Anti-inflammatory and Analgesic Effects of the Methanol Stem Bark Extract of Brachystegia eurycoma Harms (Fabaceae)

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Sub-acute Toxicity of Aqueous Fruit Pulp Extract of *Hunteria umbellata* in Albino Wistar Rats

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Summary: *Hunteria umbellata* K. Schum (Apocynaceae) is used in herbal medicine for the treatment of diabetes, peptic ulcers, piles, yaws, dysmenorrhea, fevers, infertility, and helminthic infections. The present study investigated the *in vivo* sub-acute toxicity of the aqueous fruit pulp extract of *Hunteria umbellata* (*H. umbellata*). Sub-acute toxicity was evaluated after administering daily oral doses of 200, 400 and 800 mg/kg of *H. umbellata* extract, for 28 days to the rats. Anthropometric, biochemical, hematological and histopathological parameters were assessed using standard procedures. There were significant reductions ($p<0.01$) in the pattern of weight gain in 200 and 400 mg/kg *H. umbellata* -treated rats but no significant differences in the organ weight index between control and treated animals. Hematological and biochemical analysis showed no marked differences in any of the parameters examined in either the control or treated groups but there was significant ($p<0.05$) thrombocytosis. Pathologically, neither gross abnormalities nor histopathological changes were observed. *H. umbellata* led to activation of the reticulo endothelial tissue of the spleen as evidenced by proliferation of the sinus histocytes and activation of the lymphoid aggregates in the lungs, indicating activation of the local immune system of the lungs. *H. umbellata* fruit pulp is relatively nontoxic in animals but there is increased tendency to cause thrombocytosis on prolonged use.

Keywords*: Hunteria umbellata*, Histopathology, Subacute, Thrombocytosis, Hematology

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INTRODUCTION

Hunteria umbellata K. Schum (Apocynaceae) is a shrub or small tree up to (15–22 m) tall, with colorless or milky latex in all parts; bole sinuous or straight, up to 40 cm in diameter, fluted; outer bark 1 mm thick, rough or smooth, grey to dirty brown; crown dense. Fruits consist of 2 separate globose mericarps 3-6 cm long, yellow, smooth, 8-25-seeded. Seeds oblong to ellipsoid, 1-1.5 cm long, flattened at one side (Boone, 2006).

In African folk medicine, various extracts prepared from different parts of the plant *Hunteria umbellata* (K. Schum.) Hallier f. are employed in the treatment of various human diseases such as yaws, stomach ulcers, pains and swellings, diabetes mellitus, dysmenorrhoea and to induce or augment labour (Adegoke and Alo, 1986; Elujoba, 1995; Oluwemimo and Usifoh, 2001; Raman & Mallam, 1994 Falodun et al., 2006; Igbe et al., 2009; Igbe et al., 2010). Water decoction made from the dried seeds of *H. umbellata* is highly valued in the local management of diabetes mellitus, obesity, stomach ache, pains and swellings, hypertension and as immune booster (Boone, 2006; Adeneye and Adeyemi, 2009a).

The aqueous fruit pulp extract of *H. umbellata* has been shown to be effective against acute inflammation in a dose related manner but without any significant effect on chronic inflammation (Igbe et al., 2010). The hypoglycaemic effects of the seed extract have been reported (Igbe et al., 2009a; Adeneye and Adeyemi, 2009a; 2009b). In addition, the anti-obesity and anti hyperlipidemic activities of *H. umbellata* have also been shown to be mediated via inhibitions of intestinal lipid absorption and de novo cholesterol and triglyceride syntheses (Adeneye et al., 2010a). Falodun et al. (2006) and Igbe et al. (2009) reported the oxytocic effect and antipyretic/analgesic effect of the fresh fruit pulp of *H. umbellata*, respectively. Chronic treatment with *H. umbellata* seeds have been shown to produce significant changes in haematological parameters (Adeneye et al, 2010b). Although it has previously been reported that there was no mortality after oral acute toxicity assays at 15 g/kg of the fruit pulp extract (Igbe et al., 2010), no work has been done on the toxicity of the fruit pulp on long term use, hence

the aim of the study was to determine the sub-acute toxicity of the aqueous fruit pulp extract of *H. umbellata*

MATERIALS AND METHODS

Chemicals

Chloroform (Sigma-Aldrich Corporation, Missouri), 10% formyl saline, heparinized saline, absolute alcohol (Sigma Chemicals, UK), xylene (BDH Chemicals, UK), haematoxylin and eosin (H&E) stain

Plant materials and extraction

The ripe fruits of *H. umbellata* were collected from Okhoro village in Benin City, Nigeria in the month of October, 2010. The plant was first identified by Prof. MacDonald Idu of the Department of Botany, Faculty of Life Sciences, University of Benin, Benin City and was later authenticated by the Forest Research Institute of Nigeria (Ibadan, Nigeria) where a herbarium sample with voucher number FHI 107678 has been deposited. The seeds were removed from the ripe fruits and were sun-dried to a constant weight over a 14-day period. The dried fruit pulp was then powdered using a mechanical grinder. The powdered fruit pulp (400 g) was boiled in 2 L of distilled water for 30 min. The material was then filtered, concentrated to dryness under reduced temperature and pressure in a vacuum evaporator (yield = 34%). The dried extract was stored in at 4^{o}C until use.

Experimental animals

Experiments were performed using albino wistar rats of either sex (250-280 g). The animals were obtained from animal house, Ambrose Ali College of medicine, Ekpoma, Nigeria. The animals were acclimatized for two weeks and fed with standard feed (Ewu Feeds and Flour Mills Limited, Ewu, Edo state, Nigeria) and tap water *ad libitum*. Animals were exposed to natural lighting conditions. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and complied with the NIH guidelines on handling of experimental animals

Repeated dose toxicity study

After the two weeks acclimatization period, animals were randomly allotted to four groups of rats each containing five males and five females

Group I rats were administered 4ml/kg distilled water.
Group II received 200 mg/kg of *H. umbellata*.
Group III received 400 mg/kg of *H. umbellata*.
Group IV received 800 mg/kg of *H. umbellata*.

The extract was administered daily at single doses for 28 days. The animals were closely observed for signs of toxic manifestation and toxicity. At the end of the 28-day treatment period, animals were sacrificed under chloroform anaesthesia. Blood samples were withdrawn directly from the heart chamber with a 21G needle mounted on a 10 ml syringe (Agary pharmaceutical LTD, Nigeria) into the lithium heparinized sample bottles (BD Vacutainer®, BD-Plymouth, Plymouth, U.K).

Effect of *H. umbellata* on body weight

In the course of the 28-day oral treatment, body weights of rats were regularly taken at a 7day interval and weight changes were calculated in respect of the initial body weight on day 0.

Effect of *H. umbellata* on organ weight index

The organs (heart, lungs, liver, spleen, and kidney) were carefully dissected out and freed from adjoining supporting connective tissues. The organs were gently rinsed in normal saline, blotted with filter paper (Whatman's No. 1 filter paper, Whatman international Ltd Maidstone, England) and weighed. The organ index was then calculated using the formula:

$$\text{Organ weight index} = \frac{\text{Organ weight}}{\text{Body weight}}$$

(Tofovic & Jackson, 1999)

Haematological assay

The Blood samples collected into the lithium heparinized sample bottles was for determination of the red cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), platelet count (PLT),Erythrocyte indices, total white blood cell counts and its differentials using Automated Haematology System (Diatron® Abacus junior hematology analyzer).

Biochemical assay

Biochemical analysis was performed on serum obtained after centrifugation of whole blood (without Anticoagulant) at 2500 rpm for 5 min. Standardized diagnostic kits (Randox® by Randox laboratories LTD., United kingdom) were used for spectrophotometric (INICO® 1200 spectrophotometer) determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, alkaline phosphatase, urea, bicarbonate, total proteins, albumin, total and conjugated bilirubin.

Histopathological studies

Liver, kidney, spleen, pancreas, lungs, heart were fixed immediately in 10% formalin for routine histopathological examination. The tissues were embedded in paraffin and then sectioned, stained with hematoxylin and eosin and were examined under light microscope. Histopathological evaluations were performed by a pathologist. Photomicrographs of the microscopic sections were taken with the help of a photomicroscope (Motic, Canada) provided with Motic Images Plus 2.0 software.

Statistical analysis

Data were expressed as the mean ± SEM. The data were analysed using one way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical analysis was performed using GraphPad Prism V. 4.01 where $p<0.05$ was considered statistically significant

RESULTS

Effect of 28-days oral treatment with *H. umbellata* on body weight

Sub-acute treatment with 200 and 400 mg/kg/day of *H. umbellata* caused significant (p<0.01) reduction in the pattern of weight gain in the both sexes of the rats when compared to the control (Table 1).

Effect of 28-days oral treatment with *H. umbellata* on the organ weight index

Sub-acute treatment with 200-800 mg/kg/day caused no significant (p>0.05) difference in the organ weight index of the heart, kidney, liver, spleen and lungs in either sex of the animals (Table 2).

Effect of 28-day oral treatment with *H. umbellata* on haematological parameters

There was a significant (p<0.05) increase in platelet counts in both the male and female rats but they were not significantly different from each other. (Tables 3).

Effect of 28-day oral treatment with *H. umbellata* on biochemical parameters

There was no significant change in biochemical parameters at the end of the repeated dose

Effect of 28-days oral treatment with 200-800 mg/kg/day of *H. umbellata* on the histopathology of selected organs

Figures 1a-1b is representative sections of normal rat heart, and 800 mg/kg *H. umbellata* -treated rat cardiac muscles, respectively. At an oral dose of 800 mg/kg, thickening of the coronary artery was

Table 1: Effect of sub-acute oral treatment with 200-800 mg/kg of *H. umbellata* on body weights

		Change in body weights (g)				
Groups	**Dose(mg/kg)**	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Female						
Control	-	252.0 ± 3.39	20.0 ± 5.83	36.0 ± 8.46	41.0 ± 8.46	33.0 ± 9.35
H. umbellata	200	243.0 ± 3.39	6.0 ± 4.00*	6.0 ± 4.84*	18.0 ± 5.09*	12.0 ± 4.74*
	400	266.0 ± 8.77	6.0 ± 2.43*	5.0 ± 1.22*	8.0 ± 4.35*	10.0 ± 5.28*
	800	231.0 ± 9.27	16.0 ± 4.28	26.0 ± 6.01	35.0 ± 18.80	36.0 ± 9.60
Male						
Control	-	260.0 ± 5.28	18.0 ± 4.80	32.0 ± 9.51	40.0 ± 7.70	41.0 ± 2.38
H. umbellata	200	255.0 ± 1.19	10.0 ± 2.10*	8.0 ± 1.68*	17.0 ± 5.62*	15.0 ± 4.83*
	400	268.0 ± 6.70	8.0 ± 5.43	6.0 ± 2.24*	7.0 ± 2.55*	11.0 ± 3.50*
	800	252.0 ± 7.67	20.0 ± 1.08	29.0 ± 8.22	31.0 ± 9.50	35.0 ± 10.10

Values are expressed as mean ± S.E.M (n=5), Control group received 4ml/kg distilled water. *P < 0.01 compared to ontrol

Table 2: Effect of sub-acute oral treatment with 200-800 mg/kg of *H. umbellata* on organ weight index

	Organ weight index			
	Control	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**
Female				
Liver	0.0230±0.0001	0.0258±0.0003	0.0260±0.0004	0.0266±0.0010
Kidney	0.0031±0.0001	0.0030±0.0021	0.0031±0.0001	0.0030±0.0001
Heart	0.0034±0.0001	0.0033±0.0031	0.0029±0.0001	0.0031±0.0001
Lung	0.0060±0.0003	0.0060±0.0001	0.0063±0.0002	0.0061±0.0001
Spleen	0.0033±0.0001	0.0038±0.0005	0.0040±0.0001	0.0041±0.0021
Male				
Liver	0.0260±0.0011	0.0288±0.0008	0.0290±0.0014	0.0276±0.0010
Kidney	0.0031±0.0001	0.0031±0.0001	0.0029±0.0001	0.0030±0.0001
Heart	0.0033±0.0001	0.0033±0.0001	0.0031±0.0001	0.0032±0.0001
Lung	0.0062±0.0003	0.0063±0.0001	0.0068±0.0004	0.0062±0.0003
Spleen	0.0032±0.0001	0.0040±0.0006	0.0042±0.0006	0.0035±0.0004

. Values are expressed as mean ± S.E.M (n=5), Control group received 4ml/kg distilled water

Table 3. Effect of sub-acute oral treatment with 200-800 mg/kg of *H. umbellata* on haematological parameters

	Group			
	Control	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**
Female				
RBC ($x10^6$/µl)	8.3±1.11	7.6±0.35	7.1±0.12	7.5±1.20
Hb (g/dl)	12.8±0.44	12.1±0.33	11.1±0.20	12.2±0.21
HCT (%)	42.0±0.83	41.1±1.66	40.8±1.05	42.2±1.68
WBC ($x10^3$/µl)	15.1±2.10	16.9±4.51	13.9±1.21	14.9±2.78
PLT ($x10^3$/µl)	1483.2±613.8	3836.3±238.5*	4093.0±289.0*	3888.0±361.9*
MPV(fL)	5.9±0.03	6.1±0.51	6.0±0.05	5.7±0.07
LY %	56.1±3.0	58.2±2.80	57.0±0.15	56.5±2.10
MI %	13.3±1.51	14.5±1.05	14.2±1.52	15.0±0.74
GR %	38.5±1.90	36.0±2.53	37.4±0.60	38.4±1.90
MCV (fL)	60.2±1.20	61.0±1.88	58.2±0.50	58.0±2.20
MCH (pg)	18.0±0.06	18.2±1.04	16.8±0.55	17.0±0.05
MCHC (g/dl)	31.4±0.04	30.0±0.88	29.0±1.22	28.6±1.08
RDWc (%)	14.4±0.38	14.0±0.06	15.6±0.44	15.2±1.02
Male				
RBC ($x10^6$/µl)	7.1±0.13	7.1±0.25	7.5±0.27	8.2±0.20
Hb (g/dl)	12.4±0.43	11.5±0.63	12.1±0.28	12.1±0.71
HCT (%)	41.4±1.23	40.1±1.96	41.8±1.55	40.9±2.65
WBC ($x10^3$/µl)	13.0±1.13	15.5±4.56	10.9±1.00	14.2±0.72
PLT ($x10^3$/µl)	1834.2±613.8	4331.3±280.5*	4313.0±396.0*	3437.0±401.9*
MPV(fL)	6.4±0.43	6.3±0.50	5.8±0.20	5.8±0.47
LY %	48.1±3.15	51.9±5.89	47.9±6.15	55.5±3.90
MI %	11.3±1.77	15.5±2.05	12.5±0.92	16.1±1.79
GR %	40.6±3.91	32.6±6.33	39.7±5.63	28.4±4.91*
MCV (fL)	58.7±0.88	56.4±1.08	55.8±1.50	52.0±1.25
MCH (pg)	17.6±0.46	16.2±0.34	16.2±0.57	15.6±0.35
MCHC (g/dl)	30.0±0.58	28.7±0.48	29.1±0.82	29.5±0.38
RDWc (%)	16.7±0.34	16.9±0.44	18.2±0.76	18.2±0.80

Values are expressed as mean ± SEM (n = 5). Hb, hemoglobin; HCT, hematocrit; RBC, red blood cells; WBC, white blood cells; PLT, platelets; MPV, mean platelet volume; GR, granulocytes count; MI, monocytes/eosinophils; LY, lymphocytes; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; RDWc, red cell distribution width. Control group received 4ml/kg distilled water. *p <0.05 compared with control.

Table 4. Effect of sub-acute oral treatment with 200-800 mg/kg of *H. umbellata* on biochemical parameters

	Group			
	Control	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**
Female				
Creatinine (mg/dl)	0.38±0.01	0.41±0.10	0.38±0.05	0.29±0.02
Urea (mg/dl)	31.0±1.21	28.8±0.50	28.4±1.02	27.1 ±1.0
Bicarbonate (mMol/L)	28.0±0.08	28.6±2.10	26.0±0.20	26.2±1.31
Total protein (mg/dl)	7.1±0.04	7.4±1.03	7.0±0.16	6.6±0.34
Total albumin (mg/dl)	2.4±0.02	3.3±0.11	3.3±0.10	3.1±0.22
TB (mg/dl)	0.21±0.01	0.12±0.04	0.20±0.04	0.22±0.04
CB (mg/dl)	0.11±0.01	0.10±0.05	0.10±0.03	0.12±0.01
ALP (IU/L)	18.2±1.01	20.0±0.66	17.0±1.33	18.4±0.10
AST (IU/L)	135.4±1.10	133.0±0.02	132.6±0.66	134.0±1.10
ALT (IU/L)	40.2±2.30	41.0±0.94	45.0±5.10	45.4±2.14
Glucose (mg/dl)	92.54±6.12	96.40±2.20	100.42±2.68	102.12±0.40
Male				
Creatinine (mg/dl)	0.30±0.02	0.35±0.02	0.30±0.05	0.31±0.02
Urea (mg/dl)	31.8±2.21	29.8±3.51	27.4±1.72	27.0 ±1.44
Bicarbonate (mMol/L)	24.0±0.89	28.6±1.08	25.2±1.24	24.2±1.74
Total protein (mg/dl)	7.4±0.41	6.0±0.33	6.0±0.16	5.6±0.45
Total albumin (mg/dl)	2.2±0.44	3.2±0.18	3.3±0.16	3.0±0.23
TB (mg/dl)	0.20±0.02	0.15±0.02	0.21±0.04	0.20±0.04
CB (mg/dl)	0.10±0.01	0.13±0.03	0.10±0.03	0.11±0.03
ALP (IU/L)	17.5±2.01	21.8±0.86	20.0±2.30	17.8±2.10
AST (IU/L)	133.9±4.00	138.2±2.02	138.4±0.74	135.0±1.20
ALT (IU/L)	42.0±3.34	31.6±2.94	42.0±4.06	48.3±6.13
Glucose (mg/dl)	106.67±2.65	98.47±6.60	102.22±6.65	100.44±2.40

Values are expressed as mean ± SEM (n = 5); Control group received 4ml/kg distilled water.ALP, Alkaline Phosphatase; AST, Aspartate transaminase; ALT, Alanine transaminase; TB, Total Bilirubin; CB, conjugated Bilirubin.

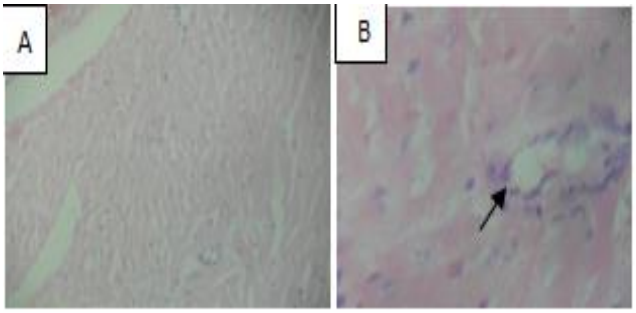

Figure 1. A cross-sectional representation of (A) normal rat heart showing normal cytoartichecture and (B) 800 mg/kg *H. umbellata*-treated rat heart showing mild interstitial oedema and thickening of the coronary artery (Haematoxylin & Eosin stain x40 magnification).

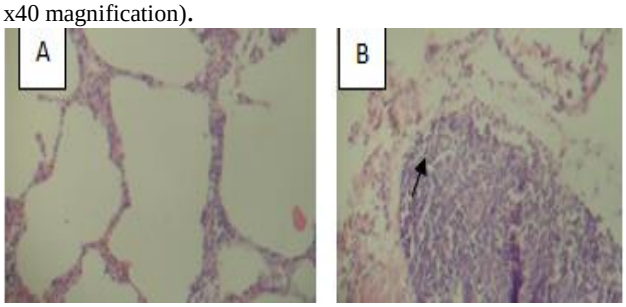

Figure 2. Cross-sectional representation of (A) normal rat lungs showing well expanded alveoli and intervening interstitial space; (B) 800 mg/kg *H. umbellata*-treated rat lungs showing moderate activation of lymphoid follicle (Haematoxylin & Eosin stain x40 magnification).

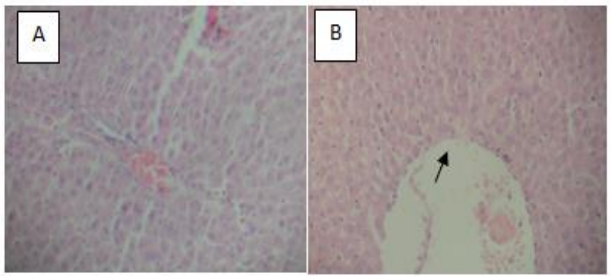

Figure 3. Cross-sectional representation of (A) normal rat liver showing hepatocytes, Sinusoids and portal veins. (B) 800 mg/kg *H. umbellata* -treated rat liver showing dilated central vein (Haematoxylin & Eosin stain x40 magnification).

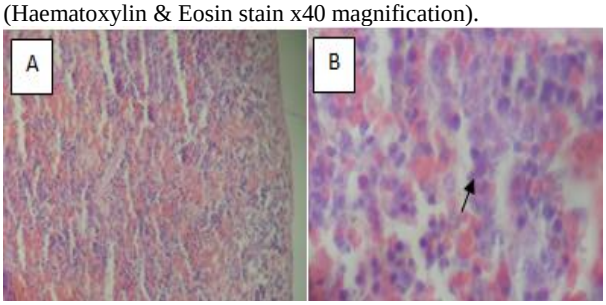

Figure 4. Sectional representation of (A) normal rat spleen showing capsule, red pulp and white pulp (B) 800 mg/kg *H. umbellata* treated rat spleen showing hyperplastic sinus histiocyte (Haematoxylin & Eosin, x40 magnification)

cardiac muscles, respectively. At an oral dose of 800 mg/kg, thickening of the coronary artery was observed. In the lungs, 800 mg/kg of extract showed moderate activation of lymphoid follicle (Figure 2b). In the spleen**,** sub-acute *H. umbellata* treatment produced hyperplastic sinus histiocytes (Figure 3a) in comparison to the normal splenic red and white pulp (Figure 3b).

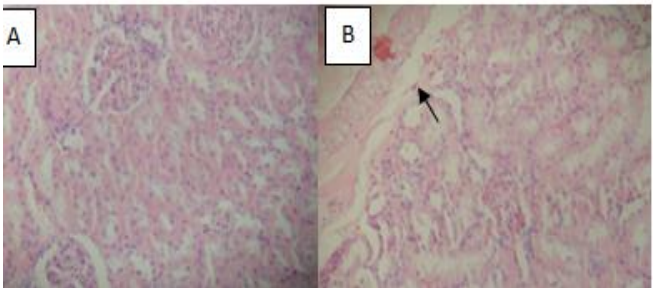

Figure 5: A cross-sectional representation (A) normal rat cortical glomeruli and renal tubules separated by interstitial space. (B) 800 mg/kg *H. umbellata* treated rat kidney showing mild interstitial oedema and thickening of arcuate arteries (Haematoxylin & Eosin stain, x40 magnification).

Sub- acute *H. umbellata* treatment at 800 mg/kg produced mild interstitial oedema and thickening of arcuate arteries in the kidney.

DISCUSSION

The safety of these herbal remedies is still doubtful though effectiveness of some has been validated through research and clinical studies (Kaufman et al., 2002). Experimental screening method is therefore important in order to ascertain the safety and efficacy of herbal products (Sim et al., 2010). *H. umbellata* at oral doses of 200, 400 and 800 mg/kg showed
significant difference ($P<0.01$) in the pattern of change in body weight over the 28 day period when compared to the control. Reduction in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to a toxic substance (Tofovic & Jackson, 1999; Raza et al. 2002; Teo et al., 2002). There was no significant difference in the organ weight index of all selected organs in both treatment and control groups indicating that the reduction in body weights may be due to decrease in food consumption (decreased appetite) by the animals due to the extract.

Analysis of blood parameters is relevant to risk evaluation, as the changes in the hematological system have a higher predictive value for human toxicity when data are translated from animal studies (Olson et al., 2000). After 28 days of treatment, there was a significant ($P<0.01$) increase in platelet count (thrombocytosis) thus increasing the risk for thrombotic complications such as venous thromboembolism. Significant changes in enzymes such as ALP, AST and ALT represent liver impairment, since these are important indices of liver toxicity (Hayes, 1989). *H.Umbellata* has no deleterious effect on liver functions as it can be seen that no significant alterations occurred on the liver enzymes. This is a strong indication of the oral safety of *H. umbellata* on liver function. Similarly, the effect of *H. umbellata* on serum electrolytes, urea and creatinine which are reliable markers of renal function is also a strong indication of the extract's safety on the renal function and the possible inherent nephroprotective potential of the extract (Kachmar &

Grant, 1982). Since there was no effect on the levels of transaminases (ALT, AST) and creatinine, which are good indicators of liver and kidney functions, respectively, it is reasonable to suggest that the *Hunteria umbellata* extract did not induce any damage to the liver and the kidneys. This is further confirmed by the histological assessment of these organs. In all organs, mild chronic inflammation as demonstrated by mild oedema, vasodilatation, and congestion, thickening of arterial walls and mild infiltrates of chronic inflammatory cells suggest a protective response of the tissues. Activation of the reticuloendothelial tissue of the spleen at 800mg/kg as evidenced by proliferation of the sinus histocytes, indicates the activation of the immune system by *H. umbellata*. At all doses, there is activation of the lymphoid aggregates in the lungs, indicating activation of the local immune system of the lungs. However, this was more prominent at 800mg/kg. In conclusion, at the oral doses tested, our data suggested that subacute oral administrations of the aqueous fruit pulp extract of *H. umbellata* is relatively nontoxic in animals but there is increased tendency to induce thrombocytosis on prolonged administration.

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