JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH

RESEARCH ARTICLE

Protective Effects of Garcinia kola Stem Barks Aqueous Extract on Metabolic Disorders and Oxidative Stress in Dexamethasone-Induced Insulin Resistance in Rat

SÉVÉRIN OWONO EBELA[1], AURELIE NGUIMMO-METSADJIO[2], NYEMB NYUNAÏ[2*], THEODORA KOWA KOPA[3], FABRICE MBA MEDOU[2], ESTHER NGO LEMBA TOM [1]

[1] Department of Biological Sciences, Higher Teachers' Training College, University of Yaoundé I, P.O Box 47, Yaoundé, Cameroon

[2] Medical Research Centre, Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O Box 13033, Yaoundé, Cameroon, E-mail: nyunain@yahoo.fr

[3] Centre for Medicinal Plants Research and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O Box 13033, Yaoundé, Cameroon

**Corresponding Author*

ABSTRACT

Aim: To evaluate how the Garcinia kola stem bark aqueous extract impacted dexamethasone-induced insulin resistance in male rats.

Method: This work was completed in 2020. The extract was first subjected to phytochemical analysis. Using acute and subacute studies (11 days), the effect of the extract was evaluated at 200 mg/kg and 400 mg/kg, on dexamethasone-induced hyperglycemic rats. Glycemia was measured before and after treatment in both studies, while histological examination for isolated liver, kidneys, and pancreas was performed. Body weight and some internal organs weights were determined, and biochemical assays, for blood samples were achieved only for the subacute study.

Results and Conclusion: The existence of alkaloids, phenolic compounds, flavonoids, saponins, coumarins, anthocyanins, and anthraquinones was discovered in the plant's chemistry. G. kola extract decreased the Area Under Curve induced by dexamethasone injection, in the acute and in the subacute test. The extract and glibenclamide decrease the alanine aminotransferase activity induced by DEXA. In the glibenclamide and extract groups, total protein levels increased. In comparison to control, the uric acid level in dexamethasone-treated rats remains elevated. Rats treated with the extract or glibenclamide exhibited an increase in superoxide dismutase activity. Dexamethasone induced islet hypertrophy in the pancreas, vascular congestion, cytolysis, and vacuolation of hepatocytes. The kidney presented leucocyte infiltration and a decrease in glomerular cell density. All of these abnormalities were remedied by the administration of glibenclamide or Garcinia kola extract.

KEYWORDS:
Garcinia kola, stem barks, dexamethasone, antioxidant, diabetes, insulin resistance

ARTICLE HISTORY:
Received May 06, 2021
Accepted June 14, 2021
Published July 25, 2021

DOI:
10.5455/jcmr.2021.12.02.24

VOLUME: 12
ISSUE: 2
ISSN: 2146-8397

INTRODUCTION

Garcinia kola Heckel otherwise called "Bitter kola", or known as "African wonder nut" belongs to the Clusiaceae family and is observed ordinarily within the woodland zone of the Republics of Sierra Leone, Ghana, and Cameroon (Eka, 1971; Uko et al., 2001). In Cameroonian vernacular languages, it is commonly called Ognié in Ewondo and Gnèè in Bassa.

The plant is employed in folk medicine for the treatment of liver, larynx, and bronchi complaints, as well as gonorrhea (Dao et al., 2020). Bark extracts yield xanthone, a brownish-yellow natural resin that is utilized as a tint in industry. Seed is sometimes used to promote the secretion of saliva and is

additionally suggested for the control of sickle cell anemia (Ilondu et Enwa, 2013). It is also employed in the treatment of diabetes and as a poison neutralizer in Africa (Iwu, 2014). The stem bark has purgative properties, and the crushed bark is served to treat cancerous tissues, the sap is employed to cure dermatosis symptoms and therefore the latex is applied outwardly on recent wounds to stop bacterium contamination (Esiegwu et al., 2014). The bark of the G. kola tree is said to have aphrodisiac benefits (Singh et al., 2012). The leaves are used to cure lung ailments and as typhoid medication (Omonike et Edith, 2010; Chinyere et Ebakota, 2013).

Many secondary metabolites have been found in G. kola, the middle fingers of which are kolaviron and garcinia bioflavonoids (Madunagu et al., 1991; Terashima et al., 1995; Nmaju et al., 2014). The seed and the stem bark yielded garcinia biflavonoid 1, garcinia biflavonoid 2, garcinia biflavonoid 1a, kolaflavanone, and their glycosides. Garcinia kola's roots produced phlobatannins, anthraquinones, glucosides; garcinia; garcifuran-A, garcinifuran-B, and two aryl benzofurans (Madunagu et al., 1991; Terashima et al., 1995; Kelly et al., 1997). Carbohydrates and a few fatty acids were extracted from Garcinia kola seed (Seanego et Ndip, 2012; Ukaoma et al., 2013). Seeds and hulls yielded many types of mineral ions (Aniche et Uwakwe, 1990). The study of chemical compounds of G. kola revealed that the seed and the hull produced fatty acid and amino acid derivatives (Eleyinmi et al., 2006).

Many studies have found that various parts of Garcinia kola have antimicrobial, antiviral, hepatoprotective, antiarthritic, anti-ulcer, anti-cancer, and anti-hypertensive activities (Buba et al., 2016; Tene et al., 2016). Furthermore, the xanthone of G. kola has been shown to have anti-asthma properties (Chen et Kang, 1997).

Garcinia kola root saponin extract demonstrated significant antioxidant activity, by inhibition of malondialdehyde production and important anti-free radical enzyme activities (Smith et Adanlawo, 2014). Among the ME1 to ME5 fractions of G. kola seed methanolic extract, ME4 fraction containing biflavonoids GB1 and GB2, garcinol, and garcinoic acid exhibited the best inhibition of free radicals in macrophage cells models (Tebekeme, 2009). The hypoglycemic and hypolipidemic activities of G. kola and fractions from kolaviron respectively have been demonstrated in normal rats and different diabetic rat models (Adaramoye et Adeyemi, 2006; Nwangwa, 2012). Quercetin from Garcinia kola seed protects against hyperglycemia-induced impairment in endothelial progenitor cells (Zhao et al., 2014). Garcinia kola seed extract was found to have hypoglycemic and hypolipidemic effects on diabetic albino rats in a recent report (Etim et al., 2020).

As far as we could know, no research has been done on the protective effect of Garcinia kola in type 2 diabetes; therefore, we are interested in evaluating the protective effects of Garcinia kola stem barks aqueous extract on metabolic disorders and oxidative stress in dexamethasone-induced insulin resistance rats.

MATERIALS AND METHODS

Plant Material Collection and Extraction

Garcinia kola fresh barks collected in the locality of Koumou I (Mbalmayo suburbs) were recognized at the National Herbarium of Cameroon in comparison to sample No. 27839/SRF-Cam in August 2019. The samples were then cut up and dried out of direct sunlight. They were then reduced to powder by grinding in the mill. 500 grams of dried powder were mixed with 3 liters of distilled water and heated for 30 minutes. Whatman paper No. 3 was used to filter the ensuing decoction. Subsequently, the filtrate was lyophilized and the aqueous extract of stem bark of Garcinia kola was obtained, yielding 3.30% w/w (16.52 g).

Phytochemical Screening

The phytochemical analysis was done according to the method of Harbone (1998) and that of Evans (2009).

Experimental Animals

The investigation was conducted in January 2020 and carried out on Wistar male rats weighing between 170 g and 260 g. They were obtained at the Yaoundé Higher Teachers' Training College (ENS) (Cameroon). During this time, they were fed a conventional rat diet and had unlimited access to water. They were kept at a controlled temperature of 25 °C ± 2°C with half ± 10% relative humidity and with a 12 h light/12 h dark cycle. The animal study was carried out at the Endocrinology and Radio-Isotopes Laboratory of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon and was performed out with the consent of the Institutional Animal Ethics Committee.

Drugs and Chemicals

Mahalakshmi chemicals (Hyderabad, India) supplied the glibenclamide. Diethyl ether (Ether Gifrer Solution) was obtained from a local pharmacy with a labeled quantity of FL/400ML. The remaining reagents were of analytical quality and were used exactly as received.

Acute Effects of the Aqueous Extract of Garcinia Kola Stem Barks on Dexamethasone-Induced Insulin Resistance

Twelve-hour fasted rats were randomly assigned into five categories of six animals (n = 6). Group I (Normal control): received gavage of vehicle (distilled water at 10 ml/kg) and normal saline 1 ml/kg i.p., Group II (DEXA): received vehicle (10 ml/kg, per os) and dexamethasone (8 mg/kg i.p), Group III (GLIB + DEXA): received gavage of glibenclamide 5 mg/kg and dexamethasone (8 mg/kg i.p), Group IV (GK200 + DEXA) and Group V (GK400 + DEXA) received respectively gavage of 200 mg/kg or 400 mg/kg of Garcinia kola extract and dexamethasone (8 mg/kg i.p.) for each group. Normal saline or dexamethasone was administered 30 min after oral gavage of vehicle, glibenclamide, or extract. After 4 h of dexamethasone injection, animals had been subjected to an Oral Glucose Tolerance Test (OGTT). Glycemia was measured at 30, 60, and 120 minutes after administration of glucose (2

g/kg).

Subacute Study of the Protective Effects of the Aqueous Extract of Garcinia Kola Stem Barks on Dexamethasone-Induced Insulin Resistance

Experimental Procedure

Rats have been randomized divided into five groups of six animals (n = 6) each and were subjected to various treatments once a day for 11 days. In all experiments, dexamethasone was administered intraperitoneally at 8 mg/kg. Group I (Control) received vehicle 10 ml/kg and normal saline 1 ml/kg i.p., Group II (DEXA) received vehicle 10 ml/kg and dexamethasone, Group III (GLIB + DEXA) was given glibenclamide (5 mg/kg) and dexamethasone, and Group IV (GK200 + DEXA) and Group V (GK400 + DEXA) received respectively gavage of Garcinia kola extract 200 mg/kg or 400 mg/kg, and dexamethasone. Dexamethasone was given to Group II to Group V rats from the 7th day to the 11th day of the study. Dexamethasone was provided 30 minutes after the vehicle or extract gavage (Nayak, 2017).

At the end of the experiment, the animals fasted for 12 hours before undergoing an Oral Glucose Tolerance Test (OGTT). Blood samples were collected from rats under light ether anesthesia via retro-orbital plexus puncture, with a capillary tube and centrifuged at 2500 RPM for 25 minutes. Biochemical assays have been performed on the isolated serum. The rats were sacrificed at the end of the OGTT after being anesthetized with ether. Each rat's pancreas, adrenal glands, kidney, and liver were dissected and weighed, and then fixed in formalin (10%), to conduct further histopathology studies, except for the adrenal. Throughout the experiment, the animals' body weights were registered daily and the percentage change on the day was calculated as follow:

$$\frac{(\text{Body weight on the day} - \text{Initial Body weight (Day 1)}) \times 100}{\text{Initial Body weight}}$$

Oral Glucose Tolerance Test (OGTT)

On the eleventh day, the animals were fasted for 12 hours following the last treatment, after which the first blood glucose level was estimated, using a CERA-CHEK TM glucometer on blood taken from the tail. Then, the rats were given an oral glucose excess (2 g/kg). The blood glucose levels were then measured 30, 60, and 120 minutes post-ingestion of the glucose solution.

Assays of Biochemical Parameters

Serum transaminases (alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT)), and uric acid assays were carried out by commercial kits provided by Biolabo S.A.S., Les Hautes Rives, 02160 Maizy, France, and following the procedure indicated. Superoxide dismutase (SOD) and catalase activities were determined according to the methods of Misra et Fridovish (1972) and that of Sinha (1972) respectively. The total protein level was determined using the method of Gornall et al. (1949).

Histopathology Examination

Following the macroscopic observation, using Mayer's technique (Mayer, 1896), representative fragments of the liver, left kidney, and pancreas was trapped in a 10% solution of buffered formalin (pH 7.4) and enveloped in paraffin, with slight modifications, and analyzed under a light microscope. For tissue changes study, sections with a thickness of five micrometers were gotten, stained with Hematoxylin-Eosin (HE), and visualized under an optical microscope (100 X).

Statistical Analysis

The data were presented as a mean ± standard error of the mean (SEM). The statistical analyses were performed using GraphPad Prism software version 5.03. The ANOVA-One-Way test was used to compare the effects, followed by Dunnett's post-test. When the p-value was less than 0.05, the result was considered to be significantly different.

RESULTS

Phytochemical Screening

The existence of alkaloids, phenolic compounds, flavonoids, saponins, coumarins, anthocyanins, and anthraquinones was discovered in Garcinia kola stem bark extract (Table 1).

Table 1: phytochemical screening of Garcinia kola stem bark aqueous extract

Phytochemicals classes	Result of the test
Alkaloids	+
Phenolic compounds	+++
Triterpenes	-
Steroids	-
Flavonoids	+
Glucosides	-
Saponins	+
Gallic tannins	+++
Coumarins	+
Anthocyanins	+
Anthraquinones	+

Legend: -: Absent; +: present; ++: moderate presence; +++: abundant.

Acute Effects of Garcinia Kola Stem Bark Aqueous Extract on Dexamethasone-Induced Insulin Resistance

Oral Glucose Tolerance Test (OGTT) Curve revealed that blood glucose is a function of time in the different groups (Figure 1A). A significant increase in the Area Under the Curve (AUC) of dexamethasone-group (DEXA) treated rats compared to the control group (14380 ± 156.9 to 18120 ± 631.1; p <0.001) was noted. There was a substantial decrease in the AUC of the GLIB + DEXA group, in comparison to the DEXA group (18120 ± 631.1 versus 12970 ± 313.8; p <0.001). AUC was drastically reduced following the extract (200 mg/kg) administration (18120 ± 631.1 vs. 16095 ± 184.4 (p <0.01) in comparison to the DEXA group. The AUC of the rats given 400 mg/kg of extract stayed the same as that of rats treated with dexamethasone only (17059 ± 123.4 and 18120 ± 631.1 respectively) (Figure 1B).

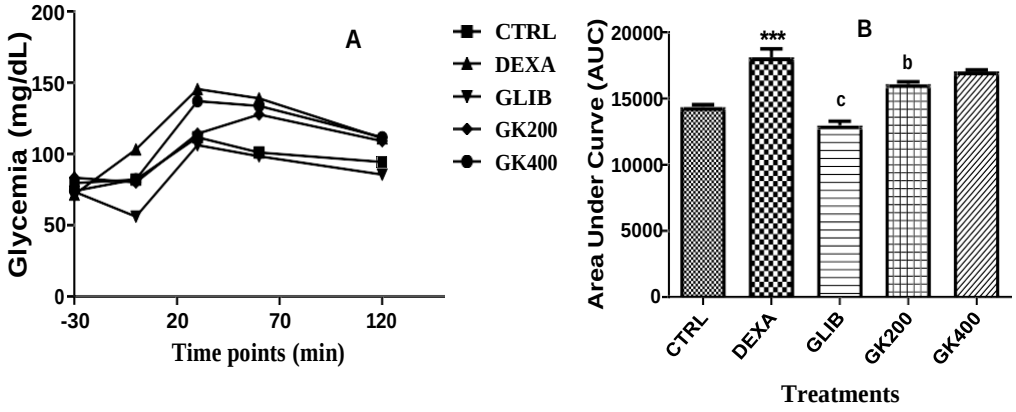

Figure 1: Oral Glucose Tolerance Test (OGTT) in Acute Study (A) and the Area Under the Curve (AUC) associated with this Test (B).

Legend: CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone; GLIB: rats treated with glibenclamide (5 mg/kg) and dexamethasone; GK200: rats treated with the extract at 200 mg/kg and dexamethasone; GK400: rats treated with the extract at 400 mg/kg and dexamethasone. Curve points and bars of AUC represent the mean±ESM, n = 6. *** p <0.001; a significant difference compared to rats in the CTRL group, b p <0.01; c p <0.001; a significant difference compared to rats in the DEXA group.

Protective Effects of Garcinia Kola Stem Bark Aqueous Extract on Dexamethasone-Induced Insulin Resistance During Subacute Study

Garcinia kola extract exerts blood glucose-lowering effects in insulin-resistant rats

The Area Under the Curve (AUC) associated with the Oral Glucose Tolerance Test (OGTT) showed a significant increase in rats treated with dexamethasone (DEXA) (17667 ± 276.0 vs. 25151 ± 605.0) in comparison to the rats of the normal control group (CTRL). Administration of Garcinia kola aqueous extract at 200 or 400 mg/kg resulted in a significant decrease in AUC associated with the OGTT test (15855 ± 132.8 and 1727.0 ± 763.0 respectively; p < 0.001 for both doses) as compared to the DEXA group (25151 ± 605.0) (Figure 2B).

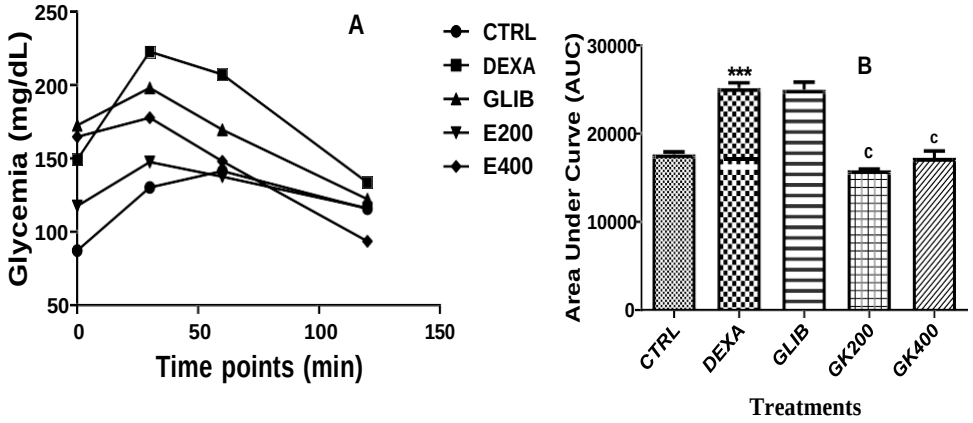

Figure 2: Oral Glucose Tolerance Test (OGTT) in the Subacute Study (A) and the area under the curve (AUC) associated with this test (B).

Legend: CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone (8 mg/kg); GLIB: rats treated with glibenclamide (5 mg/kg) and dexamethasone; GK200: rats treated with the extract at 200 mg/kg and dexamethasone; GK400: rats treated with the extract at 400 mg/kg and dexamethasone. Curve points and bars of AUC represent the mean ± ESM, n = 6. *** p <0.001; significant difference compared to rats in the CTRL group, c p <0.001; a significant difference compared to rats in the DEXA group.

Effect of Garcinia kola stem bark aqueous extract on body weight

The administration of dexamethasone caused a significant reduction in body weight loss (from day 8 until day 12 as opposed to the normal control group ($p < 0.05$ to $p < 0.001$) (Table 2). The body weight change increase in the group of rats receiving Garcinia kola extract at 400 mg/kg, in comparison with rats treated with dexamethasone only (1.34 ± 2.73 to 8.97 ± 4.03; $p < 0.05$) on day 8.

Table 2: Effect of Garcinia kola stem bark aqueous extract on body weight (Weight change (%))

	CTRL	DEXA	GLIB	GK200	GK400
Day 1	0.00	0.00	0.00	0.00	0.00
Day 3	6.10±2.33	7.01±0.95	5.53±2.27	3.70±2.13a	8.33±2.68
Day 5	7.52±3.52	7.94±2.18	7.69±4.40	9.35±2.06	11.77±3.39
Day 7	9.41±5.48	8.55±2.32	8.64±6.65	9.66±2.42	13.75±3.32
Day 8	8.76±6.68	1.34±2.73*	4.22±5.58	5.51±2.94	8.97±4.03a
Day 9	7.12±7.87	-2.33±1.79**	-0.93±4.33	-0.20±3.48	3.56±4.31
Day 10	8.25±6.60	-5.84±2.27***	-6.74±4.17	-5.06±2.80	-0.87±3.07
Day 11	7.33±7.44	-9.08±2.89***	-9.02±3.39	-9.53±3.32	-5.36±3.66
Day 12	2.88±6.28	-13.37±3.23***	-14.02±3.35	-13.99±2.25	-11.21±3.29

Legend: CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone; GLIB: rats treated with glibenclamide (5 mg/kg) and dexamethasone; GK200: rats treated with the extract at 200 mg/kg and dexamethasone; GK400: rats treated with the extract at 400 mg/kg and dexamethasone. Each value represents the percentage change of body weight mean with ESM per group of rats n = 6; * p <0.05; ** p <0.01; *** p <0.001; a significant difference compared to rats in the CTRL group. a p <0.05; a significant difference compared to the DEXA group.

Effect of Garcinia kola stem bark aqueous extract on the relative weight of organs

Increased liver weight was noted in rats treated with dexamethasone ($p < 0.01$), as compared to the control. An increase in the weight of the kidneys was also seen in rats treated with 200 mg/kg of extract ($p < 0.05$). Administration of dexamethasone-induced a noteworthy diminution in adrenal relative weight in the dexamethasone group ($p < 0.01$), in contrast to rats of the control group (Table 3). Rats treated with 400 mg/kg of extract exhibited a significant decrease in the relative weight of their adrenals ($p < 0.05$).

Table 3: Relative organ weights of rats in the treated groups (% bw)

Organs	CTRL	DEXA	GLIB	GK200	GK400
Liver	2.75 ± 0.13	3.97 ± 0.30 **	4.04 ± 0.13	3.64 ± 0.21	3.45 ± 0.16
Kidneys	0.63 ± 0.03	0.78 ± 0.04	0.82 ± 0.04	0.94 ± 0.12a	0.85 ± 0.04
Pancreas	0.42 ± 0.06	0.35 ± 0.03	0.33 ± 0.05	0.40 ± 0.06	0.40 ± 0.05
Adrenals	0.017 ± 0.002	0.012 ± 0.001**	0.013 ± 0.001	0.012 ± 0.001	0.011 ± 0.001a

Legend: CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone; GLIB: rats treated with glibenclamide (5 mg/kg) and dexamethasone; GK200: rats treated with the extract at 200 mg/kg and dexamethasone; GK400: rats treated with the extract at 400 mg/kg and dexamethasone. The values represent the means of the relative weights of the ESM organs. n = 6; ** p <0.01; a significant difference compared to rats in the CTRL group. a p <0.05; a significant difference compared to the DEXA group.

Effects of Garcinia kola stem bark aqueous extract treatment on liver function and metabolic markers

Table 4 indicates a significant rise in serum Alanine Amino Transferase (ALAT) activity of rats of the DEXA group, in contrast to the control group (CTRL) (70.85 ± 14.70 vs. 152.9 ± 5.94; $p < 0.001$). In contrast to the DEXA group (152.9±5.94), the extract at doses of 200 mg/kg (92.71±1.11), 400 mg/kg (84.70±3.39), and glibenclamide (101.3±6.42) induced respectively a significant decrease in ALAT activity ($p < 0.001$).

A significant increase in serum Aspartate Amino-Transferase (ASAT) activity was observed in rats given dexamethasone only in contrasted to the control group, from 59.03 ± 3.67 to 217.2 ± 8.89 ($p < 0.001$) (Table 4). There was a significant diminution in ASAT activity in rats pretreated with glibenclamide, in contrast with the DEXA group (from 217.2 ± 8.89 to 156.0 ± 9.19). The extract groups exhibit high ASAT activities compared to the DEXA group ($p < 0.001$) (279.9 ± 5.42 for 200 mg/kg and 272.2 ± 14,98 for 400 mg/kg vs. 217.2 ± 8.89 for the DEXA group) ($p<0.001$ for both) (Table 4).

The total protein levels in rats are shown in Table 4. In comparison with DEXA group, glibenclamide or extract pretreated groups resulted in a considerable increase in serum protein levels (2.86 ± 0.01 for DEXA group to 3.90 ± 0.07 for

glibenclamide treated group, 3.08 ± 0.07 at 200 mg/kg ($p<0.05$) and 3.35 ± 0.03 at 400 mg/kg ($p<0.001$) for extract respectively.

Rats pretreated with dexamethasone exhibited a significant rise in uric acid level, in comparison with the control group (CTRL) (12.07 ± 0.73 to 18.50 ± 0.57) ($p <0.001$). When the extract and glibenclamide groups were compared to the dexamethasone-only treated group, there was no substantial difference in uric acid concentration.

Table 4: Effect of Garcinia kola stem bark aqueous extract on liver function and metabolic markers of dexamethasone-induced insulin-resistance rats.

Biochemical Parameters	CTRL	DEXA	GLIB	GK200	GK400
ALAT (UI/L)	70.85±14.70	152.9±5.94***	101.3±6.42 c	92.71±1.11 c	84.70±3.39 c
ASAT (UI/L)	59.03±3.67	217.2±8.89***	156.0±9.19 c	279.9±5.42 c	272.2±14.98 c
TOTAL PROTEIN (mg/L)	3.03±0.08	2.86±0.01	3.90±0.07 c	3.08±0.07 a	3.35±0.03 c
URIC ACID (mg/L)	12.07±0.73	18.50±0.57***	20.64±0.10	17.59±2.68	16.34±0.10

CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone (8 mg/kg); GLIB: rats given glibenclamide (5 mg/kg) and dexamethasone; GK200: rats treated with the extract at 200 mg/kg and dexamethasone; GK400: rats treated with the extract at 400 mg/kg and dexamethasone. All values are expressed as mean±SEM, n = 6. One-way ANOVA followed by Dunnet's post-test. *p <0.05; ***p <0.001 significant difference compared to rats in the CTRL group, a p <0.05, b p <0.01, c p <0.001 significant difference compared to DEXA group.

Treatment with Garcinia kola stem bark aqueous extract improves anti-oxidant enzymes activities

Dexamethasone administration resulted in a substantial decrease in superoxide dismutase activity, in comparison to control group (0.19 ± 0.00 to 0.15 ± 0.00; $p <0.05$). Pretreatment with glibenclamide (0.18 ± 0.00) or plant extract (0.21 ± 0.00 for 200 mg/kg or 0.18 ± 0.00 for 400 mg/kg) induced significantly elevated SOD activity ($p <0.001$), when compared with the dexamethasone-treated rats (0.15 ± 0.00) (Table 5).

Concerning catalase activity, a significant rise was noted only in the glibenclamide group (28.59 ± 1.11 to 36.21 ± 1.84; $p <0.01$) (Table 5).

Table 5: Effect of Garcinia kola stem bark aqueous extract on antioxidant enzymes in dexamethasone-induced insulin resistance rats.

Enzymes Parameters	CTRL	DEXA	GLIB	GK200	GK400
SOD (U/mg protein)	0.19±0.00	0.15±0.00*	0.18±0.00 c	0.21±0.01 c	0.18±0.00 c
CATALASE (MMol of H2O2)	27.43±1.04	28.59±1.11	36.21±1.84 b	29.61±2.00	32.70±1.23

CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone (8 mg/kg); GLIB: rats given glibenclamide (5 mg/kg) and dexamethasone; GK200: rats treated with the extract at 200 mg/kg and dexamethasone; GK400: rats treated with the extract at 400 mg/kg and dexamethasone. All values are expressed as mean±SEM, n = 6. One-way ANOVA followed by Dunnet's post test. *p <0.05 significant difference compared to rats in the CTRL group, b p <0.01, c p <0.001 significant difference compared to rats in the DEXA group.

Effects of Garcinia kola aqueous extract on the histology of the pancreas, liver, and kidneys

Figure 3 shows the photomicrographs of the pancreatic tissue of the rats of different groups at the end of the treatments.

When compared to the rats of the control group, dexamethasone-treated rats showed pancreatic islet hypertrophy (Figure 3B). No alterations were observed in the rats of the other groups.

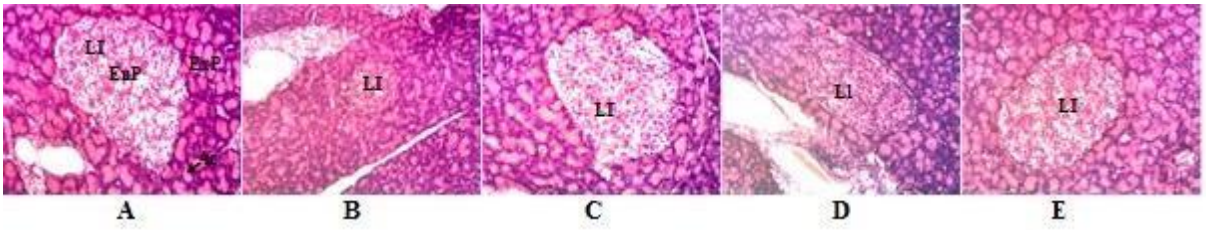

Figure 3: Photomicrographs of the pancreas. (H&E, 100X).

Legend: A = CTRL; B = DEXA; C = GLIB; D = GK200; E = GK400; Pancreas; LI = Langerhans Islets; Ac = Acini Cell; EnP = endocrine pancreas; ExP = exocrine pancreas. CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone; GLIB: rats treated with glibenclamide (5 mg/kg) and dexamethasone; GK200: rats treated with extract at 200 mg/kg and dexamethasone; GK400: rats treated with extract at 400 mg/kg and dexamethasone.

The liver tissue of the rats in the normal control group, as well as those treated with glibenclamide (GLIB) or plant extract at 400 mg/kg, appeared normal (Figure 4C and Figure 4E). Histological examination of the liver in dexamethasone-treated rats showed vascular congestion, cytolysis, and hepatocyte vacuolation (Figure 4B). Treatment at 200 mg/kg shows an alteration marked by the presence of lipid vacuoles (Figure 4D).

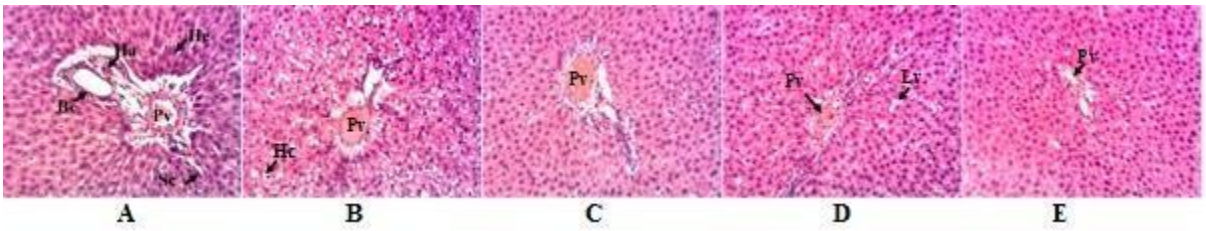

Figure 4: Photomicrographs of the Liver. (H&E, 100X).

Legend: A = CTRL; B = DEXA; C = GLIB; D = GK200; E = GK400; Pv = portal vein; He = Hepatocyte; Sc = Sinusoidal capillary; Ha= hepatic artery; Bc = Biliary canal; Hc = Hepatocyte cytolysis; Lv: lipid vacuoles. CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone; GLIB: rats treated with glibenclamide (5 mg/kg) and dexamethasone; GK 200: rats treated with extract at 200 mg/kg and dexamethasone; GK 400: rats treated with extract at 400 mg/kg and dexamethasone.

Histological exploration of the kidneys showed normal renal parenchyma in rats in the control groups (CTRL), those treated with glibenclamide (GLIB), and the plant extract. The kidneys of the rats in the dexamethasone-treated group showed leukocyte infiltration and a decrease in glomerular cell density (Figure 5B).

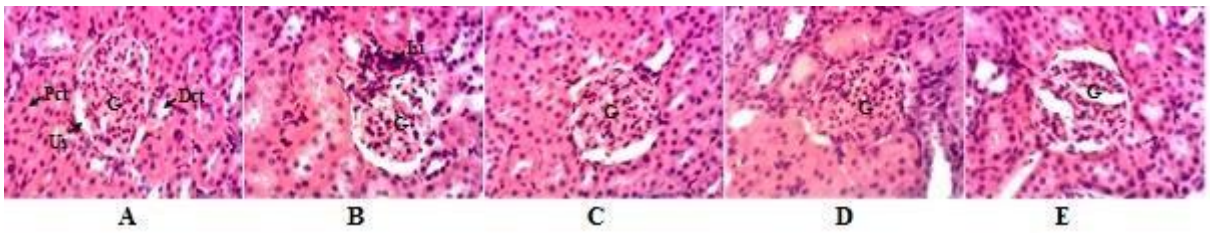

Figure 5: Photomicrographs of the kidney. (H&E, 200X).

Legend: A = CTRL; B = DEXA; C = GLIB; D = GK200; E = GK400; Kidney; G = Glomerulus; Us = urinary space; Dct = distal collecting tubule; Pct = proximal collecting tubule; Li = Leukocyte infiltration. CTRL: rats given distilled water and 0.9% NaCl; DEXA: rats given distilled water and dexamethasone; GLIB: rats treated with glibenclamide (5 mg/kg) and dexamethasone; GK200: rats treated with extract at 200 mg/kg and dexamethasone; GK400: rats treated with extract at 400 mg/kg and dexamethasone.

DISCUSSION

This study aimed to assess the protective effects of Garcinia kola stem bark aqueous extract on metabolic disorders and oxidative stress in dexamethasone-induced insulin resistance in the rat.

The results indicated that in the acute study, glibenclamide (5 mg/kg), as well as G. kola extract at 200 mg/kg, decreases the Area Under Curve (AUC) of the OGTT curve while in the subacute study, only the extract decreases the AUC related to the blood glucose curve of the dexamethasone-induced insulin-resistance rats, unlike glibenclamide. This observation suggests that the extract could act by a mechanism other than that of glibenclamide (stimulating insulin secretion by beta-pancreatic cells). One or more compound classes detected by phytochemical studies, such as alkaloids, phenolic compounds, flavonoids, saponins, coumarins, anthocyanins, or anthraquinones may be responsible for the antihyperglycemic effect of Garcinia kola stem bark aqueous extract. The anti-diabetic activity of some of these compounds classes has been previously reported (Sancho et Pastore, 2012; Asgar, 2013; Aba, 2018).

Elevation of transaminases indicates impaired liver function or cytolysis (Giboney, 2005; Pariente, 2013). In contrast to the rats in the control group, rats treated with dexamethasone had significantly higher levels of Alanine Amino-Transferase and Aspartate Amino-Transferase activities. This would be due to an impairment of hepatocyte cells as well as muscle cells. Indeed, although these two enzymes are markers of hepatic function; ALAT is a specific marker of the liver, and ASAT transaminase is more specific to skeletal and cardiac muscles. Muscle disorders induce an increase in ASAT greater than that of ALAT (Knight, 2005). This can be seen in the loss of body weight of the rats treated with the extract, indicating that the increase in ASAT activity was not prevented. The aqueous extract of Garcinia kola inhibited the increase of ALAT activity in serum, thus showing the inhibitory effect of the plant extract on protein catabolism. This result could suggest that G. kola would have hepatoprotective properties. This hepatoprotective effect previously demonstrated by Iwalokun

et al. (2006) may be linked to the polyphenolic compounds present in the plant extract. The treatment with dexamethasone causes degradation and inhibition of muscle protein synthesis, thus, leading to atrophy of the skeletal muscles, which may justify the loss of body weight (Sandri, 2013). The decrease in weight obtained on day 3 with 200 mg/kg of Garcinia kola extract can be considered, as an artifact as long as it is not observed with the highest dose of extract used (400 mg/kg) and there had not yet been an injection of dexamethasone; however, the large increase in the weight change obtained at 400 mg/kg can be taken into account as a real effect of the extract, but which did not last, since it disappeared over the next few days and just after DEXA injection. Neither treatment with glibenclamide, nor that with the plant extract affected dexamethasone-induced body weight loss.

The body weight loss is accompanied by a decrease in total protein level. Indeed, in the rats of the DEXA group a low level of total protein was observed compared with the control rats. This reduction would be due to the stimulation of gluconeogenesis to meet energy needs and a decrease in protein synthesis normally stimulated by insulin. In rats treated with dexamethasone, G. kola aqueous extract prevented a substantial reduction in total protein levels, probably by inhibition of gluconeogenesis, or by stimulation of protein synthesis.

The production of uric acid is due to the increased breakdown of purines and their excretion in the urine. The presence of more uric acid in the blood suggests a decrease in renal function. Several experimental and epidemiological studies are also in favor of a direct or indirect role of hyperuricemia in the pathogenesis of most of the clinical abnormalities involved in the constitution of the metabolic syndrome. Mitochondrial oxidative stress caused by excess intracellular uric acid leads to disturbance of energy metabolism, the inflammatory state in adipose and liver tissue, dysfunction of vascular endothelial cells, dyslipidemia, fatty liver, and insulin-resistance (Schlienger, 2016). A rise in the blood level of uric acid was observed in the rats treated only with dexamethasone, indicating a decrease in uric acid clearance by the kidneys, as previously hypothesized by Ejaz et al. (2007). The plant extract at 400 mg/kg reduced uric acid levels by 20%. The aqueous extract of G. kola bark might have an important influence on urea production or improve renal function by increasing urea excretion (Iwu, 2014). This action could be also related to its beneficial effect against insulin resistance.

Oxidative stress is caused by an imbalance between oxidants and antioxidants that favors oxidants. Hyperglycemia induced by dexamethasone has been linked to oxidative stress as a primary source of complications (Rolo et Palmeira, 2006). Antioxidants are generated by cells in response to oxidative stress, which helps them to provide a variety of defenses. Among which are superoxide dismutase (SOD) and catalase (CAT). In the present study, as related to the control group, dexamethasone-treated rats had significantly lower SOD activity. However, in rats pretreated with the extract, SOD activity was significantly increased compared to the dexamethasone group. SOD trap free radicals produced by proteolysis, preventing them from accumulating in the bloodstream. These results are in agreement with those obtained by Omolola (2014), who demonstrated the antioxidant effect of garcinia biflavonoids and kolaflavanone contained in Garcinia kola seeds extract.

A previous study in rats treated for 14 days, after being rendered insulin-resistant with dexamethasone found that glibenclamide increased the amount of hepatic catalase, which was decreased by dexamethasone (Tsubanova et Berdnyk, 2020). This may be explained by glibenclamide's antioxidant activity, like an increase in catalase levels, which would not be the case with Garcinia kola stem bark extract.

The change (increase or decrease) in the relative organ's weight is recognized as an indicator of xenobiotics toxicity. Based on the results obtained, increased liver, kidney, and adrenal weights were seen in some groups of rats treated with dexamethasone, therefore, reflects hepatorenal and adrenal toxicity linked to the administration of dexamethasone. The enlarged liver may be due to either fatty liver disease (accumulation of fat) or the metabolism of dexamethasone (Jiao et al., 2020). The extract did not inhibit the weight increase of the kidneys and liver in the doses given. Dexamethasone-treated rats only demonstrated a decrease in adrenal gland weight, when compared to control group rats. This may be explained by the fact that supraphysiological doses of glucocorticoids suppress the hypothalamic-pituitary-adrenal axis, preventing the adrenal glands from producing enough cortisol, this results in hypotrophy of the adrenals and thus leading to atrophy of the adrenal glands (Nicolaides et al., 2020).

The histological study revealed cytolysis and hepatocytes degeneration of the rats treated with dexamethasone only. In this same group, pancreatic islets hypertrophy, leukocyte infiltration, and degeneration of the renal glomeruli were observed. The rats pretreated with G. kola stem bark aqueous extract did not show the various abnormalities observed in the DEXA group. Treatment of rats given dexamethasone associated with G. kola bark extract improves liver, pancreatic and renal tissue histology, which supports the work of Smith et Adanlawo (2014). According to these authors, saponins isolated from the roots of G. kola protect the structural integrity of liver cells. The reduced size of the vacuoles of the liver at 200 mg/kg of extract would therefore reflect a regressive phase of hepatic steatosis, rather than the beginning of this process. The aqueous extract of the bark of G. kola demonstrated its protective role in the liver, pancreas, and kidneys against the deleterious effects of dexamethasone. These results are in agreement with the work of Omolola (2014), which demonstrated the anti-hepatotoxic properties of kolaviron (a Garcinia biflavonoid), through its ability to significantly modify the action of hepatotoxins.

CONCLUSIONS

As a result of the analyses, Garcinia kola stem barks aqueous extract shows antihyperglycemic, hepatorenal protective, and antioxidant properties. These properties could be attributed to the antioxidant potential of the phytochemicals found in this extract.

CONFLICT OF INTEREST

There are no potential conflicts of interest for the authors to disclose.

ETHICAL STATEMENT

The research was conducted in agreement with the Cameroon National Ethical Committee's recommendations (Ref No. FW-IRB00001954, 22 October 1987) and was carried out with the Institutional Animal Ethics Committee's approval.

CONSENT FOR PUBLICATION

N/A

AVAILABILITY OF DATA AND MATERIAL

All of the data used in this paper can be obtained by contacting nyunain@yahoo.fr.

SOURCE OF FUNDING

There was no financial support for this study.

AUTHORS' CONTRIBUTION

SOE carried out the majority of the experiments and assisted with data processing. The biochemical analysis was assisted by ANM and FMM. NN was involved in the study's design, analysis, and manuscript revision. The phytochemical examination of GK extract was facilitated by TKK. ENLT was involved in the study's analysis and design. The manuscript's content has been approved by all of the authors.

REFERENCES

1. Aba, P.E. et Asuzu, I.U. (2018). Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants: A review. Indian Journal of Natural Products and Resources 9(2), 85-96
2. Adaramoye, O.A. et Adeyemi, E.O. (2006). Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron. a bioflavonoid complex from Garcinia kola in streptozotocin-induced diabetes mellitus rats. Journal of Pharmacy and Pharmacology 58(1), 121-128.
3. Aniche, G.N. et Uwakwe, G.U. (1990). Potential use of Garcinia kola as hop substitute in larger beer brewing. World Journal of Microbiology & Biotechnology 6, 323-486.
4. Asgar, A. (2013). Anti-diabetic potential of phenolic compounds: A Review. International Journal of Food Properties 16 (1), 91-103.
5. Buba, C.I., Okhale, S.E. et Muazzam, I. (2016). Garcinia kola: the phytochemistry. pharmacology and therapeutic applications. International Journal of Pharmacognosy 3(2), 67-81.
6. Chen, Y.W. et Kang, J.J. (1997). Mechanism of vasorelaxation of thoracic aorta caused by xanthone. European Journal of Pharmacology 336 (1), 23-28.
7. Chinyere, C.E. et Ebakota, O.D. (2013). Antibacterial activity of Garcinia kola seed and leaf extracts on some selected clinical isolates. Science Journal of Microbiology 2013, 1-8. Article ID sjmb-298, doi: 10.7237/sjmb/298.
8. Dao, J.P., Kouakou, K.L., Kouakou, C., Cherif, M., Ouedraogo, M.H., Koffi, K.K., Bi, I.A.Z. (2020). Effect of Leafy and Leafless Greenwood, Softwood and Hardwood Cuttings Success of Garcinia kola (Heckel). Agricultural Sciences 11 (10), 897-911.
9. Ejaz, A.A., Mu, W., Kang, D.H., Roncal, C., Sautin, Y.Y, Henderson, G., Tabah-Fisch, I., Keller, B., Beaver, T.M., Nakagawa, T., Johnson, R.J. (2007). Could uric acid have a role in acute renal failure? Clinical journal of the American Society of Nephrology 2, 16-21.
10. Eka, O.U. (1971). Chemical composition and uses of kolanuts. Journal of the West African Science Association 16, 167-169.
11. Eleyinmi, A.F., Bressler, D.C., Amoo, I.A., Sporns, P., Oshodi, A.A. (2006). Chemical composition of bitter kola (Garcinia kola) seed and hulls. Polish Journal of Food and Nutrition Sciences 15(4), 395-400.
12. Esiegwu, A.C., Okoli, I.C., Emenalom, O.O., Esonu, B.O., Udedibie, A.B.I. (2014). The Emerging nutraceutical benefits of the African wonder nut (Garcinia kola): A Review. German Journal of Advanced Scientific Research 2(2), 170-183.
13. Etim, II., Etukudoh, N.S., Olumide, O.B., Uchejeso, O.M., Lucy, N.L., Bwotle, F.Y. (2020). Hypoglycemic and Hypolipidemic Effect of Bitter Kola (Garcinia kola) Seed Extract on Alloxan-Induced Diabetic Albino Rats. Journal of Biosciences and Medicines 8, 127-134.
14. Evans, W.C. (2009). Trease & Evans' Pharmacognosy (16th Ed.). Elsevier Health Sciences.
15. Giboney, P.T. (2005). Mildly Elevated Liver Transaminase Levels in the Asymptomatic Patient. American Academy of Family Physicians 71 (6), 1105-1110.
16. Gornall, A., Bradwill, C. et David, M. (1949). Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry 77, 167-182.
17. Harbone, J.B. (1998). Phytochemical methods. A guide of modern techniques of plants (3rth Ed.). Springer Science & Business Media.
18. Ilondu, E.M. et Enwa, F.O. (2013). Commonly used medicinal plants in the management of sickle cell anemia and diabetes mellitus by the local people of Edo State. Nigeria. International Journal of Pharmaceutical, Biological and Chemical Sciences 2(2), 14-19.
19. Iwalokun, B.A., Efedede, B.U., Alabi-Sofunde, J.A., Oduala, T. (2006). Hepatoprotective and antioxidant activities of Vernonia amygdalina on acetaminophen-induced hepatic damage in Mice. Journal of Medicinal Food 9(4), 524-530.
20. Iwu, M.M. (2014). Handbook of African Medicinal Plants. (2nd Ed.). CPC Press.
21. Jiao, T., Yao, X., Zhao, Y., Zhou, Y., Gao, Y., Fan, S., Chen, P., Li, X., Jiang, Y., Yang, X., Gonzalez, F J., Huang, M., Bi, H. (2020). Dexamethasone-Induced Liver Enlargement Is Related to PXR/YAP Activation and Lipid Accumulation but Not Hepatocyte

Proliferation. Drug Metabolism and Disposition 48, 830-839.
22. Kelly, T.R., Szabados, A. et Lee, Y.J. (1997). Total synthesis of garcifuran B. Journal of Organic Chemistry 62(2), 428-429.
23. Knight, J.A. (2005). Liver function tests: their role in the diagnosis of hepatobiliary diseases. Journal of Infusion Nursing 28 (2), 108-17.
24. Madunagu, B.E., Ekpe, E.D. et Otung, I.N. (1991). Microbiological exploitation of cardiac glucosides and alkaloids from Garcinia kola. Borreria ocymoides. Kola nitida and Citrus aurantifolia. Journal of Applied Microbiology (71): 398-401.
25. Mayer, P. (1896). Hematoxylin and Eosin (H and E) staining protocol. Mitt Zool Stn Neapel.;12 (303).
26. Misra, H. et Fridovish, I. (1972). Determination of the level of superoxide dismutase in whole blood, vol. 1. Yale University Press New Haven (p 101–109).
27. Nayak, I.M.N. (2017). Comparison of pioglitazone and metformin efficacy against glucocorticoid-induced atherosclerosis and hepatic steatosis in insulin-resistant rats. Journal of Clinical and Diagnostic Research 11(7), FC06-FC10.
28. Nicolaides, N.C., Pavlaki, A.N., Alexandra M., Chrousos, G.P. (2019). Glucocorticoid Therapy and Adrenal Suppression. [Updated 2018 Oct 19]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279156/ [acces date: 15.05. 2021].
29. Nmaju, A.U., Biosong, S.A., Nwankwo, A.A., Joshua, J.E., Osim, E.E. (2014). Comparative effects of G. kola and coffee diets on learning and memory in mice. British Journal of Medicine and Medical Research 4(2), 731-746.
30. Nwangwa, E.K. (2012). Effects of Garcinia kola on the lipid profile of alloxan-induced diabetic Wistar rats. African Journal of Biochemistry Research 3(2), 39-42.
31. Omolola, R.A. (2014). Effect of kolaviron a Garcinia kola biflavonoid on biochemical and histological parameters in streptozotocin-induced diabetes and diabetic complications (nephrotoxicity and hepatotoxicity) in male Wistar rats [Thesis submitted for the Doctor of Technology: Biomedical Technology, Cape Peninsula University of Technology]. http://etd.cput.ac.za/bitstream/20.500.11838/1512/1/Ayepola_OR_DTECH%20THESIS%202014%20CPUT.pdf .
32. Omonike, O.O. et Edith, O.A. (2010). Traditional management of tuberculosis in Ogun state of Nigeria. The practice and ethnobotanical survey. African Journal of Traditional, Complementary, and Alternative Medicines 7(1): 79-84.
33. Pariente, A. (2013). Cytolyse hépatique (augmentation des aminotransférases) chez l'adulte. Hépato-Gastro & Oncologie Digestive 20, 630-639.
34. Rolo, A.P. et Palmeira, C.M. (2006). Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. Toxicology and Applied Pharmacology 212 (2), 167-178.
35. Sancho, R.A.S. et Pastore, G.M. (2012). Evaluation of the effects of anthocyanins in type 2 diabetes. Food Research International 46(1), 378-386.
36. Sandri, M. (2013). Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome. The International Journal of Biochemistry & Cell Biology 45 (10), 2121-2129.
37. Schlienger, J.L. (2016). Chronic hyperuricemia: Factor or predictor of cardio-metabolic risk? Médecine des maladies Métaboliques 10 (3), 280-284.
38. Seanego, C.T. et Ndip, R.N. (2012). Identification and antibacterial evaluation of bioactive compounds from Garcinia kola (Heckel) seeds. Molecules; 17, 6569-6584.
39. Singh, R., Singh, S., Jeyabalan, G., Ashraf, A. (2012). An overview of traditional medicinal plants as an aphrodisiac agent. Journal of Pharmacognosy and Phytochemistry 1(4): 43-56.
40. Sinha, K. (1972). Colorimetric essay of catalase. Analyze biochemistry 47: 389-394.
41. Smith, Y.T. et Adanlawo, I.G. (2014). In-vitro and in-vivo antioxidant activity of saponins extracted from the root of G. kola (bitter kola) on alloxan-induced diabetic rats. World Journal of Pharmaceutical Sciences 3 (7), 8-26.
42. Tebekeme, O. (2009). In-vitro antioxidant and free radical scavenging activities of Garcinia kola seeds. Food and Chemical Toxicology 47(10), 2620-2623.
43. Tene, T.O., Signe, M., Seukep, A.J., Ngouafong Tatong, F. (2016). Review on traditional uses. phytochemical and pharmacological profiles of Garcinia kola Heckel. Merit Research Journal of Medicine and Medical Sciences 4(11), 480-489.
44. Terashima, K., Aqil, M. et Niwa, M. (1995). Garcinianin. a novel biflavonoid from the root of Garcinia kola. Heterocycles 41(10), 2245-2250.
45. Tsubanova, N.A. et Berdnyk, O.H. (2020). The antidiabetic activity of the new composition "Thigliben" on the experimental dexamethasone diabetes mellitus model in rats. Clinical pharmacy 24 (1), 6-13.
46. Ukaoma, A.A., Ukaoma, V.O., Okechukwu, R.T., Iwuagwu, M. (2013). Phytochemical screening and antibacterial properties of G. kola. Journal of Phytopharmacology 2(3), 34-38.
47. Uko, O.J.A, Usman, A. et Ataja, A.M. (2001). Some biological activities of Garcinia kola in growing rats. Veterinarski Arhiv 71, 287-297.
48. Zhao, L-R., Du, Y-J., Chen, L., Liu, Z-G., Pan, Y-H., Liu, J-F., Liu, B. (2014). Quercetin protect against high glucose-induced damage in bone marrow-derived endothelial progenitor cells. International Journal of Molecular Medicine 34, 1024-1031.