WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 4, 810-822. Research Article ISSN 2277– 7105

EVALUATION OF *MIMOSA PIGRA* ROOTS ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF ALBINO RATS

Shorinwa Olusayo A.[1]*, Etozuo Nnamdi[1], Afieroho Ozadheoghene E.[2]

[*1]Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

[2]Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

Article Received on
26 Jan 2016,
Revised on 17 Feb 2016,
Accepted on 08 March 2016
DOI: 10.20959/wjpr20164-5843

***Correspondence for Author**
Dr. Shorinwa Olusayo A.
Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

ABSTRACT

To investigate the effect of sub-acute doses of the ethanol extract of *mimosa pigra* on haematological and biochemical indices in albino rats. Animals of both sexes were divided into four groups of six each. The extract treated groups were administered with oral doses of 250mg/kg, 500mg/kg and 1000mg/kg of the extract while the control received distilled water for 28 days consecutively. Blood samples were collected from the rats on the 29th day for evaluation of haematological (pcv, rbc and hb) and biochemical (ast, alt, alp, total protein, cholesterol, urea, creatinine and total bilirubin) parameters. The results of the study showed that there was a statistically significant ($p<0.05$) increase in packed cell volume, haemoglobin and red blood cell count at doses of 500mg/kg and 1000mg/kg. The extract produced no significant changes in the levels of total bilirubin, total cholesterol, total protein, aspartate aminotransferase, and alkaline phosphatase in all of the treated groups. But, a marginal statistically significant increase and a significant increase ($p<0.05$) in the level of alanine transaminase at doses of 500mg/kg and 1000mg/kg of the extract respectively were observed. Serum levels of urea, creatinine were not affected. The findings of this study has shown that the roots of *mimosa pigra* may be safe at doses below 500mg/kg but may pose toxicological risks at doses greater than 500mg/kg with the liver being the most affected on prolonged usage.

KEYWORDS: *Mimosa pigra*, Sub-acute, Toxicity, Haematological, Biochemical.

INTRODUCTION

Medicinal plants have been the most important source of life saving drugs for the majority of the world's population.[1] It is clear that medicinal plants will continue to be employed in the treatment of diseases especially in rural settings and economically modest societies as they are easily sourced and are affordable as compared to orthodox treatment.[2] However while orthodox medicines have side effects and adverse effects associated with their use in disease treatment and management, data and information concerning these unpleasant effects are reported as part of pharmacovigilance efforts. As such, timely and up-to-date information regarding the toxicity of these drug substances are readily made available in standard references for physicians, pharmacists and all members of the health team. Also, strict quality control measures are employed to ensure the sustained quality, uniformity of content and ratio of active substance contained in a given drug formulation. In contrast to orthodox drugs, traditional medicines which contain crude medicinal principles have no well-regulated standardization, quality control and assurance measures, and most importantly comprehensive information about their safety/toxicity profiles. Hence, consumers of traditional medicine are unaware of the potential adverse effects of the medicines they consume.

*Mimosa pigra Linn. (*Fabaceae*)*, the giant sensitive plant, is a prickly leguminous shrub that can reach up to 6m in height.[3] Different parts of *Mimosa pigra* are used in traditional medicine in Madagascar, tropical Africa, South America and Indonesia for various troubles including head colds, toothaches, eye medicine, diarrhoea, weak heart or weak pulse, and also for its antimicrobial activity.[4,5] Leaves and stems of this plant are commonly prescribed by the folk medicinal practitioners (Kavirajes) of Bangladesh for lowering of blood sugar in diabetic patients and for alleviation of pain.[6] The root of this plant has been reported to possess anti-diabetic, anti-inflammatory and analgesic activities by.[7, 8] There exists little information concerning the safety profile of Mimosa pigra roots. Hence, this study was aimed at evaluating the toxicological effect of ethanol extracts of the roots of *Mimosa pigra* on haematological and biochemical indices in albino rats.

MATERIAL AND METHODS

Plant Materials

The roots of *M. pigra* were collected from Chaza village, Suleja, Niger State of Nigeria, by Mallam Mu'azam of National Institute for Pharmaceutical Research and Development

(NIPRD), Abuja, Nigeria. The identification and authentication were done by Jemilat Ibrahim, a staff of same institute, where a voucher specimen (NIPRD/H/6405) was deposited at the herbarium for reference.

Extraction of plant materials

The roots of *Mimosa pigra* Linn. were cleaned, washed under running tap water, air dried under shade, for about a month and pounded into fine powder using mortar and pestle. A 1kg quantity of the powder was macerated using 10L of absolute ethanol, for 72 hours. The menstruum was filtered through Whatmann No. 1 (Whatmann International Ltd, Maidstone, UK) paper and evaporated under reduced pressure using a rotary evaporator. The resulting concentrate was then evaporated to dryness over a water bath set at 40ºC. The dried root extract was weighed, percentage yield determined and stored in an airtight container, refrigerated and used for the study.

Chemicals and Reagents

Absolute ethanol manufactured by Sigma–Aldrich, USA was exclusively used for the extraction.

Phytochemical screening

Phytochemical screening was carried out on the ethanol extract of the *Mimosa pigra* roots for the detection of various plant constituents including: alkaloid, flavonoids, tannins, phlobatannins, anthraquinones and saponins using standard procedures.[9]

Animals

Adult wistar rats of both sexes maintained at the animal house of the Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria were used for the study. The animals were fed ad libitum with standard feed (Guinea feeds, Nigeria PLC) and had free access to water. They were maintained under standard conditions of humidity and temperature. The animals were acclimatized for two weeks before the commencement of the study. All the standard ethical requirements for experimental animals were complied with.

Acute toxicity study

The acute toxicity of the ethanol extract of *M.pigra* Linn. was evaluated by[7] and the LD_{50} was reported to be greater than 5000mg/kg according to[10] method.

Sub-acute toxicity study

Twenty four rats were selected by randomization and then divided into four groups of six rats each. They were designated as groups A, B, C and D. The first group (A) served as control, and were given only distilled water (5ml/kg body weight), while the remaining three groups B, C, and D were given 250, 500, and 1000 mg/kg body weight of ethanol extract of *M. pigra* Linn respectively which corresponded to $1/20^{th}$,$1/10^{th}$ and $1/5^{th}$ of the 5000mg/kg dose. The first day of dosing was taken as D_0 whereas the day of sacrifice was designated as D_{28}.

Weekly body weight

The body weight of each rat was assessed using a sensitive balance once during the acclimatization period, once before commencement of dosing, every seventh day during the dosing period and once on the day of sacrifice.

Relative organ weight

On the 28^{th} day (D_{28}) of the dosing period, all the animals were euthanized by exsanguinations under diethyl ether-induced anaesthesia. The organs namely the liver, and kidneys were carefully dissected out and weighed in grams (absolute organ weight) as described by.[11] The relative organ weight of each animal was then calculated using the equation:

$$Relative\ Organ\ Weight = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100 \quad \ldots . 1$$

Haematological assay

Blood samples collected in the heparinized tubes were used to investigate white blood cells (WBC), red blood cells (RBC), packed cell volume (PCV) and haemoglobin (Hb).

Biochemical Analysis of Serum

Blood samples from the exsanguinated rats were collected into non heparinized tubes and centrifuged at 3000 revolutions per minute (rpm) for 10 min. The serum thus obtained was analyzed to determine the level of the liver enzymes Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), using the method of.[12] Serum urea, uric acid, creatinine, bilirubin and protein were evaluated by the method of[13] and total serum cholesterol by the method of.[14]

Statistical analysis

Results were expressed as the mean ± standard error of mean (SEM). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) and Student's t-test to analyse the differences between the control group and treatment groups. P-values <0.05 were considered to be statistically significant.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the extract of *M. pigra* Linn revealed the presence of tannins, phlobatannins, flavonoids, triterpenes and saponins in the extracts, as shown in table 1. However alkaloids, anthraquinones and phenolic compounds were absent.

Table 1: Phytochemical screening of ethanol extract of roots of *M. pigra* Linn.

Chemical constituent	Observation in extract
Alkaloids	-
Tannins	+
Anthraquinones	-
Phlobatannins	+
Flavonoids	+
Triterpenes	+
Saponins	+

+ shows presence of chemical constituent

- shows absence of chemical constituent

Weekly body weight

During the 28 days of study and observation, there were no statistically significant (P>0.05) changes in the bodyweights of the treated rats compared to the control rats, and also no statistically significant difference between day 0 (D_0) and day 28 (D_{28}) as shown in Table 2.

Table 2: Mean bodyweight of rats after daily oral treatment (28 days) with *M. pigra* Linn ethanol extract.

Group	Dose	Day				
	(mg/kg)	0	7	14	21	28
Control	5ml	204.50 ± 7.63	222.83 ± 8.12	231.83 ± 6.98	241.33 ± 9.82	260.50 ± 4.45
Extract	250	135.40 ± 4.09	155.33 ± 2.76	161.60 ± 2.77	171.17 ± 5.21	172.25 ± 5.71
Extract	500	188.50 ± 5.91	210.83 ± 6.44	221.60 ± 8.38	229.60 ± 6.04	237.25 ± 4.23
Extract	1000	142.50 ± 6.40	173.33 ± 6.10	178.17 ± 7.37	175.00 ± 5.68	180.50 ± 8.77

Values are expressed as Mean ± Standard error of mean of 6 rats treated for 28 days.

Relative Organ Weight

There were statistically significant ($P<0.05$) changes in the relative weights of the liver specifically between the control group and the group of rats treated with 1000mg/kg of the extract. There was a statistically significant ($P<0.05$) increase in the relative kidney weight of the group treated with 250mg/kg of the extract (Table 3).

Table 3: Effects of the ethanol root extracts of *M. pigra* Linn. on relative organ weights of rats

Group	Dose (mg/kg)	Liver	Kidney
Control	5ml	3.21 ± 0.16	0.35 ± 0.04
Extract	250	4.17 ± 0.64	0.64 ± 0.05*
Extract	500	3.81 ± 0.35	0.35 ± 0.02
Extract	1000	3.79 ± 0.10*	0.37 ± 0.02

** Significantly different from controls (P<0.05). Values are expressed as Mean ± Standard error of mean of 6 rats treated for 28 days*

Haematology

There were no significant (P>0.05) changes in white blood cell count of all the treated groups. There was however, a statistically significant(P<0.05) increase in packed cell volume (fig 1), haemoglobin (fig 2) and red blood cell (fig 3) count between the control and rats given 500mg/kg and 1000mg/kg of the extract.

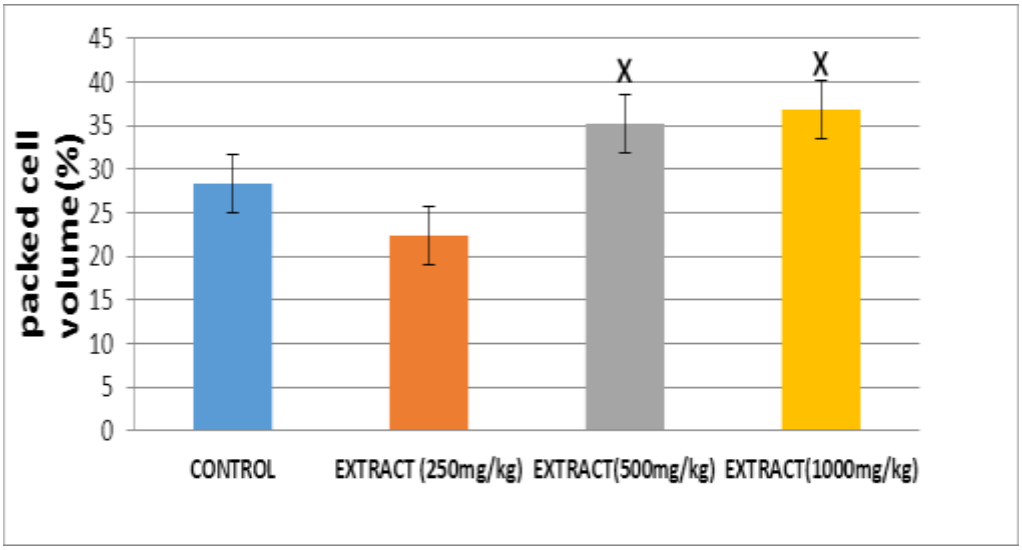

Figure 1: Effects of the ethanol root extract of *M. pigra* Linn on packed cell volume.

Values represent the mean ± SEM (n=6); x = P<0.05, significantly different from control

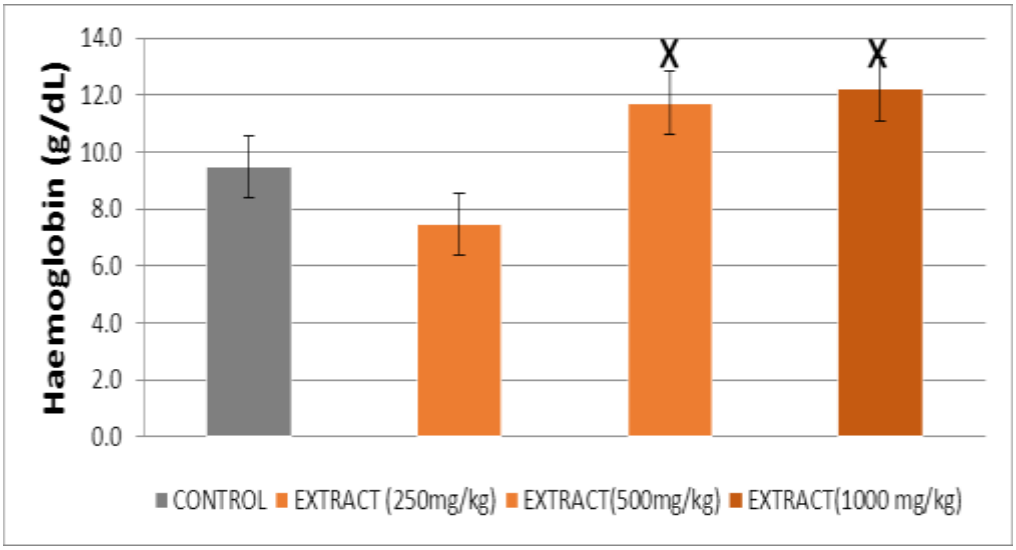

Figure 2: Effects of the ethanol root extract of *M. pigra* Linn on haemoglobin.

Values represent the mean ± SEM (n=6); X= P<0.05, significantly different from control

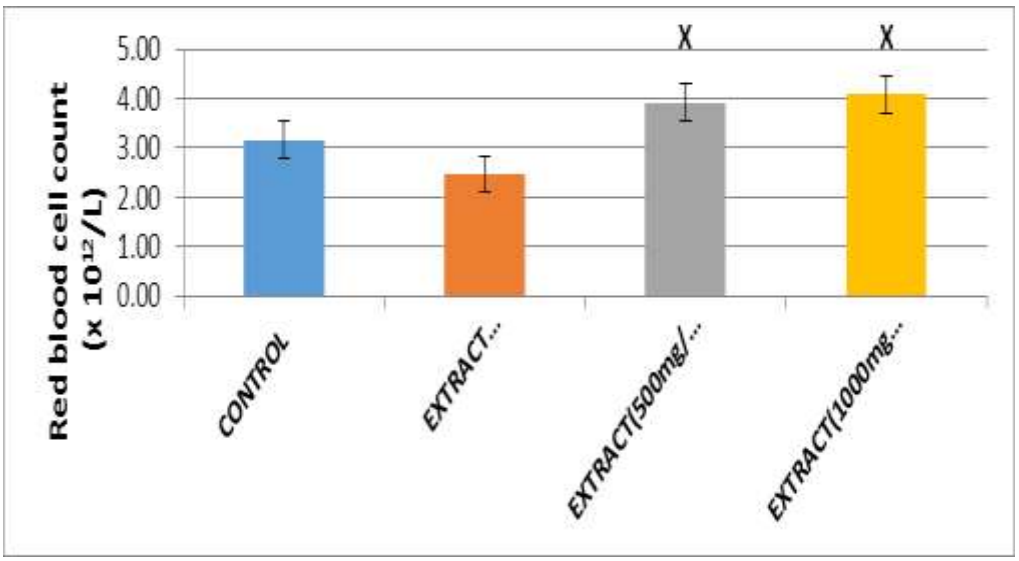

Figure 3: Effects of the ethanol root extract of *M. pigra* L. on red blood cell count.

Values represent the mean ± SEM (n=6); x = P<0.05 significantly different from control

Effects on Hepatic Function Indices

The extract produced no significant (P>0.05) changes in the levels of total bilirubin, total protein (table 4), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in any of the treated groups. But, a marginally significant(P<0.1) increase in the level of alanine transaminase (ALT) in the group treated with 500mg/kg of the extract and a significant increase as well, in the group treated with 1000mg/kg of the extract in relation to the control were observed (figures 4 – 6).

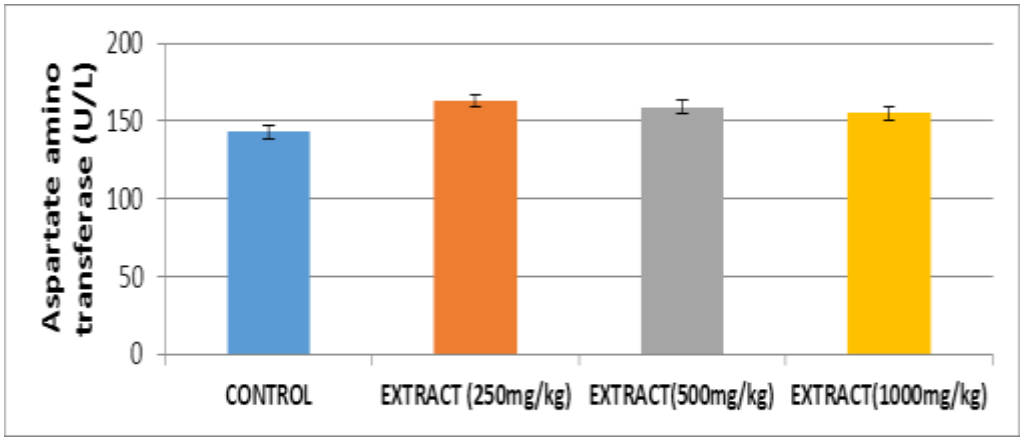

Figure 4: Effects of the ethanol extract of *M. Pigra* Linn. on liver aspartate amino transferase enzyme levels in rats.

Values represent the mean ± SEM (n=6)

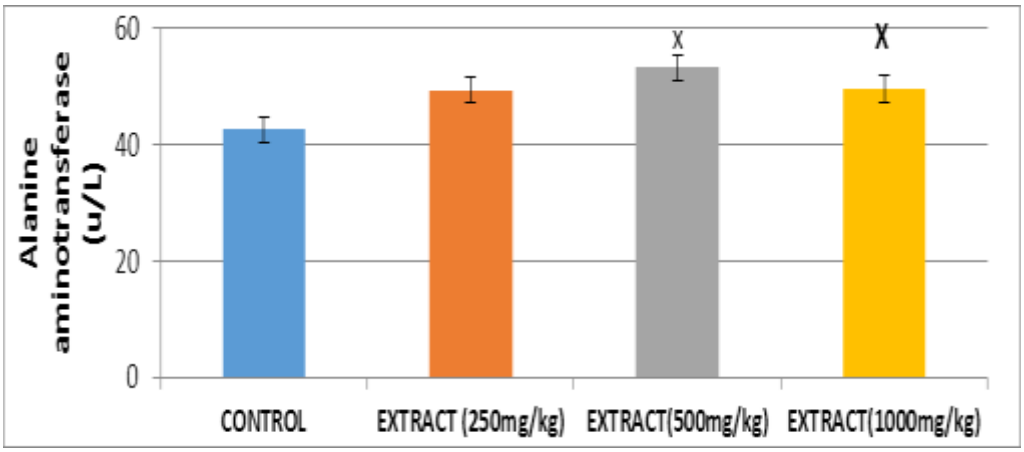

Figure 5: Effects of the ethanol extract of *M. Pigra* Linn. on liver alanine amino transferase enzyme levels in rats

Values represent the mean ± SEM (n=6); X= p<0.05. Significantly different from control

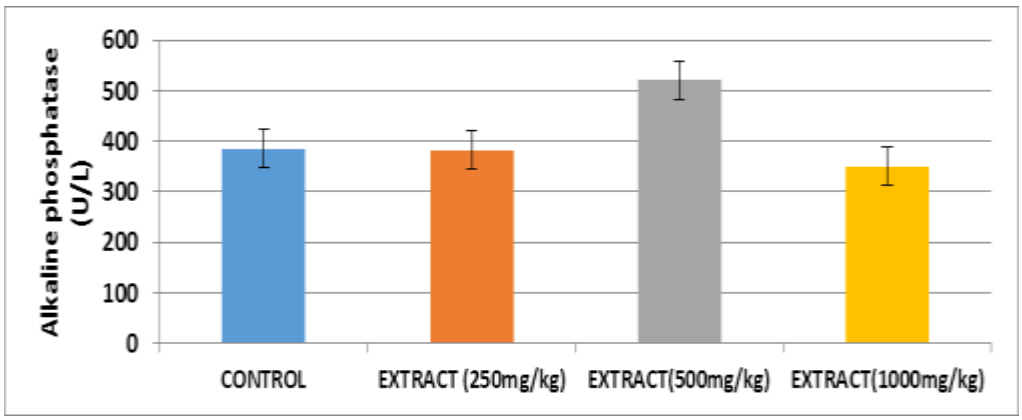

Figure 6: Effects of the ethanol extract of *M. Pigra* Linn. on liver alkaline phosphatase enzyme levels in rats.

Values represent the mean ± SEM (n=6)

Effects on renal function

There was no statistically significant (P>0.05) alteration in the blood urea and creatinine of all the treated animals in relation to the control group.

Effects on cholesterol levels

There was no statistically significant (P>0.05) difference in cholesterol levels between the control and treated groups (table 3).

Table 3: Effects of the extract of *M. pigra* Linn. on total protein, total cholesterol and total bilirubin in rats*

Group	Dose (mg/kg)	Total Protein (g/L)	Total Cholesterol (mmol/L)	Total Bilirubin (µmol/L)
Control	0	63.00 ± 2.32	2.38 ± 0.20	3.60 ± 0.51
Extract	250	59.67 ± 1.86	2.13 ± 0.27	3.33 ± 0.33
Extract	500	65.75 ± 3.33	2.68 ± 0.41	2.25 ± 0.25
Extract	1000	58.20 ± 1.66	2.46 ± 0.33	3.40 ± 0.24

**Values represent the mean ± SEM (n=6)*

DISCUSSION

The purpose of this study was to look at the effect of root extract of *M. pigra* Linn on haematological and biochemical indices following sub-acute exposure using experimental animals. A wide variety of adverse effects can be detected from sub-acute toxicity studies. The result from such studies can provide information, which will aid in selecting dose level, and regimen.[15] Acutely nontoxic compounds may be toxic on prolonged exposure even at low dose levels due to accumulation, changes in enzyme level and disruption of physiological and biochemical homeostasis.[15]

The phytochemical screening of the plant extract showed the presence of saponins, flavonoids, triterpenes, tannins and phlobatannins. This can be related to a study conducted by,[16] which revealed the presence of saponins and tannins in the ethanol extract of *M. pigra.*

There were no statistically significant changes in the bodyweights of the treated rats compared to the control rats, indicating that the ethanol root extract of *M. pigra* did not have any adverse effects on the body weight, which is an important parameter used to assess the response to drug therapy and to indicate adverse effects of drugs.[17]

A statistically significant increase in packed cell volume, haemoglobin and red blood cell count between the control and groups C (500mg/kg) and also D (1000mg/kg) indicate that the ethanol extract of *M. Pigra* may affect the hematopoietic system. The results of biochemical analysis of hepatic function showed that the extract produced no significant changes in the levels of total bilirubin. Elevation of bilirubin suggests increase in haemolysis.[18] The extract of *M. pigra* did not alter the bilirubin level of the treated rats. There were no statistically significant changes in total cholesterol and total protein. There were also no statistically significant changes in AST, and ALP in any of the treated groups. But, a marginally significant ($P<0.1$) increase in the level of ALT in the group treated with 500mg/kg of the extract and a significant increase as well, in the group treated with 1000mg/kg of the extract in relation to the control. Serum ALT and AST are useful indices for identifying inflammation and necrosis of the liver.[19] A rise in the activities of ALT and AST is a sensitive indicator of damage to cytoplasmic and mitochondrial membranes and their relative plasma activities may help to indicate the type of hepatic damage whenever it manifests.[20] ALT has its highest concentration in the liver with kidney and skeletal muscles having lesser activity of the enzyme. ALT measurements are however more liver specific than the AST and its activity is usually greater than AST activity at early or acute hepatocellular disease. AST on the other hand tend to be released more than the ALT in chronic liver diseases such as cirrhosis.[21] There were no adverse effects on renal function since there was no significant alteration in the blood urea and creatinine level of all the treated animals in relation to the control group. The findings of this study corroborates the reports of [22] which stated that the acetone extract of *Crinum jagus* bulbs did not produce significant changes in the levels of bilirubin, total cholesterol and total protein even though it affected some of the examined biochemical parameters. The phytochemical screening of the plant extract showed the presence of saponins. Saponins have been reported to cause disruption of biological membranes by[23] and generation of free radicals by[24] that cause lipid peroxidation.[25] Saponins make the lipid bi-layer permeable to macromolecules[26] by inducing pore-like structures.[23] The subsequent increase of membrane fluidity was supposed to be one of the key steps in saponin-induced hepatotoxicity.[23] Consequently, changes in some biochemical parameter levels on both sides of the membrane (electrolytes, enzyme substrates and enzymes) may emerge as signs of saponin-induced biological effects. Thus, the observed changes in the biochemical parameters may be attributed to the presence of saponin as a phytoconstituent of this plant. This suggests that the extract is not nephrotoxic at the doses studied.

CONCLUSION

The results of this study have shown that the root extract of *M. pigra* may be safe at doses below 500mg/kg. But doses above 500mg/kg may pose toxicological risks, with the liver being the most affected on prolonged usage.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest.

REFERENCES

1. Tripathi L, Tripathi JN. Role of Biotechnology in Medicinal Plants. Trop J Pharm Res, 2003; 2(2): 243-53.
2. Fasola TR, Egunyomi A. Nigerian Usage of Bark in Phytomedicin Ethnobot Res Appl, 2005; 3: 073-7.
3. Lonsdale WM, Miller IL, Forno IW *Mimosa pigra* L. The Biology of Australian Weeds. Groves RH, Shepherd RCH, Richardson RG (Eds.). RG & FJ Richardson, Melbourne: 1995; 169 – 188.
4. Grosvenor PW, Supriono A, Gray DO. Medicinal Plants from Riau Province, Sumatra, Indonesia. Part 2: Antibacterial and Antifungal Activity. J Ethno-Pharmacol, 1995; 45: 97–111.
5. Rosado-Vallado M, Brito-Loeza W, Mena-Rejon GJ, Quintero-Marmol E, Flores-Guido JS .Anti- Microbial Activity Of Fabaceae Species Used In Yucatan Traditional Medicine. Fitoterap, 2000; 7(5): 570–573.
6. Toma TT, Rahman S, Jahan S Md, Haque M, Agarwala B Md, Shelley MR, Hossain S, Mahal MJ Md, Hossain S, Rahmatullah M. Antihyperglycemic and antinociceptive activity of Fabaceae family plants – an evaluation of *Mimosa pigra* L. Leaves. Adv Nat Appl Sci, 2012; 6(8): 1552.
7. Shorinwa OA, Onwuka CK, Ukwueze SE. Evaluation of the anti-diabetic potential of ethanol root extract of Mimosa pigra Linn (Fabaceae) in alloxan induced diabetic albino rats. Int J Curr Res 2015; 7(5): 15577-81.
8. Shorinwa OA, Ubele C, Ukwueze SE. Evaluation of the analgesic and anti-inflammatory activities of ethanol extract of the root of *Mimosa pigra* linn (Fabaceae) in albino rats. Int J Pharm Pharm Sci, 2015; 7(7): 376-79.
9. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd Ed. London: Chapman and Hall; 1998.

10. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983; 54(4): 275-87.
11. Uma RB. Acute and Sub-Acute Toxicity of *Amalakyadi churna*. Pharmacol Online, 2010; 1: 625-33.
12. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX, Ngongang J. Evaluation of acute and Sub-acute toxicities of aqueous ethanol extract of leaves of *Senna Alata* (L.) Roxb (Ceasalpiniaceae). Afri J Biotechnol, 2006; 5(3): 283- 89.
13. Aniagu SO, Nwinyi FC, Akumka DD, Ajoku GA, Dzarma S, Izebe S, Ditse M, Patrick E, Nwaneri C, Wambebe C, Gamaniel K Toxicity Studies in Rats Fed Nature Cure Bitters. Afri J Biotechnol, 2005; 4(1): 72-8.
14. Taga I, Kamseu P, Nganzie O, Ack, F.X., Ngongang J Etude comparative des méthodes de dosage chimique et enzymatique de quelque paramètres biochimiques: 16 cholestérol, glucose acide urique et phosphatase alcaline. *Biosciences* proceeding. Annal publ Cameroon Biosci Soc, 1998; 251: 258.
15. Gandhare B, Kavimani S, Rajkapoor B. Acute and sub-acute toxicity study of methanol extract of *Ceiba pentandra* (Linn.) Gaertn. on rats. J Sci Res, 2013; 5(2): 315-24.
16. Mbatchou VC, Ayebila AJ, Apea OB. Antibacterial activity of phytochemicals From *Acacia nilotica , Entada africana and Mimosa pigra* L. on *Salmonella* typhi. J Animal Plant Sci, 2011; 10(1): 1248-58.
17. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V . A 90- day oral gavage toxicity study of D- methyl penidate and DL methyl penidate Sprague – Dawley rats. Toxicol, 2002: 179: 183.
18. Orisakwe OR, Afonne OJ, Chude MA, Obi E, Dioka CE. Sub- chronic toxicity studies of the aqueous extract of *Boerhavia diffusa* leaves. J Health Sci, 2003; 49(6): 444-7.
19. Tilkian SM, Conover MB, Tilkian AG .Clinical Implications of Laboratory Tests; C.V. Mosby Company; St Louis. Toronto. London, 1979; 3-44; 117-132; 154-159.
20. Crook MA. Clinical Chemistry and Metabolic Medicine. 7th ed. London: Edward Arnold Publishers; 2006; 253.
21. Whitby LG, Smith AF, Becket GJ Lecture Notes on Clinical Chemistry; Fourth Ed; Blackwell Scientific Publications; Oxford, London, Edinburgh, Boston, Melbourne, 1989; 38 – 178.
22. Shorinwa OA, Ebong OO, Obianime AW, Siminialayi IM. Acetone extract of *Crinum jagus* bulbs (Liliaceae): an acute and sub chronic toxicological evaluation in albino rats. Int Res J Pharm, 2014; 5 (7): 560-64.

23. Francis G, Kerem Z, Makkar HPS, Becker K The Biological Action of Saponins in Animal Systems: A Review. British Journal of Nutrition, 2002; 88(6): 587–605.
24. Nand N, Aggarwal HK, Jai D, Sharma M. Indigenous Drug Induced Nephropathy. In: Sahay BK (Ed). Medicine Update 2006; 16: 458-62.
25. Babu SP, Sarkar D, Ghosh NK, Saha A, Sukul NC, Bhattacharya S. Enhancement of membrane damage by saponins isolated from *Acacia Auriculiformis.* Japanese J Pharmacol, 1997; 75(4): 451–54.
26. Baumann E, Stoya G. Völkner A, Richter W, Lemke C, Linss W Hemolysis of human erythrocytes with saponin affects the membrane structure. Acta Histochem, 2000; 102(1): 21–35.