EFFECTS OF ETHANOLIC SHOOTS EXTRACT OF *Borassus aethiopum* ON SOME HAEMOSTATIC PARAMETERS AND LIVER HISTOLOGY IN WISTAR RATS

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ABSTRACT

Background: *Borassus aethiopum* shoot is called *muruchi* in Hausa, used as *Aphrodiasic*, antibiotic as food locally consumed and cultivated in many parts of Africa. Haemostasis is an important mechanism for the control of bleeding through the use of vWF, F V, F VII or F VIII (Kalot *et al.*, 2022). Liver is an important organ for detoxification (Mujahid *et al.*, 2017).

Aim: The study aimed at determine the effects of ethanolic shoots extract of *B. aethiopum* on Haemostatic Parameters and Liver histology in wistar rats.

Methodology: For the acute toxicity study, the median lethal dose (LD_{50}) was determined using Lorke's method while for Sub acute toxicity study, twenty (20) males Wistar rats weighed 120-200g were divided into four groups of five rats (n=5) each. The groups 1 (control), 2, 3 and 4 were received 2 ml Distilled water, 300, 600 and 1,200 mg/kg of extract for 28 days. Body weight recorded daily. The haemostatic parameters were analyzed using ELISA (Enzyme linked immunosorbent assay) method. Liver histology were examined using method of Microscopy.

Results: The LD_{50} was determined at greater than (>5,000) mg/kg. No mortality was recorded after toxicity Study. No significant change in the body weight. There was no significant increase in haemostatic parameters; von Willebrand factor (vWF), Clotting Factor V (Labile factor) (FV), Clotting Factor VII (Tissue factor) (FVII) and Clotting Factor VIII (Antihaemophilic factor) in the treated groups compared to control group ($p>0.05$). The histology of Liver microscopy showed histopathological changes with periportal inflammations, unremarkable hepatocytes compared to the control group.

Conclusion: This finding shows that the ethanolic shoots extract of *B. aethiopum* did not exert significant changes in the haemostatic parameters compared to the control group. However, histopathological changes were observed in the liver of Wistar rats.

Key words: *Borassus aethiopum*, Haemostatic Parameters, Liver Histology, Wistar Rats.

INTRODUCTION

The *Borassus aethiopum* shoot is a young germinating seed of *B. aethiopum* tree (Merlin *et al*., 2019). The tree is a genus of five species; *Borassus aethiopum* found in Africa, *B. flabellifer* found in Asia, *B. akeassii* found in central Africa, *B. heineanus* found in New Guinea and *B. madagascariensis. Borassus aethiopum* (African palmyrah fan palm) belongs to the family Aracaceae, found in Nigeria, can grow up to 30 m height (Merlin *et al*., 2019). The tree is called *Giginya* in Hausa, *Ubiri* in Igbo and *Agbonolodu* in Yoruba language with dioecious seed having male and female flower from separate tree, the fruits contain 1 to 3 seeds (cotyledon) (Bayton, 2007 and Ahmed *et al.,* 2023).

The truck part of the tree is used in house roofing, leaves used in making basket, the fruits as food, the seeds as soft drinks; sugar drink, alcohol (common name) and medicinal. The seed germinate between 7 to 8 weeks (Balami *et al.*, 2016). The shoot is called *muruchi* in Hausa reported to possess medicinal and non-medicinal important where the boiled or raw shoots are consumed as antibiotic, *Aphrodisiac* for men and *Libido* for women to stimulate sexual performance or consumed as food in most parts of Nigeria especially the North (Akinniyi *et al*., 2012).

Figure 2.1: The *B. aethiopum* shoots (*muruchi or Gazari)* (A) The *B. aethiopum* Tree (B)

(Muhammad *et al*., 2019).

Haemostasis is a physiological process within vascular tissues, occur in response to injury and normal blood flow. Components of Haemostasis; Primary Haemostasis (platelet activation), Secondary Haemostasis (coagulation cascade activation) and Tertiary Haemostasis (activation of fibrinolytic system) (Acharya *et al*., 2004). Normal haemostasis involve maintenance of normal blood flow or a series of steps in response to injury through conversion of fibrinogen to fibrin clot (Osmosis.org, 2023).

von Wilebrand Factor (vWF) is protein produced by endothelium and platelet, deficiency of vWF is called von Wilebrand Disease (vWD); Type1vWD, Type2 or Type3, cause by inherited mutated gene or acquired, result to bleeding disorder, von Willebrand disease is indicating deficiency of vWF (Bharati and Prashanth, 2011 and Kalot *et al.*, 2022). Clotting Factor V (Labile factor or proaccelerin) is a coagulation factor, synthesized in the liver, play role in activation of prothrombin to thrombin for conversion of fibrinogen to fibrin. FV deficiency may be acquired (Liver disease) or due to genetic mutation in (gene of 1q23) called Factor V leiden (Batt *et al*., 2021).

Researchers found that F5 Leiden mutation resist inactivation by protein S and protein C inhibitors, leading to clots formation to thrombosis (Huang and Koerper, 2008). Clotting Factor VII (contact factor) on activation become FVIIa and activate F Xa leading to different steps and form fibrin clot. Clotting Factor VIII (Antihaemophilic factor) is a cofactor in a complex form as a key mechanism for haemostasis, it is a plasma protein important for normal blood clot, its deficiency called haemophilia A disease, due to x-link genetic mutation, leading to uncontrol bleeding in small injury (Jace, 2021). According to United State (U.S) Centers for Disease Control (CDC), (2022) about 1 in 10,000 peoples in U.S has Haemophilia A, males are more affected than female that inherited heterozygote carrier gene (Tanako *et al*., 2023). Disease of haemophilia A is a deficiency of clotting factor VIII, Haemophilia B deficiency of clotting factor IX and Haemophilia C is a deficiency of clotting factor X (Osmosis.org, 2023). Pathophysiology of haemostasis result to thrombosis or bleeding disorder (Kalot *et al.*, 2022).

With global increase in use of herbal medicine, there is paucity of data on effects of ethanolic shoots extract of *B. aethiopum* on some haemostatic parameters and liver histology. Despites its use in medicinal as antibiotic, *aphrodisiac* and *libido* for sexual performance or as food, even though it may have some effect on the liver enzymes as reported by Tata *et al*., (2021), as most of the clotting factors are produced in the liver. Therefore, this study was aimed to determine the effects of ethanolic shoots extract of *B. aethiopum* on some haemostatic parameters and liver histology in wistar rats.

MATERIALS AND METHODS

Plant Preparation

Fresh shoots of *B. aethiopum* were procured from Toro town market (Lat. 10.0596 º N, Long. 9.0709º E), Toro Local Government Area of Bauchi State. Plant sample was identified and authenticated with a Voucher number: PCG/UDUS/ARC/0001 by a Botanist in the Herbarium of the Department of Pharmacognosy, Usmanu Danfodiyo University, Sokoto. The Barks and fibers of the shoots were removed and peeled, the shoots were washed and cut using a stainless knife, then allowed to air dry under shade. The shoots were *pulverized* into powdered form using mechanical grinder.

Plant Extraction

The *Borassus aethiopum* shoots weighed 22.4 kg and the shoots powder of 1.6 kg was used in the extraction using 8 L of absolute ethanol by cold maceration method for 48 hours with periodic shaking. The solution was filtered using filter paper to remove the marc. The filtrate was air dried in hot air oven at 50ºC. The shoots extract of 64.7g was extracted with percentage yield of 4.04% and stored before analysis at Pharmacognosy laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

Ethical clearance

Ethical approval was obtained on the use of animal, from Departments of Pharmacology Usmanu Danfodiyo University, Sokoto with approval code: PTAC/Ba/(Ee)/OT/65-23.

Experimental design

Thirty-two (32) healthy male Wistar rats of twelve (12) for LD_{50} and twenty (20) for treatment, weighed about 120-200g using weighing balance (ATOM®-A110C) were purchased from Ahmadu Bello University Zaria and kept to *acclimatize* with the environment for two weeks (14 days). The animals were fed with standard diet (Chikun® Broiler feed) and water *ad libitum* for 28 days at Animal house, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto (UDUS).

Acute toxicity study (determination of LD_{50} of ethanolic shoots extract of *B. aethiopum*)

Total of twelve (12) Wistar rats were used for LD_{50}.

In phase I, nine rats were divided into three groups of three rats (n=3) each. Group 1, 2 and 3 received oral tea doses of 10, 100 and 1,000 mg/kg and were observed for 24 hours. In the second phase II, three rats were divided into three groups of one rat (n=1) each. Group 1, 2 and 3 received oral tea doses of 1,600, 2,900 and 5,000 mg/kg, then observed for 24 hours for any signs of toxicity or mortality and weight recorded by Lorke's method (1983).

Sub-acute toxicity study (administration of ethanolic shoots extract of *B. aethiopum*)

Total of twenty (20) Wistar rats were grouped into four groups of five rats (n=5) each, were administered as group 1 (control), 2, 3 and 4 received oral tea doses of 2ml of distilled water, 300, 600 and 1,200 mg/kg oral doses of ethanolic shoots extract using canula with daily record of body weight.

Blood Sample Collection

Wistar rats were sacrificed on day 28 after exposed to chloroform vapour. Blood sample was collected through cardiac puncture using syringe and needles, whereby 3ml of blood were collected into K_3EDTA containers and plasma obtained by centrifugation for clotting factors assay. The Liver organs were harvested using surgical blade and collected into universal container with 10% formalin as fixative for histology of liver microscopic examination.

Laboratory Analysis

Determination of haemostatic parameters in Wistar rats

The haemostatic (clotting factors) von Willebrand Factor (vWF) with Lot no. PRS-30683Ra, Factor V (F5) with PRS-30168Ra, Factor VII (F7) with PRS-31023Ra and Factor VIII (F8) with PRS-30904Ra were determined by Nanjing Pars Biochem Elisa reagent kits, China, using Rayto RT 2100C, Elisa plate reader machine (Dacie and Lewis 1950).

Principle of the assay procedure of the clotting factors (haemostatic parameters)

The parameters have the same principle as vWF, Factor V (F5), Factor VII (F7) and Factor VIII (F8) Rat Plasma samples. Purified antibody specific to each clotting factor coated on microplate wells, formed solid-phase antibody. In addition of plasma samples combined with HRP (Horseradish Peroxidase) labelled enzyme, formed antibody-antigen-enzyme-antibody complex. After complete washing, addition of TMB (Tetra Methyl Benzidine) substrate, formed blue colour. Then Sulphuric acid solution added and stopped the reaction, turned blue colour to yellow and measured spectrophotometrically at wave length of 450 nm.

Determination of histology of Liver microscopy on Wistar rats

Samples were analyzed in Histopathology Laboratory, Usmanu Danfodiyo University Teaching Hospital (UDUTH) of Usmanu Danfodiyo University Sokoto, where the tissues were processed, stained with (H and E) and examined microscopically.

Data analysis

Data were analyzed using IBM SPSS (Statistical Package for the Social Sciences) version 27.0. The haemostatic parameters were analyzed using one-way analysis of variance (ANOVA) as (Mean±SEM). The P-value ($p \leq 0.05$) was considered statistically significant.

RESULTS

Effects of Acute toxicity (LD_{50}) of ethanolic shoots extract of *B. aethiopum* in Wistar rats

Median Lethal dose (LD_{50}) of ethanolic shoots extract of *B. aethiopum*: In phase I and phase II, indicated no signs of toxicity and no mortality was recorded after 24 hours. Therefore, LD_{50} was determined to be greater than 5,000 mg/kg.

Table 1: Effects of acute toxicity LD_{50} on ethanolic shoots extract of *B. aethiopum*

Doses (mg)	Inference	
	Phase I groups	Phase II groups
10	0/3	-
100	0/3	-
1,000	0/3	-
1,600	-	0/1
2,900	-	0/1
5,000	-	0/1

The LD_{50} was >5,000mg/kg body weight at the administered doses in Wistar rats.

Key: 0/3 and 0/1; 0 indicated no death recorded, 1 and 3 indicated number of rats per group

Table 2: Effects of Ethanolic Shoots Extract on the Body weight of Wistar rats

	Mean±SEM			
Weight	Control	300mg/kg	600mg/kg	1200mg/k
WEEK0 (g)	123.20±3.652	124.40±2.462	128.40±2.544	130.20±4.198
WEEK1(g)	140.20±4.716	157.80±6.065	153.20±3.238	157.80±3.196
WEEK2 (g)	133.30±3.203	148.80±5.869	152.00±2.804	151.20±4.502
WEEK3 (g)	134.40±3.210	142.20±6.659	146.40±4.379	147.20±5.722
WEEK4 (g)	122.40±2.438	134.60±6.925	135.80±7.850	131.00±2.322

There were no statistically significant changes in the body weights of the treated group when compared with the untreated (control) group ($p>0.05$).

Key: Values express as Mean±SEM (standard error of mean), (n=5), one-way ANOVA (analysis of variant), Group 1 control (2ml Distilled water), group2 (300), group3 (600) and group 4 (1,200 mg/kg).

Effects of Ethanolic shoots extract of *B. aethiopum* on haemostatic parameters in Wistar rats.

The results of haemostatic parameters showed no significant increase among the treated groups compared to untreated (control) group

Table 3: Effects of Ethanolic Shoots Extract of *B. aethiopum* on Haemostatic parameters (Clotting factors) in Wistar Rats

	Groups (Mean±SEM)					
Paramesters	Control	300mg/kg	600mg/kg	1200mg/k	F	P-value
vWF (pg/L)	212.67±7.39	225.42±8.59	236.95±12.83	210.86±7.41	1.616	0.228
FV (ng/L)	22.90±2.68	29.91±1.31	29.21±1.93	24.41±2.71	2.587	0.092
FVII (ng/L)	2.95±0.12	3.08±0.11	3.21±0.13	2.96±0.03	1.206	0.342
FVIII (pmol/ml)	0.64±0.36	0.69±0.47	0.78±0.54	0.66±0.02	2.099	0.143

There was no significant increase in haemostatic parameters (Clotting factors), $P> 0.05$ of treated Wistar rats compared to control group except vWF at group 4 higher dose showed no significant decrease.

Key: Values express as Mean±SEM (standard error of mean), one-way ANOVA (analysis of variant), vWF (vonWillebrand factor), FV (Clotting factor 5), FVII (7), FVIII (8), Group 1 control (2ml Distilled water), group2 (300), group3 (600) and group 4 (1,200 mg/kg).

Effect of ethanolic shoots extract of *B. aethiopum* on histology of Liver microscopy

The result showed histological changes of liver microscopy with periportal inflammation, unremarkable hepatocytes and ductular congestion compared to the control group.

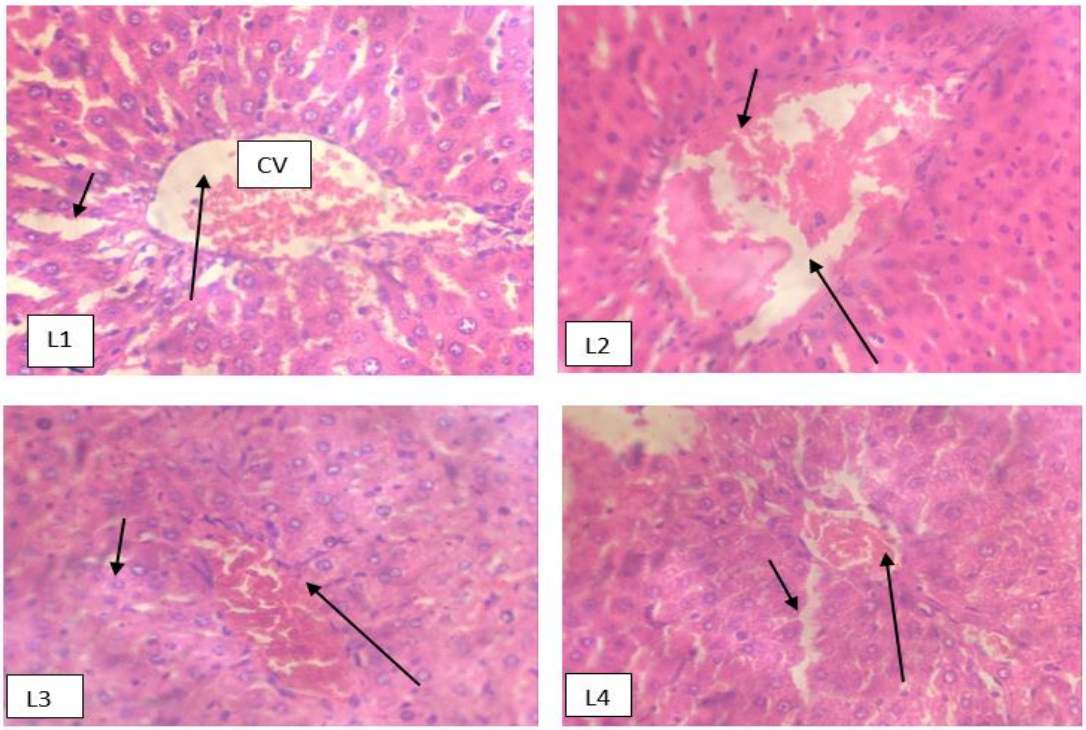

Plate 1: Photomicrographs of Liver section showing effects of ethanolic shoots extract of *B. aethiopum* in Wister rat H and E (×400).
The photomicrographs of Liver sections L1 (control) showing normal liver with regular hepatocytes and portal tracts (short arrow) and central vein (CV) (long arrow). L2, 3 and 4 (low dose) with hepatic vascular congestion and periportal inflammation (long arrow) and mild ductular reaction with unremarkable hepatocytes (short arrow).

DISCUSSION

Finding on toxicity from this study showed no toxicity and no mortality were recorded from the Median Lethal dose (LD_{50}) of ethanolic shoots extract of *B. aethiopum* in both Phases after 24 hours observed each phase. Therefore, LD_{50} was greater than 5,000 mg/kg. This is similar to the report by Muhammad *et al*., (2019) and Ramalan *et al*., (2022) of LD_{50} on the effects of shoots extract of *B. aethiopum* on Wistar rats. There were no significant changes in the body weights of the treated groups compared with the untreated (control) groups ($p>0.05$). This is in agreement with the report by Muhammad *et al*., (2019) and Tata *et al*., (2021) who reported on similar findings.

The findings on effect of ethanolic shoots extract of *B. aethiopum* on haemostatic parameters showed no significance increase ($p>0.05$) in vonWillebrand factor (vWF), clotting Factor V (F5), Factor VII (F7) and Factor VIII (F8) to all the treated groups compared to the control, except for vWF which showed no significance decrease ($p>0.05$) at group 4 (high dose) compared to the control.

Therefore, the reason for this slight increase in the clotting factors suggested that the shoot has coagulation activity to stop haemorrhage. The is similar to a report by Orwa indicated Young seedlings and leaves of *B. aethiopum* usually consumed as vegetables are reported to stop haemorrhage by Orwa *et al*., (2016).

This Finding on Liver microscopy examination showed Plate L1 (control) with normal regular hepatocytes, portal tracts (short arrow) and central vein (CV) (long arrow). Plate L2, 3 and 4 (low dose) with hepatic vascular congestion, periportal inflammation (short arrow) and mild ductular reaction (long arrow).

Many plant toxins have been reported to cause 20–40% of hepatic failure (Geresu *et al.*, 2022).

Finding from this study is in agreement with the report of Muhammad *et al*., (2019) and Tata *et al*., (2021) and in contrast with finding from Ramalan *et al*., (2022) except for Direct bilirubin which showed Significant increase in AST, ALT and ALP ($p<0.05$) on similar finding. Similar report showed histopathological changes on Sub chronic lead toxicity study in Wistar rats Usman *et al*., (2022). In this finding, ethanolic shoots extract of *B. aethiopum* showed histopathological changes in Liver organ but did not affect blood clotting factors assayed in Wistar rats.

CONCLUSION

Findings of this study revealed there was no mortality recorded from the LD_{50} in the Wistar rats. No changes observed on body weight of the treated groups. Similarly, there was no effect observed on some clotting factors assayed compared to control group. The shoot extract showed histopathological changes in liver histology, indicating that the shoots extract of *B. aethiopum* have toxic effect in the Liver.

Recommendations

Further study on Median Lethal doses (LD_{50}) for chronic toxicity to assess effect of shoots for the damage organs which may cause mortality. Further investigation on coagulation pathways to ascertain effect of the shoots on other clotting factors. Further investigation to assess the mechanism that lead the organ changes and advance for special stain to assess reticulin fibre of the organ are recommended.

Conflict of Interest: The authors have declared no conflict of interest.

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