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SUB-ACUTE TOXICOLOGICAL EVALUATION OF THE AQUEOUS STEM BARK EXTRACT OF *Brachystegia eurycoma* (HARMS)

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ABSTRACT

Brachystegia eurycoma is a mainstay in the treatment of malaria, diabetes, hypertension, microbial infections and inflammatory conditions in folkloric medicine in Eastern Nigeria. Thus, the present study investigated the *in vivo* sub-acute toxicity of its aqueous stem bark extract. Toxicological evaluation was done using organ weight index, haematological, biochemical and histopathological parameters, following daily oral administration of 100, 400 and 800 mg/kg of the extract to albino Wistar rats for 14 days. The extract at all doses caused no significant changes in the organ weight index of selected organs except for 100 mg/kg which caused a significant ($p < 0.05$) increase in the weight of lungs compared to control. There was significant elevation of white blood cells and lymphocytes observed at all doses of extract tested. However, *B. eurycoma* caused mild periportal infiltrates of chronic inflammatory cells and Kupffer cell activation in the liver, lymphoid aggregate activation in the lungs as well as activation of lymphoid follicle and sinus histiocyte in the spleen, thus indicating activation of the immune system. Overall, the results suggest that aqueous extract of *B. eurycoma* stem bark is not toxic in rats, although it has a tendency to activate the immune system.

Keywords: *B. eurycoma*, immunity, spleen, toxicity.

INTRODUCTION

Brachystegia eurycoma is an economic tree whose parts have been used for both food and medicine (Ndukwu, 2009). Extracts from the seeds is valuable as a thickener for traditional soups in Eastern part of Nigeria (Okwu and Okoro, 2005). Various secondary plant metabolites such as alkaloids, saponins, tannins and flavonoids have been detected in extracts of different parts of the plant (Adekunle, 2000; Okwu and Okoro, 2005; Okoli *et al*, 2015). Whereas pure compounds with biological/pharmacological activity have been isolated from the crude extract of *B. eurycoma* (Ogunbinu *et al*, 2006), the crude extract of different parts of the plant has continued to be of value in folkloric medicine in Nigeria and other parts of Africa. Extracts from the plant either alone or in combination with other plant are widely used in Nigeria for malaria, diabetes, hypertension bone fractures and inflammatory conditions (Ayinde and Abada, 2010). The aqueous and ethanol crude extract of the bark have been reported to have anti-fungal activity (Adekunle, 2000). The ethanol extract of the dried seeds and bark have been reported to be

effective against carrageenan induced acute inflammation and formalin induced chronic inflammation (Igwu and Okwu, 2013). Evidence that suggest the effectiveness and ineffectiveness of the aqueous leaf extract on acute and chronic inflammation respectively have also been reported (Igbe and Inarumen, 2013). These scientific studies partly validate the medicinal usefulness of the crude extract of *B. eurycoma* in folkloric medicine. However, while the crude extracts and pure isolates from *Brachystegia eurycoma* have been screened for various biological activities as highlighted by the studies reviewed (Igwu and Ejeme, 2013; Igwu and Okwu, 2013), very little attention has been paid to their toxicological screening. Acute toxicity studies of the methanol stem bark extract showed neither mortality nor signs of toxicity in male swiss abino mice (Igbe *et al*, 2012). Nevertheless, the need for more extensive toxicological screening of the crude extract has become imperative given its widespread use as food and medicine. To this end, this study was done to determine the sub-acute toxicity of the aqueous stem bark extract of *Brachystegia eurycoma*.

MATERIALS AND METHODS

Plant Collection and Extraction

The stem barks of *Brachystegia eurycoma* were collected in the month of June, 2014 from Iwo, Osun state and were identified by a botanist in the Department of Pharmacognosy, University of Benin, Benin City. The fresh barks were air-dried for a period of five days followed by oven drying at 50 °C for 6 hours. Thereafter, they were reduced to powder form by an electric mill. The powdered stem bark (500g) was boiled in distilled water for 30 minutes, allowed to cool and then filtered (Igbe *et al*, 2012). The resulting filtrate was then dried at 50 to a constant weight of 24.1g (4.8%). The dried extract was stored in airtight glass jar at 4 prior to use.

Experimental Animals and Design

All experiments were performed using Wistar albino rats (140-225g) of either sex. The animals were sourced from the Faculty of Pharmacy Animal House, University of Benin, Benin City. The animals were allowed to acclimatize for two weeks and allowed access to standard feed (Ewu Feeds and Flour Mills Limited, Ewu, Edo State) and tap water *ad libitum*. Animals were exposed to natural lighting conditions and handled in accordance with the international principles guiding the use and handling of experimental animals (National Institute of Health, USA, 2002) and approved by the Faculty of Pharmacy Animal Ethics Committee.

Following acclimatization period which lasted for two weeks, animals were randomly divided into four groups of rats with each group containing five males and five females. The extract was administered orally at 100, 400 and 800 mg/kg different groups while control group received distilled water daily for 14 days.

Body Weights and Organ Weight Index

The body weights of rats were taken at day 7 and 14 during the course of the 14 days treatment. Weight changes were calculated in respect of the initial body weight of the rats at day 0. The organs were carefully dissected out, gently rinsed in normal saline and weighed. The organ weight index was then evaluated.

Haematological Assay

Red blood cell count (RBC), packed cell volume (PCV), haemoglobin (Hb) concentration, platelet count (PLT), erythrocyte indices, total white blood cell counts and its differentials were done with blood samples collected into lithium heparinised sample bottles using automated haematology systems (Sysmex Haematology Systems, 2008).

Biochemical Assay

Serum obtained following centrifugation of whole blood (without anti-coagulant) at 2,500 rpm for 5 minutes was used for biochemical analysis. Biochemical parameters; aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total and conjugated bilirubin were determined spectrophotometrically using standardized diagnostic kits (Randox by Randox Laboratories Ltd, United Kingdom).

Histopathological Studies

Lungs, spleen, heart, kidney and liver were fixed as soon as they were removed from the rats in 10% formalin for histopathological examination. The organs were embedded in paraffin and then sectioned, stained with hematoxylin and eosin. This was followed by examination under the light microscope and histopathological evaluation was done by a pathologist. Photomicroscope (Motic, Canada) provided with Motic Images Plus 2.0 software was used for taking photomicrographs of the microscopic sections.

Statistical Analysis

Statistical analysis was done using graph pad prism version 5. The data were analysed using one way analysis of variance (ANOVA) followed by turkey post hoc test. Data represent mean± SEM. Statistical significance was considered at $p < 0.05$.

RESULTS

Body Weight Values of Rats treated with *B. eurycoma* for 14 days

Sub-acute treatment with 100-800 mg/kg/day of *B. eurycoma* did not cause significant ($p > 0.05$) difference in the pattern of weight gain in both sexes of the rats when compared to the control (Table 1).

Organ Weight Index of Rats treated with *B. eurycoma* for 14 days

Similarly, sub-acute treatment with 100-800 mg/kg/day of *B. Eurycoma* did not cause significant ($p > 0.05$) difference in the organ weight index of the heart, kidney, liver and spleen versus control. However, only sub-acute treatment with 100mg/kg caused a significant increase ($p < 0.05$) in the weight of the lungs versus control (Table 2).

Biochemical Parameters

Sub-acute treatment with 100-800 mg/kg/day *B. eurycoma* did not significantly change the serum concentrations of alanine transaminase, aspartate transaminase, alkaline phosphatase, total bilirubin, and conjugated bilirubin versus control (Table 3).

Heamatology

Sub-acute treatment with *B. eurycoma* at all doses tested in the study caused an elevation in the white blood cells and lymphocytes. However, there was no significant effect on other parameters at all doses tested (Table 4).

Histopathology

Treatment with 200, 400 and 800mg/kg of *B.eurycoma* for 14 days caused vascular congestion, mild periportal infiltration of chronic inflammatory cells and kupffer cell activation in the liver of treated animals. There was also interstitial congestion and lymphoid aggregate activation in all other selected organs (Fig 1 - 10)

Table 1: Effect of Sub-acute oral treatment with *B. eurycoma* on body weights

Groups	Dose(mg/kg)	Change in body weight (g)		
		Day 0	Day 7	Day 14
Control	-	191.0 ± 6.6	26.0 ± 2.9	25.0±3.8
<i>B.eurycoma</i>	100	178.0 ± 8.2	26.0 ± 3.8	27 ± 8.7
	400	179.0 ± 6.0	30.0 ± 3.5	33.0 ± 7.1
	800	180.0 ± 5.4	28.0 ± 1.3	23.0 ± 22.8

Control group received 4 ml/kg distilled water. Values represent mean ± SEM (n=10).

Table 2: Effect of Sub-acute oral treatment with *B. eurycoma* on the organ weight index

	Organ weight index			
	Control	100mg/kg	400mg/kg	800mg/kg
Liver	0.0261±0.0006	0.0294±0.0013	0.0269±0.0011	0.0279±0.0007
Heart	0.0029±0.0001	0.0034±0.0001	0.0030±0.0001	0.0032±0.0001
Kidney	0.0027±0.0001	0.0032±0.0001	0.0029±0.0001	0.0030±0.0001
Spleen	0.0036±0.0003	0.00417±0.0005	0.0035±0.0004	0.0036±0.0003
Lungs	0.0066±0.0004	0.0085±0.0001*	0.0068±0.0005	0.0072±0.0003

Control group received 4 ml/kg distilled water. Values represent mean ± SEM (n=10). *P < 0.05 versus control.

Table 3: Effect of Sub-acute oral treatment with *B. eurycoma* on biochemical parameters

	Groups			
	Control	100mg/kg	400mg/kg	800mg/kg
AST (IU/L)	30.0 ± 2.3	36.9 ± 8.7	33.0 ± 4.9	36.7 ± 1.6
ALT (IU/L)	9.7 ± 1.2	11.6 ± 1.8	10.8 ± 2.5	9.9 ± 1.2
ALP (IU/L)	41.3 ± 1.7	39.8 ± 2.5	36.8 ± 3.8	39.3 ± 1.7
TB (mg/dl)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2
CB (mg/dl)	0.1 ± 0.1	0.1±0.1	0.1±0.01	0.1±0.2

Control group received 4 ml/kg distilled water; AST= Aspartate transaminase; ALT= Alanine transaminase; ALP= Alkaline phosphatase; TB= Total Bilirubin; CB=Conjugated Bilirubin.

Table 4: Effect of Sub-acute oral treatment with *B. eurycoma* on some haematological parameters

	Groups			
	Control	100mg/kg	400mg/kg	800mg/kg
RBC (x10⁶/µl)	6.2 ± 0.5	6.6 ± 0.5	6.9 ± 0.4	7.4 ± 0.3
HGB (g/dL)	11.2 ± 0.6	11.9 ± 0.6	12.4 ± 0.6	13.3 ± 0.3
HCT (%)	35.4 ± 2.3	38.1 ± 2.6	40.3 ± 2.2	42.9 ± 1.2
MCV (fL)	57.6 ± 1.1	57.4 ± 1.1	58.0 ± 1.1	58.2 ± 1.5
MCH (pg)	18.2 ± 0.6	18.1 ± 0.5	17.9 ± 0.3	18.1 ± 0.5
MCHC (g/dL)	31.6 ± 0.8	31.6 ± 0.8	30.8 ± 0.5	31.1 ± 0.4
WBC (x10³/µl)	5.3 ± 1.3	8.4 ± 1.5	9.3 ± 0.7	8.0 ± 1.6
LYM (%)	57.9 ± 12.1	67.5 ± 10.0	66.3 ± 10.4	64.9 ± 11.4
NEUT (%)	39.9 ± 12.2	32.5 ± 10.0	33.7 ± 10.4	37.6 ± 13.6
PLT (x10³/µl)	607.8 ± 39.4	647.0 ± 46.0	658.7 ± 56.5	658.3 ± 69.6

Control group was administered 4 ml/kg distilled water; RBC= Red blood cell count; HGB= haemoglobin concentration; HCT= Haematocrit; MCV= Mean corpuscular volume; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin concentration; WBC= White blood cell count; LYM%= lymphocytes percentage; NEUT%= neutrophils; PLT= platelet count.

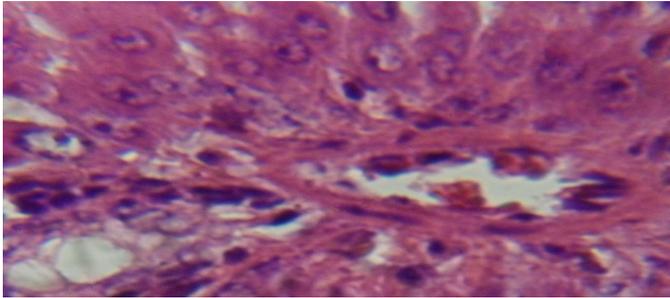


Fig 1: Control: Rat Liver composed of portal triad A, hepatocytes B separated by sinusoids C (H&E x 40)

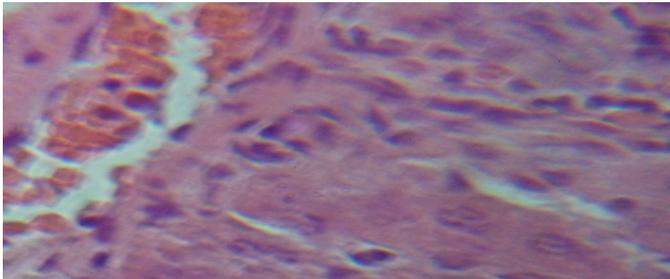


Fig 2: Rat Liver treated with 800mg/kg *B. eurycoma* for 14 days showing mild vascular congestion A, mild periportal infiltrates of chronic inflammatory cells B and kupffer cells activation C (H&E x 40)

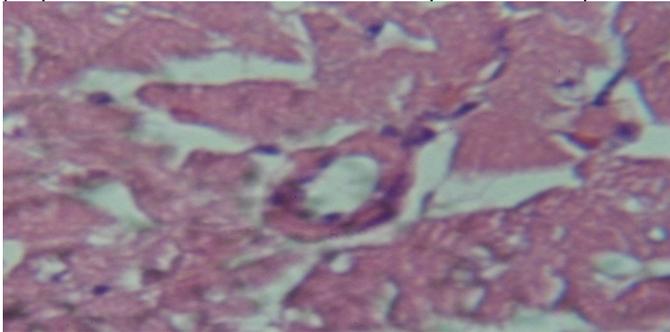


Fig 3: Control: Rat Heart composed of bundles of myocytes A separated by interstitium B and pierced by coronary vessel C (H&E x 40)

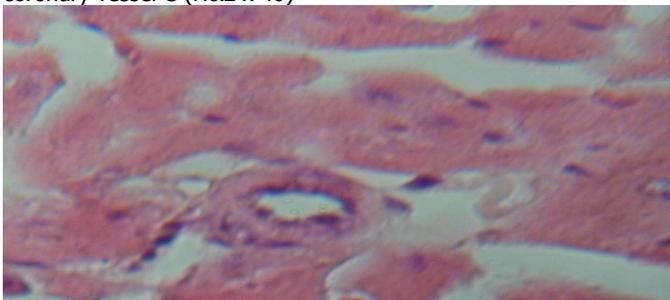


Fig 4: Rat Heart treated with 800mg/kg *B. eurycoma* for 14 days showing mild tissue separation A (H&E x 40)

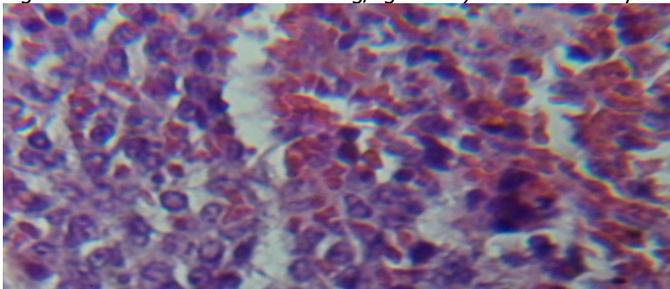


Fig 5: Control: Rat Spleen composed of white pulp (lymphoid follicles) A and red pulp B (H&E x 40)

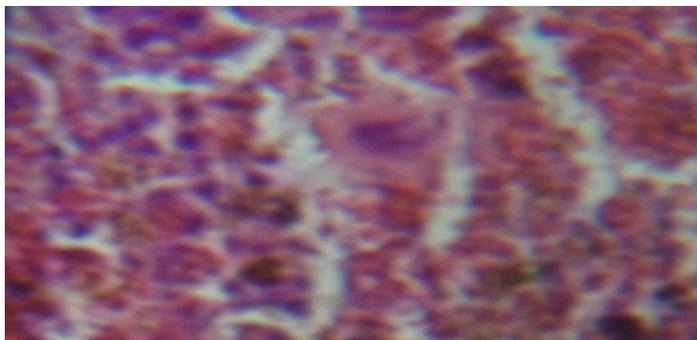


Fig 6: Rat Spleen treated with 800mg/kg *B. eurycoma* for 14 days showing mild activation of lymphoid follicles A and sinus histiocytes activation B (H&E x 40)

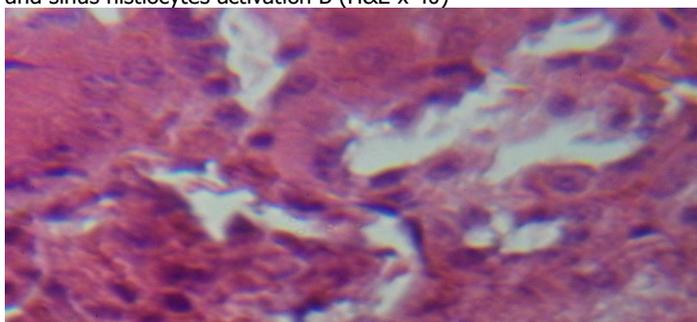


Fig 7: Control: Rat Kidney composed of cortical glomeruli A and tubules B separated by interstitium C (H&E x 40)

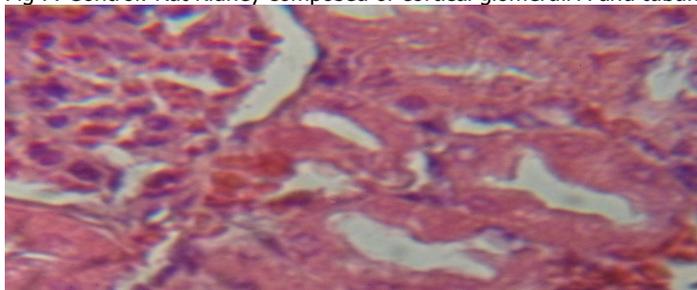


Fig 8: Rat Kidney treated with 800mg/kg *B. eurycoma* for 14 days showing mild interstitial congestion A (H&E x 40)

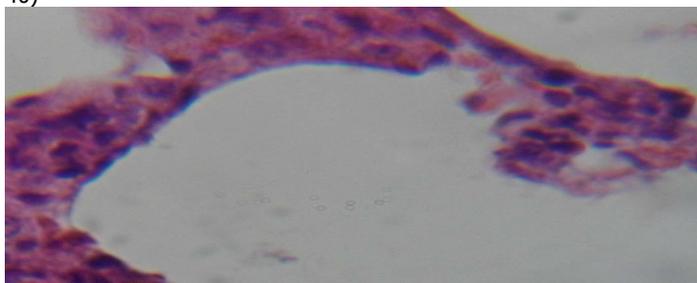


Fig 9: Control: Rat Lungs composed of alveoli A surrounded by interstitial space B (H&E x 40)

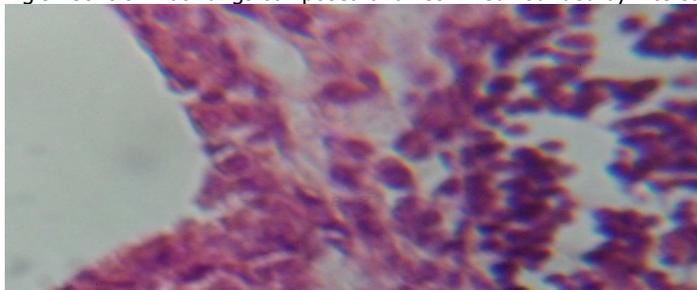


Fig 10: Rat Lungs treated with 800mg/kg *B. eurycoma* for 14 days showing mild interstitial congestion A and lymphoid aggregate activation B (H&E x 40)

DISCUSSION

Herbal medicines have become increasingly popular in modern societies around the world, and several studies have been conducted on herbal medicine's pharmacological properties to establish its scientific evidence. Evidence of therapeutic materials is assessed throughout various sources including toxicity, pharmacological properties, clinical trials, and systematic reviews (Eddy, 2005).

The reduction in body and organ weights is an important and sensitive indicator of chemically induced changes to the organs and body, and as such sensitive indicators of toxicity following exposure to a toxicant (Teo *et al*, 2002; Michael *et al*, 2007). The non significant differences in the body weights and organ weight index among *B. eurycoma* treated groups suggest that the phyto-chemicals present in the extract did not change the mass composition of the body and organs of the rats either by not reducing food intake or not interfering with biochemical processes that determine body and organ weight.

Since significant increase in the levels of liver enzymes AST, ALP and ALT indicate hepatic cell damage and hence liver function impairment (Hayes, 1989), the non-significant changes in the serum levels of these enzymes caused by all doses of *B. eurycoma* tested strongly suggest that the extract is not hepatotoxic and thus does not impair liver function when administered orally. Similarly, given that elevation of total and conjugated bilirubin is also an important indicator of hepatic cell injury and hence hepatic impairment (Edem and Usoh, 2009), the non-significant alterations in the serum levels of total and conjugated bilirubin caused by all doses of the extract tested lends further credence to the oral safety of the extract on liver function.

The slight elevation of white blood cells and lymphocytes levels caused by all doses of the extract tested suggest a tendency by the extract to activate the immune system. This observation is in consonance with other findings which showed that the extract at all doses caused activation of lymphoid follicle and sinus histiocyte in the spleen as well as activation of lymphoid aggregate in the lungs, which are all evidences suggesting activation of the immune system by the extract.

Contrary to the evidence which suggest that the extract is not deleterious to liver function, histological assessment of the extract treated rats' liver which

showed mild periportal infiltration of chronic inflammatory cells, suggest mild injury or damage to the liver cells, bearing in mind that cell injury or damage is a major trigger of inflammatory response. However, given that the Kupffer cells are immune cells residing in the liver whose activation produces cytokines that trigger inflammatory reactions (Wheeler *et al*, 2003); infiltration of chronic inflammatory cells in the liver may also be attributed to the activation of Kupffer cells by the extract. This evidence again indicates a tendency by the extract to activate the immune system thus lending further credence to earlier evidences in this regard. In the light of this evidence, the need for future research to screen *B. eurycoma* extracts for both chronic toxicity and immuno-toxicity has become imperative. This is more so, bearing in mind serious implications that activation of immune system may portend for people predisposed to auto-immune disease such as rheumatoid arthritis, using *B. eurycoma* extract for either culinary or medicinal purposes. Mild interstitial congestion produced by all doses of the extract in the kidney and mild vascular congestion produced by the extract in the heart may be due to protective inflammatory response of the tissues.

CONCLUSION

The results suggest that sub-acute oral treatment with aqueous stem bark extract of *B. eurycoma* at the doses tested is relatively safe in rats; however, the extract may have a tendency to activate the immune system.

Authors' Contributions

This work was carried out in collaboration between all authors. Authors II and ABA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author IS managed the literature searches, analyses of the study. Author ENA and OA managed the experimental process. All authors read and approved the final manuscript.

Conflict of Interests

Authors have declared that no conflict of interests exists.

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