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RESEARCH ARTICLE

Safety Evaluation of Rhodomyrtus Tomentosa Leaf Extract: Sub-Acute Toxicity Assessment in A Murine Model

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ABSTRACT

Rhodomyrtus tomentosa (Aiton) Hassk. has been widely exploited in traditional medicine for the treatment of many diseases and disorders. There have been no scientific reports on safety information of R. tomentosa leaf extract. The present study attempted to assess in vivo toxicity profile of R. tomentosa extract in order to provide information on the safety margin of the extract to support its pharmacological and medical applications. The extract at a dosage of 0.5 to 3.0 g/kg body weight of mice was orally administered daily up to 14 days. Behavioural and functional integrity were assessed by functional observational battery (FOB) analysis. Histopathological and ultrastructural changes were visualized under light microscopy and transmission electron microscopy, respectively. The extract at the concentration up to 3.0 g/kg body weight per day for 14 days caused no mortality. The extract at 0.5 to 2.0 g/kg body weight showed no adverse effects or signs of toxicity. It is evident that R. tomentosa extract is safe up to the dosage of 2.0 g/kg body weight of mice per day which is equivalent to 20.0 g/day for 70 kg person. According to Economic Cooperation Development (OECD) Guidelines, the lethal dose of R. tomentosa extract falls under class four values with no signs of toxicity at 2.0 g/kg body weight. The results demonstrated that R. tomentosa extract is considered safe as there was no adverse effect related to its use. This study assures R. tomentosa extract as of high value for potential applications as alternative remedies or natural supplements.

KEYWORDS:
Rhodomyrtus tomentosa, Toxicological study, Safety profile, Functional observational, battery, Histopathology, Behaviour

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INTRODUCTION

Since ancient times, plant-based products play an important role in human health care. According to World Health Organization (WHO), 80% of today's world population is turning towards the Ayurvedic system of medication and 30% of the commercialized drugs possess active constituents that were isolated from medicinal plants.1-2 A medicinal plant, Rhodomyrtus tomentosa belonging to the family Myrtaceae, is commonly known as 'Kratu' in Thailand, 'Harendong sabrang' in Indonesia, 'Karamunting' in Malaysia, 'Sim' in Vietnam, 'Sragan' in Cambodia, and 'rose myrtle' in Philippines.3 The plant is widely exploited in traditional medicine for the treatment of diarrhea,4 gynaecopathy, abscesses, hemorrhage, and dysentery.5 The potential use of R. tomentosa in traditional medicine has been scientifically supported by the isolation and identification of numerous active principles including acyl phloroglucinols, flavonoids, tannins, and triterpenes.6-8 During the past decade, the excellent antibacterial activity of the plant extract and rhodomyrtone, its principle compound against Gram-positive bacteria have been reported.9-10 Moreover, the extract exhibits anti-biofilm,11-12 and anti-quorum sensing activities.11 The plant extract has been used in preparing cosmetic formulations such as skin-whitening, anti-ageing, and skin-beautifying gels/lotion.13 Strong evidence of being a potent drug for the treatment of several diseases such as staphylococcal cutaneous infections,14 acnes,15 psoriasis,16 bovine mastitis,17-19 potential applications in aquaculture,20 and food industry,21-22 have been documented.

Despite the widespread use of the R. tomentosa in indigenous

traditional medicine with remarkable pharmacological activities and promising application potential from many previous studies, no adequate toxicological studies of this plant extract have been reported. Toxicological evaluations are immensely important to establish and commercialize safe and effective therapeutic treatment. The cytotoxic effect of the ethanol extract on human dermal fibroblasts demonstrated the IC50 values of 476 mg/ml, which is approximately 15-fold higher than its respective MIC. It was suggested that the extract exhibited very low cytotoxicity and could be applied as a safe topical agent for anti-acne treatment.23 Unfortunately, no drugs are clinically approved without subjecting to clinical trial as well as toxicological studies in animal models.24 Functional observational battery (FOB) is a set of tests to evaluate behavioral changes which include sensory, neuromuscular, and autonomic functions. FOB proposed to provide dose and time-dependent response of a drug exposure, which is helpful to decide its toxicology potential leading to further studies for risk assessment.25 Besides, the assessment of pathological alterations in model animals by novel drug candidates signifies their safety measurement.26 This preliminary study which is based largely on conventional histopathological techniques provides direct evidence of tissue pathology and represents a major contribution to the development of safe and novel therapeutics for the treatment of human diseases.27-28

Hence, the present study focused on exploring the safety level of R. tomentosa leaf extract to validate its pharmacological activities by analysis of FOB data and histopathological changes in mice following sub-acute exposure.

MATERIALS AND METHODS

Plant Collection and Extraction

Rhodomyrtus tomentosa leaves were collected from the Singha Nakorn District, Songkhla, Thailand. The specimen (Exsiccata registration number: A. Hiranrat 001) was identified by J. Wai and deposited in the Herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Thailand. The collected leaf material was washed with sterile distilled water to remove all debris, dried in an oven at 60oC for 48 -72 h and ground in an electric blender. The plant extract was prepared by soaking 100 g of R. tomentosa dried leaf powder (mesh size 200 µm) in 500 mL of 95% acetone and keeping in the dark at room temperature to avoid the light for 7 days. The content was filtered using Whatman filter paper, NC 45, pore size 0.45 µm and concentrated by evaporating the solvent using a rotary evaporator (BUCHI Rotavapor R-114, Buchi Labortechnik AG, Flawil, Switzerland). After evaporation, the percentage yield of 7.5%, w/w was obtained. The standardization of the extract was confirmed by High-performance liquid chromatography (HPLC) concerning for the peak of rhodomyrtone, a principal compound, according to previously described work.29 The extract was stored at -20 oC until use. As the plant extract was partially soluble in water, the plant extract was dissolved in 1% dimethyl sulfoxide (DMSO) on the day of oral administration to mice. The administration of DMSO as a vehicle and its tolerence in mice was well documented.30

Experimental Animals

Adult male Swiss albino mice (Mus musculus) weighing 28±2 g were obtained from the Laboratory Animal Facility Unit, Faculty of Science, Prince of Songkla University, Thailand. Most of the behavioral studies exclude the use of female mice as they may cause greater data variability as their estrous cycle could get in the way. A group of six mice per cage was housed in stainless-steel cages with a relative humidity of 50-60%, and artificial illumination between 06:00 and 18:00 h. The cages were kept in an air-conditioned room (25±2°C). Commercial diets and drinking water were provided. The animal study carried out in the present work was approved by the Institutional Committee for Ethical Use of Experimental Animals at Prince of Songkla University, Thailand (Ethical Clearance number: Prince of Songkla University MOE 0521.11/103).

R. tomentosa Dosing (Oral gavage) in Mice and Sub-acute Toxicity Study Protocol

Sub-acute toxicity experiment was carried out as described by Organization for Economic Cooperation Development (OECD) Guidelines, 2001.31 Briefly, mice were separated into control and treatment groups (one control group and six treatment groups) comprising of four animals in each group. The animals were fasted for 4 h before the experiment. The R. tomentosa leaf extract was orally administered to six test groups at dose levels of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g per kg body weight per day. Atmost care was taken not to exceed 0.5 mL of dosage volumes to administer the plant extract. The mice oral gavage procedure was continuously executed for 14 days, daily in the morning ~between 10:00 – 11:00 AM. Based on the previous reports of potent biological activities of plant extract at the dose of 0.5 g per kg body weight, the starting dose was determined for the present study. Mice in the control group received 1% DMSO (10 mL/kg body weight).

In-Life Observation

The general behaviour of mice, signs of toxicity, and the latency of death were continuously monitored hourly for a period of first 24 h, and then daily for 14 days.32 Body weight and food consumption were measured daily throughout the study period.

Functional Observational Battery (FOB)

FOB tests on the experimental animals provide a detail description of their appearance, behaviour, and functional integrity.33 The tests were assessed through observations at three stages i.e., (i) in the home cage, (ii) while handling, and (iii) in an open field. Procedural details and scoring criteria for FOB were considered according to the published protocol.34 FOB tests were performed in all groups of animals at the end of the study period. Evaluated parameters in three stages of FOB are represented (Table 1).

Organs Collection

On day 15, mice in control and treatment groups were anaesthetized with chloroform and sacrificed by decapitation. Their vital organs including liver, kidney, spleen, and heart were carefully dissected out and weighed. Portions of the liver and the kidney tissues of control and the extract-treated animals (2.0 and 3.0 g/kg body weight) were collected to assess histopathological and ultrastructural changes.

Examination of Histological and Ultrastructural Changes

A small slice (1 cm in length) from freshly harvested tissue was aseptically cut, washed three times in normal saline, fixed in 10% formalin, and embedded in paraffin. Micrometer sections (5-6 µm) cut by a microtome (MT-XL RMC, USA) were stained with haematoxylin-eosin and observed under light microscope, 35 Photographs of the samples were taken and any histological alterations, compared with the normal structure, were recorded.

For ultrastructure examination, samples (1-2 mm) were fixed in 4% paraformaldehyde buffered with pH 7.3, 0.1 M phosphate at 4°C overnight. The fixed specimens were subsequently post-fixed in phosphate buffer containing 1% v/v osmium tetroxide (Merck) for 4 h at room temperature and rinsed with 0.1 M phosphate buffer. Dehydration was performed by soaking the samples in successive ethanol series; 50% and 70% for 30 min each, 95% and 100% for 1 h each; subsequently immersed in propylene oxide for 15 min, twice. The tissues were infiltrated twice each with epoxy resin/propylene oxide mixture in the ratio of 1:1 for 30 min, 3:1 for 30 min, and pure epoxy resin for 30 min. The specimens were embedded in beam capsule size 00 and cured at 80°C, overnight. Specimen blocks were trimmed and semi-thin sections (60-90 nm) were cut using an ultramicrotome (MT-XL RMC, USA). The sections were mounted on uncoated copper grids (EMS, 200 mesh), and stained with uranyl acetate and lead citrate. The mounted sections were photographed under transmission electron microscopy (JEM-100 CX II, JEOL, Japan).35-36

Statistical Analysis

Values of each parameter were expressed as mean ± standard error mean (SEM). Comparisons among different groups were performed by one-way analysis of variance (ANOVA). When significant differences existed, Dunnett's multiple range tests were applied to compare the means. A probability of $p < 0.05$ was considered significant.

RESULTS

Daily Observation for Symptoms of Toxicity

Daily observation data for symptoms of the animals treated with R. tomentosa extract are summarized (Table 2). Administration of the extract up to the dose level of 2.0 g/kg body weight, daily for 14 days did not produce any signs of toxicity. However, two animals in treatment groups with the extract of 2.5 and 3.0 g/kg body weight demonstrated mild symptoms of toxicity such as rough fur, lethargy, loss of appetite, and lacrimation. Mortality was not observed in any treatment groups.

Feed Intake and Body Weight

No significant decrease in food intake (Figure 1) and body weight in Table 3 were observed in R. tomentosa extract administered groups of 0.5 to 2.0 g/kg body weight. By day 14, the average body weight of each mice administered with the extract up to 2.0 g/kg body weight was comparable to the control group. However, a slight loss of body weight was noted in the extract-treated groups of 2.5 and 3.0 g/kg body weight during day 6 to 10 after the treatment. An improvement was observed from day 12 until the end of the study period (Table 3).

Functional Observational Battery

FOB was analyzed after the completion of the treatment period. The data are shown in Table 4 and Table 5. Among home cage observation, parameters such as activity, palpebral closure, tonic movements, and biting that were not observed in the animals received R. tomentosa extract of 0.5 to 2.0 g/kg body weight. In hand held observation, no significant alterations in the tested parameters including the behaviour of the animal while removing from the cage, lacrimation and fur appearance in the R. tomentosa extract administered groups of 0.5 to 2.0 g/kg body weight were noticed (Table 4). On the other hand, a slight increase in lacrimation and decrease in the percentage of normal fur appearance was noted in the R. tomentosa extract-treated groups of 2.5 and 3.0 g/kg body weight (Table 4). In open field observation, no changes in evaluated parameters such as mobility, faecal boluses, fore and hind limb grip strength, touch, approach, and tail pinch responses were observed in the R. tomentosa extract-treated groups of 0.5, 1.0, 1.5, and 2.0 g/kg body weight when compared to the control group. Besides, analysis of rearing, gait, mobility and arousal showed no significant decrease in these parameters in the extract-treated group (0.5 to 2.0 g/kg body weight) (Table 5). However, administration of R. tomentosa extract at 2.5 and 3.0 g/kg body weight of mice caused marginal impairment in mobility and reactivity to stimuli.

Relative Organ Weight

Oral administration of R. tomentosa leaf extract at all doses for 14 days caused no significant changes in weight of all organs including the liver, right and left kidney, spleen, and heart, compared to the control mice ($^*P > 0.05$) (Table 6).

Histological and ultrastructural examination

Light microscopic photographs of the liver and kidney tissues of the control and treated groups are given (Figure 2). Histological features of liver in both control and treatment group with the R. tomentosa extract of 2.0 g/kg body weight did not demonstrate any adverse effect on histoarchitecture of hepatocytes and nephrons. However, mice treated with the R. tomentosa extract of 3.0 g/kg body weight showed neutrophil

infiltration at portal tract area of hepatocytes and caused an alteration in the histoarchitecture of nephrons, showing inflammation and damage at convoluted tubule. The ultrastructural examination of the liver and kidney of the animals are represented in Figure 3. Both liver and kidney of the control and 2.0 g/kg of R. tomentosa extract-treated group did not demonstrate any signs of changes. Hepatocytes of the 2.0 g/kg of R. tomentosa extract treated animals appeared normal, demonstrating prominent nucleus and sinusoids. In the animals treated with the extract of 3.0 g/kg body weight, infiltration of neutrophils and erythrocytes penetrating the cell membrane into the cytoplasm of hepatocyte and glomerulus containing endothelial cells with irregular borders with bleb formation and nucleolus with dense chromatin were observed.

DISCUSSION

Herbal medication as alternative therapeutics has become current and future perspectives in ethnopharmacology. However, plants or their derived products can be toxic if wrong plant parts or wrong concentrations are used.37 In vivo toxicological evaluation of plant products assists to predict the safe dosage for human consumption.38 Many studies have been designed to give extract 1-200 mg/kg/day to mice or rats. In the present study, observations were made on male mice following sub-acute exposure to R. tomentosa leaf extract for 14 days. No signs of adverse effects were noted up to the dosage level of 2.0 g/kg body weight of the animals which is equivalent to 20.0 g/day for 70 kg person. The LD50 of the extract is above 2.0 g/kg which falls under class four values according to the OECD guidelines. Similar safe dosage of R. tomentosa leaf extract was reported in oxidative stress-induced mice,39 and hepatic damage-induced rats.40 In earlier studies, the extracts of R. tomentosa up to 200 and 400 mg/kg demonstrated antiulcer activity with no sign of toxicity in acute, and chronic ulcer-induced rats,41-42 respectively.

It has been considered that change in body weight is a measure to indicate the adverse effects of drugs and chemicals. Mice administrated with R. tomentosa leaf extract up to 2.0 g/kg demonstrated no significant decrease in food intake and body weight loss. It is to be noted that an imperceptible decrease in the body weight was found in mice receiving the R. tomentosa extract of 2.5 and 3.0 g/kg body weight during day 4 to 10 of treatment. However, an increase in the body weight was observed on day 12 to 14, comparable to the control group. Similarly, organ weight of the treated groups remained unchanged. The results attested to the non-lethality of the plant extract at doses used since it is known that reduction in body weight gain and internal organ weight are simple and sensitive indices of toxicity after exposure to a toxic agent.43

Functional observational battery test was adopted to appraise behavioural changes of mice in the extract-treated group. In all sets of FOB assessment including home cage, hand held, and open field observations, R. tomentosa extract up to 2.0 g/kg body weight showed no alteration in the tested parameters. It is well-documented that neurotransmitter systems, dopaminergic and serotonergic pathways, as well as neuronal cell surface receptors are responsible for regulating locomotion.44-45 In the present work, the extract up to 2.0 g/kg body weight did not affect the locomotor behaviour of mice, whereas, administration of R. tomentosa extract at 2.5 and 3.0 g/kg body weight of mice caused marginal impairment in mobility and reactivity to the stimulus. A possible explanation for the impairment in the locomotion may be due to the alteration in neurotransmitter systems by a high dose of R. tomentosa extract treatment resulted in the inconsequential alteration in the mobility of the experimental animals.

Histological and ultrastructural changes provide direct evidence of pathology in the vital organs.27 Numerous reports have implied the need for histopathological studies to assess the adverse effect of the plant extract before being used for the treatment of patients. Therefore, we assessed whether the tested concentrations of R. tomentosa extract produced any adverse effects on the internal organs of mice. As a result, no obvious effects were observed in liver and kidney of all animals administrated with 2.0 g/kg of body weight. Hepatocytes and glomerulus of mice remained normal. However, with 3.0 g/kg of the extract, some changes were noted. Immunomodulatory effects on THP-1 human monocyte cell line led to the enhanced killing of methicillin-resistant Staphylococcus aureus.45 It is envisaged that infiltration of neutrophils in hepatocytes and inflammation of convoluted tubule in nephrons may be due to some phytochemicals of the extract which stimulate an immune-modulatory hypersensitivity reaction (resulted in infiltration and inflammation.)

Behavioural studies on laboratory animals and histopathology of its vital organs play an important role in evaluating the safe dosage of newly developed therapeutics. The results from the present study provided information regarding its dosage and toxicological effects in mice, which is advantageous for the safe use of this plant. R. tomentosa leaf is safe when administered up to the dose level of 2.0 g/kg body weight of mice per day which is equivalent to 20.0 g/day for 70 kg person. According to OECD guidelines, the lethal dose of R. tomentosa extract falls under class four values with no signs of toxicity at 2.0 g/kg body weight. Moreover, the results from histopathology supported that R. tomentosa leaf is safe when administered up to the dose level of 2.0 g/kg body weight of mice per day. It is evident that the plant extract is safe and suitable for pharmaceutical applications with high commercial value.

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Table 1: Evaluation of clinical symptoms in each case of a functional observational battery

Stages of functional observational battery tests		
Home cage observations	Hand-held observations	Open field observation
Normal body posture	Reactivity to being held	Body posture
Involuntary motor movements	Lacrimation	Rearing
Clonic	Salivation	Gait
Tonic	Normal fur appearance	Mobility
Vocalizations	Palpebral closure	Arousal
		Stereotypy
		Defecation
		Urination
		Approach response
		Touch response
		Click response
		Tail pinch response
		Pupillary reflex
		Pinna reflex
		Righting reflex
		Extensor thrust reflex
		Fore-limb grip strength
		Hind-limb grip strength
		Landing foot splay

Table 2: Daily observation of mice orally administered with R. tomentosa leaf extract.

Dose of R. tomentosa (g/kg body weight)	No. of deaths per total no. of animal	Symptoms of toxicity	Severity of the symptoms	Duration of symptoms
0	0/4	None	None	-
0.5	0/4	None	None	-
1	0/4	None	None	-
1.5	0/4	None	None	-
2.0	0/4	None	None	-
2.5	0/4	Lethargic	Mild	Day 6 to 14
		Rough fur	Mild	Day 6 to 14
		Lacrimation	Mild	Day 7 to 14
		Loss of appetite	Mild	Day 7 to 14
3.0	0/4	Apathetic	Mild	Day 7 to 14
		Rough fur/piloerection	Mild	Day 4 to 14
		Lacrimation	Mild	Day 7 to 14
		Loss of appetite	Mild	Day 7 to 14
		Tremors	Mild	Appeared once (day 1), 2 h after dosing and lasted for 30 min

Table 3: Mean body weights (g) of mice orally administered with R. tomentosa leaf extract

Day	Dose of R. tomentosa leaf extract (g/kg)						
	0	0.5	1.0	1.5	2.0	2.5	3.0
0	34.5 ± 2.15	32.2 ± 1.26	32.8 ± 1.54	35.6 ± 2.52	33.7 ± 1.16	34.3 ± 1.02	35.4 ± 1.05
2	35.6 ± 1.08	33.4 ± 1.01	36.5 ± 1.52	37.2 ± 2.11	35.1 ± 1.07	32.2 ± 1.25	35.4 ± 1.19
4	36.4 ± 1.44	35.6 ± 1.24	37.5 ± 2.18	36.3 ± 1.64	36.1 ± 1.51	31.2 ± 1.02*	35.2 ± 1.28
6	36.6 ± 1.56	34.4 ± 1.31	37.3 ± 2.67	37.3 ± 2.49	37.4 ± 2.82	32.2 ± 1.33*	32.7 ± 1.96*
8	37.2 ± 2.14	35.8 ± 1.05	37.1 ± 2.42	37.6 ± 2.51	37.6 ± 2.08	31.5 ± 1.26*	33.5 ± 2.11*
10	39.4 ± 1.00	39.5 ± 1.14	40.7 ± 2.18	40.2 ± 2.16	39.7 ± 1.37	33.3 ± 1.04*	36.4 ± 2.33*
12	40.0 ± 1.62	40.3 ± 1.42	40.6 ± 1.86	39.1 ± 1.19	40.4 ± 1.86	36.1 ± 1.56	39.3 ± 1.73
14	40.2 ± 1.91	40.2 ± 1.32	40.3 ± 1.95	39.5 ± 1.25	40.3 ± 1.48	36.4 ± 1.83	39.6 ± 1.18

Values are expressed as Mean ± SEM of four rats in each group. * indicates significantly ($P<0.05$) different from control at 5% level. Data were analyzed by one way ANOVA followed by Dunnett's test.

Table 4: Home cage and hand-held observation of behavioural changes in mice orally administered with the leaf extract of R. tomentosa assessed by functional observational battery tests

Measures observed	Dose of R. tomentosa leaf extract (g/kg)						
	0	0.5	1.0	1.5	2.0	2.5	3.0
Home cage observations							
Activity (R)	1.0	1.0	1.0	1.0	1.0	3.5*	4.0*
Normal body posture (D) (%)	100	100	100	100	100	95*	95*
Involuntary motor movements							
Clonic movements (R)	-	-	-	-	-	2.5*	3.0*
Vocalizations (%)	-	-	-	-	-	10.2*	11.3*
Hand-held observations							
Ease of removal from the cage (R)	3.0	3.5	4.0*	3.0	3.5	1.0*	1.0*
Reactivity while handling (R)	4.0	4.0	4.0	3.5	3.5	2.0*	2.0*
Lacrimation (R)	1.0	1.0	1.0	1.0	1.0	2.0*	2.0*

Normal fur appearance (D) (%)	100	100	100	100	100	90*	80*

Descriptive (D) data expressed as a percentage of incidence; ranked (R) data expressed as the mean score of the scale used. Data were analyzed by one way ANOVA followed by Dunnett's test; *p≤0.05 compared to the control group.

Table 5: Open field observation of behavioural changes in mice orally administered with R. tomentosa leaf extract assessed by functional observational battery test.

Measures observed	Dose of R. tomentosa leaf extract (g/kg)						
	0	0.5	1.0	1.5	2.0	2.5	3.0
Activity level (R)	1.0	1.0	1.0	1.0	1.0	3.5*	4.0*
Rearing (R)	2.0	2.0	2.0	2.0	2.0	1.5*	1.0*
Gait (R)	1.0	1.0	1.0	1.0	1.0	2.0*	2.0*
Mobility (R)	1.0	1.0	1.0	1.0	1.0	2.0*	2.0*
Arousal (R)	4.0	4.0	4.0	4.25	4.5	2.5*	2.0*
Faecal boluses (C)	2.0	2.5	2.0	2.0	2.5	4.0*	6.0*
Urine pools (C)	2.0	2.0	2.5	2.0	2.5	X*	X*
Approach response (R)	2.0	2.0	2.0	2.0	2.0	1.5*	1.25*
Touch response (R)	2.0	1.5	2.0	1.5	2.0	1.0*	1.0*
Click response (R)	2.5	2.5	2.0	2.0	2.5	1.5*	1.0*
Tail pinch response (R)	3.0	3.0	3.0	3.0	2.5	2.0*	2.0*
Righting reflex (R)	1.0	1.0	1.0	1.0	1.0	2.0*	2.0*
Fore limb grip strength (D) (%)	100	100	100	100	100	95*	90*
Hind limb grip strength (D) (%)	100	100	100	100	100	90*	80*
Landing foot splay (mm)	32.1	30.0	34.1*	32.4	31.5	42.6*	45.1*

All values are expressed as mean. R = Ranked, C = Counts, and D = Descriptive data in a percentage; X = overlapping urine pools; * indicates significantly (P<0.05) different from control at 5% level. Data were analyzed by one way ANOVA followed by Dunnett's test.

Table 6: Mean organ weights (g) of mice orally administered with the leaf extract of R. tomentosa

Dose administered (g/kg body weight)	Organ weight (g)				
	Liver	Right kidney	Left kidney	Spleen	Heart
0	2.72 ± 0.52	0.46 ± 0.03	0.43 ± 0.03	0.20 ± 0.02	0.27 ± 0.04
0.5	2.32 ± 0.10	0.48 ± 0.05	0.52 ± 0.06	0.15 ± 0.01	0.21 ± 0.02
1.0	2.56 ± 0.32	0.42 ± 0.02	0.48 ± 0.02	0.19 ± 0.03	0.25 ± 0.03
1.5	2.72 ± 0.26	0.44 ± 0.05	0.41 ± 0.04	0.20 ± 0.04	0.23 ± 0.03
2.0	2.19 ± 0.13	0.42 ± 0.03	0.38 ± 0.01	0.15 ± 0.02	0.23 ± 0.04
2.5	2.38 ± 0.11	0.45 ± 0.04	0.48 ± 0.02	0.13 ± 0.01	0.22 ± 0.06
3.0	2.19 ± 0.18	0.37 ± 0.02	0.40 ± 0.03	0.14 ± 0.05	0.21 ± 0.02

Values are expressed as Mean ± SEM of four rats in each group. Data were analyzed by one way ANOVA followed by Dunnett's test. There was no significant difference between the groups.

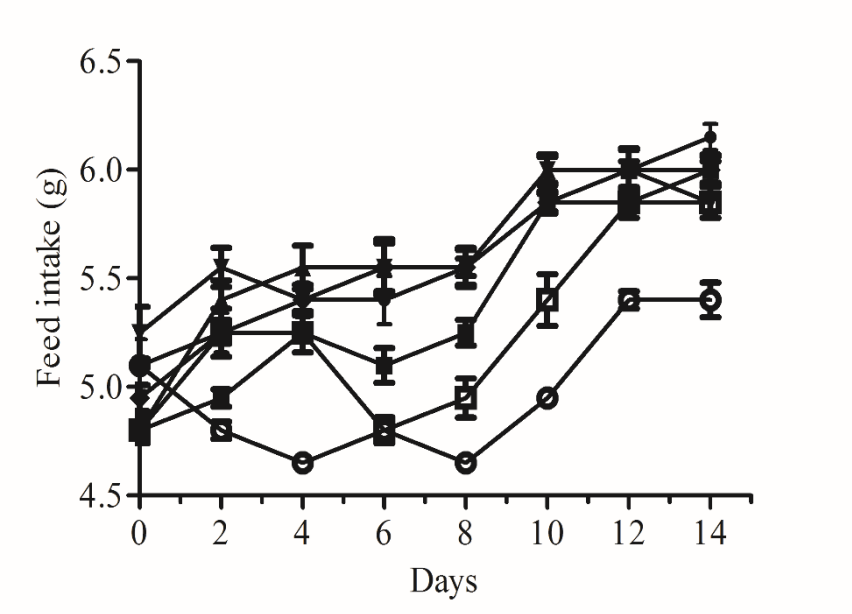

Fig.1: Effects of administration of Rhodomyrtus tomentosa leaf extract by oral gavage in mice. The plant extract was fed for 14 days to the groups of Swiss albino mice (n = 4 per group) at the doses of 0 (●), 0.5 (■), 1.0 (▲), 1.5 (▼), 2.0 (◆), 2.5 (○), and 3.0 (□) Values are expressed as mean ± SEM, P >0.05.

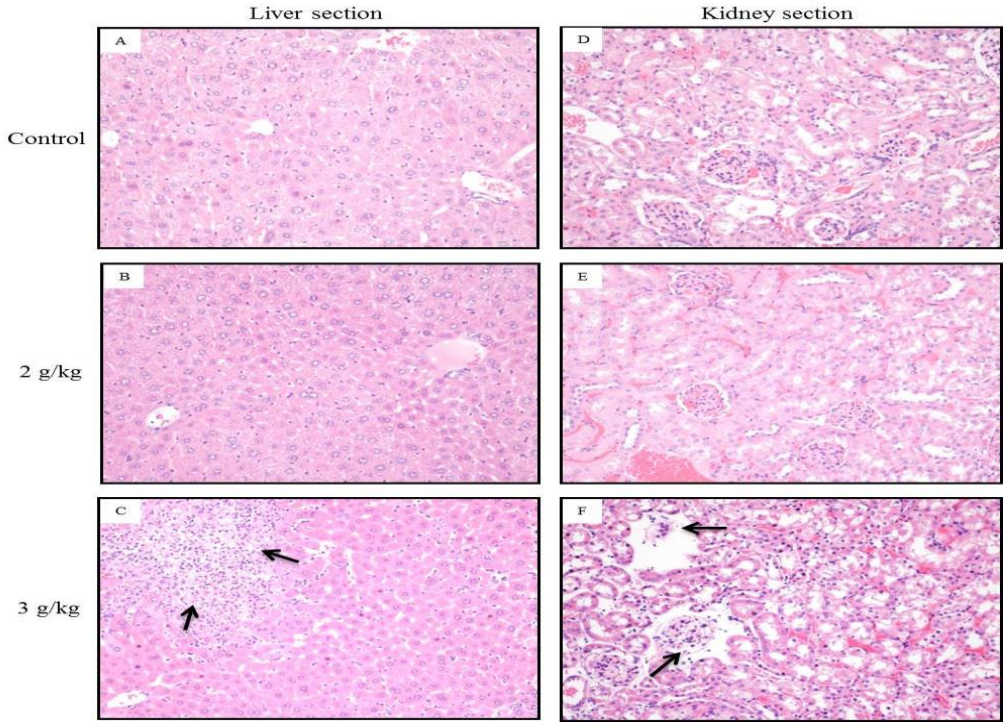

Fig.2: Light microscopic photographs showing histology of the liver and kidney of mice. The liver section of control mice (A) and mice treated with 2.0 g/kg of Rhodomyrtus tomentosa leaf extract (B) showing normal histoarchitecture of hepatocytes, mice treated with 3.0 g/kg of the extract showing marked neutrophils infiltration at portal tract area (arrow) (C). The kidney section of control mice (D) and mice treated with 2.0 g/kg of the extract (E) showing normal histoarchitecture, mice treated with 3.0 g/kg of the extract showing inflammation and damage at convoluted tubule (arrow) (F). Magnification × 200.

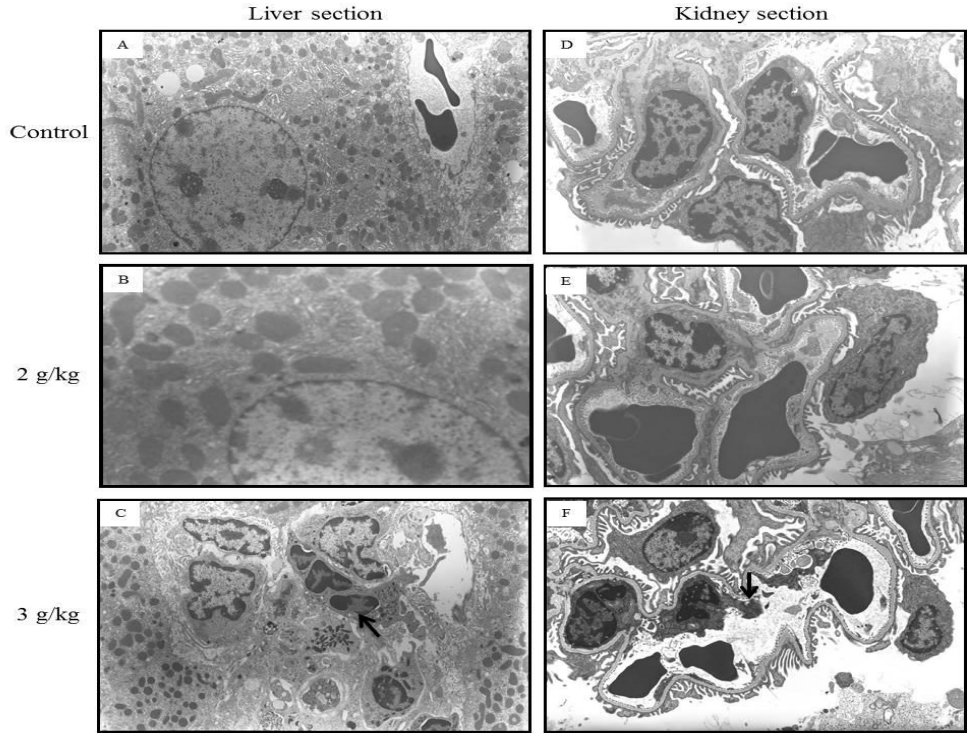

Fig.3: Ultrastructural images of the liver and kidney sections disclosed by transmission electron microscopy. The liver section of control mice (A) and mice treated with 2.0 g/kg of Rhodomyrtus tomentosa leaf extract (B) showing normal histoarchitecture of hepatocytes, mice treated with 3.0 g/kg of the extract showing infiltration of neutrophils at sinusoids (arrow) and erythrocytes penetrating the cell membrane into the cytoplasm of hepatocyte (C). The kidney section of control mice (D) and mice treated with 2.0 g/kg of the extract (E) showing normal histoarchitecture, mice treated with 3.0 g/kg of the extract showing glomerulus containing endothelial cells with irregular borders with bleb formation (arrow) and nucleolus with dense chromatin (F). Magnification × 5000.