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ORIGINAL ARTICLE **OPEN ACCESS**

Subchronic toxicity studies of the aqueous stem bark extract of *Bridelia ferruginea* in Wistar rats

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ABSTRACT

The aqueous stem bark extract of Bridelia ferruginea was used to assess its effects in wistar rat after a daily administration during 90 days. This study was carried out according the protocol described by the OECD guideline 408 for testing chemicals. General behavior, mortality, animal body weight, food and water consumption were observed throughout the study period. At the end of the experiment, the blood was collected for hematological and biochemical parameters. Bridelia ferruginea used at the doses of 100, 200 and 400 mg/kg b.w, did not show any toxicity effects in rats after 90 days of daily treatment. In experiment period, there were no significant changes in body weight. There were some significant changes ($p<0.05$, $p<0.01$, $p<0.001$) in food and water intake in both sex. Bridelia ferruginea did not modify hematological parameters significantly ($p>0.05$) in the test groups. The extract did not modify significantly, the lipid profile, kidney and liver function parameters. At the high dose (400 mg/kg) of Bridelia ferruginea, there was a significant decrease ($p<0.05$) in relative kidney weight in female group. The results obtained in this study, showed that the aqueous extract of Bridelia ferruginea did not cause any mortality. This extract did not modify the body weight, lipid profile, the liver and kidney functions in rats after daily administration for 90 days. It decreased the relative kidney weight in the high dose in female group and the histopathological analysis did not show any tissue damage.

Keywords : *Bridelia ferruginea*, toxicity, Clinical biochemistry, Kidney, Liver

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INTRODUCTION

The herbal medicine plays a very important role in health care and it is used in the treatment of several pathologies. According to Potterat and Hostettmann [1], the active compounds from plants represent 25% of prescription drugs. But the use of medicinal plants is not without danger to health and poses problems of toxicity, overdose and side effects [2]. One of the problems found in medicinal plants is the absence of clinical, toxicological and pharmacological studies [3] and it is generally thought that herbs are considered 'Natural' and thus are considered as free from risk [4]. From the perspective of drug development to enhance the traditional pharmacopoeia, we undertook the study of *Bridelia ferruginea* (Euphorbiaceae) which is a common savannah of genus *Bridelia*.

Its habitat is the savannah, especially in the moister region extending from Guinea to Zaire through Angola. It is usually a gnarled shrub, which sometimes reach the size of a tree when grown in a suitable environment. The bark is dark grey, rough and even markedly scaly [5].

This plant is known traditionally as a purgative, diuretic, aphrodisiac and anti-gonococcal [6].

Nene-Bi *et al.* [7] have demonstrated that the aqueous extract of stem bark of *Bridelia ferruginea,* do not disrupts the markers of renal function (urea and creatinine) in rats after a 24 hour treatment.

The aim of the present study is to evaluate the subchronic toxic effects of the aqueous stem bark extract of *Bridelia ferruginea* in Wistar rats.

MATERIALS AND METHODS

Plant material

The plant material of the present study, *Bridelia ferruginea* stem-bark, was obtained at market from Yopougon (Ivory Coast). These stem barks were identified by an expert, Mr. Assi Jean, a botanist of the National Floristic Center of University Félix Houphouët-Boigny (Ivory Coast). A voucher specimen (herbarium No. 17148, 2015) has been deposited in this center.

Preparation of extract

The stem barks were dried in the shade at room temperature between 26 to 30°C and powdered with a micro-crusher. The powder obtained (100 g) was macerated for 24 hours in a 1 liter of distilled water using magnetic stirrer. The supernatant was filtered with Whatman No 1 filter paper and it was evaporated using rotating evaporator (Buchi, France). The concentrate was frozen at -30°C before and lyophilized at -45°C using a lyophilisator (Telstar, Spain). A brown colored powder was obtained.

Animals

Healthy Wistar rats weighing 150-250 g, were procured from the animal house, J.S.S. College of Pharmacy, Udhagamandalam, India. The animal house was well ventilated and animals had 12 ± 1 h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the experimental period and room temperature was maintained at 25 ± 1°C. The animals were fed with standard rat feed and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (JSSCP/IAEC/PH.COLOGY/PH.D/01/2015-16).

Sub-chronic Toxicity Study

The sub-chronic toxicity was conducted according to OECD guidelines (Organization for Economic Cooperation and Development, Guideline-408, adopted on 21st September 1998). The animals were randomly divided into four groups of 8 animals each (8; 4/sex). Animals of group I served as control and received the vehicle (distilled water) only by gavage (10 ml/kg of body weight) while those of groups II, III and IV were treated daily by gavage with aqueous extract of *Bridelia ferruginea* at 100, 200 and 400 mg/kg body weight respectively for 90 days. The body weight, consumption of food and water were measured weekly throughout the study period.

Pre-clinical Observations

General physical condition of each animal was observed during the experimental period. All animals were observed twice daily for mortality. Physical observations were made throughout the study period. Examination included observation of fur, eyes, nose, abdomen and external genitals; occurrence of secretions and excretions, autonomic nervous system.

Hematological Analysis

Hematological analysis was carried out at the end of the study period. Whole blood was collected by retroorbital bleeding under light ether anesthesia in eppendorffs tubes with EDTA as anticoagulant (1mg/ml of blood). The blood sample was analyzed for hemoglobin, red blood cells (RBC), white blood cells (WBC) and platelet count in clinical laboratory using cellanalyzer (Medonic CA 530, Oden).

Clinical Chemistry

Blood was collected on 91st day by retro-orbital bleeding under light ether anesthesia in eppendorffs tubes without anticoagulant; serum was obtained by centrifugation at 3000 rpm for 15 minutes. Serum glucose, cholesterol, triglycerides (TGs), high density lipoproteins (HDL), alanine amino transferase (ALT), aspartate amino transferase (AST), total protein, bilirubin (total and direct), alkaline phosphatase, albumin, urea, uric acid and creatinine levels were measured by biochemical assay kits (Erba Mannheim, Transasia Bio-Medical Ltd., Nalagarh Road, Village Malpur, Baddi, dist. Solan, (HP)- 173205, India) using auto analyzer Alisa (Infinte M200 Pro, TECAN).

Histopathology

The animals of all the groups were sacrificed after the study period and subjected to gross and histopathological examination. The liver and kidney will be dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in Xylene, and embedded in paraffin wax. The section which are 5-6 µm thick, will be the prepared using rotary microtome (Leica RM 2125 RTS, Singapore) and stained with hematoxylin and eosin dye for microscopic observation of histopathological changes in these organs. The relative organ weight of the kidney, heart, liver and lung was calculated as (organ/body weight) x 100 %.

Statistical Analysis

The experimental results have been expressed as the mean ± S.E.M. Significant differences were determined using Tukey-Kramer's multiple comparison tests (graphpad 5 software, inc. California, USA) and differences were considered significant at $p<0.05$.

RESULTS

Pre-clinical observations

The animals were examined before to start the experiment and once a day during three months for signs of morbility and mortality (toxic effect). The clinical appearance of all rats was normal or did not show any treatment-related adverse effects during 13 weeks.

Body weight

The gains of body weight were observed in control group (52.66 % in male and 12.75 % in female). Not significant increase ($p > 0.05$) in of body weight was seen in test groups (male and female) compared to the control (Figure 1).

Food and water intake

There was some variations in food consumption during the period of experiment in male and female groups (Figure 2).

In respect male rats, *Bridelia ferruginea* at the dose of 100 mg/kg b.w, provokes a significantl decreased of 15.84 % ($p<0.001$) in food consumption in week 1 compared to the control group. At the same dose, the food intake increased in week 4, week 7, week 8, week 9 and week 12, respectively of 27.47 % ($p<0.001$), 12.23 % ($p<0.001$), 13.48 % ($p<0.001$), 12.55 % ($p<0.001$) and 11.10 % ($p<0.05$). *Bridelia ferruginea* at the dose of 200 mg/kg in male group, decreased the food consumption of 14.11 % ($p<0.001$), 14.93 % ($p<0.001$) and 16.17 % ($p<0.001$) respectively in week 1, week 10 and week 11, compared to the control group. In the high dose group of the extract (400 mg/kg), the food intake in male group decreased of 7.97 % ($p<0.05$) in week 10 (Figure 2 A).

Concerning the female group, *Bridelia ferruginea* at the dose of 100 mg/kg b.w, decreased the food consumption of 13.94 % ($p<0.01$) in week 2. In the group II (200mg/kg) of female, the food intake decreased of 41.31 % ($p<0.05$), 26.17 % ($p<0.001$) and 15.48 % ($p<0.05$), respectively in week 1, week 2 and week 3, compared to the control group. In the high dose (400 mg/kg b.w) of female group, the food consumption decreased in week 2, week 3, week 9, week 10 and week 12, respectively of 34.98 % ($p<0.001$), 13.58 % ($p<0.05$), 8.72 % ($p<0.05$), 8.78 % ($p<0.05$) and 11.16 % ($p<0.05$) (Figure 2 B).

The water consumption was calculated in each group of animals weekly (Figure 3).

In group II (100 mg/kg) of male, *Bridelia ferrginea* decreased the water intake of 32.42 % ($p<0.05$) in week 3. At the same dose, water consumption increased of 25.26 % ($p<0.001$), 26.36 % ($p<0.001$), 24.51 % ($p<0.01$), 15.99 % ($p<0.01$), 19.14 % ($p<0.01$) and 23.69 % ($p<0.001$), respectively in week 7, week 8, week 9, week 10, week 11 and week 12. In group III of male rat treated with *Bridelia ferruginea* at the dose of 200 mg/kg, the water consumption increased in week 7 and week 8, respectively of 28.03 % ($p<0.001$) and 26.37 % ($p<0.001$). The decrease of 16.62 ($p<0.01$) and 21.50 ($p<0.01$) in water intake was observed in group III (200 mg/kg), respectively in week 10 and week 11. In group IV (400 mg/kg), this paramater was increased significantly ($p<0.05$) of 15.51 % in week 8 (Figure 3 A).

In respect female group, the effect of *Bridelia ferruginea* on water consumption was evaluated (Figure 3 B). In the low dose (100 mg/kg), *Bridelia ferruginea* decreased water intake of 29.78 % ($p<0.01$) and 21.88 % ($p<0.001$) respectively in week 1 and week 2. In group II (200 mg/kg), the extract decreased water intake in week 1 and week 2 with the respective percentage of 36.28 % ($p<0.01$) and 24.58 % ($p<0.001$). For the dose of 400 mg/kg, *Bridelia ferruginea* provoked the decrease of water consumption in week1, week 2, week 3 and week 5 respectively of 41.37 % ($p<0.001$), 29.13 % ($p<0.001$), 19.22 % ($p<0.05$) and 23.36 % ($p<0.01$).

Hematological analysis

The effect of *Bridelia ferruginea* on the hematological parameters in rats, was carried out. There was no significant changes in haemoglobin, red and white blood cells and platelets in the test groups compared to control (Table 1).

Clinical chemistry

There was no significant ($p>0.05$) differences in the lipid profile (Table 2). The parameters (urea, uric acid and cratinine) of kidney function did not show any changes in all groups (male and female) compared to the control groups (Table 3). In liver function parameters, there was no statistically ($p>0.05$) differences in AST, ALT, total and direct bilirubin, alkaline phosphatase and albumin, but a significant ($p<0.05$) increase was observed in total protein level in low dose group (100 mg/kg) of male (Table 4).

Relavite organ weight

The effects of the aqueous stem bark extract of *Bridelia ferruginea* during 90 days were measured on the relative organ weight (Table 5). There was no significant ($p>0.05$) changes in the relative kidney, heart, Liver and lung weights in male group. But in the high dose group (400 mg/kg) of female, there was a significant decrease of 13.73 % ($p<0.05$) in relative kidney weight when compared to the control.

Histopathological analysis

The effects of aqueous stem bark extract of *Bridelia ferruginea* on the histopathological studies of liver and kidney in treated and control animals are shown in figures 4 and 5. The results of this studies showed no abnormalities in liver and kidney in both sex compared to the control, related to the treatment with *Bridelia ferruginea*.

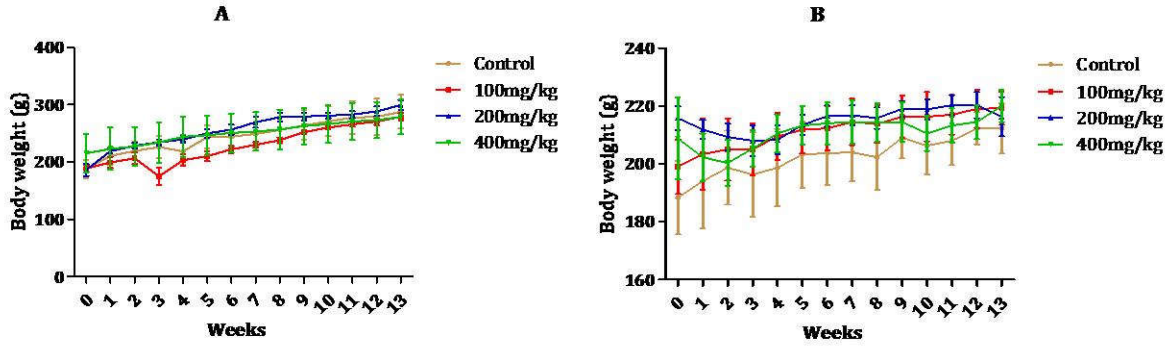

Figure 1 : Body weight changes in male (**A**) and female (**B**) rats during the 13 weeks toxicological assessment. No significant differences were detected between the treated and control groups. Data are presented as meanas the mean ± SEM, n= 8.

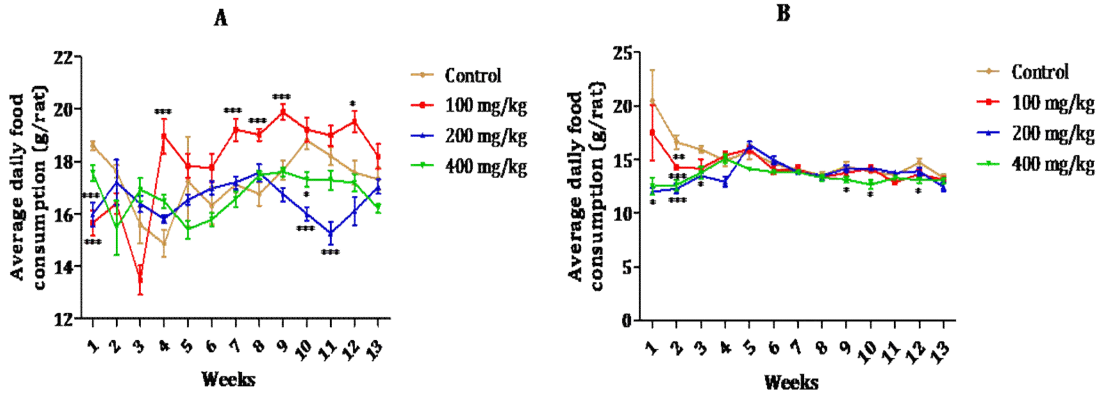

Figure 2 : Effects of the aqueous extract of *Bridelia ferruginea* on the food consumption in male (**A**) and female (**B**) rats. Values are expressed as mean ± SEM, n=8. (*p<0.05, **p<0.01, ***p<0.001 as compared to the control group).

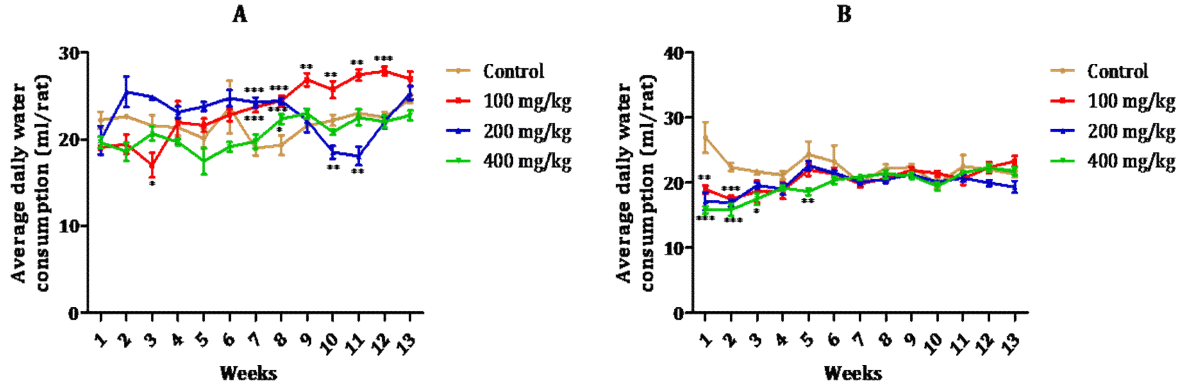

Figure 3 : Effects of *Bridelia ferruginea* on daily water intake in male (**A**) and female (**B**) rats. Results are presented as mean ± SEM (n=8), *p<0.05, **p<0.01, ***p<0.001 compared to the control group.

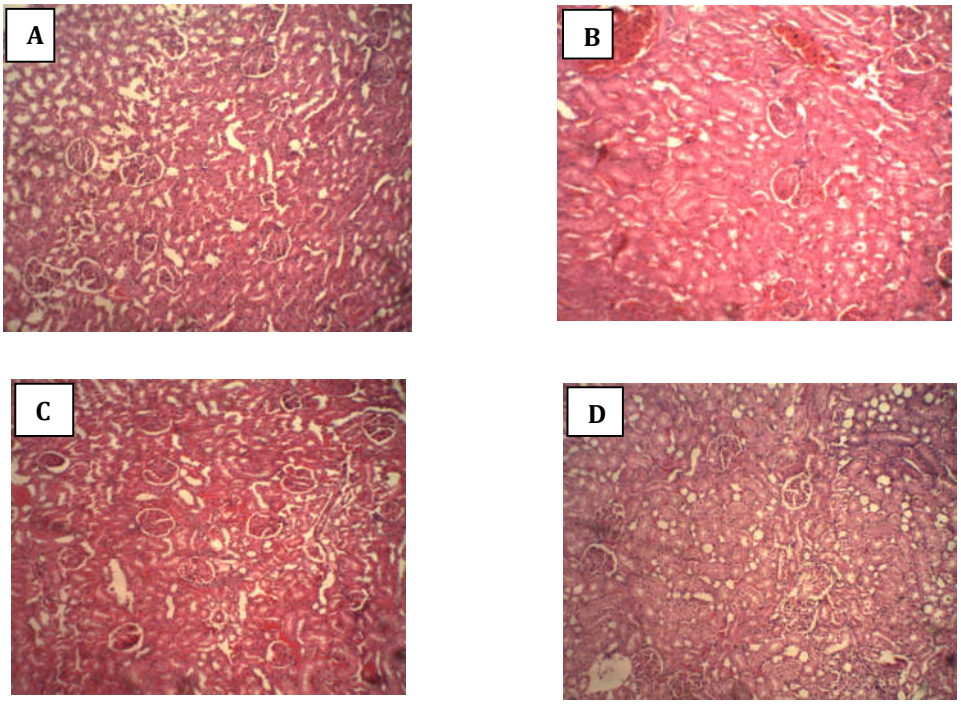

Figure 4: Effect of *Bridelia ferruginea* on the microscopy of kidney in male (a, b) and female (c, d) rats. (**a**) Control male, (**b**) 400 mg/kg bw male, (**c**) control female, (**d**) 400 mg/kg bw female.

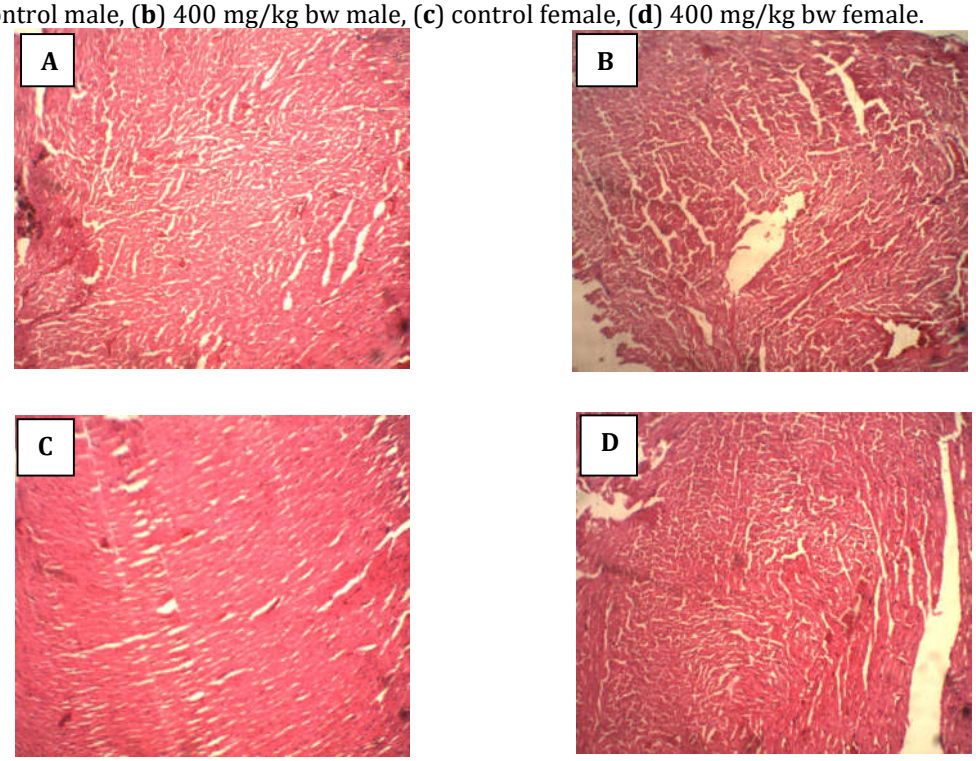

Figure 5: Histological appearance of the liver in male (a, b) and female (c, d) rats treated with *Bridelia ferruginea*. (**a**) control male, (**b**) 400 mg/kg bw male, (**c**) control female, (**d**) 400 mg/kg bw female.

DISCUSSION

The sub-chronic toxicity study of the aqueous stem-bark extract of *Bridelia ferruginea* was carried out according the method described by the Organization for Economic Cooperation and Development (OECD) guideline 408 for testing chemicals. These studies provide information on target organ toxicity and are designed to identify no-observable adverse effect level [8]. The rats received daily during 90 days the

extract of *Bridelia ferruginea* to provide a comprehensive assessment of the risk of possible prolonged use of this medicinal plant.

In toxicological study, the changes in body weight are one of the first critical parameters [9] and serve as a sensitive indication of the general health status of animals [2]. The results obtained for this study showed that the aqueous extract of *Bridelia ferruginea* does not affect the body weight in rats after daily administration during three months. This extract provokes in rats, the variations in food and water intake in both sex. These variations of water and food intake did not affect the growth of body weight. These findings are similar to those of some authors who used the methanol extract of *Gouania longipetala* in rats to carry out acute and sub-chronic toxicity profile of this plant [10].

The aqueous extract of *Bridelia ferruginea* did not disturb the the hematological parameters. The prolonged use did not affect the hematopoetic system that performs importants functions and furnishes vital informations in treated animals.

The prolonged use of the aqueous extract of this plant did not modify significantly the glucose level, lipid profile, kidney function. This extract increased significantly (7.81 ± 0.27 vs 6.65 ± 0.23 g/dl control) the protein level in the low dose (100 mg/kg) in male group. According to Singh et *al*. [11], the normal range of total protein is 6.0 to 8.3 g/dl. Considering the work of these authors, this increase is in the normal range of total protein level. The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), used as biochemical markers for hepatotoxicity [12], were not statistically different compared to the control. The aqueous extract of *Bridelia ferruginea* did not disturb liver function in treated animal groups.

In toxicology studies, the organ weights are widely accepted in the evaluation of test article-related effects [13]. There were no significant changes in the relative organ weights of the liver, kidney, heart and lung in male group. However, there was a decrease in relative kidney weight in female group at the dose of 400 mg/kg. It is known that, in toxicity studies, the kidney shows major pathology and according some authors, the changes in kidney weight after administering a chemical or drug, may reflect renal toxicity, tubular hypertrophy or chronic progressive nephropathy [14]. Similar results were obtained by Maronpot et *al*. [15] in female Sprague-Dawley rat treated with 5% myricitrin during 90 days. According to these authors, The decreased organ weights may be related to reduced caloric intake or nutrient displacement. The histopathological analysis of kidney, and liver did not show any gross pathological lesion in all samples. Considering, the findings of Maronpot et *al*. [15], the decrease of relative kidney weight in our study, would be linked to the reduction of food intake and not the toxic effect of *Bridelia ferruginea* in the group IV of female (400 mg/kg).

Table 1: Effect of *Bridelia ferruginea* on the hematological parameters

Groups	Dose (mg/kg p.o)	Haemoglobin (g/dL)	Red blood cell (10^6/mm^3)	White blood cell (10^3/mm^3)	Platelets (10^3/mm^3)
Male					
Group I	Control	15.65 ± 0.65	9.80 ± 0.10	13.05 ± 0.16	357.3 ± 10.11
Group II	100 mg/kg	15.30 ± 0.40	9.65 ± 0.35	13 ± 0.10	371.7 ± 7.27
Group III	200 mg/kg	15.50 ± 0.50	9.70 ± 0.10	12.90 ± 0.10	376.7 ± 12.02
Group IV	400 mg/kg	15.45 ± 0.45	9.75 ± 0.25	13.13 ± 0.38	370 ± 11.55
Female					
Group I	Control	15.10 ± 0.20	8.30 ± 0.20	12.19 ± 0.19	403.3 ± 8.82
Group II	100 mg/kg	15.15 ± 0.85	8.33 ± 0.43	12.25 ± 0.75	396.7 ± 6.67
Group III	200 mg/kg	15.05 ± 0.25	8.40 ± 0.20	12.20 ± 0.70	390 ± 5.77
Group IV	400 mg/kg	15.20 ± 0.30	8.43 ± 0.27	12.49 ± 0.42	396.7 ± 7.27

Values are presented as mean ± SEM, n=8

Table 2 : Effect of aqueous extract of *Bridelia ferruginea* on glucose and lipid profile

Groups	Dose (mg/kg p.o)	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)
Male					
Group I	Control	139.1 ± 6.70	110.7 ± 10.08	50.50 ± 1.01	63.12 ± 3.67
Group II	100 m/kg	117.8 ± 11.15	112.2 ± 0.35	49.06 ± 0.69	61.61 ± 3.45
Group III	200 mg/kg	120.9 ± 9.30	92.76 ± 1.94	50.91 ± 1.01	60.60 ± 3.22
Group IV	400 mg/kg	90.16 ± 10.94	95.50 ± 8.99	48.96 ± 2.23	63.83 ± 2.25
Female					
Group I	Control	85 ± 6.46	86.78 ± 5.36	56.27 ± 2.53	59.72 ± 1.15
Group II	100 m/kg	91.50 ± 8.60	83.27 ± 3.76	53.31 ± 1.45	62.59 ± 0.92
Group III	200 mg/kg	85.93 ± 12.43	81.77 ± 1.85	59.14 ± 2.64	63.15 ± 2.07
Group IV	400 mg/kg	96.37 ± 15.70	77.42 ± 3.79	52.40 ± 1.93	60.77 ± 1.08

Values are presented as mean ± SEM, n=8

Table 3 : Effect of aqueous extract of *Bridelia ferruginea* on kidney function parameters

Groups	Dose (mg/kg p.o)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Male				
Group I	Control	46.33 ± 5.19	6.35 ± 1.91	1.15 ± 0.05
Group II	100 m/kg	45.44 ± 1.54	4.81 ± 0.23	1.29 ± 0.11
Group III	200 mg/kg	51.82 ± 0.52	3.78 ± 0.18	1.20 ± 0.10
Group IV	400 mg/kg	55.15 ± 4.84	5.95 ± 0.78	1.32 ± 0.09
Female				
Group I	Control	40.06 ± 2.16	4.60 ± 0.28	1.37 ± 0.06
Group II	100 m/kg	36.23 ± 1.87	3.99 ±0.16	1.26 ± 0.13
Group III	200 mg/kg	37.30 ± 1.25	4.41 ± 0.44	1.21 ± 0.08
Group IV	400 mg/kg	39.99 ± 1.86	4.33 ± 0.18	1.04 ± 0.10

Results are presented as mean ± SEM, n=8

Table 4 : Effect of aqueous extract of *Bridelia ferruginea* on liver function parameters

Groups	Dose (mg/kg p.o)	AST (IU/l)	ALT (IU/l)	Total protein (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Albumin (g/dl)	ALP (IU/l)
Male								
Group I	Control	42.05 ± 2.84	91.68 ± 2.59	6.65 ± 0.23	2.40 ± 0.26	1.75 ± 0.15	2.56 ± 0.14	53.67 ± 11.03
Group II	100 m/kg	43.92 ± 1.83	93.79 ± 2.79	7.81 ± 0.27*	0.26 ± 0.06	1.54 ± 0.06	2.51 ± 0.07	69.61 ± 18.96
Group III	200 mg/kg	40.74 ± 2.98	92.55 ± 2.25	6.98 ± 0.16	2.62 ± 0.48	1.52 ± 0.06	2.88 ± 0.12	58.73 ± 8.23
Group IV	400 mg/kg	43.12 ± 2.16	93.49 ± 1.50	7.70 ± 0.28	2.58 ± 0.38	1.85 ± 0.17	2.28 ± 0.18	67.79 ± 12.23
Female								
Group I	Control	33.88 ± 2.41	93.77 ± 2.34	7.20 ± 0.25	2.17 ± 0.07	1.80 ± 0.05	2.93 ± 0.16	41.55 ± 6.61
Group II	100 m/kg	33.17 ± 2.31	87.17 ± 2.47	7.66 ± 0.18	2.11 ± 0.02	1.66 ± 0.02	2.97 ± 0.09	50.34 ± 10.61
Group III	200 mg/kg	34.75 ± 1.70	89.44 ± 1.55	7.82 ± 0.05	2.08 ± 0.03	1.43 ± 0.03	3.18 ± 0.07	45.71 ± 4.10
Group IV	400 mg/kg	32.66 ± 3.17	86.80 ± 4.07	7.67 ±0.18	2.25 ± 0.06	1.78 ± 0.15	2.68 ± 0.13	51.17 ± 10.27

The results showed a significant difference in total protein level in group II (100 mg/kg) of male. Values are expressed as mean ± SEM, n=8, *p<0.05 compared to the control group.

Table 5 : Effect of aqueous extract of *Bridelia ferruginea* on relative organ weights of animals.

Groups	Dose (mg/kg p.o)	Relative organ weight (%)			
		Kidney	Heart	Liver	Lung
Male					
Group I	Control	0.66 ± 0.02	0.42 ± 0.01	3.57 ± 0.47	0.82 ± 0.07
Group II	100 m/kg	0.67 ± 0.05	0.45 ± 0.02	3.89 ± 0.51	0.83 ± 0.09
Group III	200 mg/kg	0.64 ± 0.01	0.45 ± 0.02	3.93 ± 0.04	0.84 ± 0.05
Group IV	400 mg/kg	0,78 ± 0,10	0.48 ± 0.07	3.94 ± 0.41	1.13 ± 0.10
Female					
Group I	Control	0.71 ± 0.02	0.48 ± 0.03	3.82 ± 0.28	1.10 ± 0.14
Group II	100 m/kg	0.67 ± 0.03	0.43 ± 0.01	4.15 ± 0.21	1.10 ± 0.02
Group III	200 mg/kg	0.69 ± 0.01	0.46 ± 0.04	4 ± 0.13	1.07 ± 0.07
Group IV	400 mg/kg	0.61 ± 0.02*	0.47 ± 0.02	4.13 ± 0.12	1.18 ± 0.15

All values are presented as mean ± SEM (n=8), *p<0.05 compared to the control group.

CONCLUSION

In this study, the aqueous stem-bark extract of *Bridelia ferruginea* used for the doses of 100, 200 and 400 mg/kg bw, did not cause any mortality in both sex of Wistar rats. This treatment did not affect the body weight of the treated animals after daily administration for 90 days. The treatment did not disturb lipid

profile, liver and kidney functions but decreased the relative kidney weight in female group (400 mg/kg b.w) and the histopathological analysis did not show any tissue damage.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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