Effects of Aqueous Stem Extract of *Massularia Acuminata o*n Some Liver Function Indices of Male Rats

Musa ToyinYakubu [*1], *Babasoji Percy Omoniwa* [2]

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ABSTRACT

Background: *Massularia acuminata* has been claimed to be used in managing several ailments in folk medicine and in some instances substantiated with scientific data. This however has been without recourse to its safety. Therefore, aqueous stem extract of *M. acuminata* was evaluated for its effects on some function indices of the liver of male rats.

Methods: Sixty, male rats were grouped into 4 (A, B, C and D) such that Group A (control) was orally administered 1cm^3 of distilled water while those in groups B, C and D received orally 1 cm^3 of extract corresponding to 250, 500 and 1000 mg/kg body weight respectively. Some biochemical parameters of liver function were evaluated in the animals after 1, 7 and 21 daily doses.

Results: The extract significantly decreased ($P<0.05$) the activity of alkaline phosphatase in the liver of rats throughout the experimental period. This decrease was accompanied by corresponding increase in the serum enzyme. In contrast, all the doses of the extract increased the activities of both the AST and ALT in the liver and serum aspartate aminotransferase and alanine aminotransferase as well as the concentrations of serum total bilirubin, protein and albumin.

Conclusion: This study has revealed that the aqueous stem extract of *Massularia acuminata* at the doses of 250-1000 mg/kg body weight hampered the normal functioning of the liver of male rats and is therefore not safe for oral consumption at the doses investigated.

Keywords: Functional Indices, Hepatotoxicity, *Massularia acuminata*, Rubiacea, Safety.

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INTRODUCTION

Many of these indigenous plants have been used by man since time immemorial for curing various ailments and thus lessening human sufferings healthwise. They contain substances in one or more of their organs with not only chemopreventive and/or therapeutic effects on ailments but can also be used for the synthesis of useful drugs. These substances referred to as bioactive agents include alkaloids, flavonoids, phenolics, anthraquinones, terpenes and saponins. The continuous indiscriminate use of these plants has therefore necessitated investigations into their safety. One of such plant is *Massularia acuminata*.

Massularia acuminata (family-Rubiacea), is otherwise known as chewing stick (English), *Pako Ijebu* (Yoruba-Western Nigeria), and *Igbo* (Igbo-Eastern Nigeria) is a medium sized shrub that grows up to 5 m high. It is primarily found in the understorey of the closed-forest of western Africa. The leaves are large, practically stalkless, elliptic and acuminate. The flowers, usually red, borne in short axillary cymes, appear around January. The fruits which are narrowly ovoid, beaked and yellowish-white in

1. Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.
2. Department of Science Laboratory Technology, University of Jos, Jos, Nigeria.
*Corresponding Author Email: tomuyak@yahoo.com

colour are 5 cm long. The stems are used as chewing sticks in southern Nigeria (1). The pulped roots are claimed to be used as enema for dysentery, aphrodisiac and anticancer. The fruit juice is also used as antibiotic for eye drop in Sierra Leone (2).

The aqueous stem extract of *M. acuminata* have been reported to contain alkaloids (0.22%), saponins (1.18%), anthraquinones (0.048%), flavonoids (0.032%), tannins (0.75%) and phenolics (0.066%) (3). Several studies have shown that *M. acuminata* at concentrations less than 10% is capable of inhibiting the growth of *Bacteriodes gingivalis, B. assacharolyticus* and *B. melaninogenicus* (4-6). They have also reported that the alkaloidal content of the plant extract have anti-inflammatory activity and is effective in preventing against gingivitis and periodontitis. Furthermore, the androgenic and gonadotropic effects as well as aphrodisiac activity of the aqueous stem extract of *M. acuminata* at the doses of 250, 500 and 1000 mg/kg body weight have also been reported in separate studies using male rats as model (3,7). Despite all these studies, there is dearth of information on the effect of the extract on some liver function parameters of male rats. Therefore, this study was designed to provide information on the effect of aqueous stem extract of *Massularia acuminata* at the doses of 250-1000 mg/kg body weight on some biochemical indices of liver damage in male rats.

MATERIALS AND METHODS

Plant materials and authentication

The plants which were obtained from herbsellers at Ijebu Ode, Ogun State, Nigeria were authenticated at the Federal Research Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria. A voucher specimen (FHI107644) was deposited at the Herbarium of the Institute.

Animals

Sixty, male albino rats(164.50 ± 9.20 g) of *norvegicus* strain were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Assay kits

Assay kits for alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total and conjugated bilirubin were products of Randox Laboratories, Co-Antrim, United Kingdom. All other reagents used were of analytical grade and were prepared in all glass distilled water.

Animal grouping

The rats were housed in standard cages and allowed to acclimatize for 7 days. They were fed with normal rat pellet and tap water throughout the experimental period. Rats were grouped into 4: A, B, C and D. Animals in Group A which served as the control were orally administered with $1cm^3$ of distilled water while those in groups B, C and D received orally $1cm^3$ of the extract corresponding to the doses of 250, 500 and 1000 mg/kg body weight respectively. The administration was done once daily. The animals were handled according to the guidelines of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (8). The 250, 500 and 1000 mg/kg body weight doses were prepared by dissolving 2.05 g, 4.10 g and 8.20 g of extract concentrate respectively in 50 cm^3 of distilled water. The animals were sacrificed 24 hours after 1 (single dose), 7 (seven daily doses) and 21 (twenty one daily doses) days of administration.

Preparation of aqueous stem extract of Massularia acuminata

The stem of the plant was first weighed after which it was sliced into pieces and oven-dried at $40^{\circ}C$ to a constant weight. This was then pulverized using an electric grinding machine. A known amount (500 g) was percolated in 1 litre of

distilled water with intermittent shaking and kept in the refrigerator for 48 hours. The solution was thereafter filtered using Whatman No 1 filter paper and the filtrate concentrated on a water bath to give a yield of 25.50 g representing a percentage yield of 5.10%.

Preparation of serum and liver homogenates

The rats were anaesthetized in a jar containing cotton wool soaked in diethyl ether. When the animals became unconscious, they were quickly brought out of the jar and their jugular veins were cut. Blood samples were collected into sample bottles and allowed to clot at room temperature for 20 minutes and thereafter centrifuged at 1398 x g 15 minutes. Clear colourless serum was collected with the aid of Pasteur pipette. The animals were quickly dissected and the liver removed, blotted, weighed and homogenized in ice-cold 0.25M sucrose solution (1:5w/v). The homogenates were kept frozen and used for the biochemical analysis within 24 hours of preparation.

Determination of liver function indices

The liver function indices were determined as described for alkaline phosphatase (E. C. 3.1.3.1) (9), aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) (10), total protein (11), total and conjugated bilirubin (12) and albumin (13). All measurements were done using spectronic 21 digital Spectrophotometer (Bausch and Lomb, Rochester NY).

Statistical analysis

Data were expressed as the mean ± SEM of five replicates. The data were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) and complemented with Duncan's Multiple Range Test. Statistical difference was set at $p<0.05$.

RESULTS

The extract significantly ($P<0.05$) decreased the activity of ALP alkaline phosphatase in the liver of rats throughout the experimental period. This decrease was accompanied by corresponding increase in the serum enzyme (Table 1).

In contrast, the extract at all the doses investigated significantly increased the activities of both the AST and ALT in the liver and serum of the animals. The increase in serum enzyme activity manifested at all the days of intervention (Tables 2 and 3).

Table 1. Alkaline phosphatase activity of male rat liver and serum orally administered with aqueous stem extract of *Massularia acuminata*

		Liver			Serum		
		Days after administration					
Test Samples	**Doses (mg/kg)body weight**	**1**	**7**	**21**	**1**	**7**	**21**
Control	1ml	33.25 ± 0.17^{a}	33.16 ± 0.11^{a}	32.20 ± 0.12^{a}	5.93 ± 0.23^{a}	5.96 ± 0.21^{a}	5.95 ± 0.29^{a}
M. acuminata stem extract	250	19.40 ± 0.28^{b}	20.10 ± 0.29^{b}	27.10 ± 0.14^{b}	8.04 ± 0.48^{b}	8.65 ± 0.28^{b}	8.55 ± 0.19^{b}
"	500	19.20 ± 0.16^{b}	13.90 ± 0.16^{c}	12.20 ± 0.32^{c}	9.18 ± 0.16^{c}	9.28 ± 0.07^{c}	10.60 ± 0.06^{c}
"	1000	14.00 ± 0.14^{c}	23.40 ± 0.16^{d}	10.90 ± 0.50^{d}	9.20 ± 0.12^{c}	11.70 ± 0.19^{d}	12.50 ± 0.74^{d}

n=5± SEM; values carrying superscripts different from the control for each day are significantly different ($P<0.05$).
Enzyme activities are expressed in UI

Table 2. Aspartate aminotransferase activity of male rat liver and serum orally administered with aqueous stem extract of *Massularia acuminata*

		Liver			Serum		
		Days after administration					
Test Samples	**Doses(mg/kg) body weight**	**1**	**7**	**21**	**1**	**7**	**21**
Control	1ml	5.50 ± 0.20[a]	5.47 ± 0.29[a]	5.51 ± 0.17[a]	1.42 ± 0.11[a]	1.38 ± 0.18[a]	1.40 ± 0.11[a]
M. acuminata stem extract	250	14.20 ± 0.41[b]	11.35 ± 0.23[b]	13.08 ± 0.13[b]	1.57 ± 0.05[b]	1.72 ± 0.03[b]	2.40 ± 0.01[b]
“	500	14.00 ± 0.16[b]	11.50 ± 0.22[b]	18.05 ± 0.11[c]	1.57 ± 0.04[b]	1.75 ± 0.01[b]	4.50 ± 0.04[c]
“	1000	17.50 ± 0.19[c]	18.25 ± 0.07[c]	24.00 ± 1.08[d]	1.75 ± 0.03[c]	2.91 ± 0.02[c]	5.80 ± 0.03[d]

n=5± SEM; values carrying superscripts different from the control for each day are significantly different (P<0.05).
Enzyme activities are expressed in UI

Table 3. Alanine aminotransferase activity of male rat liver and serum orally administered with aqueous stem extract of *Massularia acuminata*

		Liver			Serum		
		Days after administration					
Test Samples	**Doses(mg/kg) body weight**	**1**	**7**	**21**	**1**	**7**	**21**
Control	1ml	4.48 ± 0.07[a]	4.46± 0.09[a]	4.45± 0.10[a]	0.50 ± 0.09[a]	0.51 ± 0.09[a]	0.48 ± 0.10[a]
M. acuminata stem extract	250	5.71 ± 0.05[b]	6.82± 0.04[b]	8.39 ± 0.01[b]	0.48 ± 0.01[a]	1.40 ± 0.02[b]	1.04 ± 0.22[b]
“	500	6.25 ± 0.03[c]	8.30± 0.02[c]	14.80 ± 0.71[c]	1.03 ± 0.03[b]	1.51 ± 0.05[c]	1.62 ± 0.36[c]
“	1000	8.51 ± 0.01[d]	8.35± 0.04[c]	12.80 ± 0.36[d]	0.95± 0.04[c]	1.28 ± 0.71[d]	1.60 ± 0.08[c]

n=5± SEM; values carrying superscripts different from the control for each day are significantly different (P<0.05).
Enzyme activities are expressed in UI

All the doses of the extract significantly increased the concentration of the total bilirubin in the serum of the animals throughout the days of intervention. These increases were however not dose related (Table 4). This pattern of increase was not the same for the conjugated bilirubin in the serum of the animals as the effect manifested only in the 1000 mg/kg body weight of the extract treated animals (Table 4).The extract at the doses of 250, 500 and 1000 mg/kg body weight significantly increased the concentration of total protein in the serum of the animals (Table 5).

Similarly, the extract also increased the serum albumin content in the animals with the increase manifesting only in the animals administered with a single dose (day 1) of 500 and 1000 mg/kg body weight treated animals whereas the seven (day 7) and 21 daily administration (day 21) of all the doses of the extract significantly increased the serum albumin content of the animals (Table 5).

Table 4. Serum total and conjugated bilirubin contents of the serum of male rats orally administered with aqueous stem extract of *Massularia acuminata*

		Serum total bilirubin			Serum conjugated bilirubin		
		Days after administration					
Test Samples	**Doses (mg/kg) body weight**	**1**	**7**	**21**	**1**	**7**	**21**
Control	1ml	2.00 ± 0.01[a]	1.96 ± 0.06[a]	1.98 ± 0.05[a]	1.00 ± 0.03[a]	1.04± 0.00[a]	1.00 ± 0.03[a]
M. acuminata stem extract	250	3.00 ± 0.16[b]	3.60 ± 0.33[b]	6.60 ± 0.78[b]	1.00 ± 0.04[a]	0.98± 0.06[a]	1.00 ± 0.01[a]
"	500	3.00 ± 0.74[b]	6.25 ± 0.64[c]	5.50 ± 0.41[c]	1.00 ± 0.07[a]	1.05 ± 0.01[a]	1.00± 0.06[a]
"	1000	3.00± 0.02[b]	4.80 ± 0.01[d]	8.40 ± 0.01[d]	1.25 ± 0.36[b]	1.00 ± 0.07[a]	2.10 ± 0.03[b]

n=5± SEM; values carrying superscripts different from the control for each day are significantly different ($P<0.05$).
Concentrations were expressed in µmol/L

Table 5. Serum total protein and albumin contents of the serum of male rats orally administered with aqueous stem extract of *Massularia acuminata*

		Serum total protein			Serum albumin		
		Days after administration					
Test Samples	**Doses (mg/kg) body weight**	**1**	**7**	**21**	**1**	**7**	**21**
Control	1ml	61.00 ± 0.71[a]	59.27± 1.51[a]	60.16 ± 0.84[a]	33.00 ± 0.00[a]	29.60 ± 3.51[a]	31.28 ± 2.02[a]
M. acuminata stem extract	250	70.50 ± 0.57[b]	70.10 ± 0.12[b]	69.90 ± 0.28[b]	34.04 ± 0.04[a]	35.58± 0.10[b]	40.00 ± 0.64[b]
"	500	70.20 ± 0.22[b]	70.50 ± 0.25[b]	65.00 ± 0.14[c]	36.30 ± 0.06[b]	43.75 ± 0.21[c]	44.60 ± 0.87[c]
"	1000	65.00 ± 0.75[c]	70.70 ± 0.29[b]	69.00 ± 0.35[b]	39.00 ± 0.06[c]	35.50 ± 0.46[b]	40.30 ± 0.71[b]

n=5± SEM; values carrying superscripts different from the control for each day are significantly different ($P<0.05$).
Concentrations were expressed in g/L

DISCUSSION

The liver is prone to xenobiotic-induced injury because of its central role in the metabolism of foreign compounds and its portal location within the circulatory system (14). The biochemical parameters investigated in the present study are indices routinely used to assess the normal functioning of the liver. These functions may be synthetic, secretory and excretory. Enzyme pattern in the tissues may be used to assess liver dysfunction and serum enzyme may be used to corroborate the physiological state of the organs.

ALP is located in the plasma membrane and the membrane of the endoplasmic reticulum (15). It is required in certain amounts for proper functioning

of organs (16). Therefore, the reduction in the ALP activity of the liver accompanied by corresponding increase in serum enzyme suggest permeability changes leading to leakage of the ALP from the liver to the serum (17). Such pattern indicates hepatotoxic effect of the aqueous stem extract of *M. acuminata* at these doses. Interestingly, the cytosolic enzymes (AST and ALT) would have been expected to follow the same trend as the ALP, but the contrary is the case in the present study. Although, the serum AST and ALT were elevated corroborated the leakage, but the reason behind the increase in these liver enzymes is unclear. It is however possible that the rate of induction of these enzymes was far more than the amount that leaked into the serum (18). All the same, the reduction in the liver ALP as well as the raised levels of AST and ALT are suggestive of *M. acuminata* induced hepatoxicity. It is also possible that these enzymes in some organs not investigated in the present study might have contributed to the elevated levels of the transaminases in the serum of the animals.

Assessment of albumin and protein in the liver could be used as important indicator of synthetic function of the organs whereas bilirubin (total and conjugated) could be used to assess the excretory function of the liver (19,20). Severe hemolysis causes the release of more bilirubin into the blood which manifests as elevated levels of unconjugated and total bilirubin (21). Unconjugated and total bilirubin can also increase in the event of low bilirubin conjugation (21). Therefore, the elevated levels in the albumin, total protein and bilirubin may be due to increase in the functional activity of the organ. The extract might have stimulated the liver to increase the synthesis of the albumin and protein to a level that far exceeded that required by the animals. Similarly, the increase in the total and conjugated bilirubin may not only indicate obstruction of the biliary duct but also suggest an effect on the normal excretory function of the liver. This may have consequential effect on the conjugation process in the animals. Such elevated levels of these biomolecules are indicators of toxic effect of the extract on the organ at the doses investigated.

CONCLUSION

The aqueous stem extract of *Massularia acuminata* at the doses of 250, 500 and 1000 mg/kg body weight have caused functional toxicity of the liver in the animals. Therefore, the extract at these doses is hepatotoxic and should be used with caution.

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