Reperfusion (I/R) Injuries in Animal Ovaries and Testes

Torsion of the ovary is an emergency situation in normal individuals or the presence of an ovarian mass. The conservative therapy of twisted ovary is detorsion. However, detorsion has some disadvantages such as the huge amount of reactive oxygen species (ROS) (such as hydrogen peroxide, superoxide anion, hydroxyl radical, and singlet oxygen) produced after detorsion and reperfusion. In the reperfusion phase, xanthine dehydrogenase is converted to XO, which is an enzyme producing huge amounts of ROS, by degrading ATP, ADP, and AMP. Celik *et al.* [6] reported the protective effect of CAPE on rat ovaries injured by I/R. They analyzed GSH, XO, and malondialdehyde (MDA), a lipid peroxidation end product, in the ovarian homogenates obtained from torsion/detorsion (T/D) rats. The MDA level of T/D group was significantly higher compared to those of the control groups. CAPE significantly reduced MDA levels after T/D. CAPE treatment reduced the pathological findings (i.e. acute PMN infiltration, diffuse hemorrhage, edema, and vascular dilatation) detected in the T/D group. Celik *et al.* [6] concluded that CAPE attenuates reperfusion injury in the ovary by decreasing MDA and XO and by increasing GSH. Therefore, the authors suggested the use of CAPE in ovarian T/D injury as well as possibly in some other pathologic conditions related with increased ROS production.

In another study, the role of CAPE was studied in rabbit ovaries T/D injury model by examining the changes in the lipid peroxidation, antioxidant system, and by pathologically [7]. Administration of CAPE one hour before I/R significantly increased ovarian GSH-Px activity and GSH level, on the other hand significantly decreased CAT activity and thiobarbituric acid reactant substances (TBARS). Ovaries of the I/R group that were not treated with CAPE mostly represented grade IV pathological alterations with severe hemorrhage, edema, leukocyte infiltration, and vascular congestion within and around the ovarian medulla. CAPE treatment diminished the pathological changes and reduced the deteriorating changes to Grade 0-III, caused by I/R. As it alleviated the degenerative changes in the ovaries, it was concluded that CAPE might have a therapeutic use additional to the surgery.

To explore the effects of CAPE on I/R model in testis, we have conducted a study using albino rats [8]. In CAPE plus detorsion group, CAPE was applied intra-peritoneally (10 μmol/kg) 30 min before the detorsion. The rats were killed, and bilateral orchiectomy was performed 4 hours after the detorsion. NO levels were analyzed in both twisted ipsilateral and non-twisted contralateral testes. Results of the mentioned study indicate testicular T/D induces significant changes in NO level in the ipsilateral testis while NO level in the contralateral non-T/D testis is not affected. Ischemia led to increases in the concentrations of testicular NO by inducing NOS activity or migration of neutrophils to the testis [9]. Our study also revealed that levels of NO started to diminish with the detorsion process. After the CAPE treatment, NO levels became higher than that of the sham-operated rats with an unknown mechanism, which may suggest the possible protecting role of CAPE in the testicular injury. The possibilities for this finding may be; CAPE directly increases NOS activity, CAPE might have activated the synthesis of cofactors related with NO production or selectively activated neutrophil migration to the testis, which may increase NO synthesis. The most probable and logical explanation for the effect of CAPE is the scavenging effect on ROS together with the other antioxidant enzymes and its preventive effects on the inhibition of NO. Whatever the mechanisms are, the final conclusion is that testicular torsion increases NO level in testis and CAPE administration before testicular ischemia prevents the decrease of NO synthesis during reperfusion. Accordingly, CAPE may be a useful treatment strategy in the I/R injuries of testes.

In another study, the histopathological changes in testicular I/R injury of Wistar rats and the protective effect of CAPE (10 μmol/kg) was investigated [10]. I/R caused an increase in the expression of testicular inducible nitric oxide synthase (iNOS) and myeloperoxidase (MPO) enzymes. CAPE attenuated the increases of MPO and iNOS, leading to normalization of these parameters. The appearance of testicular tissues under the light microscope was normal in CAPE administered group whereas I/R without CAPE administration caused inflammatory infiltration, edema, necrosis, and congestion. These results have a potential to show the pathophysiology of testis I/R injury and also suggest that inhibition of MPO and iNOS activities by CAPE may be a new therapeutic strategy to avoid testicular damage.

I/R Model in Testicular Injury as Remote Organ

I/R injury damages not only in the related initial ischemic tissues and organs, but also in remote unrelated areas [11-13]. To date, the underlying mechanisms of remote organ injury are poorly clarified. In this respect, the effect of oxidative damage on testis following the I/R myocardial injury and also the protective effects of CAPE were reported by Esrefoglu *et al.* [14]. The authors demonstrate that CAPE treatment significantly decrease the elevated serum NO and MDA levels caused by the myocardial I/R. Many testicular pathological changes such as disorganization of the seminiferous epithelium, hyalinization, degeneration, and tubular atrophy were detected in myocardial I/R. Degenerated sertoli cells that were present in the atrophic tubules contained acidophilic cytoplasm and

fragmented nuclei. In the CAPE group, testes demonstrated generally the normal structure of sertoli and germinal cells. CAPE significantly reduced the number of degenerating cells. The authors suggested that involvement of increased NO synthesis in the testicular injury caused by myocardial I/R was a novel finding and more importantly, that inhibition of NO production by potential new agents like CAPE might be a new pharmacological strategy for the prevention of cell damage.

Angiogenesis in Ovaries

In recent years, several studies report that a number of phytochemical substances or their synthetic derivatives represent angiogenesis inhibitory effects, which is very important for preventing or delaying cancer by suppressing its neovascularization [15]. Apers *et al.* [16] recently reported the novel derivatives of caffeic acid esters by biomimetic dimerization in order to obtain antiangiogenic lignans. Similarly, Basini *et al.* [17]. 2012 studied the potential antiangiogenic effect of a synthetic CAPE derivative, benzo (k,l) xanthene lignan synthesized through the biomimetic dimerization in ovarian cell line and an angiogenesis bioassay. Granulosa cells of swine ovaries were aseptically harvested by aspiration of large follicles, and they were grown in culture medium to test the synthesis of vasculoepithelial growth factor (VEGF). CAPE-derived lignan significantly inhibited the secretion of VEGF by granulosa cells, which suggested this compound as a novel potential angiogenesis inhibitor.

Estrogens have a vital role on the reproductive tissues in respect to growth, differentiation, and function. Additional to the reproduction system, estrogen receptors are also present in other tissues, for example estrogen receptor β (ERβ) has important functions in the differentiation of epithelial cells and it has been reported that ERβ is the major ER expressed in colon [18]. Estrogen loss during the postmenopausal period is related with physiological changes and an increase in ROS that may be associated with several pathologic conditions [19,20]. Ovariectomy itself is used as an experimental model for oxidative stress [21,22]. Finally, evidence indicates that some kind of ROS such as hydrogen peroxide and superoxide anion are involved in the pathogenesis of inflammatory bowel disease [23]. The effect of CAPE has been studied in an inflammatory bowel disease model produced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) in ovariectomized rats [24]. CAPE at the doses of 10 and 30 mg/kg significantly diminished the colon damage caused by TNBS compared to that of the vehicle-treated group. Levels of GSH, CAT, and MDA were significantly altered in the CAPE group compared to colitis and vehicle control groups. Authors concluded that CAPE had those effects through anti-inflammatory and antioxidant mechanisms and that it might be used as an adjunct therapy in colitis in ovariectomized female rats.

Effects of Smoking on Testicular Functions and Oxidant/Antioxidant Balance

Testicular tissue has been known as a highly vascular tissue and because of the blood supply cigarette smoke may deteriorate the balance between oxidant and antioxidant enzyme systems. Oxidative stress can cause the production of abnormal spermatozoa and affect sperm functions [25]. The assessment of infertility of males is usually based on the evaluation of several semen parameters which can easily be affected by smoking [26]. Histological changes in the seminiferous tubules, sperm counts, and sperm morphology are seriously affected by smoking [27]. In one previous report, 21 rats were exposed to cigarette smoke, intra-peritoneal CAPE was applied for 60 days, and testicular NO, SOD, GSH-Px, catalase, and MDA were studied [28]. It was found that CAT and SOD activities were significantly high and GSH-Px activity was significantly low in smoking group whereas they were normalized in CAPE applied group. Increased MDA and NO levels in the testicular tissue in the smoking group were reversed by CAPE application, showing the protective role of CAPE on smoking-related damage.

Effects of Pesticides on Testes

Pesticides have a toxic effect on testes mostly due to induction of oxidative stress because of production of high levels of ROS. Spermatozoa is especially affected by oxidative stress because of high levels of polyunsaturated fatty acids in the membranes [29] and low levels of antioxidant enzymes in their cytoplasms. λ cyhalothrin (LC) is a new generation of insecticide, which is effective against a large variety of arthropods. Abdallah *et al*. studied the adverse effects of LC on reproductive organs and fertility in male rats and evaluated the protective role of CAPE [30]. The authors studied testicular oxidative status, epididymal sperm characteristics, and testicular pathology. LC declined the sperm quality by increasing oxidative stress. CAPE treatment reduced testicular oxidative stress and the deleterious effects of LC on male fertility due to its antioxidant properties.

Effect of Methotrexate (MTX) on Testes

Methotrexate is a widely used chemotherapeutic agent for the treatment of different diseases such as several cancer types (osteosarcoma, acute lymphoblastic leukemia, lymphoma, head and neck cancer, bladder cancer, and breast cancer) and non-malignant diseases (psoriasis, rheumatoid arthritis, and graft versus host disease).

The mechanism of MTX toxicity is related with oxidative stress. Testicular toxicity is one of the important adverse effects of MTX. It may infertility via the inhibition DNA synthesis by the increased production of ROS [31], decreasing GSH level [32], SOD, CAT and GSH-Px activities [33,34]. MTX may cause chromosomal changes which may result in oligozoospermia [35]. The rich polyunsaturated fatty acid content of the testicular tissue makes this organ more vulnerable to oxidative damage [36]. MTX decreases antioxidant mechanisms, alters the function of pro-inflammatory cytokine system, and increases the formation of ROS due to the stimulation of phagocytic cells [33]. In one study, authors aimed to evaluate whether there is any change in the ROS production and oxidative stress by MTX administration in rat testes and whether CAPE treatment

stops this abnormal condition [34]. Mean body and testicular weight, antioxidant enzyme activities, lipid peroxidation parameters were studied to test this hypothesis. The level of lipid peroxidation and the activities of SOD were significantly higher in the MTX group. These were decreased after CAPE administration. CAT activity in MTX group decreased insignificantly although its activity was significantly increased by CAPE administration. Altogether, it was concluded that CAPE administration with MTX treatment has a protective effect on MTX-induced oxidative injury on testes.

Effect of MK-801-induced Psychosis on Testicular Oxidative Balance

Gonadal functions have been reported to be abnormal in schizophrenic men. On the other hand, none of the studies have been conducted to show the association between schizophrenia and oxidative stress on testicular tissues in relation to gonadal dysfunction. ROS may have a role in the pathophysiology of neuropsychiatric disorders because it is involved in membrane pathologies in the central nervous system. Oxidative stress in blood, cerebrospinal fluid, and postmortem brain tissues has been extensively studied in the literature. MK-801 (dizocilpine) has been used to create animal models of schizophrenia. Pharmacologically, it is an uncompetitive antagonist of the N-Methyl-D-aspartate receptor. Ozyurt *et al*. [37] reported the oxidative changes in the testicular tissues of MK-801-induced schizophrenia model in rats for the first time. Biochemical parameters of oxidative damage and pathological changes in testicular tissues have been studied in this schizophrenia model. A significant increased oxidative stress has been noted in testicular tissues of rats in response to MK-801-induced psychosis. Treatment of the animals with CAPE decreased the oxidative stress and normalized the histological changes caused by the MK-801 administration (disorganization and degeneration of the seminiferous epithelium, hyalinization, and tubular atrophy). CAPE treatment reduced the disorganization, degeneration of the germinal cells, and tubular atrophy.

CONCLUSION

In general, it is obvious that a significant oxidative stress has been noted in all of the pathological models of the reproductive organs such as ovary I/R, testicular I/R, methotrexate-induced testicular injury, MK-801-induced testicular injury, and cigarette smoke-induced injury. The administration of some harmful compounds as well as I/R injuries in both reproductive organs cause the elevation of oxidative stress and pretreatment with CAPE has protecting effects on the oxidative stress in testis and ovaries. Overall, the findings of the aforementioned studies clearly display that CAPE has a protective effect on testicular and ovarian I/R as well as medicine-induced injury, and that this effect is shared by two of the most possible pathways; inhibition of neutrophil-mediated injury, and scavenging of ROS extensively by CAPE [Figure 2]. Clinical studies are needed to validate the correct usage of CAPE either alone or in combination with existing alternative therapies.

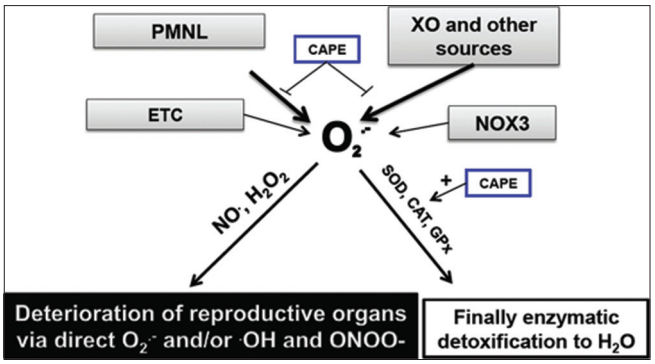

Figure 2: Proposed mechanism for the relationship between oxidative stress and the protective effect of caffeic acid phenethyl ester on reproductive organs

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