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Toxicological assessment of the methanolic leaf extract of *Bridelia ferrugelia*

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ABSTRACT

This study evaluated the acute and subacute toxicity effects of *Bridelia ferrugelia* leaf extract. Observation of the acute group showed that LD₅₀ of the extract is greater than 2000 mg/kg. The subacute investigation was determined by administering 200 mg/kg, 400 mg/kg and 600 mg/kg of the methanolic leaf extract to male Wistar rats for 28 days with distilled water as a control. Haematological and biochemical parameters, as well as lipid levels of vital organs, were examined. Toxicological evaluation of the extract did not produce any significant change in haematological and biochemical parameters in rats. In addition, blood lipids levels were not significantly affected, while dyslipidaemia effect observed in some vital organs were found to be non-lipotoxic. Administration of *Bridelia ferrugelia* at a dose of 200, 400 and 600 mg/kg for 28 days resulted in reduction of cardiac cholesterol level by 37.16%, 39.36% and 17.64% respectively, reduction of pulmonary cholesterol by 22.17%, 28.08% and 6.24 % respectively and dose-dependent decrease in pulmonary triglyceride level by 16.17, 29.14 and 54.25% respectively. This study indicates that *Bridelia ferrugelia* extract administered at 200, 400 and 600 mg/kg did not show any toxic effect on the parameters investigated in rats. Thus, the extract can be considered safe when administered orally.

Keywords: *Bridelia ferrugelia*, Toxicological Evaluation, Plant extract.

INTRODUCTION

Many human and animal diseases are getting treated with the use of herbal medicine majorly in West Africa countries^[1]. Not less than, 8 out of 10 residence of developing countries rely solely on traditional medicines for their treatment in which plants and its derivatives represent 25% of the drugs in use^[1].

Bridelia ferrugelia is one of the most studied plants because of its traditional uses and its pharmacological properties^[2, 3]. It is a tropical plant located mostly in Savannah regions and a member of Euphorbiaceae family^[4] Although the plant is a shrub, it can grow to the size of a tree if the condition is right, it is native to some West Africa countries such as Benin, Nigeria, Burkina Faso and Ivory Coast. As a medicinal plant it is used to treat series of diseases, for example, decoction of its leaves is used to treat diabetes and purgative^[5], the bark extract is used for water treatment^[6] while the roots are effective against intestinal and bladder disorder and skin diseases^[7].

The research finding has revealed that the extract of the plant has trypanocidal^[4], molluscicidal^[8], antimicrobial^[9], anti-inflammatory properties^[10] and antioxidant properties^[11]. The effectiveness of various bark extracts of *B. ferruginea* has been established against some pathogenic organisms^[12]. The therapeutic potentials of *B. ferruginea* have been linked to secondary metabolites, such as terpenoids, glycosides, saponins, anthraquinones, quinones, alkaloids, sterols, and polyphenols that are present in the plant^[13, 14].

However, despite the widespread use of *B. ferruginea* in the treatment of different conditions such as diabetes, information on its toxicity is lacking^[2]. There is a growing need to conduct toxicity study on herbal products to determine their safety for consumption^[15]. Intake of medicinal plants without adequate information on its safety can lead to organs damage and usually, liver and kidney are affected due to their involvement in metabolism and excretion of compounds. Renal damage is particularly associated with the consumption of medicinal plants for treatment of diseases^[16].

Therefore, the aim of the present study was to evaluate the safety of *Bridelia ferrugelia* for consumption by determining its toxicity potential after acute and sub-acute administration of a methanolic extract of the plant to rats.

MATERIALS AND METHODS

Reagents

Folin-Ciocalteu reagent, thiobarbituric acid, sodium carbonate, sodium chloride, sodium hydroxide, trichloroacetic acid, Triton-X100, heparin and manganese chloride were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). All other reagents and chemicals used were of analytical grade.

Plant Material (*Bridelia ferrugelia*)

Fresh leaves of *Bridelia ferrugelia* were obtained from Ilorin and authenticated at Department of Pure and Applied Biology of Ladoko Akintola University of Technology, Ogbomoso, by Prof. A.J. Ogunkunle and a specimen was deposited in the herbarium.

Preparation of *Bridelia ferrugelia* Extract

The powdered sample of *Bridelia ferrugelia* (250g) was soaked in 1100 mL of methanol for 72 hours. The extract was filtered, and the solvent was removed from the extract with a vacuum rotary evaporator at 45°C. The concentrated dried methanolic extract was then stored at -20°C before use.

Animals

Thirty (30) male Wistar strain albino rats with body weights between 200 and 220 g were bought from the University of Ibadan animal house. They were housed in the Ladoko Akintola University of Technology, (LAUTECH) animal house. They were allowed fourteen (14) days to acclimatize before the commencement of the experiment. The animals were maintained on a standard pellet diet throughout the acclimatization and administration period.

Acute toxicity test

The rats were fasted overnight prior to dosing. A rat was weighed each time and given 5000 mg/kg body weight of *B. ferrugelia* by gavage but due to mortality of the rats at this dosage, the experiment was repeated using 2000mg/kg dosage. The animals were closely observed for two hours and subsequently daily for up to 14 days, for gross morphological, physiological, behavioural changes and mortality. All observations were recorded and individual records for each rat were maintained.

Sub-acute toxicity test

Twenty rats were divided into 4 groups of 5 animals each. Group A was given distilled water for 28 days and taken as a control, while Group B, C and D were intubated daily with 200mg/kg, 400mg/kg and 600mg/kg respectively of *B. ferrugelia* for 28 days. At the end of the *B. ferrugelia* treatment, blood was collected from the animals into heparinized tubes by cardiac puncture under light ether anaesthesia and after an overnight fast. The tissues (Liver, kidney, brain, heart, lung and spleen) were removed from the animals for biochemical analyses. The blood samples were centrifuged to separate plasma and red blood cells. All samples were stored at -20°C until analyzed.

Biochemical Analyses

Determination of ALP, AST and ALT activities in Plasma

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), in plasma were determined using enzymatic kits (CYPRESS® Diagnostics, Langdorp, Belgium) according to the manufacturer's instructions.

Preparation of liver homogenate and determination of MDA level

The liver sample was homogenized in Phosphate buffer saline (PBS) to give a 10 % (w/v) liver homogenate. The homogenate was centrifuged, and the supernatant was used to determine MDA level using the method of Draper and Hadley [17]. Briefly, 0.5 mL of liver homogenate was mixed with 2.5 mL of trichloroacetic acid (TCA, 10%, w/v) and boiled in a water bath for 15 min. After cooling, the samples were centrifuged, and 2 mL of each sample supernatant was transferred to a test tube containing 1mL of TBA solution (0.67%, w/v). Each tube was then boiled for 15 min, cooled and absorbance were taken at 532 nm with respect to the blank solution. The concentration of MDA was calculated using absorbance coefficient ($\epsilon = 1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$).

Blood lipid profiles

Plasma concentrations of total cholesterol and triglycerides were determined with commercial kits (CYPRESS® Diagnostics, Langdorp, Belgium). HDL cholesterol and triglycerides were determined in plasma with the same commercial kits for total cholesterol and triglycerides after very low-density lipoproteins (VLDL) and LDL were precipitated with a heparin-MnCl₂ solution [18].

Organ lipid profiles

Lipids in the organs (liver, kidney, heart, brain, lung and spleen) were extracted using a chloroform-methanol mixture (2:1) as described by Folch *et al.* [19]. Cholesterol content in the aliquots were determined by evaporating chloroform-methanol extract to dryness at 60°C. Twenty (20µl) of Triton X-100/ chloroform mixture was added and evaporated after which 1 ml of cholesterol kit reagent was added and mixed. Cholesterol content was determined spectrophotometrically after incubation in the dark for 30 min, [20]. Triglyceride concentrations were determined using the procedure described by Kriketos *et al.* [21]. Aliquot of the chloroform-methanol extract was evaporated to dryness at 60°C, cooled after which 200 µl of ethanol (97%) was added. One (ml) of triglyceride kit was added and mixed. Triglyceride content was determined in spectrophotometer after incubating in the dark at room temperature for 20 min.

Statistical Analysis

Results are expressed as mean \pm S.E.M. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p values < 0.05 were considered statistically significant.

RESULT

Acute Toxicity Test

There was death of rats given 5000 mg/kg body weight of *B. ferrugelia* extract within 24 hours. But when the dosage was reduced to 2000 mg/kg body weight, there were no deaths of rats. However various toxicity behavioural signs were observed such as restlessness, narrowing of the eyelids, irritation, abnormal posture, tachypnoea and anorexia. The LD₅₀ was observed to be higher than 2000 mg/kg body weight/oral route.

Sub-Acute Toxicity Test

Effects of *B. ferrugelia* extract on haematological parameters in rats.

The data in Table 1 show the effects of methanolic leaf extract of *B. ferrugelia* on haematological parameters. At all doses examined, the extract showed non-significant changes in haematological parameters in rats when administered daily for 28 days.

Table 1: Effects of methanolic leaf extract of *B. ferrugelia* on haematological parameters in rats.

Parameter	Control	200 mg/kg	400 mg/kg	600 mg/kg
White blood cell (x 10 ⁹ /L)	4.33a	4.80a	4.52a	4.74a
Red blood cell (x10 ¹² /L)	7.27a	7.46a	6.90a	6.61a
Haemoglobin (g /dL)	13.30a	14.77a	13.80a	12.76a
Haematocrit	40.05a	41.37a	41.43a	38.10a
Mean corpuscular volume (fl)	53.57a	53.40a	53.87a	56.60a
Mean cell haemoglobin (pg)	17.75a	18.97a	18.43a	19.60a
Mean cell haemoglobin concentration (g/dl)	33.25a	35.60a	34.33a	34.80a
Red cell distribution width standard deviation (%)	30.05a	27.63a	26.63a	29.90a
Red cell distribution width count volume (%)	16.93a	14.50a	13.46a	14.80a
Platelet volume (×10 ⁹)	5.89a	5.91a	5.64a	5.38a
Mean platelet volume (%)	6.43a	6.97a	7.27a	7.20a
Platelet width volume (%)	15.25a	15.50a	16.26a	16.00a
Platelet count percent	0.46a	0.42a	0.49a	0.38a

Each value represents the Mean of 5 rats. Values within a row with different alphabets for each group are significantly different at P< 0.05

Effect of *B. ferrugelia* on hepatic antioxidant enzyme activities and MDA levels

Table 2 shows the effects of the ethanol leaf extract of *B. ferrugelia*

on liver enzymes and hepatic lipid peroxidation in rats. The extract did not elicit any significant effect on various biochemical parameters and hepatic lipid peroxidation.

Table 2: Effects of methanolic leaf extract of *B. ferrugelia* on AST, ALT, ALP and MDA

Parameter	Control	200 mg/kg	400 mg/kg	600 mg/kg
AST	121.25 ± 3.74a	123.59 ± 2.41a	118.35 ± 3.22a	125.37 ± 2.82a
ALT	73.52 ± 2.11a	71.63 ± 1.47a	75.72 ± 3.14a	74.48 ± 2.92a
ALP	92.42 ± 3.81a	96.25 ± 3.63a	90.93 ± 2.74a	94.53 ± 3.44a
MDA (x10 ⁻⁶)	1.84 ± 0.20a	1.75 ± 0.24a	1.53 ± 0.26a	1.82 ± 0.22a

Each value represents the Mean of 5 rats. Values within a row with different alphabets for each group are significantly different at P< 0.05

Effects of methanolic leaf extract of *B. ferrugelia* on blood lipids in rats

As presented in Table 3 sub-acute administration of *B. ferruginea*

extract to rats for 28 days did not significantly (*p* < 0.05) affected both cholesterol and triglyceride levels of plasma, HDL and VLDL+ LDL when compared with the control animals.

Table 3: Effects of methanolic leaf extract of *B. ferrugelia* on blood lipids in rats

Parameter	Control	200 mg/kg	400 mg/kg	600 mg/kg
Plasma Cholesterol	83.69 ± 4.56a	83.01 ± 1.40a	93.10 ± 1.96a	76.70 ± 2.80a
Plasma Triglyceride	79.30 ± 5.68a	80.42 ± 6.98a	76.67 ± 2.40a	73.75 ± 2.17a
HDL Cholesterol	41.15 ± 3.33a	37.50 ± 6.50a	39.38 ± 1.08a	35.00 ± 1.44a
HDL Triglyceride	49.72 ± 3.88a	47.72 ± 2.43a	45.48 ± 1.82a	44.93 ± 4.86a
LDL+VLDL Cholesterol	42.55 ± 2.15a	45.51 ± 2.78a	53.73 ± 0.88a	41.70 ± 1.36a
LDL+VLDL Triglyceride	25.58 ± 3.34a	32.70 ± 1.22a	27.19 ± 0.97a	21.82 ± 0.63a

Each value represents the Mean of 5 rats. Values within a row with different alphabets for each group are significantly different at P< 0.05

Effects of methanolic leaf extract of *B. ferrugelia* on tissue lipids in rats

Results of tissue lipids analysis are presented in Table 4. Administration of *B. ferruginea* for 28 days produced no significant changes in the liver, spleen, kidney cholesterol and triglyceride levels. However, all doses of *B. ferruginea* extract (200, 400 and 600 mg/kg)

significantly decrease the cardiac cholesterol level by 37.16%, 39.36% and 17.64% respectively while cardiac triglyceride level was reduced in a dose-dependent manner. Similarly, there was a dose-dependent decrease in pulmonary triglyceride by 16.17%, 29.14% and 54.25% respectively while all the three doses caused a reduction in pulmonary cholesterol by 22.17%, 28.08% and 6.24 % respectively.

Table 4: Effects of methanolic leaf extract of *B. ferrugelia* on tissue lipids in rats

Tissues Lipid	Control	200 mg/kg	400 mg/kg	600 mg/kg
Hepatic cholesterol	0.78 ± 0.05a	0.66 ± 0.02a	0.63 ± 0.11a	0.79 ± 0.09a
Hepatic triglyceride	2.21 ± 0.26	1.69 ± 0.10a	1.53 ± 0.01a	1.74 ± 0.19a
Renal cholesterol	2.57 ± 0.16a	2.28 ± 0.13a	2.58 ± 0.21a	2.52 ± 0.25a
Renal triglyceride	2.00 ± 0.10a	1.91 ± 0.22a	2.09 ± 0.15a	2.37 ± 0.06a
Cardiac cholesterol	9.07 ± 0.38a	5.70 ± 0.30b	5.50 ± 0.15b	7.47 ± 0.27c
Cardiac triglyceride	7.60 ± 0.72a	5.45 ± 0.24b	4.36 ± 0.29b	3.22 ± 0.30c
Pulmonary cholesterol	6.09 ± 0.33a	4.74 ± 0.31b	4.38 ± 0.31b	5.71 ± 0.34a
Pulmonary triglyceride	13.42 ± 0.45a	11.25 ± 0.76a	9.51 ± 0.32b	6.14 ± 0.72c
Splenic cholesterol	7.49 ± 0.23a	8.86 ± 0.48a	8.08 ± 0.23a	7.10 ± 0.41a
Splenic triglyceride	6.50 ± 0.36a	6.45 ± 0.22a	5.95 ± 0.09a	5.81 ± 0.28a

Each value represents the Mean of 5 rats. Values within a row with different alphabets for each group are significantly different at P< 0.05.

DISCUSSION

The interest in the use of medicinal plants to treat ailments is on the increased because of the belief that it is safe and has no side effects [22]. The surge in the number of people that take medicinal plants couple with a lack of data on the safety of many medicinal plants demands that toxicity study need to be conducted on herbal products [15]. Acute oral toxicity test is usually the first test to be done when assessing the toxicity of any plant [23], this is to ascertain the adverse reactions to either a single dose or overdose of the agent [24]. In this study, *B. ferruginea* did not produce any mortality at 2000 mg/kg, although this dosage alters the behavioural patterns of the rats. This indicates that the oral LD₅₀ of *B. ferruginea* extract is greater than 2000 mg/kg body weight and can be considered to be non-toxic [25]. The high safety value obtained in this study may suggest the reason why *B. ferruginea* is a popular medicinal plant.

Subacute toxicity studies were carried out in this study to know the organs which may be affected by plant extract. Evaluation of haematological parameters can be used to determine the effect of plant extract on blood functions [26]. Fluctuation in the value of plasma constituents could indicate haematotoxicity [27]. The physiological and

pathological status of a man and animals can be determined through blood parameters such as white blood cell count, haemoglobin, packed cell volume and platelets count [28]. Ingestion of toxic plants can alter the normal range of these parameters [29, 30]. These blood indices were all measured after oral administration of *B. ferruginea* for 28 days with no significant change when compared with control values, suggesting that the extract did not affect the erythropoiesis, osmotic fragility and morphology of the red blood cells [31].

The clinical chemistry analysis was carried out to evaluate the possible alterations in hepatic functions of the extract treated rats compared to controls. *B. ferruginea* methanolic leaf extract did not produce any significant changes in biochemical parameters after 28-day of treatment. Evaluation of liver function is very important when analysing toxicity of drugs and plant extracts because of its relevance for the survival of the organism [32]. High levels of alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) are an indicator of hepatotoxicity or liver diseases [33]. The non-significant changes in the values of AST, ALT and ALP indicate that subacute administration of *B. ferruginea* leaf extract has no adverse effect on hepatocyte function. In addition,

analysis of lipid peroxidation level in hepatocyte indicates no significant increase in this parameter was observed when the treated group were compared to the control group. A rise in lipid peroxidation level is only identified if there is oxidative damage due to the increase in free radical generation.

The effects of sub-acute administration of *B. ferruginea* extract for 28 days on blood lipid was investigated in this study. The analysis of blood lipid is important because high levels of lipids have been linked to atherosclerosis (hardening of arteries) and by implication, increase in the risk of heart disease and stroke [34]. In this study, cholesterol and triglyceride levels of plasma, HDL and VLDL+ LDL were not significantly affected by all the three doses of *B. ferruginea* use in this study when compared with the control animals. High-Density Lipoprotein-Cholesterol (HDL-C) is known as the good cholesterol because of it relevant to the cardiovascular system. When HDL-C is lower, there is a higher chance of cardiovascular disease [35]. LDL is the main source of blood cholesterol [36]. LDL acts as the main transporter of cholesterol and cholesteryl esters and makes up more than half of the total lipoprotein in plasma [37]. Increase in the level of lipids in the plasma could lead to atherosclerosis which could progress to coronary heart disease [34]. Therefore, non-significant changes in blood lipids observed in this study suggest a wide safety margin of *B. ferruginea* extract.

The effects of administration of *B. ferruginea* extract for 28 days on tissues lipids were also investigated in this study. This study is important because evidence abounds which indicated that lipid accumulation in the liver is associated with Non-alcoholic fatty liver disease (NAFLD) [38, 39]. Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and several studies have also linked NAFLD to an increased risk of cardiovascular disease [40, 41]. Increasing evidence also suggests that triglyceride accumulation in the liver plays an important role in the pathogenesis of obesity, diabetes and cardiovascular disease [42]. Also, cholesterol and triglyceride accumulation in the kidney may lead to kidney disease, including metabolic syndrome [43], chronic glomerulopathy, nephrotic syndrome, obesity-associated renal disease and acute kidney injury [44]. In this study, there were no significant differences in the liver, spleen and kidney cholesterol and triglyceride levels of the treated and control groups indicating that the plant is safe for use at the proposed doses. However, both 200 and 400 mg/kg doses of *B. ferruginea* extract significantly decrease the cardiac cholesterol level while cardiac triglyceride level was reduced in a dose-dependent manner. Similarly, there was a dose-dependent decrease in pulmonary triglyceride while all the three doses also caused a reduction in pulmonary cholesterol. The reduction in cardiac cholesterol and triglyceride observed in this study suggests the usefulness of *B. ferruginea* in helping to ameliorate various cardiac conditions that are precipitated by high cholesterol and triglycerides levels.

CONCLUSION

The findings of the present study indicate that acute administration of *B. ferruginea* extract did not cause mortality in rats. The results obtained in respect to the subacute toxicity study suggest that the leaf extract is relatively safe when administered orally. and lack of toxic effect renders *B. ferruginea* a candidate for bioassay-guided isolation of compounds which can use for drug development against diseases.

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