Liver and Kidney Function Indices of Male Wistar Rats after Oral Administration of Aqueous Extract of *Massularia acuminata* Root

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Received: March 14, 2017; Revised: January 22, 2018; Accepted: February 6, 2018

Abstract

This study was aimed at evaluating the effects of administration of aqueous extract of *Massularia acuminata* roots on the function indices of the liver and kidney of male Wistar rats. Male rats were completely randomized into four groups, A, B, C and D. Animals in group A (Control) received orally 1ml of distilled water while those in groups B, C and D (test groups) were treated like the control except that the male rats received 1 ml that corresponded to 50, 100 and 200 mg/kg body weight of the extract respectively, once daily for 21 days. The results revealed that the extract significantly (P<0.05) increased the liver and serum alkaline phosphatase (ALP) activities and reduced the kidney ALP activity. The reduction (P<0.05) in the activity of γ-glutamyl transferase (γ-GT) in both the liver and kidney was accompanied by corresponding increase in the serum γ-GT activity. The serum contents of total bilirubin, globulin and K^+ increased significantly (P<0.05) on all the days of intervention, whereas the increases in creatinine and urea did not manifest until after seven daily doses of the extract. In contrast, the extract decreased (P<0.05) the serum Na^+ concentration, liver- and kidney-body weight ratios. Ca^{2+}, albumin and conjugated bilirubin in the serum of the animals increased at specific doses and days of exposure. Overall, the aqueous extract of *M. acuminata* roots at the doses (50, 100 and 200 mg/kg body weight) and the duration (21 days) investigated in this study has caused functional toxicity with adverse effects on the secretory and synthetic functions of the hepatocytes as well as tubular and glomerular dysfunction of the nephrons of the male rats. Therefore, the extract at these doses is not completely safe as oral remedy.

Keywords: *Massularia acuminata*, hepatotoxicity, nephrotoxicity, rubiacea, function indices

1.0 Introduction

Herbal remedy is gaining more popularity than ever as majority of the rural and urban dwellers are depending heavily on herbal medicine, which occasionally has been reported to be less toxic and cheaper compared to the synthetic orthodox medicines [1]. The general acceptability of herbal medicine is however limited by adulteration, inappropriate formulation, lack of understanding of plant and drug interactions and inadequate toxicity data to inform on the safety or otherwise of these medicinal herbs. Thus, toxicological evaluation of herbal remedies to establish their safety for oral remedy and their likely mechanisms/modes of toxicity is required to promote their acceptability and suitability [2].

Massularia acuminata (G. Don) Bullock ex Hoyl (Rubiaceae) known as *pako ijebu* or *orin Ijebu* (Yoruba), is a tree that grows up to 5 m high. It is distributed from Sierria Leone through Nigeria to Democratic Republic of Congo. The large leaves are practically stalkless, elliptic, acuminate and almost glabrous. The stem and the fruit juice have been acclaimed to be used as chewing stick for oral hygiene in southern Nigeria and antibiotics for the treatment of eye infections in Sierra Leone respectively. The decoction or infusion of the stem and root has also been claimed to be used as an aphrodisiac and anticancer as well as for the treatment of cough and management of mental illness [3].

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The secondary metabolite constituents of *M. acuminata* roots have been reported to include alkaloids (0.036%), anthraquinones (0.031%), saponins (0.0207%), phenolics (1.090%), flavonoids (0.038%) and tannins (0.720) [4]. It has also been reported that aqueous extracts of the root and stem of *M. acuminata* exhibited sex-enhancing effects on male rats as evidenced from the higher mount frequency, intromission frequency, testosterone, follicle stimulating and lutenising hormone levels, reduced mount latency, intromission latency, ejaculation frequency and post-ejaculatory interval [4,5]. Yakubu *et al.* [6] have also reported that the aqueous extract of *M. acuminata* stem increased the testes-body weight ratio, testicular protein, glycogen, sialic acid, cholesterol, testosterone, luteinizing and follicle stimulating hormone concentrations throughout the 21 days period of administration and concluded that the extract has androgenic potential which may stimulate sexual maturation and enhance normal testicular function in the male rats. According to Taiwo *et al.* [7], aqueous extracts from *Vernonia amygdalina*, *Fagara zanthoxyloides* and *M. acuminata* showed activities against bacteria that are significant to periodontal disease. Furthermore, Tedwins *et al* [8], reported that the ethanol extract of *M. acuminata* exhibited bactericidal activity against *Lactobacilus species*, *Streptococcus oralis* and *Neissera sicca* at a concentration of 50 mg/Ml and recommended that the extract can be adopted as mouth wash for periodontal infections. In another related antimicrobial study, Bankole *et al.* [9] concluded that all the bacterial and fungal test organisms were susceptible to the inhibitory properties of both the aqueous and ethanolic extracts of *M. acuminata* and *Distemonanthus benthamianu* with that of *M. acuminata* exhibiting better antimicrobial activity. The ethyl acetate, acetone, ethanol and methanol root bark extract of *M. acuminata* has also been found to be active against the clinical isolates, *Salmonella spp*., *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* but at varied level [10].

On the toxicity of *M. acuminata*, Yakubu and Omoniwa [11,12] have reported that aqueous extract of *M. acuminata* stem at 250, 500 and 1000 mg/kg body weight decreased the liver alkaline phosphatase (ALP) activity and increased the liver and serum aspartate and alanine aminotransferases (AST, ALT), kidney and serum γ-glutamyl transferase (γ-GT) activities, serum total bilirubin, total protein, albumin, uric acid, creatinine and electrolytes. In addition, the saponins from *M. acuminata* stem at 25, 50 and 100 mg/kg body weight increased the levels of serum potassium, sodium, phosphate, urea, creatinine, total bilirubin and conjugated bilirubin, kidney, liver and serum ALP and acid phosphatase activities, liver and kidney ALT and decreased the testicular body-weight ratio, serum ALT, uric acid, globulin, albumin and calcium ions with preserved structure of the organs [13].

Despite all these pharmacological and toxicological studies on *M. acuminata*, there are no reports, at least to the best of our knowledge, on the toxicity profile of aqueous extract of *M. acuminata* root in male rats with respect to the normal functioning of the liver and kidney. This study thus evaluates the effects of administration of aqueous extract of *M. acuminata* roots on the function indices of the liver and kidney of male Wistar rats.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant Material and Authentication

Fresh samples of the plant obtained from herb sellers at Ijebu-Ode, Ogun State, Nigeria, were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan. The voucher specimen that was deposited in the Herbarium of the Institute for future reference was assigned FHI107644.

2.1.2 Experimental Animals

Sixty male albino rats (*Rattus norvegicus*), weighing172.36 ± 4.87 g obtained from the Animal Facility of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, were allowed to acclimatize in clean plastic cages. The cages were placed in well-ventilated housing conditions (temperature: 28 ± 3˚C; photoperiod: 12 h light/dark; humidity: 45-50%). The animals were allowed free access to rat pellets (Premier Feeds, Ibadan, Nigeria) and contaminant-free tap water.

2.1.3 Assay Kits

The assay kits for ALP, γ-GT, albumin and bilirubin were products of Randox Laboratories, Co Atrim, United Kingdom while those of urea, creatinine, sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) were manufactured by Quinica Clinica Applicada, S.A., Amposta, Spain.All other reagents used in the present study were from Sigma-Aldrich Inc., St. Louis, USA.

2.2 Methods

2.2.1 Preparation of Aqueous Extract of Massularia acuminata Root

Thin slices of the roots of *M. acuminata* were oven-dried at 40^{o}C for 48 h until a constant weight was obtained. The dried root slices were then pulverized using an electric blender (Euro Premium Mixer Grinder, India). An amount of the powder (300 g) was extracted in 2 L of distilled water for 48 h, filtered and lyophilized to yield 35.9 g (11.97%) of the starting material. Calculated amount of the yield were reconstituted in distilled water (vehicle) to give the equivalent doses of 50, 100 and 200 mg /kg body weight of the extract. The doses of 50, 100 and 200 mg/kg body weight of the extract used in the present study were adopted from a previous study by Yakubu *et al.* [4] that investigated the pro-sexual effects of *Massularia acuminata* roots in male Wistar rats.

2.2.2 Grouping of the Male Rats and Extract Administration

The male rats were completely randomized into four groups, A, B, C and D of 15 animals each. Male rats in Group A (control) were administered 1 ml of distilled water (the vehicle) while those in groups B, C and D received 1 ml of the extract that corresponded to 50, 100 and 200 mg/kg body weight. The administration was done orally, once daily for 21 days at same point time of between 09:00-10:00hrs with the aid of metal oropharyngeal cannula. The animals were handled humanely according to the Principles of Good Laboratory Procedure of the Basic Organization for Economic Co-operation and Development [14].

2.2.3 Preparation of Serum and Tissue Supernatants

The serum and tissue supernatants were prepared by adopting the procedure described by Yakubu *et al.* [15]. Blood samples obtained from the jugular veins of diethyl ether anaesthetized animals were collected into clean, dry centrifuge tubes. The blood samples were left to clot at 28°C for 10 minutes and thereafter centrifuged (Hermle Bench Top Centrifuge Model Hermle, Z300, Hamburg, Germany) at 685 x *g* for 15 minutes. The liver and the kidneys were blotted with blotting paper, weighed, homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v) and afterwards centrifuged at 1398 x *g* for 15 minutes to obtain their respective supernatants. The serum and the supernatants were used within 24 hours of preparation for the analyses of function indices of the liver and kidney of male rats.

2.2.4 Determination of the Liver and Kidney Function Indices of Male Rats

The total protein concentration was assayed using Biuret reagent as described by Gornall *et al*. [16], while the concentrations of albumin, bilirubin, urea and creatinine in the serum of the animals were assayed according to the procedures described by Doumas *et al*. [17], Jendrassik and Grof [18], Veniamin and Varkirtzi [19] and Bartels *et al*. [20] respectively. The concentration of globulin was obtained from the difference between the concentrations of serum total protein and serum albumin [21]. ALP and γ-GT activities were determined using the standard procedures described by Wright *et al.* [22] and Szazs [23] respectively. The procedures described by Tietz [21] were adopted for the determination of concentrations of sodium, potassium and calcium ions in the serum of the animals. The liver- and kidney-body weight ratios were also computed as previously described [6].

2.3 Data Analysis

Data were expressed as the mean ± SEM; n = 5. Means were analyzed with one-way Analysis of Variance followed by Duncan Multiple Range Test using Statistical Package for Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

3.0 Results

Administration of all the doses of aqueous extract of *M. acuminata* root significantly ($P<0.05$) and progressively increased the ALP activity in male rat liver throughout the 21 days exposure period (Figure 1). The increases in the liver ALP activity were not dose-dependent. In contrast, all the doses of the extract significantly ($P<0.05$) decreased the activity of ALP in the kidney of the male rats (Table 1); the reduction ($P<0.05$) in the activity of kidney ALP was accompanied by corresponding increase ($P<0.05$) in the activity of ALP in the serum of the male rats (Table 1). Furthermore, the extract reduced ($P<0.05$) the activity of γ-GT in the liver (Figure 2) and kidney of the male rats (Table 1). The decrease ($P<0.05$) in the activity of γ-GT was however accompanied by corresponding increase ($P<0.05$) in the activity of γ-GT in the serum of the animals (Table 1).

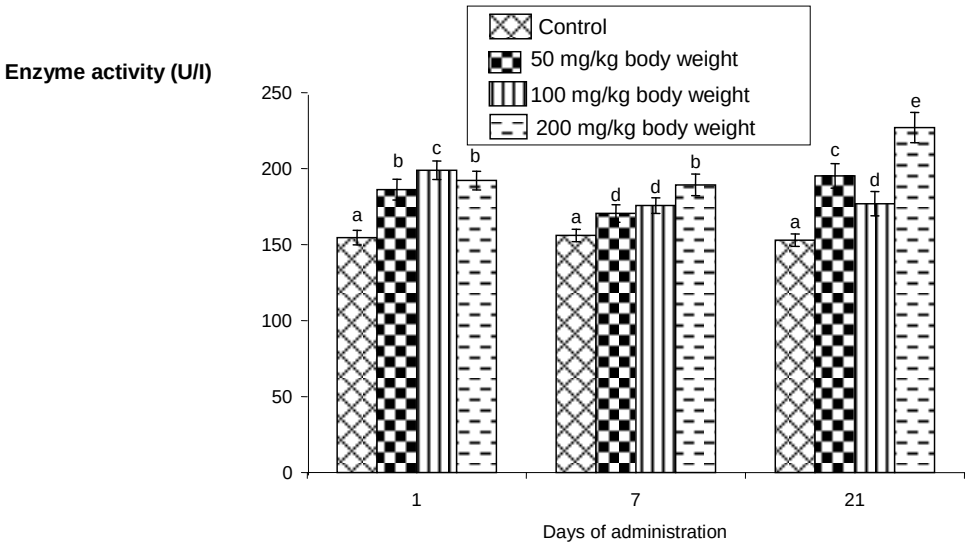

Fig 1: Effect of administration of aqueous extract of *Massularia acuminata* root on the male rat liver alkaline phosphatase activity

Table 1: Selected kidney function indices of male rats administered aqueous extract of *M. acuminata* root

Treatments	DAY 1				DAY 7				DAY 21			
	A	B	C	D	A	B	C	D	A	B	C	D
Kidney ALP (U/l)	235.60 ± 9.35^{a}	172.80 ± 8.43^{b}	164.80 ± 7.34^{c}	190.60 ± 8.14^{d}	233.60 ± 9.12^{a}	92.40 ± 5.15^{d}	94.80 ± 4.02^{d}	75.50 ± 4.05^{e}	237.50 ± 9.05^{a}	127.40 ± 6.05^{d}	80.60 ± 4.04^{e}	77.40 ± 3.76^{e}
Serum ALP (U/l)	41.50 ± 2.11^{a}	83.50 ± 5.18^{b}	84.60 ± 4.20^{b}	76.10 ± 3.06^{c}	41.20 ± 2.04^{a}	69.60 ± 6.08^{d}	82.90 ± 5.42^{b}	114.10 ± 7.03^{d}	43.00 ± 1.15^{a}	79.60 ± 3.34^{b}	106.50 ± 4.02^{e}	124.30 ± 7.02^{d}
Kidney GGT (U/l)	92.80 ± 7.51^{a}	93.40 ± 6.98^{a}	50.40 ± 2.99^{b}	64.20 ± 3.10^{c}	93.80 ± 6.01^{a}	81.90 ± 5.20^{d}	46.10 ± 3.03^{b}	62.80 ± 5.81^{c}	92.70 ± 7.14^{a}	54.80 ± 4.58^{b}	36.40 ± 2.02^{e}	78.20 ± 5.07^{d}
Serum GGT (U/l)	27.40 ± 1.08^{a}	46.10 ± 3.31^{b}	40.90 ± 2.35^{c}	45.80 ± 3.06^{b}	29.30 ± 1.05^{a}	25.20 ± 1.04^{a}	67.50 ± 5.02^{d}	41.10 ± 3.35^{b}	29.40 ± 2.61^{a}	26.90 ± 1.72^{a}	37.60 ± 2.89^{b}	104.40 ± 9.28^{e}
Serum Creatinine (mmol/L)	59.80 ± 4.18^{a}	60.00 ± 5.23^{a}	61.50 ± 4.26^{a}	64.70 ± 3.04^{a}	58.90 ± 3.05^{a}	97.20 ± 7.08^{b}	110.10 ± 7.06^{c}	113.50 ± 8.02^{c}	59.60 ± 4.33^{a}	75.40 ± 5.89^{d}	81.10 ± 6.04^{e}	87.40 ± 5.93^{e}
Serum Urea (mmol/L)	6.20 ± 0.16^{a}	6.40 ± 0.10^{a}	5.80 ± 0.05^{a}	6.10 ± 0.03^{a}	6.10 ± 0.09^{a}	8.20 ± 0.31^{b}	9.70 ± 0.41^{c}	10.20 ± 0.53^{c}	6.10 ± 0.22^{a}	8.10 ± 0.47^{b}	8.10 ± 0.36^{b}	10.30 ± 0.61^{c}
Serum Na^{+} (mmol/L)	135.30 ± 9.22^{a}	115.80 ± 8.55^{b}	106.60 ± 9.76^{c}	101.90 ± 5.04^{c}	136.20 ± 9.78^{a}	117.30 ± 6.26^{b}	112.20 ± 6.24^{b}	105.20 ± 5.21^{c}	130.20 ± 9.23^{a}	97.20 ± 3.74^{d}	82.30 ± 3.05^{e}	80.30 ± 4.83^{e}
Serum K^{+} (mmol/L)	34.20 ± 1.35^{a}	40.10 ± 2.64^{b}	42.30 ± 2.75^{b}	48.10 ± 2.78^{c}	32.20 ± 1.08^{a}	48.30 ± 3.07^{c}	58.20 ± 3.51^{d}	60.30 ± 2.68^{d}	34.30 ± 1.18^{a}	59.10 ± 3.52^{d}	62.30 ± 4.30^{d}	64.10 ± 4.62^{d}
Serum Ca^{2+} (mmol/L)	16.21 ± 0.56^{a}	15.24 ± 0.71^{a}	15.51 ± 0.63^{a}	15.32 ± 0.81^{a}	16.18 ± 1.01^{a}	17.21 ± 0.81^{a}	21.12 ± 1.06^{b}	21.10 ± 0.72^{c}	16.13 ± 0.83^{a}	15.13 ± 1.01^{a}	17.24 ± 1.02^{a}	19.20 ± 1.05^{c}
Kidney-body weight ratio	0.81 ± 0.03^{a}	0.69 ± 0.02^{b}	0.61 ± 0.02^{c}	0.66 ± 0.03^{d}	0.80 ± 0.03^{a}	0.52 ± 0.01^{d}	0.50 ± 0.01^{d}	0.49 ± 0.02^{d}	0.80 ± 0.02^{a}	0.51 ± 0.01^{d}	0.48 ± 0.01^{d}	0.50 ± 0.04^{d}

Data are mean of five replicates ± SEM. Test values with superscripts different from the control across the treatment group for each parameter are significantly different ($P<0.05$) A: Distilled water (control). B: 50 mg/kg body weight of extract; C: 100 mg/kg body weight of extract; D: 200 mg/kg body weight of extract.

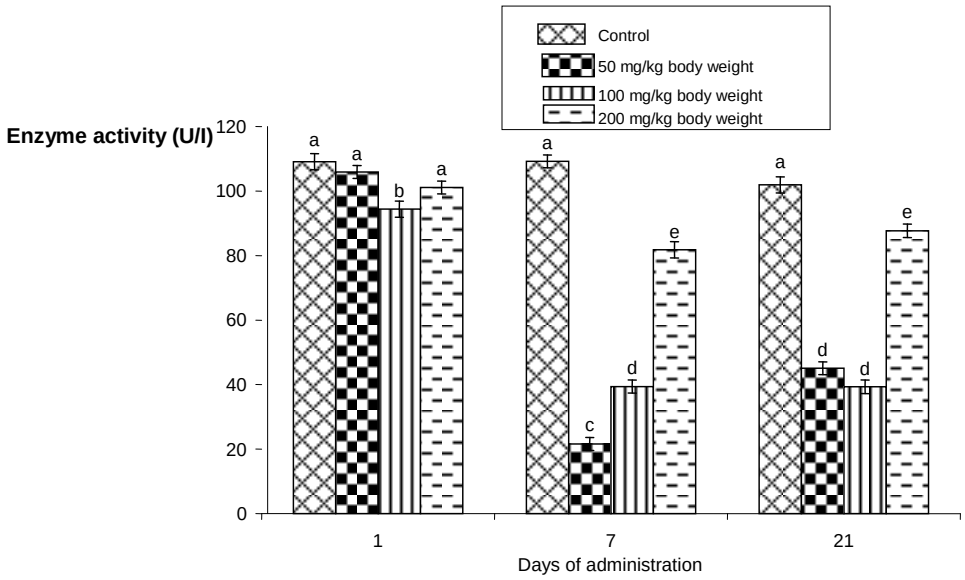

Fig 2: Effect of administration of aqueous extract of *Massularia acuminata* root on Gamma glutamyl transferase activity of male rat liver

The significant increase (P<0.05) in serum albumin content of the male rats was not sustained beyond the first day of administration of the extract, since albumin concentration was not significantly (P>0.05) altered on other days of intervention, except the increase (P<0.05) produced by the male rats that received the 200 mg/kg body weight of the extract after the 7 daily doses of the extract (Figure 3). In contrast, the extract also increased (P<0.05) the serum concentration of globulin of the male rats on all the days of intervention (Figure 4). Furthermore, the extract at 50 and 100 mg/kg body weight did not significantly (P>0.05) alter the levels of serum total bilirubin after a single dose (Day 1) whereas the single dose of 200 mg/kg body weight of the extract increased (P<0.05) the serum concentration of total bilirubin of the male rats. This increase in serum total bilirubin concentration at the 200 mg/kg body weight was extended to the other days of intervention (Days 7 and 21) after treatment with all the doses of the extract (Figure 5). The male rats also produced dose-specific response on the serum conjugated bilirubin content throughout the exposure period (Figure 6).

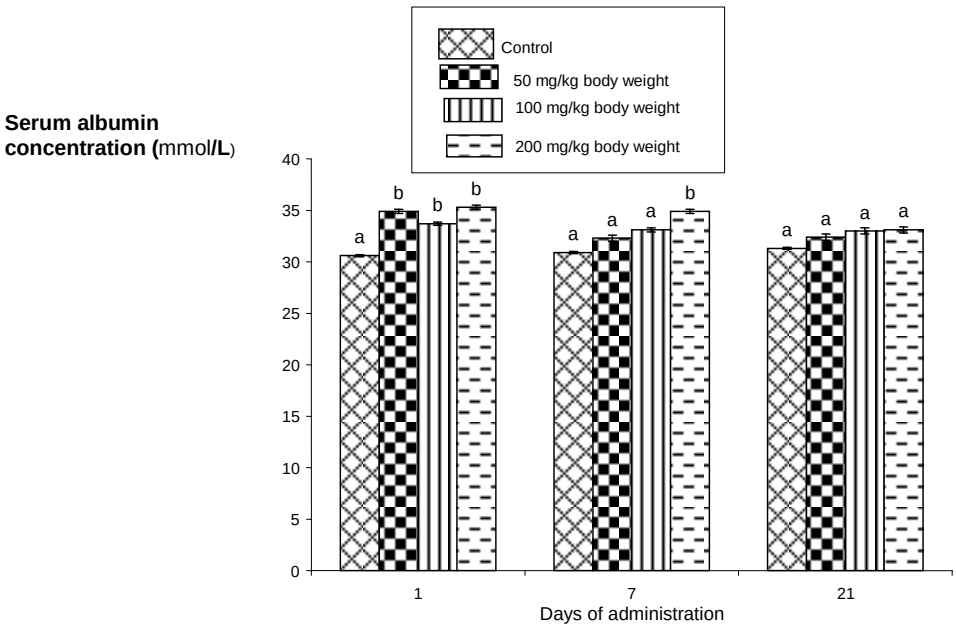

Fig 3: Effect of administration of aqueous extract of *Massularia acuminata* root on male rat serum albumin concentration

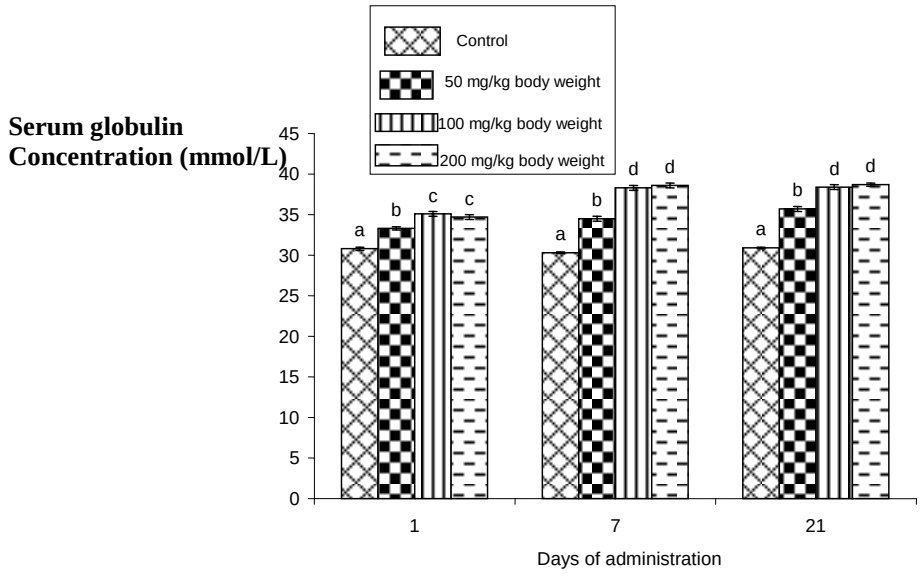

Fig 4: Effect of administration of *Massularia acuminata* root on male rat serum globulin concentration

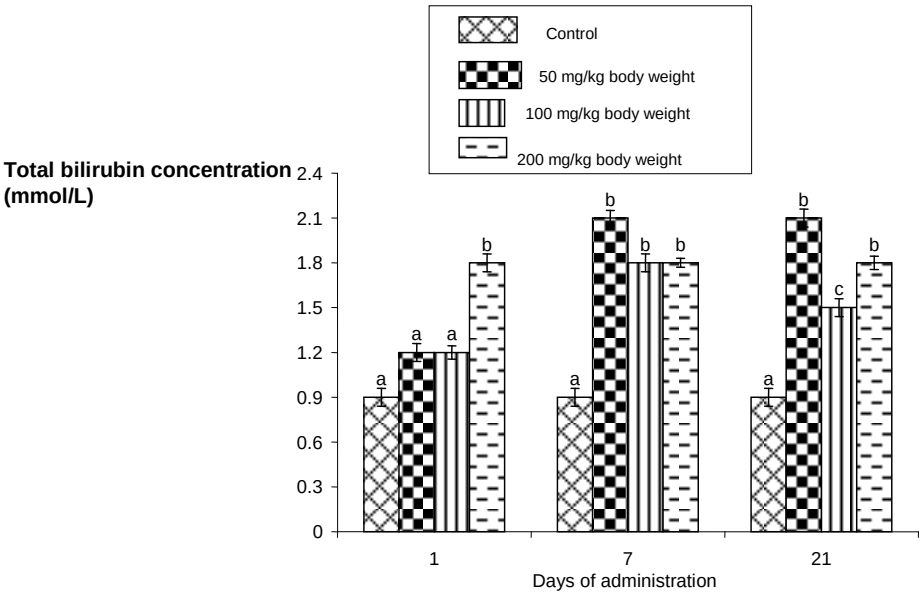

Fig 5: Effect of administration of aqueous extract of *Massularia acuminata* root on male rat serum total bilirubin concentration

The administration of the extract significantly ($P<0.05$) decreased the levels of Na^+ in the serum of the male rats (Table 1), whereas the serum concentration of K^+ increased ($P<0.05$) throughout the exposure period (Table 1). Administration of the extract of *M. acuminata* roots did not significantly ($P>0.05$) alter the levels of Ca^{2+} in the serum of the male rats except the increase ($P<0.05$) by the 100 and 200 mg/kg body weight of the extract after 7 daily doses and the 200 mg/kg body weight of the extract after 21 daily administration (Table 1). Furthermore, administration of single dose of the extract did not produce any significant ($P>0.05$) change in the levels of serum creatinine and serum urea of the male rats, whereas the 7 and 21 daily administration of all the doses of the extract significantly ($P<0.05$) increased the concentrations of serum creatinine and serum urea of the male rats (Table 1). The liver- and kidney-body weight ratios were significantly ($P<0.05$) decreased by all the doses of the extract throughout the exposure period (Figure 7 and Table 1).

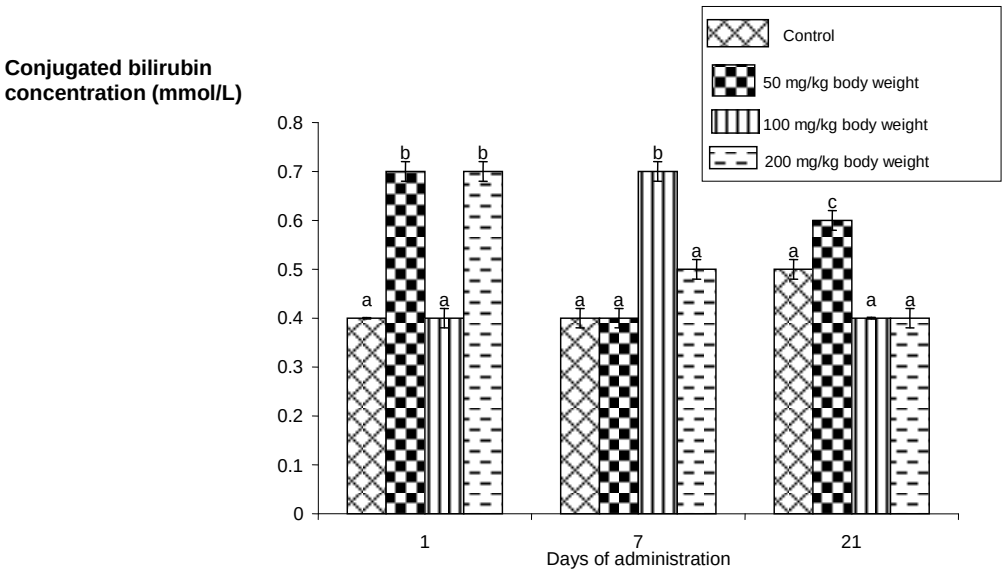

Fig 6: Effect of administration of aqueous extract of *Massularia acuminata* root on male rat serum conjugated bilirubin concentration

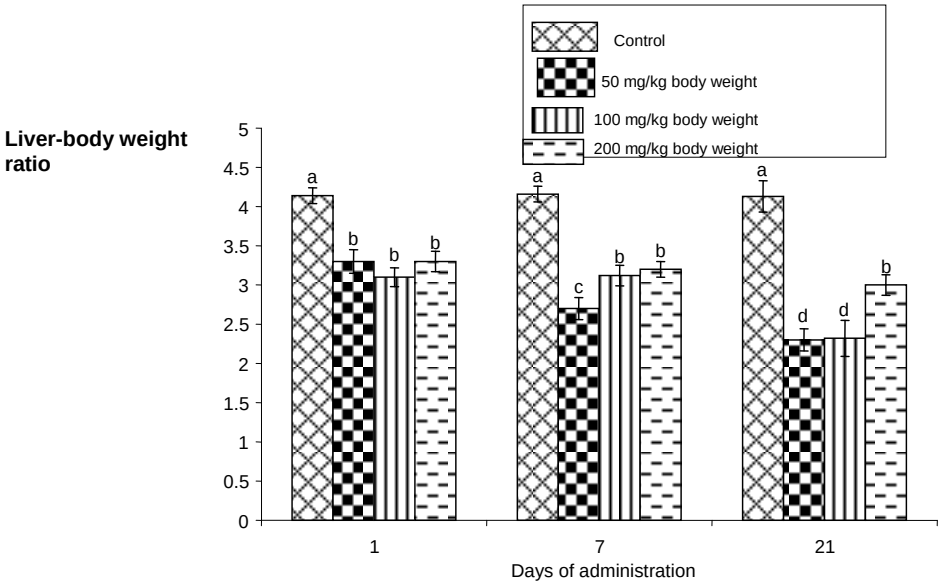

Fig 7: Effect of administration of aqueous extract of *Massularia acuminata* root on male rat liver body weight ratio

4.0 Discussion

From time immemorial, plants have been used as alternative medicine for bringing about relief from several ailments and diseases due to the assumed efficacy, acceptability, availability and affordability with little or zero cases of side effects. Currently, about 80% of the world population in developing countries still relies heavily on herbal medicine for their primary health care needs [24]. However, the use of these medicinal plants as decoction (boiling of plant material for a long period of time), infusion (pouring of boiled water over an herb), tincture (alcohol or water extract), maceration (soaking of fresh or dried plant material in cold water overnight), poultices (applying herbs directly on the skin), compresses (soaking a cloth in a prepared infusion) and baths and bathing remedies (adding medicinal plants to bath water and soaking the patient in it) are not without their side effects. Generally, medicinal plants have been reported to cause diverse side effects which include but not limited to dermatitis, growth depression, respiratory distress, ascending paralysis of the central nervous system, cancer and even death [25]. Some of these side effects are consequences of major alterations in the metabolic pathways and biomolecules as well as structure and function of the organs in the biological system. Therefore, the need to investigate the toxicity profile of *M. acuminata* roots is imperative.

Determination of both the specific activities of relevant enzymes and the concentrations of metabolic products in tissues and biological fluids of animals can be used to monitor the cellular integrity/damage, functionality and clinical conditions of organs in a biological system. Alkaline phosphatase is a 'marker' enzyme of damage to the plasma membrane and endoplasmic reticulum and is often employed to assess the integrity of the plasma membrane [26]. The significant and progressive increase in the activity of ALP in the liver of the male rats after oral administration of aqueous extract of *M. acuminata* roots may be a consequence of increase in the functional ability of the liver leading to induction in the synthesis of ALP probably by *de novo* means [27]. Such increase in liver ALP activity may threaten the existence of cells that depend on a variety of phosphate esters for their vital process since there may be indiscriminate hydrolysis of phosphate ester of the tissues. The increase in the activity of liver ALP after the administration of *M. acuminata* root contrast that previously reported by Yakubu and Omoniwa [11] on the effect of *M. acuminata* stem on liver ALP activity of male rats. Furthermore, since the reduction in kidney ALP activity was accompanied by a corresponding increase in the serum ALP activity, it will be logical to submit that the extract of *M. acuminata* roots in the present study might have compromised the cell membrane of the nephrons resulting in the loss of membrane components (including ALP) into the extracellular fluid, the serum in this instance [28]. Reduction in the activity of kidney ALP as recorded in the present study might adversely affect the transport of inorganic phosphorus in the nephrons.

GGT, the most sensitive enzymatic indicator of hepatobiliary disease, is a liver and bone disease marker enzymes [29]. The decrease in the activity of GGT in the liver of the male rats after the administration of aqueous extract of *M. acuminata* roots might be due to either inhibition of the enzyme activity at the cellular level or inactivation of the enzyme molecule *in situ* since the findings on the liver ALP activity did not support compromise of the integrity of the plasma membrane of the hepatocytes. In addition, similar decrease in the activity of kidney GGT by *M. acuminata* root extract might be attributed to leakage of the enzyme from the kidney as evidenced from the reported compromise of the plasma membrane of the kidney and the corresponding increase in the activity of the serum GGT. Extract-induced injury to the hepatic cells and the nephrons may be important for the interpretation of the marked reduction in the activity of GGT in both the liver and the kidney of the male rats. Consequently, this may affect the transfer of amino acids across the cellular membrane. The findings with respect to reduction in the activity of GGT in the kidney of male rats after administration of *M. acuminata* roots in the present study did not agree with the increase in kidney GGT activity after the administration of aqueous extract of *M. acuminata* stem as previously reported by Yakubu and Omoniwa [12]. The reason for the differences in findings at the moment is unclear, but it may not be unconnected with the different parts of the plant which might have accumulated different biomolecules in varying amount and the different doses used.

Determination of the levels of albumin, globulin, and bilirubin (total and conjugated) in the serum of animals after the administration of plant extracts and natural products can be used to assess the effect of the extract on the normal functioning of the liver. In specific terms, albumin and globulin are useful indices of the synthetic/secretory function of the liver whereas bilirubin (total and conjugated) are indicators of excretory capacity of the liver [30].The elevated levels of albumin after the first daily dose of aqueous extract of *M. acuminata* roots may not be of toxicological concern since increase in albumin is normally not associated with any naturally occurring condition except when there is decreased plasma water. However, the similar increase by the 200 mg/kg body of the extract after seven daily doses may be a physiological attempt to adapt to the assault of the extract by the male rats leading to the increased hepatic synthesis of the protein. Furthermore, the increase in the levels of globulin throughout the 21 daily doses of the aqueous extract of *M. acuminata* roots may imply an increase in the synthetic activity of the liver leading to enhanced rate of synthesis of albumin. Furthermore, reduced hepatocyte uptake, impaired conjugation of bilirubin and reduced hepatocyte secretion of bilirubin may be responsible for the interpretation of the marked increase in the serum total bilirubin concentration after the administration of aqueous extract of *M. acuminata* roots to the male rats. The impaired conjugation of bilirubin became evident with the dose-specific response of the male rats on serum conjugated bilirubin concentration to the aqueous extract of *M. acuminata* roots. The findings in the present study with respect to serum albumin, total bilirubin and conjugated bilirubin after the administration of aqueous extract of *M. acuminata* roots are similar with those previously reported by Yakubu and Omoniwa [11] following the administration of aqueous extract of *M. acuminata* stem.

Serum levels of some electrolytes such as potassium, calcium and sodium as well as urea, and creatinine have been used as important indices for the evaluation of toxic effects of plant extracts on the kidney [30,31]. Such determination can also indicate kidney damage at both glomerular and tubular levels. It has also been reported that a wide variety of renal disease with different permutation of glomerular, tubular, interstitial, or vascular damage can cause an increase in serum urea and creatinine [32]. Therefore, the significant increase in the level of creatinine after the 7 and 21 daily doses of the extract may be an indication of impaired kidney function resulting into poor clearance of creatinine by the kidney. It may also be an indication of deficient or impaired glomerular filtration. In addition, the increase in the levels of urea in the serum of the male rats suggest that the balance between the synthesis of urea by the liver and the

excretion by the kidney has been impaired by the aqueous extract of *M. acuminata* roots. Consequent upon this, the increase in the serum urea levels might be adduced to impaired glomerular filtration. Furthermore, since K^+, Cl^- and HCO_3^- are reabsorbed at the distal tubules, the increase in the levels of K^+ after the administration of the extract might be an indication of renal damage resulting into tubular dysfunction [33]. In contrast, the decrease in the levels of Na^+ after the administration of aqueous extract of *M. acuminata* roots might suggest reduction in tubular reabsorption of the ion whereas the non-specific pattern of effect on the Ca^{2+} by the aqueous extract of *M. acuminata* roots may be the consequence of physiological response by the male rats to either counteract the effect of the extract or attempt to recover from the assault of the extract. It also suggests that the normal functioning of the kidney at both the glomerular and tubular levels have been compromised. All biochemical processes that are dependent on these ions will be adversely affected. Finally, the loss of the enzymes and metabolites from the liver and kidney in the present study may explain the marked decreases in the computed liver- and kidney-body weight ratios after the administration of the aqueous extract of *M. acuminata* roots. The findings in the present study with respect to increase in the level of K^+ and absence of definite pattern of effect of the extract on Ca^{2+} are similar to those earlier reported by Yakubu and Omoniwa [12] after the administration of aqueous extract of *M. acuminata* stem whereas those on the Na^+ in the present study contrast the earlier report by Yakubu and Omoniwa [12] on the same ion.

5.0 Conclusion

Overall, the aqueous extract of *Massularia acuminata* roots at the doses investigated has caused functional toxicity on the liver and kidney of male rats. The consumption of the extract at 50, 100 and 200 mg/kg body weight as oral remedy is of toxicological concern. Therefore, the extract at these doses is not completely safe as oral remedy.

6.0 Conflict of Interest

The authors declare no conflict of interest. The authors alone are responsible for the writing and content of the paper.

7.0 Acknowledgement

This research was supported by the International Foundation for Science (IFS), Stockholm, Sweden, through a grant to Yakubu, M. T. (Grant Number F/3977-1 and F/3977-2).

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