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Research Article
Sub-chronic (28-days) Toxicity Study of Hydroalcohol Stem Bark Extract of *Bridelia ferruginea* (Euphorbiaceae) on Wistar Rat

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Abstract

Background and Objective: *Bridelia ferruginea* is a widely plant used in Togo for traditional healing of many diseases such as sickle cell anemia, bladder disorders, malaria, rheumatism and diabetes. Many parts of the plant are then, used for this purpose. This study aims to investigate the subacute toxicity of the stem bark of *Bridelia ferruginea* by repeated oral dose of 28 days on Wistar rats. **Materials and Methods:** Three groups of 8 rats were used to evaluate the subacute toxicity of *B. ferruginea* stem bark extract. The first group served as control and received distilled water and the two other groups received, respectively 500 and 1000 mg kg^{-1} of extract. The solutions were administrated orally to rats for 28 days and each animal received 10 mL kg^{-1} of solution. At the end of the study, hematological and biochemical parameters were recorded as well as the histological study of some organs. **Results:** The 28 day repeated oral administration of *B. ferruginea* stem bark extract at 500 and 1000 mg kg^{-1} did not induce significant changes in hematological and biochemical parameters except for creatine kinase when compared to the control group. No architectural changes were observed on organ histological data in all groups. **Conclusion:** The hydroalcoholic extract of *B. ferruginea* stem bark is relatively safe for oral administration in Wistar rats as no toxic effect was observed during the 28 days of administration.

Key words: *Bridelia ferruginea*, subacute-toxicity, hematological parameters, biochemical parameters, Wistar rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The world population, more particularly African, uses plants for the treatment of various diseases. Then in Africa, more than 80% of the population uses plants for their health care problems[1].This is an ancient practice which is maintained to nowadays due to a generation to generation transmission. Unfortunately, the use of the medicinal plant in traditional healing often is made regardless of plants toxicity. Thus, many plants are incriminated in intoxication cases[2].

Bridelia ferruginea belonging to the Euphorbiaceae family's, is widely used plant in Togo for its different properties: bladder disorders, anti-dysentery, anti-arterial hypertension, anti-rheumatism pain[3], anti-diabetic[3-6], anti-bacterial[4,7], anti-inflammatory, laxative, antispasmodic, anti-malarial[8], antioxidant[7,9-11], anti-sickle cell anemia[11] activities. Many parts of the plant are used in traditional healing such as root and stem bark, leaves or fruits.

In Togo, Bakoma *et al.*,[12] studied the acute and sub-chronic toxicity of hydroethanolic extract from the root bark of *B. ferruginea*. The study of Bakoma *et al.*[12] done in the department concerned the root bark of *B. ferruginea* and the present study looked at the stem bark of the same plant.

The purpose of this study was to investigate the sub-chronic toxicity of the stem bark of *B. ferruginea* by repeated oral dose of 28 days on Wistar rats.

MATERIALS AND METHODS

Collection and extraction of plant materials: Fresh stembark of *B. ferruginea* was collected in October, 2019 at Noépé, situated at 24 km northwest of Lomé (coordinates 6°17'21.7"N 1°05'19.3"E). Botanical identification has been made by the Department of Botany, Faculty of Sciences, University of Lomé, Togo. A voucher specimen was deposited at the herbarium of University of Lomé.

The stem bark of *B. ferruginea* was washed, dried, crushed and macerated at laboratory temperature in a hydro-ethanolic solution (50/50) for 72 hrs. The macerate was then filtered and evaporated at 45° by rotavapor.

Animals: Wistar male rats of average weight of 145 g were used for this study. These rats were acclimated in the pet store of the Faculty of Sciences (24-25°, alternating 12 h of light and 12 hrs of darkness) for 2 weeks.

Phytochemical screening: The qualitative screening was done according to conventional methods[13,14].

Repeated dose 28-day oral toxicity study: The rats were grouped into 3 balanced batches of 8 rats each, including a control batch. The control batch received distilled water containing 2% of Twin 80. The treated groups received, respectively 500 and 1000 mg kg^{-1} of *B. ferruginea* stem bark extract. The extract was dissolved in distilled water with 2% Twin 80.

Solutions were administered orally every day at 8 am for 28 consecutive days to each rat depending on the batch. Each rat received 10 mL kg^{-1} of preparation solution. Every three days during the study, each rat is weighed and its weight is recorded.

On the 29th day, after observing a 12 hrs fasting the day before, each rat undergoes anesthesia using ether in a closed vase. A blood sample is taken using a capillary tube at the retro-orbital level and collected on a dry tube and a tube containing EDTA. The animal is then sacrificed and some organs (heart, kidney, spleen, liver and testicle) are carefully isolated and then weighed and their relative weights expressed. A histological study of each organ follows.

Hematological analysis: The tubes containing EDTA were used for this analysis. Red Blood Cell (RBC) count, Haemoglobin (HGB), White Blood Cell (WBC) count, Neutrophil (PN) and Platelet (PLT) were determined on automated haematology analyser (BC 6800, Mindray, China).

Biochemical analysis: The dry tubes were used for this analysis. The tubes were centrifuged at 3000 g for 10 min. On the serum obtained, the following parameters were studied: Urea (UREA), Glucose (GLU), Creatinine (CREA), Calcium (CA), Magnesium (MG), Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatases (ALP), Sodium (NA), Potassium (K), Chlorine (Cl), Creatinine kinase (CK). Cobas c311 analyzer, Roche, USA was used for the analysis.

Statistical analysis: The results were expressed as mean±SEM (standard error of the mean). The data were subjected to one-way analysis of variance (ANOVA) test and the different between groups was determined by Tukey's test, using the graph pad prism statistical software (Graph Pad Software Inc., USA). Results were considered significant at $p<0.05$.

RESULTS

Extraction and phytochemical screening: From the evaporation 24,7% of dry extract was obtained. The phytochemical screening revealed the presence of tannins,

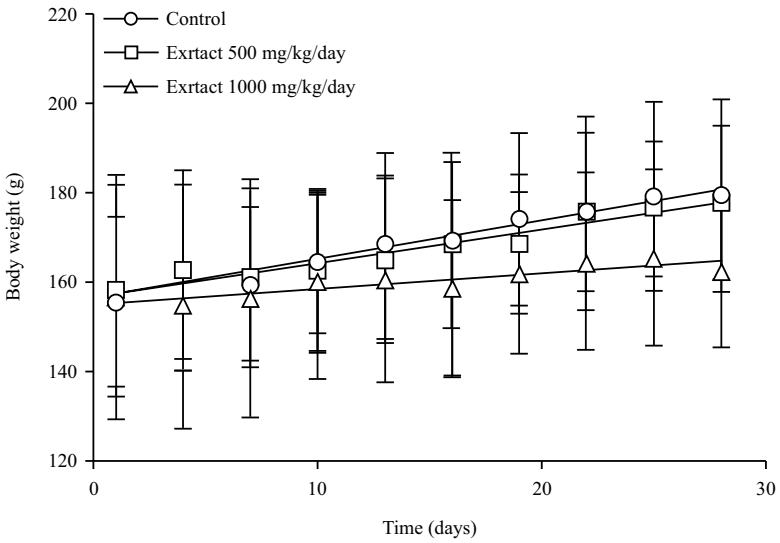

Fig. 1: Mean body weigh tot rats during 28 days treatment with hydroethanolic extract of *B. ferruginea* stem dark

Table 1: Effects of aqueous stem bark extract of *B. ferruginea* on relative weight of organs of rat

Parameters	Control	500 (mg kg^{-1})	1000 (mg kg^{-1})
Heart	0.412±0.024	0.360±0.013	0.384±0.013
Liver	3.089±0.150	2.775±0.105	2.869±0.119
Kidney	0.645±0.027	0.610±0.015	0.641±0.019
Spleen	2.055±1.849	0.171±0.010	0.190±0.007
Testicle	0.664±0.227	0.639±0.203	0.616±0.204

n = 8, data is represented as mean±SEM

Table 2: Haematological parameters of rats after 28 days treatment with hydroethanolic stem dark of *Bridelia ferruginea*

Parameters	Control	500 (mg kg^{-1})	1000 (mg kg^{-1})
RBC (×10^{12})	7.66±0.19	7.79±0.23	7.50±0.08
HGB (g dL^{-1})	12.81±0.27	13.39±0.27	12.61±0.98
WBC (×10^{9})	6.66±0.95	6.50±0.71	7.61±0.98
PN (×10^{9})	1.90±0.26	1.52±0.18	2.42±0.51
PLT (×10^{9})	581.00±55.60	638.63±81.07	523.38±18.66

Results are presented as Mean±SEM (n = 8), RBC: Red blood cell, HGB: Haemoglobin, WBC: White blood cell, PN: Neutrophil and PLT: Platelet, no significant difference if results are compared to the control

flavonoids, reducing sugars and absence of alkaloids, saponins, anthracenes, cardiotonic heterosides, sterols and terpenoids in the stem bark hydro-alcoholic extract of *B. ferruginea*.

Repeated dose 28 days oral toxicity

Mortality and behavioural changes: The aqueous stem bark extract of *B. ferruginea* did not produce any mortality and no behavioural changes when administered orally at 500 or 1000 mg kg^{-1} for 28 days.

Body weight: There were no significant differences in the weight of animals during the experimentation when compared with control (Fig. 1).

Organ relative weight and macroscopic observation: Some variations in organ weights were noted, but these variations were not significant in comparison with the control (Table 1).

Haematological parameters: No significant differences were observed according to evaluated parameters when compared treated groups to control after 28 days of administration. The values are in the Table 2.

Biochemical parameters: The data in Table 3 show the effects of aqueous stem dark extract of *B. ferruginea* on biochemical parameters. No significant changes were find in the clinical chemistry parameters measured between the treated and controls groups except for Creatine kinase.

Table 3: Biochemical parameters of rats after 28 days treatment with hydroethanolic stem dark of *B. ferruginea*

Parameters	Control	500 (mg kg^{-1})	1000 (mg kg^{-1})
Urea (mg dL^{-1})	41.75±2.87	42.13±3.24	39.88±4.01
GLU (mg mL^{-1})	61.88±3.68	65.50±3.16	57.50±5.61
CREA (mg mL^{-1})	0.41±3.68	0.39±0.02	0.40±0.00
CA (mg dL^{-1})	10.20±0.16	9.93±0.07	9.85±0.12
MG (mg dL^{-1})	2.60±0.11	2.43±0.08	2.13±0.05
AST (UI L^{-1})	120.13±11.35	110.13±12.42	118.50±10.66
ALT (UI L^{-1})	45.75±5.95	43.38±5.41	40.00±3.74
GGT (UI L^{-1})	0.13±0.12	0.76±0.31	0.26±0.25
ALP (UI L^{-1})	100.13±11.55	64.25±9.13	60.50±4.04
Na (mmol L^{-1})	144.63±0.38	143.50±0.53	143.63±0.42
K (mmol L^{-1})	11.43±6.09	4.75±0.17	4.95±0.10
Cl (mmol L^{-1})	101.00±0.60	100.88±0.35	102.00±0.38
CK (UI L^{-1})	459.83±61.62	312.29±66.46***	310.17±39.70***

Results are expressed as Mean±SEM, ***p<0.001 vs control, Urea: Urea, GLU: Glucose, CREA: Creatinine, CA: Calcium, MG: Magnesium, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatases, NA: Sodium, K: Potassium, Cl: Chlorine, CK: Creatinine kinase

Histological studies: No obvious histological changes are observed in organs (heart, kidney, spleen, liver and testicle) of animals received an extract of stem bark of *B. ferruginea* compared to controls.

DISCUSSION

The root or stem bark, leaves and fruits of *B. ferruginea* are widely used in Africa because of their many properties[7].

The stem bark extracts of *B. ferruginea* at 500 and 1000 mg/kg/day did not produce signs of toxicity in the treated rats, similarly, no dead animals were identified during the 28 days.

The variations in weight observed during the 28 days between the treated animals and the control did not give any statistically significant difference. These results are in contradiction with those of Bakoma *et al.*[12], who had used the root bark. There would therefore be a difference in the composition of the extracts of root and stem bark.

Toxicity testing is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when data are translated from animal studies[15]. Awodele *et al.*[16] reports that according to Degruchy, RBC and Hb are very important in transferring respiratory gases. Thus, in this study, the non-significant effect on RBC and HGB in the test groups, compared to the control, implies that there was no change in the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following the administration of various doses of the extract to the test animals. Obtained results are comparable to those of Awodele *et al.*[16]. The increase in Hb is in contrast to the findings of Olarewaju *et al.*[17], who reported a decrease in Hb in the test groups administered aqueous extract of *B. ferruginea* stem bark, compared to control.

The total of white blood cells is slightly high at the administration of high dose of extract compared to the control, but this difference is not statistically significant. The number of neutrophils is following the same trend. This same observation is made by Owodele *et al.*[16], who believe that the extract stimulate neutrophils to promote phagocytosis (cellular ingestion of offending agents). High neutrophil counts can be the result of many factors that include bacterial infection, acute inflammation, stress response effect from some drugs and splenectomy, among others[18].

The biochemical parameters explored did not make it possible to find toxicity with regard to the liver, the kidneys, or electrolyte disorders. The same results were observed by Bakoma *et al.*[12] and Awodele *et al.*[16].

The reduction of glucose observed with the administration of a high dose of extract confirms the anti-glycemic property of the plant. This reduction is less significant with the extract of the stem bark than that of the root bark[12].

Significantly lower CK was found between the treated animals and the control. Bakoma *et al.*[12] using root bark extracts did not find any difference between the treated animals and the control. CK level is an indicator of heart or muscles toxicity. The histological study of the different organs removed did not find any significant histological modification. Further studies must be then carried out to investigate the effect of the extract on the heart in view to understand the decrease in CK level. Additionally long-term toxicity study must be conducted to reinforce the safety of *B. ferruginea* stem bark hydroalcoholic extract.

CONCLUSION

The study of the subacute toxicity of the aqueous extract of *B. ferruginea* stem bark did not reveal any significant

modification in terms of weight, organ structure and hematological and biochemical parameters. This could provide some security in the long-term administration of *B. ferruginea* extract. The results can be superimposed on those obtained from root bark.

SIGNIFICANCE STATEMENT

This study revealed that the stem bark hydroalcoholic extract is relatively safe then its large traditional use for healing diseases is therefore comprehensible. This study can be a background for researchers for exploring the pharmacological activities of *B. ferruginea* stem bark.

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