http://dx.doi.org/10.4314/jpb.v13i1.5
Vol. 13 no. 1, pp. 32-41 (March 2016)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Sub-acute toxicity study on the aqueous extract of *Albizia zygia* stem bark

Stephen O. Okpo[1*], Clare O. Igwealor[1] and Gerald I. Eze[2]

[1]*Department of Pharmacology and Toxicology, University of Benin, P. M. B. 1154 Benin City. Nigeria.*
[2]*Department of Anatomy, School of Basic Medical Sciences, University of Benin, P. M. B. 1154 Benin City. Nigeria.*

Received 11th February 2016; Accepted 14th March 2016

Abstract
Albizia zygia DC (Fabaceae) is widely used in herbal medicine for the treatment of bronchial diseases, fever (including malaria), diarrhea, sores, wounds and toothache. This study was aimed at determining the sub-acute toxicity of the aqueous extract of *Albizia zygia* stem bark. The sub-acute toxicity was evaluated after administering daily oral doses of 100, 200 and 500 mg/kg of extract for 42 days to rats. Morphological (body weight and organ weight indices), haematological {white blood cell (WBC), red blood cell (RBC), haemoglobin, haematocrit, and platelet counts}, biochemical {alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein, total bilirubin (TB), creatinine, urea} and histopathological parameters were assessed using standard procedures. There was no mortality up to the highest dose of 5000 mg/kg in both mice and rats on oral acute administration of *A. zygia*. Administration of *A. zygia* (500 mg/kg/day) for 42 days caused a significant ($p<0.05$) increase in WBC count, decrease in creatinine and no significant changes in relative organ weights or serum concentrations of ALT, ALP, TB, albumin and urea in the treated rats compared to the control. Histologic analysis of the various organs showed mild activation of the lymphocytes of the lungs and the liver, sinus histiocytes of the spleen and mild interstitial congestion in the kidneys, indicating activation of the local immune system of the lungs, liver, kidney and spleen. The results of the study suggest that the aqueous extract of *Albizia zygia* stem bark can be considered relatively safe on sub-acute exposure.

Keywords: *Albizia zygia*; Acute toxicity; Histopathology; Spleen

INTRODUCTION

Several orthodox drugs have been employed in the management of various diseases, but they are believed to provoke many unwanted effects. Due to these side effects, herbal medicines have emerged as an alternative treatment to available synthetic drugs for treatment of diseases possibly due to lower cost, availability, fewer adverse effects and perceived effectiveness (Rates, 2001). Though there are scientific pieces of evidence supporting the use of plant-derived formulations for treatment of diseases, there is still requirement for scientific research of efficacy and, very importantly, the safety of these herbal medicines.

Albizia zygia (DC) J. F. Macbr (Fabaceae) also known in English as West African albizia is indigenous to, and widespread in, tropical Africa, occurring from Senegal in the west to Kenya in the east and northern Angola and Tanzania in the south (Orwa *et al*., 2009). Known locally as *Nyieavu* by the Igbos of Southeast and

[*] Corresponding author. *E-mail*: steveokpo@uniben.edu *Tel*: +234 (0) 8023802411
ISSN 0189-8442

Ayinrela by the Yoruba of Southwest Nigeria, the plant has found wide usage in traditional medicine. The bark sap is instilled in the eyes to treat ophthalmia while the bark decoction is administered to treat bronchial diseases, fever, female sterility, and as a purgative, stomachic, antidote, vermifuge and aphrodisiac. Pounded or rasped bark is applied externally to treat yaws, sores, wounds and toothache. Ground roots are added to food to treat cough and as an expectorant. Leaf decoctions are used to treat pain, fever and diarrhea (Burkill, 1995). The young leaves are cooked and consumed as a vegetable, especially in soups.

Various pharmacological studies on *Albizia zygia* have demonstrated antiprotozoal (Ndjakou *et al*., 2007), anti-oxidant and anti-microbial (Oloyede and Ogunlade, 2013) and molluscicidal (Ayoub and Yankov, 1986) activities. *Albizia zygia* gum has been used as a binding agent in tablet formulations (Femi-Oyewo *et al*., 2004) showing good potential for use as compression coating for drugs targeting the colon, being capable of protecting the core tablet in the physiological environment of the stomach and small intestine.

Based on wide usage of *Albizia zygia* in traditional medicine for the treatment of various ailments and its other applications, it becomes imperative to evaluate its toxicity potential in order to caution or encourage its use. Hence this study is aimed at determining the acute and sub-acute toxic effects of the aqueous stem bark extract of *Albizia zygia* using various haematological, biochemical and pathological indices.

EXPERIMENTAL

Plant material. The fresh stem bark of *Albizia zygia* was collected in August, 2014 along Technical Road, Ugbowo, Benin City. The plant was identified and authenticated by Messrs. O. A. Ugbogu and O. S. Shasanya at the Forestry Research Institute of Nigeria (FRIN), Ibadan where herbarium specimen (FHI 106687) was deposited for future reference.

Preparation of extracts. The fresh stem barks were air-dried for 7 days, reduced to powder form and stored in an airtight container. The dried powdered plant material (270 g) was extracted with distilled water (1.5 L) by simple maceration process for 48 hours. The solution was filtered using a muslin bag and filter paper. The filtrate obtained was dried in an oven set at 40^{o}C to give a yield of 22.3 g (8.26% $^{w}/_{w}$). A stock solution of the extract (200 mg/ml) was prepared from which other concentrations were made as required.

Laboratory animals. Male Swiss albino mice (31.0±6.0 g) and Sprague-Dawley rats (250±70 g) of both sexes obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin City were used for this study. They were allowed to acclimatize for two weeks under standard conditions prior to the commencement of the experiment. The animals were fed with standard pellets and given water *ad libitum* and handled according to standard protocols for the use of laboratory animals (National Institute of health USA; Public health service policy on humane care and use of laboratory animals, 2002).

Acute toxicity studies. Rats and mice of either sex were randomly allotted to the different control and test groups with 5 animals in each group. Four doses of the aqueous extract (500, 1000, 2000 and 5000 mg/kg body weight) were administered by oral intubation and the control group received distilled water (5ml/kg). Another batch of mice was divided into five groups of 5 animals each, with one group serving as the control. The mice were subjected to the same conditions as above. The extract was administered intraperitoneally. Four groups of animals received 100, 200, 500 and 1000 mg/kg body weight of extract, respectively, while the control group received distilled

water. The general symptoms of toxicity and mortality were recorded for 24 hours and a further 2 weeks for any signs of delayed toxicity. The toxic effects observed included agility, muscular tonus, writhes, piloerection etc. The median lethal dose (LD_{50}) was determined using the method of Litchfield and Wilcoxon (1949).

Short-term toxicity studies. Rats were randomly selected into four groups of 9 rats each. The first three groups were given, by oral intubation, 100, 200 and 500 mg/kg body weight of *Albizia zygia* respectively, while the control group received distilled water only. The animals were maintained under standard laboratory conditions and had free access to food and water during the 6-week period of observation. They were observed daily for behavioural changes, any overt toxic manifestations and mortality. The body weight of each rat was measured using an electronic balance prior to treatment, and at weekly intervals for the duration of the study.

On Day 43, blood samples were collected via the abdominal aorta under mild chloroform anaesthesia for haematological and biochemical assays. Haematological parameters including red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), haematocrit (HCT), platelet count (PLT) were determined by standard laboratory procedures (Dacie and Lewis, 1991; Ghai, 1995; John, 1972) using an automated blood analyzer (SYSMEX KX-21N, UK). Biochemical parameters, including serum alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein (TP) total bilirubin (TB), urea and creatinine were also determined using standard techniques (Doumas *et al*, 1971; Doumas *et al*, 1981; King, 1965a and b; Larsen, 1972; Schumann *et al*, 2002; Taylor and Vadgama 1992).

After blood collection, animals were sacrificed by excess chloroform inhalation and the liver, lungs, kidney, heart and spleen were isolated. Relative wet weights of the respective organs were calculated based on the body weight of the animal. Each isolated organ was examined macroscopically for colour changes or any obvious lesions and sections were fixed in 10% buffered formalin for histopathological studies.

Statistical analysis. Results are presented as Mean ± SEM. The statistical significance between the groups was assessed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test (Graph-pad prism 5, Graphpad software in San Diego California). Values were considered significant at $p<0.05$.

RESULTS

Acute toxicity studies. Rats and mice given the aqueous stem back of *Albizia zygia* by the oral route showed no visible signs of toxicity. No deaths were recorded up to the highest dose of 5000 mg/kg. The animals kept under observation for two weeks showed no obvious toxic symptoms; neither food nor water intake was found to be reduced during this period. Administration of the extract via the intraperitoneal route caused sedation, increased respiratory rate and writhing in the animals, but these effects resolved within one hour. Mortalities were observed within 24 hours with the higher doses of the extract. However, no mortalities were recorded at the lowest dose of the extract and the LD_{50} was estimated as 316.2 mg/kg.

Short-term toxicity studies.

Effect of *Albizia zygia* on body weight changes. Administration of *A. zygia* (100 - 500 mg/kg/day) caused no significant body weight changes ($p>0.05$) in extract-treated rats in the first four weeks. There was, however, a significant reduction ($p<0.05$) in body weight by day 35 in the rats treated with the higher doses (200 – 500 mg/kg) of the extract relative to control rats (Figure 1).

Effect of *Albizia zygia* on organ weight indices. Administration of *A. zygia* caused no

significant changes in the organ weight indices of the treated rats at all the doses used (Table 2).

Effect of *Albizia zygia* on biochemical parameters. The effect of *A. zygia* on biochemical parameters is shown in Table 3. Administration of the extract (100-500 mg/kg/day) for 42 days did not significantly ($p>0.05$) alter the serum concentrations of ALT, ALP, TB, albumin and urea in the treated rats. However, the highest dose of 500 mg/kg significantly ($p<0.05$) reduced the concentrations of AST and direct bilirubin compared to the control group. There was a significant increase in total protein in 200 mg/kg treatment group and also a significant decrease in the creatinine of the 500mg/kg treatment group compared to the control group.

Table 1: Effect of *Albizia zygia* on body weight changes in rats

Treatment	Dose (mg/kg)	Change in body weight (g/100g)					
		Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Control (DW)	5 ml/kg	1.96 ± 1.07	-1.45 ±1.58	-1.04±1.93	3.27±2.62	4.35±3.13	2.21±4.46
Extract	100	0.78±2.82	0.64±3.18	-3.77±3.19	3.42±3.54	5.34±3.67	4.08±3.53
Extract	200	-1.45±2.45	-2.29±3.02	-3.07±3.65	-2.83±4.39	-2.46±6.09*	0.28±5.09
Extract	500	-2.57±1.28	-4.53±1.64	-4.51±2.02	-1.07±1.66	-0.48±3.88*	0.36±2.72

Values are mean ± S.E.M. (*n* = 8-9 animals/group), *p<0.05 vs. control; Negative (-) denotes loss in body weight
DW = Distilled water

Table 2: Effect of aqueous stem bark extract of *Albizia zygia* on organ weight indices of some body organs of rats

Groups	Dose (mg/kg)	Heart	Liver	Kidney	Spleen	Lungs
Control (DW)	5 ml/kg	0.34±0.02	3.12±0.17	0.63±0.04	0.40±0.06	0.75±0.04
A. zygia	100	0.36±0.02	3.00±0.08	0.62±0.02	0.45±0.04	0.83±0.04
A. zygia	200	0.37±0.01	2.96±0.12	0.66±0.04	0.52±0.08	0.83±0.05
A. zygia	500	0.34±0.02	3.35±0.14	0.70±0.02	0.66±0.10	0.88±0.04

Control group received 5 ml/kg of distilled water. Values are mean ± SEM, (n= 8-9 animals).
DW = Distilled water

Table 3: Effect of aqueous stem bark extract of *Albizia zygia* on biochemical parameters

Treat-Ment	AST (U/L)	ALT (U/L)	ALP (IU/L)	TP (g/dl)	ALB (g/dl)	TB (mg/dl)	DB (mg/dl)	Creat (mg/dl)	Urea (mg/dl)
Control (DW)	38.42±5.80	19.59±1.69	30.93±3.40	6.00±0.57	2.75±0.27	0.82±0.06	0.65±0.12	1.43±0.34	43.93±9.06
A. zygia									
100mg/kg	28.30±5.42	12.09±2.40	19.54±3.47	4.97±0.55	2.81±0.22	0.69±0.10	0.67±0.10	1.18±0.18	40.34±5.65
200mg/kg	30.40±2.52	16.45±1.53	27.13±3.87	7.57±0.47*	2.32±0.15	0.94±0.11	0.84±0.13	1.43±0.16	64.54±7.60
500mg/kg	18.36±3.19*	17.09±1.96	34.95±5.32	6.94±0.59	2.27±0.30	0.81±0.05	0.48±0.13*	0.75±0.10*	49.80±3.65

Values are mean ± SEM. *p<0.05, significantly different from control group; (n= 8-9 rats).
ALB=Albumin; ALP=Alkaline phosphatase, ALT=Alanine transaminase; AST=Aspartate transaminase;
CREAT=Creatinine; DB=Direct bilirubin; TB=Total bilirubin. DW = Distilled water (5 ml/kg)

Table 4: Effect of aqueous stem bark extract of *Albizia zygia* on some haematological parameters

Groups	Dose (mg/kg)	WBC ($10^3/\mu l$)	RBC ($10^6/\mu l$)	Hb (g/dl)	HCT (%)	PLT ($10^3/\mu l$)
Control (DW)	5 ml/kg	8.87±0.52	8.02±0.28	16.40±0.46	48.07±1.12	367.50±33.55
A. zygia	100	11.28±0.96	8.17±0.19	16.30±0.38	49.32±1.13	353.50±24.50
A. zygia	200	11.48±0.69	7.09±0.36	13.45±0.66	41.17±1.68	411.50±16.06
A. zygia	500	13.32±0.93*	6.05±0.71	11.97±1.39	42.52±3.31	427.00±61.04

Values are mean ± SEM. *p<0.05 significantly different from control group; (n= 8-9 animals)
HCT = Haematocrit; Hb = Haemoglobin count; PLT = Platelet count; RBC = Red blood cell count;
WBC = White blood cell count. DW = Distilled water

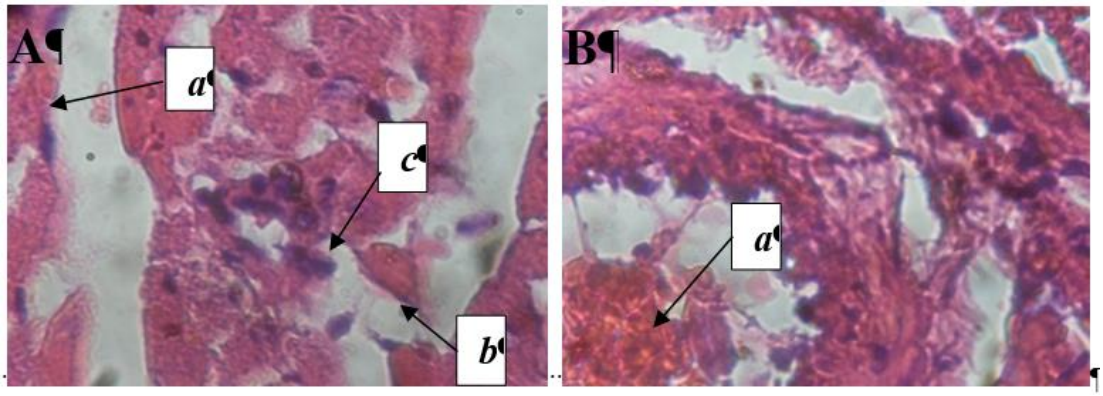

Figure 1: A cross-sectional representation of (A) normal rat heart composed of bundles of myocardiac fibres (*a*), interstitial space (*b*) coronary vessel (*c*) and (B) heart of rat given aqueous extract of *A. zygia* (500 mg/kg/day) for 42 days showing mild coronary vascular congestion (Haematoxylin & Eosin x 400 magnification)

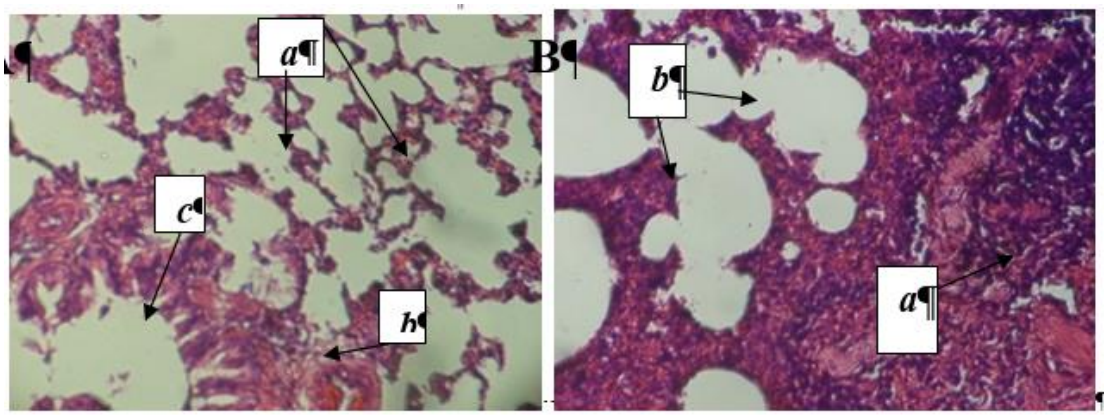

Figure 2: A cross-sectional representation of (A) normal rat lung composed of alveoli (*a*), interstitial space (*b*) terminal bronchiole (*c*) and (B) lung from rat given aqueous extract of *A. zygia* (500 mg/kg/day) for 42 days showing mild activation of lymphoid aggregates (*a*), alveoli (*b*) (Haematoxylin & Eosin x 100 magnification)

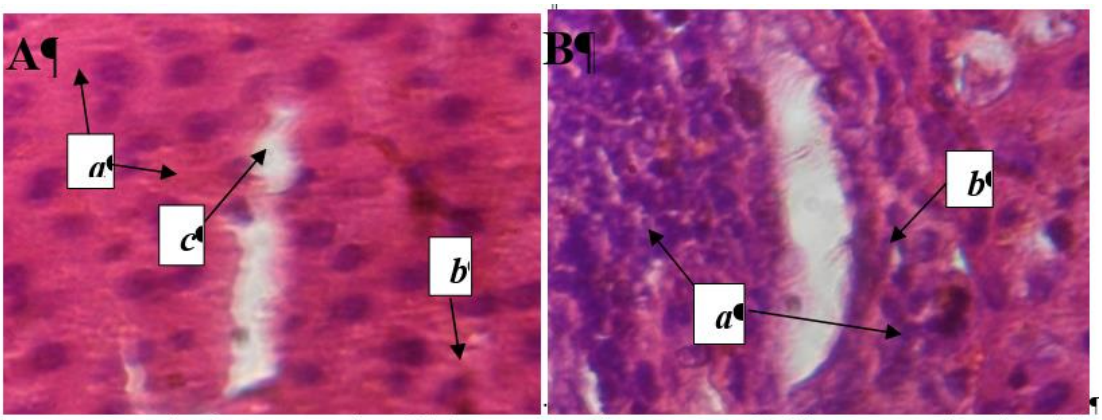

Figure 3: A cross-sectional representation of (A) normal rat liver composed of hepatocytes (*a*), sinusoids (*b*) and portal vein (*c*) and (B) liver of rat given aqueous extract of *A. zygia* (500 mg/kg/day) for 42 days showing mild periportal infiltrates of lymphocytes (*a*) and mild kupffer cell activation (*b*) (Haematoxylin & Eosin x 400 magnification).

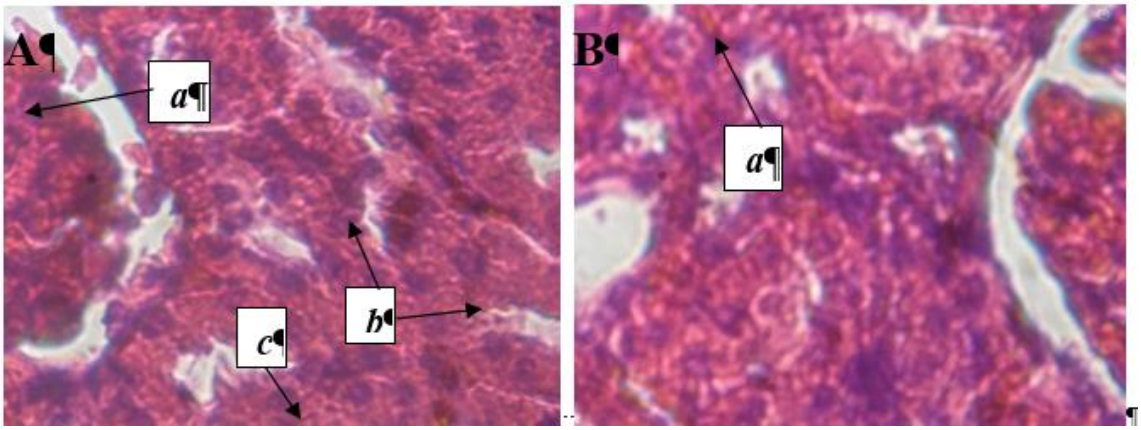

Figure 4: A cross-sectional representation of (A) normal rat kidney composed of glomerulus (*a*), tubules (*b*) interstitial space (*c*) and (B) kidney from rat given aqueous extract of *A. zygia* (500 mg/kg/day) showing mild interstitial congestion (*a*) (Haematoxylin & Eosin x 400 magnification)

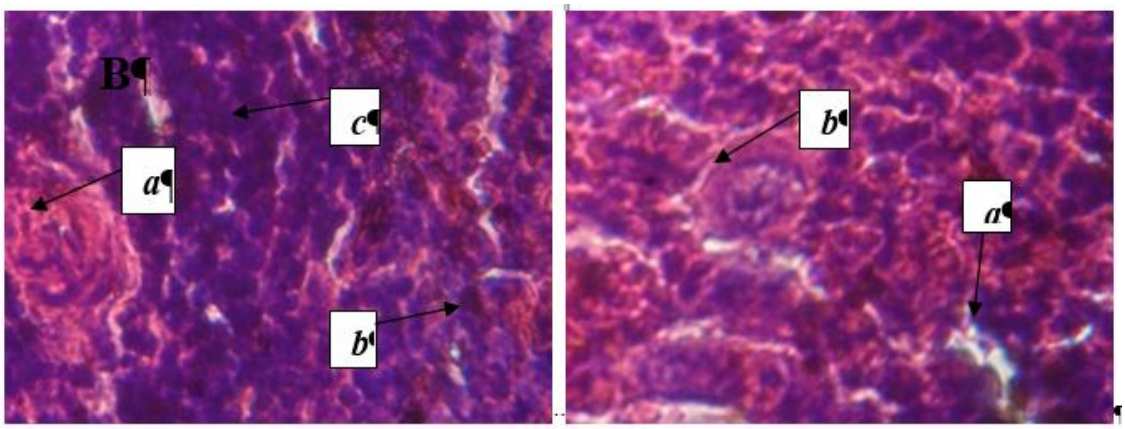

Figure 5: A cross-sectional representation of (A) spleen from normal rats composed of arteriole (*a*), red pulp (*b*) white pulp (*c*) and (B) spleen from rats treated with aqueous extract of *A. zygia* (500 mg/kg/day) for 42 days showing mild follicular (*a*) and histiocytic activation (*b*) (H&E x 400)

Effect of 42-day oral treatment with *A. zygia* on hematological indices. Treatment with different doses of *A. zygia* caused progressive elevations in white blood cell count, which was significant at 500 mg/kg ($p<0.05$) compared to the control group. RBC and haemoglobin were dose-dependently but not significantly reduced compared to control, while the two higher doses of the extract caused mild (not significant) elevations in the platelet count (Table 4).

Histopathological analysis. Histopathological examination of the various organs showed that control rats given distilled water during the 42-day study period maintained intact pericardium and myocardium (Fig 1A), normal lungs composed of alveoli, interstitial spaces and well defined bronchioles (Fig. 2A), and normal livers with central vein and radiating hepatocytes (Fig. 3A). The renal tubules (Fig. 4A) and the spleen (5A) maintained normal architecture. Rats given the extract (500 mg/kg/day) for 42 days exhibited mild coronary congestion of the heart (Fig. 1B), mild activation of lymphoid aggregates and alveoli (Fig. 2B) and mild periportal infiltrates of lymphocytes in the liver (Fig. 3B). There was mild interstitial congestion of the kidney (Fig. 4B) and mild follicular activation of the spleen (Fig. 5B).

DISCUSSION

Herbal medicines have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demand (Sushruta *et al.*, 2006). The effectiveness of some of these herbal remedies have been validated through research and clinical studies though their safety is doubtful.

The oral acute toxicity study of *Albizia zygia* extract showed reduced movement and writhes in treated mice and rats. No mortality was observed up to a maximum oral dose of 5000 mg/kg body weight, neither were there any signs of delayed overt toxicity, so the oral LD_{50} could not be determined. According to OECD-423 guidelines for acute oral toxicity, an LD_{50} dose of 2,000 mg/kg is categorized as unclassified (OECD, 2000). This suggests that the extract is relatively safe on acute single oral exposure. Complete mortality was observed in all the treated mice within 24 hours of intraperitoneal administration of the highest dose (1000 mg/kg) of the extract. Behavioural changes such as reduced movement, writhing and increased grooming were also observed at that dose. Though the extract is not used via the parenteral route, the relatively low LD_{50} of 316.2 mg/kg calls for caution in the use of the extract through other routes other than oral.

Reduction in body weight and change in internal organ weight are simple and sensitive indices of toxicity after exposure to a toxic substance (Witthawaskul *et al*., 2003). *Albizia zygia* at lower doses did not cause significant changes in the pattern of change in body weight over the 42-day period when compared to the control. This indicates that it has no adverse effects on the pattern of weight gain/loss which may mean that it lacks adverse effects on metabolic activities of the rat or does not alter appetite and feeding habits. It may also mean that *A. zygia* had no effect on normal growth of rats (Kripa *et al*., 2011). Organ weight has been implicated as a sensitive indicator of potentially harmful effects of an experimental compound, as significant differences between treated and control groups are known to occur without any morphological changes (Pfeiffer, 1968; Bailey *et al.*,2004). There were no changes observed in the relative organ/body weight ratio of liver, heart, kidney and spleen compared to control animals. This indicates absence of any form of hyperplasia, hypertrophy or organ atrophy.

Blood is an important index of physiological and pathological status in man and animals and the parameters usually measured are total red blood cell counts and its indices, haemoglobin, packed cell volume (haematocrit), platelets, total white blood cell count and its differential (Raza *et al*., 2002; Oduola *et al*., 2007). The normal ranges of hematological parameters can be altered by the ingestion of some toxic plants (Abatan and Arowolo, 1989; Adedapo *et al*., 2004). The major function of the red blood cells is to transport haemoglobin, which in turn carries oxygen from the lungs to the tissues (Mayne, 1994). Haemoglobin concentrations and haematocrit are indices for measuring the degree of anaemia (Moss, 1999). Hence frank reductions in the levels of RBC, haemoglobin and hematocrit are good indicators of anaemia. The non-significant decreases of the aforementioned haematological parameters in rats fed with the extract suggests that the integrity of the red blood cells are maintained and oxygen carrying capacity of blood is not affected. The appreciable increase in WBC count, especially at the highest dose of the extract, may be a response to infection, as leucocytes are known to increase considerably when infection occurs (Swash and Mason, 1984). It could also be a response to stress, inflammation or the toxic effect of the extract on the bone marrow. The dose dependent (though non-significant) increase in platelets in animals given the extract may indicate the

ability of the extract to precipitate thrombocytosis (a condition in which there is an excessive number of platelets in the circulatory system) especially at the high doses. This effect coupled with the leukocytosis observed might portend danger for individuals with cardiovascular disorders.

Increased levels of AST and ALT in the blood are associated with damage of hepatic cells (Witthawaskul, 2003; Bürger *et al*., 2005). The highest dose of the extract caused a significant decrease in the value of AST compared to the control group. No significant alterations occurred on other liver enzymes such as ALT, ALP as well as total bilirubin and protein concentrations. This is a strong indication of the oral safety of *A. zygia* on liver function. Serum creatinine and urea are reliable markers of renal function. Increase of blood creatinine has been shown to be a good indicator of negative impact in kidney functions (Rhiouani *et al.,* 2008). The effect of *A. zygia* on creatinine and urea is a strong indication of its safety on renal function. The significant decrease in serum creatinine concentration at the highest dose of the extract (500 mg/kg) compared to the control suggests a possible nephroprotective potential of the extract. *A. zygia* stem bark contains alkaloids and flavonoids (Oloyede *et al*., 2013) which are known to confer protection on the liver and kidneys through prevention of tissue lipid peroxidation, mediated by their antioxidant and free-radical scavenging activities (Fraga *et al*., 1987; Laughton *et al*., 1989; Sanz *et al*., 1994).

A. zygia extract caused mild congestion of the coronary vessels of the heart at the higher doses. There was evidence of activation of lymphoid aggregates indicating the activation of the local immune system of the lungs. Activation of both the kupffer cells and lymphocytes was observed in the liver, suggesting the immunostimulating effect of *A. zygia*. Also activation of the lymphoid aggregates of the arterioles in the spleen in addition to the activation of the histiocytes, indicates the activation of both inductor and effector arms of the immune system by *A. zygia*.

In summary, the study presents evidence that oral intake of the stem bark extract of *Albizia zygia* does not exhibit marked toxic effects on sub-acute exposure. Instead, there was mild activation of the local immune system in the spleen, suggesting that aqueous extract of *A. zygia* stem bark might have some immune boosting qualities. The aqueous stem bark extract of *Albizia zygia* at the dosage levels employed in this study did not exhibit marked toxicity in the animals. However, caution should be exercised in consumption of large quantities of this plant.

REFERENCES

Abatan, M. O. and Arowolo, R.O (1989). Toxicity of *Eugenia uniflora* to rats; *Nig. J. Anim. Prod.* 16, 16-19

Adedapo, A.A.; Abatan, M.O.; Olorunsogo, O.O. (2004); Toxic effects of some plants in the genus Euphorbiaceae on hematological and biochemical parameters of rats *Veterinarski Arhiv* 74(1), 53-62.

Ayoub, S. M. H. and Yankov, L. K. (1986); The molluscicidal factor of tannin-bearing plants, Int. J. Crude Drug Res. 24(1), 16-18.

Bailey, A.S.; Zidell, R. H. and Perry, R. W. (2004); Relationship between organ weight and body/brain weight in the rat: what is the best analytical endpoint? *Toxicol. Pathol.* 32, 448-466.

Bürger, C.; Fischer, D. R.; Cordenunzzi, D. A.; de Borba Batschauer, A. P.; Filho, V. C. and dos Santos Soares, A. R. (2005); Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasiliensis*) (Asteracea) in mice. *J. Pharm. Pharmaceut. Sci*. 8 (2), 370-373.

Burkill, H. M. (1995); The Useful Plants of West Tropical Africa. 2nd Edition. Families J-L. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 3, 857.

Dacie, J. V. and Lewis, S. M. (1991); Practical Haematology, 7th Ed., Churchill Livingstone, New York, pp 50-56.

Doumas, B.T.; Bayse, D.; Bornerk, K.; Carter, R. J., Peters, T.; and Schaffer, R. (1981); A candidate

reference method for determination of total protein in serum 1: Development and validation. *Clin. Chem.* 27, 1642.

Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1971); Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chim. Acta*. 31, 87-96.

Femi-Oyewo, M. N.; Adedokun, M.O. and Olusoga, T. O. (2004); *Trop. J. Pharm. Res.* 3(1), 279-284.

Fraga, C.; Martino, V.; Ferraro, G.; Coussio, J. and Boveris, A. (1987); Flavonoids as antioxidants evaluated by *in vitro* and *in situ* liver chemiluminiscence. *Biochem. Pharmacol.* 36, 717-720.

Ghai, C. L. (1995); A Textbook of Practical Physiology. Jaypee Medical Publishers (P) Ltd., New Delhi, India pp 119-202

John, M. B. (1972); Laboratory Medicine Haematology. 4th Ed, C. V. Mosby Co, St. Louis, p. 1198.

King, J. (1965a); The hydrolases-acid and alkaline phosphatases. In: Van, D. (Ed.) Practical Clinical Enzymology. Nostrand Company Limited, London. P.91.

King, J. (1965b); The transferases-alanine and aspartate transaminases. In: Van, D. (Ed.) Practical Clinical Enzymology. Nostrand Company Ltd., London p.191.

Kripa, K.G.; Chamundeeswari, D. and Thanka, J. (2011); Acute and sub-acute toxicity evaluation of ethanolic extract of *Leucas aspera* (Lamiaceae) in experimental rats; *Int. J. Drug Dev. Res.* 3(3), 339-347.

Larsen, K. (1972); Creatinine assay by reaction kinetic principle; *Clin. Chim. Acta* 41:209.

Laughton, M. J.; Halliwell, B.; Evans, P. J. and Hoult, J. R. S. (1989); Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myeicetin. Effects on lipid peroxidation, hydroxyl radical generation and bleomycin dependent damage to DNA; *Biochem. Pharmacol.* 38, 2859-2865.

Litchfield, J. T. and Wilcoxon, F. (1949); A simplified method of evaluating dose-effect experiments; *J. Pharmacol. Exper. Therap.* 96, 99-113.

Mayne, P. (1994); Clinical chemistry in diagnosis and treatment. 6th Ed., Oxford University Press, Inc., New York, USA pp. 281-323.

Moss, P. P. (1999); Blood banking: Concepts and Applications. W. B. Saunders Co., Philadelphia, USA pp. 12-34.

Ndjakou, L. B.; Vonthron-Senecheau C.; Fongang S. R.; Tantangmo. F.; Ngouela, S.; Kaiser M.; Tsamo, E. R.; Anton, R. B. and Weniger, B. (2007); *In vitro* antiprotozoal activities and cytotoxicity of some selected Cameroonian medicinal plants; *J. Ethnopharmacol.* 111, 8-12.

Oduola, T.; Adeniyi, F. A. A.; Ogunyemi, E. O.; Bello, I. S.; Idowu, T. O. and Subair, H. G. (2007); Toxicity studies on an unripe *Carica papaya* aqueous extract: biochemical and haematological effects in Wistar albino rats; *J. Med. Plant Res.* 1(1), 001-004

OECD (2000) Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment, No.24.

Oloyede, G. K. and Ogunlade, A. O. (2013); Phytochemical screening, antioxidant, antimicrobial and toxicity activities of polar and non-polar extracts of *Albizia zygia* (DC) stem-bark; *Ann. Rev. Res. Biol.* 3(4), 1020-1031.

Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R. and Anthony, S. (2009); Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya. (http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp)

Pfeiffer, C. J. (1968); A mathematical evaluation of the thymic weight parameter; *Toxicol. Appl. Pharmacol.* 13(2), 220-227.

Rates, S. M. (2001). Plants as source of drugs; *Toxicon* 39(5), 603-613

Raza, M.; Al-Shabanah, O. A.; El-Hadiyah, T. M., Al-Majed, A. A. (2002); Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice; *Sci. Pharma.* 70, 135-145.

Rhiouani, H.; El-Hilalya, J.; Israili, Z. H. and Lyoussia, B. (2008); Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *J. Ethnopharmacol.* 118, 378-386.

Sanz, M. J.; Ferrandiz, M. L.; Cejudo, M.; Terencio, M. C.; Gil, B.; Bustos, G.; Ubeda, A.; Gunasegaran, R. and Alcaraz, M. J. (1994); Influence of a series of natural flavonoids on free radical generating systems and oxidative stress; *Xenobiotica* 24, 689-699.

Schumann, G.; Bonora, R.; Ceriotti, F.; Férard, G.; Ferrero, C. A.; Franck, P. F. H.; Gella, F. J.; Hoelzel, W.; Jørgensen, P. J.; Kanno, T.; Kessner, A.; Klauke, R.; Kristiansen, N.; Lessinger, J. M.; Linsinger, T. P. J.; Misaki, H.; Panteghini, M.; Pauwels, J.; Schiele, F. and Schimmel, H. G. (2002); 725 IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37ºC, part 5; *Clin. Chem. Lab. Med.* 40, 725-733.

Sushruta, K.; Satyanarayana, S.; Srinivas, N. and Sekhar, R. J. (2006); Evaluation of the blood-glucose reducing effects of aqueous extracts of selected Umbelliferous fruits used in culinary practice. *Trop. J. Pharmaceut. Res.* 5(2), 613-617.

Swash, M. and Mason, S. (1984); Hutchison's clinical methods. 18th ed. East Sussex, Bailliere Tindall; 34.

Taylor, A. J. and Vadgama, P. (1992); Analytical reviews in clinical biochemistry: the estimation of urea; *Ann. Clin. Biochem.* 29, 245-264.

Witthawaskul, P.; Ampai, P.; Kanjanapothi, D. and Taesothikul, N. (2003); Acute and sub-acute toxicities of the saponin mixture isolated from *Schejjlera leucantha* Viguier. *J. Ethnopharmacol* 89: 115-121.