THE EFFECT OF *MANSONIA ALTISMA* ON HAEMATOLOGICAL PARAMETERS OF RAT

S.O. ASAGBA[1,*], B.U. OWHE-UREGHE[2], A. FALODUN[3] and F. OKOKO[2]

1. Department of Biochemistry, Delta State University, Abraka, Delta State, Nigeria.
2. Department of Botany and Microbiology, Delta State University, Abraka, Delta State, Nigeria.
3. Department of Pharmaceutical Chemistry, University of Benin, Benin City, Nigeria.

(*Submitted*: 26 July 2004; *Accepted*: 05 October 2006)

Abstract

The effect of ethanolic extract of *Mansonia altisima* on biochemical and haematological parameters were examined using the rat as animal model. Female wistar albino rats (105.00 ± 2.30 g) were distributed into control and three test groups, with ten rats in each group. The test rats were treated orally by intubations with 0.5, 1.0 and 2.0 g of *Mansonia* extract per kilogram body weight of rat respectively (the doses were administered in 2.5 mL of deionized water). Control rats were administered the same volume of deionized water (2.5 mL). Rats administered the highest dose of the extract had a significantly higher ($P<0.05$) activity of plasma aminotransferases as compared to the other exposure groups. The plasma alkaline phosphatase activities of rats in the test groups were not significantly ($P>0.05$) different from each other, but were significantly ($P<0.05$) reduced as compared to controls. Also, no significant ($P>0.05$) difference was observed in plasma levels of creatinine and urea of rats in all exposure groups. The plasma uric acid concentration of the test groups were not significantly ($P>0.05$) different from each other, but were significantly ($P<0.05$) increased relative to control. A significant reduction in haemoglobin (Hb) and packed cell volume (PCV) was observed in rats administered the highest dose relative to control. There was a dose dependent increase in WBC count of rats administered the extract compared to control. The study suggests that ethanolic extract of *Mansonia* may be toxic to the liver at the highest dose administered.

Keywords: Mansonia, extract, toxicity, rats.

1. Introduction

Some timbers are resistant to insects and fungal attack because of the presence of extractives. These extractives have been found to inhibit wood decay by some basidomycetes (Ejechi and Obuekwe, 1993). They contain phenolics (e.g resins, tanmins, alkaloids and terpenes) which have also been shown to be important in the resistance of plants to diseases according to the study by Matern and Knuesel (1993).

In Nigeria, the saw-dust of timbers, including very durable Nigerian species are often left in refuse dumps to rot away or are burnt. One of the many tropical Nigerian timbers generating this saw-dust is *Mansonia altisima*. Previous work has shown the extract of saw-dust of *Mansonia* inhibited the growth of *Pseudomonas aeruginosa* and *Enterococcus faecalis* (Ejechi, 1996). Ethanol and acetone extracts of *M. altisima* have also been reported to be inhibitory to *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*, both *in vivo* and *in vitro*, in a study by Oshiegbu (2001).

Besides the beneficial aspects of medicinal plants, some have been reported to have side effects on humans after consumption (Chan, 1997). The inhibitory effects of the extract of *Mansonia* on varied and diverse organisms underscore its possible use as an antibiotic agent in humans. However, no study has so far been reported on the safety of *Mansonia* extract. Therefore, the present study is a preliminary evaluation of the toxic metabolic effect of ethanolic extract of *Mansonia*. Specifically, the effect of the plant on haematological and biochemical parameters was investigated using the rat as animal model.

2. Materials and Methods

(*a*) Materials: Forty adult female albino rats of the Wister Strain with an average weight of 105 ± 2.3 g were used for the study. The animals were obtained from the animal unit of the Department of Pharmacology and Toxicology of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. They were allowed to acclimatize to laboratory conditions for two weeks during which they were subjected to initial training period called "dummy assay". *Mansonia* wood was obtained from a wood shop and identified in the Department of Botany and Microbiology, Delta State University, Abraka, Nigeria. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) reagent kits were

* *corresponding author* (email:asagbabch@yahoo.com)

products of Randox Laboratories Ltd. United Kingdom. Sodium hydroxide, p-nitrophenol, p-nitrophenyl phosphate and chloroform were obtained from May and Baker Ltd., Dagenham, England. Absolute ethanol (99% v/v), magnesium chloride and glycine were obtained from BDH, poole, England. The other chemicals used for the study were of analytical grade. The animals were fed growers mash (Bendel Feeds and Flour Mills, Ewu, Nigeria) throughout the duration of the study.

(*b*) Methods

(i) Preparation of *Mansonia* Extract

The *Mansonia* wood was milled into saw dust. The saw-dust obtained from the wood had some moisture, hence it was dried to a constant weight at 103 °C in an electric oven. Two hundred grammes (200g) of the dried saw-dust were soaked in one litre absolute ethanol (99%) for 48 hours. The ethanol extract obtained was filtered to remove particles. This was followed by evaporation in a rotatory evaporator attached to a vacuum pump at 70 °C. This temperature was used in order not only to recover the extract from ethanol but also to ensure that there is minimal microbial load. This process produced a dark brown viscous liquid substance with a weight of 8.29 g. The extract obtained was dissolved in 204 ml of de-ionized water.

(ii) Treatment of Animals

The rats were divided into four groups for the purpose of the study with ten rats in each group. Rats in groups 2, 3 and 4 were given 0.5, 1.0 and 2.0 g of the extract per Kg body weight respectively, orally by intubation. These doses were administered in 2.5 mL of deionized water. Control rats (group 1) were administered the same volume (2.5 mL) of de-ionized water. Previous investigation in our laboratory (unpublished results) indicates that the LD_{50} of ethanolic extract of Mansonia was 6 g/kg body weight of rat. Thus the doses administered in this study were sub lethal since they were below the LD_{50} for *Mansonia*. The rats were given this treatment daily for two weeks, during which the weight were recorded weekly. The daily food consumption and faecal output of the rats were also recorded. At the end of the exposure period, each rat was anaesthetized in a chloroform saturated chamber. While under anaesthesia, the abdominal and thoracic region of each rat were opened to expose the heart. Blood was obtained via cardiac puncture by means of 5 mL hypodermic syringes and needle into a heparinized tube. This was carefully swirled and left standing on ice until required. Plasma was obtained from the blood by centrifugation at 3,000 rpm for 10 minutes as previously described by Cowan and Steel (1974).

(iii) Biochemical Analysis

The plasma of each rat was analyzed for the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Other plasma constituents such as creatinine, urea and uric acids were also analyzed.

The activities of the aminotransferases were determined based on the methods of Reitman and Frankel (1957). The activities of the aminotransferases are expressed as units/mL in which one unit is defined as the amount of enzyme activity in 1 mL of serum that will lower the absorbance by 0.001 in 1 minute under the conditions of the method. Alkaline phosphatase activity was determined by the method of Annino and Giese (1976). The activity of the enzyme is expressed as units/mL in which one unit is defined as the amount of the enzyme activity that will liberate 1 mmole of p-nitrophenol per hour under the conditions of the method. The concentration of plasma creatinine, urea and uric acid were also determined by the methods of Annino and Giese (1976).

(iv) Haematological Analysis

Haemoglobin content (Hb), haematocrit (Hct) and white blood cell count (WBC) were determined in blood using a cell coulter T_{540} (Coulter Electronic Limited) based on the methods of Baker and Silverton (1978).

(v) Statistical Analysis

The values are reported as means ± S.E.M. statistical differences in the means were determined using analysis of variance (ANOVA) and Duncan's multiple range test. The significance level was 0.05.

3. Results

In this study, ethanolic extract of *Mansonia* was administered orally to rats daily for two weeks. At the end of this exposure period, the effects of *Mansonia* on some selected biochemical parameters were assessed using standard methods.

The changes in feed intake, weight gain, faecal output and feed efficiency of rats exposed to Mansonia is presented in Table 1. The results indicated that the feed intake, faecal output, weight gain and feed efficiency of rats given 0, 0.5 and 1.0 g of *Mansonia* extract per kg body weight were not significantly different. However, the rats treated with 2.0 g of the extract per kg body weight had significantly decreased feed intake and feed efficiency, but increased faecal output relative to rats in the other groups. There was also a significant decrease in body weight gain of rats treated with 2.0 g *Mansonia* per kg body weight relative to rats of the other exposure groups.

The blood parameters of the rats administered increasing doses of *M. altisima* extract are presented

Table 1: Weight gain, feed consumption, faecal output and feed efficiency of rats administered *Mansonia altisima* extract.

	Dose of extract (g/kg body weight)			
Parameters	0	0.5	1.0	2.0
Weight gain (g/day/rat)	1.60 ± 0.21^a	1.68 ± 0.32^a	1.57 ± 0.33^a	0.90 ± 0.04^b
Feed intake (g/day/rat)	24.52 ± 1.24^a	25.12 ± 1.20^a	24.86 ± 1.21^a	23.81 ± 1.32^a
Feed efficiency (g/body weight/g feed) x 10	0.7 ± 0.02^a	0.7 ± 0.03^a	0.6 ± 0.01^a	0.4 ± 0.01^b
Dry faecal output (g/day/rat)	1.20 ± 0.03^a	1.24 ± 0.03^a	1.22 ± 0.02^a	2.43 ± 0.02^b

Results are expressed as mean ± S.E.M. (n = 10).
Means of the same row followed by different letters differ significantly ($P<0.05$).

Table 2: Blood parameters of rats treated with increasing doses of *Mansonia altisima* extract.

	Dose of extract (g/kg body weight)			
Parameters	0	0.5	1.0	2.0
ALT (units/mL)	158.52 ± 22.38^a	180.48 ± 69.85^a	185.10 ± 73.10^a	225.48 ± 72.34^b
AST (units/mL)	273.84 ± 58.88^a	247.06 ± 25.84^a	284.50 ± 55.60^a	356.70 ± 10.94^b
ALP (units/mL)	282.40 ± 99.60^a	161.40 ± 52.24^b	166.30 ± 50.40^b	164.00 ± 52.43^b
Creatinine (μmol/L)	0.42 ± 0.13^a	0.36 ± 0.24^a	0.38 ± 0.21^a	0.40 ± 0.23^a
Urea (μmol/L)	8.26 ± 0.43^a	6.32 ± 1.19^a	6.49 ± 1.23^a	6.56 ± 1.27^a
Uric acid (μmol/L)	3.48 ± 0.98^a	9.60 ± 2.57^b	7.48 ± 2.60^b	6.06 ± 3.69^b
WBC count (x 10^9/L)	6.00 ± 0.24^a	7.80 ± 0.50^b	9.23 ± 0.40^c	11.21 ± 0.40^d
Hg (g/L)	112.8 ± 1.60^a	108.60 ± 2.00^a	106.49 ± 2.5^a	92.10 ± 2.1^b
PCV (%)	36.00 ± 1.00^a	36.00 ± 1.00^a	35.00 ± 2.00^a	31.00 ± 1.00^b

Results are expressed as mean ± S.E.M (n = 10)
Means of the same row followed by different letters differ significantly ($P < 0.05$).

in Table 2. No significant difference was observed in the activities of the aminotrasferases in rats treated with 0, 0.5 and 1.0 g of *Mansonia* extract per kg body weight. Conversely, rats similarly administered the highest dose of the extract had significantly higher activity of the aminotransferases as compared to the rats of the other exposure groups. Alkaline phosphatase activities of rats administered 0.5, 1.0 and 2.0 of *Mansonia* extract per kg body weight were not significantly different, but decreased significantly relative to the control. Evaluation of the data indicate that there was no significant difference in plasma urea and creatinine concentrations of rats administered 0, 0.5, 1.0 and 2.0 g of *Mansonia* extract per kg body weight. However, the plasma uric acid concentration of the test groups were not significantly different, but were increased significantly relative to control. The data for haemoglobin, haematocrit and white blood cell count are also shown in Table 2. Significant reductions in haematocrit and haemoglobin were observed only in rats administered the highest dose of the extract. The WBC counts of the rats were increased in a dose-dependent manner relative to control.

4. Discussion

The ethanolic extract of *M. altisima* has been investigated and found to be active against *Pseudonomas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Streptococcus faecalis* (Ejechi, 1996; Oshiegbu, 2001). Sequel to this finding, we investigated the safety of *M. altisima* by monitoring its effect on some biochemical parameters using the rat as animal model.

Weight changes of rats may arise from changes in feed or water consumption. Consumed food eventually translates to body energy and weight, if it is effectively absorbed and converted. As similar quantity of food was consumed by the rats in the various groups, the decreased body weight and feed efficiency of rats exposed to the highest dose of *Mansonia* (Table 1) indicate that the extract may have affected food absorption and conversion. It is possible that at the highest dose, there was interference with the digestive enzymes and food absorption leading to high faecal output (Table 1), this will lead to stress and then the observed biochemical changes.

The predominant forms of alkaline phosphatase present in the normal serum are the liver and the

bone isoforms. The enzyme catalyses the hydrolysis of phosphate monoesters of a variety of alcohol moieties, being active at the alkaline pH 10 (Maunders, 1993). The observed inhibition of plasma alkaline phosphatase activity in the test groups may be an indication of a decrease in phosphate metabolism in the liver or bone. However, the mechanism involved in the inhibition of the enzyme is presently unclear.

Increased plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels have long been associated with likely liver problems (Tegeris *et al.*, 1969; Timbrell, 1991; Nelson and Cox, 1997; Dede, 2002), as these two enzymes readily leak out of hepatic tissue when injured. No histopathological examination was done on the liver to ascertain if there was damage to this organ. However, it should be noted that the rats were exposed to the extract of *Mansonia* for two weeks, a period that may be too short to cause permanent damage, if any. So, it is possible that the observed increase in AST and ALT in the rats exposed to the highest dose of *Mansonia* (Table 2) may be due to stress occasioned by exposure to the extract rather than damage to the liver.

Increase in plasma creatinine and urea concentrations could occur when there is kidney dysfunction (Norbert, 1986). Due to their functions, the kidneys are subjected to chemically induced injury because of being the targets of the primary (in some cases) and secondary impacts (in most cases) of potentially toxic compounds and their metabolites than most other tissues (Timbrell, 1991). The results obtained for the plasma urea and creatinine concentrations suggest that *M. altisima* had no effect on kidney function of rats at the doses studied, despite the increased level of plasma uric acid (Table 2). Uric acid is the end product of purine metabolism. Its urinary excretion depends upon purine synthesis and metabolism as well as renal function. However, it has been suggested by Annino and Giese (1976) that the level of plasma uric acid may not be a reliable or sensitive guide to kidney function.

The reduction of haemoglobin and haemotocrit levels in rats administered the highest dose of *M. altisima* is an indication of anaemia, which could be due to haemolysis. The dose dependent rise in WBC may be considered as defensive mechanism triggered by the immune system in the rats.

In conclusion, though direct extrapolation of results from animal models cannot be made to humans, results obtained are however indicative of the likely trend. The present study therefore suggests that ethanolic extract of *Mansonia* is likely to be toxic to the liver at the highest dose administered. Certainly histopathology of the liver is required to confirm if there is liver damage on exposure of rats to this dose. In this regard, further studies are on to investigate the pathological effect of *Mansonia* extract on the organs of rats.

REFERENCES

Annino, J.S. and Giese, R.W., 1976. Clinical chemistry, principles and procedures. (4th Ed.), Brown and Company, Boston.

Baker, F.J. and Silverton, R.E., 1978. Introduction to medical laboratory technology (5th Ed.), The English Language Book Society and Butterworths, London.

Chan, T.Y., 1997. Monitoring the safety of herbal medicines. *Drug saf.*, 17(4), 209-213.

Cowan, S.T. and Steel, J.K.J., 1974. Manuals for the identification of medical bacteria (3rd Ed.), University Press, Cambridge.

Dede, E.B., Igboh, N.M. and Ayalogu, O.A., 2002. Chronic toxicity study of the effect of crude petroleum (Bonny light), kerosene and gasoline on rats using haematological parameters. *J. Appl. Sci. Environ. Mgt.*, 6(1), 60-63.

Ejechi, B.O. and Obuekwue, C.O., 1993. Preliminary studies on the effect of crude Opepe (*Nauciea didedrichii*) on timber biogradation. *Inter. Biodetr. Biodegr.*, 31, 11-15.

Ejechi, B.O., 1996. Growth inhibition of *Pseudomonas aeruginosa* and *Enterococcus faecalis* by crude extractives of *Mansonia altisma* timber saw dust. *Curr. Microbiol. J.*, 32,297-298.

Matern, U. and Knuesel, R.E., 1998. Phenolic compounds in plant disease resistance. *Phytoparasitica.* 16, 153-170.

Mauders, J.M., 1993. Alkaline Phosphatase In: Burrell M.M. (Ed.), Enzymes of molecular biology, Humana Press, New Jersey, pp. 331-341.

Nelson, O.L. and Cox, M.M., 1997. Lehninger Principles of Biochemistry (2nd Ed)., Worth Publishers, New York.

Norbert, W.F., 1986. Textbook of Clinical Chemistry. W.B. Saunders and Company, Philadelphia.

Oshiegbu, O., 2001. Safety test of ethanolic and acetone extracts of *Mansonia altisima* on mice and rats. B.Sc. dissertation, Department of Botany and Microbiology, Delta State University, Abraka, Nigeria.

Tegeris, A.S., Smalley, H.E. (Jr.), Earl, F.L. and Cutis, J.M., 1969. Ornithine carbamyltransferase as a liver function test. Comparative studies in the dog, swine and man. *Toxicol. Appl. Pharmacol.*, 14,54-66.

Timbrell, J.A. 1991. Principles of Biochemical Toxicology, 2nd Edition, Taylor & Francis, London.