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Acute and Sub-acute Toxicity of the Aqueous Extract of the Stem of *Masularia acuminata* (G. Don) Bullock ex Hoyle on Albino Rats and Mice

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ABSTRACT

Background: Traditional medicine also known as indigenous medicine comprises of knowledge systems that developed over generations within various societies before the era of modern medicine. **Aim:** This study is aimed at determining the toxicological effect of aqueous extract of *M. acuminata* on rodent species with specific attempt on acute and sub-acute studies. **Methods:** Fresh stems of *Masularia acuminata* were collected dried, pulverized, weighed and mixed with sterile distilled water, mixture left for 72 h and stirred at 3 hourly, filtered and concentrated in a water bath and stored in a refrigerator. Healthy, mature male rats (*Rattus norvegicus*), weighing between 170-220 g and females, weighing between 155-165 g and mature male and female mice (*Mus musculus*) weighing between 20-35 g were used for all the experiments. Histological analysis was carried out on the following organs kidney, heart, spleen and liver. **Results:** Results obtained from the acute toxicity study, indicated that *M. acuminata* administered via intraperitoneal route, produced an LD_{50} of 56.23 mg kg^{-1} while through the oral route produce no sign of toxicity or death in mice at 10,000 mg kg^{-1}, suggesting a LD_{50} of above 10,000 mg kg^{-1} via oral route. Thus, referring to the Hodge and Stemer scale, the orally administered extract of *M. acuminata* could be considered practically non-toxic. The sub-acute treatment indicated that *M. acuminata* in doses of 500, 1000 and 2500 mg kg^{-1} per day orally during a 4 weeks consecutive period did not produce any death or any clinical signs of toxicity. Analysis of blood parameters is relevant to risk evaluation of alterations of the haematological system in humans. **Conclusion:** No significant alterations of the haematological and biochemical parameters of both male and female-treated rats can be attributed to the plant extract. Histologically, there were minimal changes in the kidney comprising mainly of mild interstitial vascular congestion, tissue separation across the dosages and focal (isolated) infiltrates of lymphocytes in the cortex at 2500 mg kg^{-1}. There were minimal changes in the heart, ranging from mild vascular wall thickening (hypertrophyl) at 500, 1000 and 2500 mg kg^{-1} to mild tissue separation (oedema) at the two higher doses.

Key words: Oral, intraperitoneal, *Masularia acuminata*, toxicity, acute and sub-acute

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INTRODUCTION

Plants have been of great importance to man for its usefulness in many purposes, such as for provision of shelter, foods and beverages as effective remedies for many diseases among many others (Sofowora, 1981). Traditional medicine also known as indigenous medicine comprises of knowledge systems that developed over generations within various societies before the era of modern medicine (Dwivedi and Dwivedi, 2007). The WHO defines traditional medicine as "the health practices, approaches, knowledge and beliefs incorporating plants, animals and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain wellbeing (WHO, 1976). Pharmacological evaluation of plants used in traditional medicine has provided important advancement in therapeutic approach to several pathologies as well as extremely useful tools for the theoretical study of physiology and pharmacology (Adeshina, 1998). Traditional medicine includes herbs, herbal materials, herbal preparation and finished herbal product that contain part of plants or plan materials as active ingredients (WHO, 1976). When adopted outside of its traditional culture, traditional medicine is often called alternative medicine.

Recently, there has been a worldwide renewed interest in traditional medicine due to the realization that orthodox medicine is not widespread in poor countries whereas, healthcare has virtually been sustained by these natural alternatives (Okujagu, 2003). In some Asian and African countries, up to 80% of the population relies on

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traditional medicine for their primary healthcare needs. Also, in Britain, about 25% of the active ingredients of currently prescribed drugs were first identified in higher plants (Balandrin *et al*., 1993). Presently, higher plants continue to retain long known usefulness as important sources of novel compounds useful directly as medicinal agents, as model compounds for synthetic or semi-synthetic structure modifications and optimization as biochemical or pharmacological agents (Balandrin *et al*., 1993).

The need to study medicinal plants cannot be over emphasized for a number of reasons including rescuing traditional medicinal plants and preserving them from eminent loss as well as its need for health for all (WHO, 1976). The WHO also notes though, that inappropriate use of traditional medicines or practice can have negative or dangerous effects and that further research is required or needed to ascertain the efficacy and safety of several of the practices and medicinal plants used by traditional medicine system (WHO, 1976).

In rural communities in Africa, a lot of tropical plants have been used for the treatment of various ailments and infections especially among traditional practitioners (Okafor *et al*., 2001). World Health Organization is now pursuing a coordinated approach that is encouraging the official recognition of traditional healers on one hand and on the other hand encouraging western trained doctors and pharmacists to study the methodology and recipes of traditional healers.

Massularia acuminata (G. Don) Bullock ex Hoyl. (Rubiaceae) known as pako ijebu or orin ijebu (Yoruba) is a small tropical plant found undergrowth of closed moist forest. It is a tree, growing up to 5m high and is distributed from Sierria Leone through Zaire to Nigeria. The large leaves are practically stalkless, elliptic, acuminata and almost glaborious. The flower which is 5 cm long, narrowly ovoid beaked, torne in short auxillary limes seen in January of every year.

The juice from the fruits is used as eye drops in Sierra Leone (Gill, 1992). The stem is used as chewing stick for oral hygiene in Southern Nigeria (Ndukwe *et al*., 2004). The stem is also claimed to be used as an aphrodisiac and anticarcinogenic by making a decoction or an infusion (Gill, 1992).

M. acuminata have a pantropical distribution in the tropics of Africa, Asia and America, with their greatest diversity in Southeast Asia.

There are claims that medicinal plants or herbs possess some pharmacological properties without any scientific backing. The fresh stem of *M. acuminata* is reputed as an astringent, androgenic and aphrodisiac potentials and no literature exists on the toxicological effects of the plant either in humans or animals. This study is aimed at determining the toxicological effect of aqueous extract of *M. acuminata* on rodent species with specific attempt on acute and sub-acute studies.

MATERIALS AND METHODS

Plant material and authentication: Fresh stems of *M. acuminata* were collected from Afeye-Okpameri in Akoko-Edo Local Government Area of Edo State, Nigeria in November 2011. The plant was identified by Dr J.F. Bamidele of the department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. Immediately after collection, the stems were washed, chopped into bits and sundry for a week. The dried stems were pulverized (650 g) was mixed with distilled water (5 L) and left for 72 h. The mixture was stirred at 3 h interval using a sterile glass rod. At the end, the extract was passed through fitter paper. The filtrate was concentrated over a water bath and the concentrated extract was stored and refrigerated.

Experimental animals: Healthy, mature male rats (*Rattus norvegicus*), weighing between 170-220 g and females, weighing between 155-165 g and mature male and female mice (*Mus musculus*) weighing between 20-35 g were provided by Mr. Uwaya Dickson of the Department of Science Laboratory Technology. The animals were housed in clean wooden cages placed in well-ventilated house conditions (Temperature: 28-31°C; photoperiod: 12 h natural light and 12 h dark; humidity: 50-55%). They were also allowed unrestricted access to rat pellets (Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria.) and tap water. They were exposed to 12 h light/dark cycle and were handled according to standard protocols for the use of laboratory animals (NIH, 2002).

Acute toxicological experiment: The intraperitoneal and oral LD_{50} of the aqueous extract of *M. acuminata* were estimated by the Miller and Tainter method (Ozolua *et al*., 2010). For the oral route two rodent species were used, mice and rats. Six groups of rats and mice comprising of 3 males and 3 females per group were used. Group 1 (control) was given the vehicle for extract reconstitution. Group 2-6 were administered 1, 2, 4, 6 and 8 g kg^{-1} body weight of the extract, respectively using Orogastric tube. Signs of acute toxicity and mortality were monitored for 72 h and then 14 days. This protocol was repeated by administering extract intraperitoneally to another set of mice (6 per group) using doses 10, 100, 200, 600, 800 and 1000 mg kg^{-1}, respectively.

Sub-acute toxicological experiment: Rats of both sexes were selected into four groups of control (n = 6) and treated (n = 6), such that the number of both sexes were the same in control and treated groups. The treated

groups were given 0.5, 1 and 2 g kg^{-1} b.wt. (P.O) of extract daily for 28 days, the control group was given 10 mL kg^{-1} of distilled water (P.O) daily for 28 days. The doses of the extract chosen were based on the method of alloxan-induced rats (Reyes *et al*., 2006).

On the 28th day, the animals were anaesthetized with chloroform in a chamber and blood collected from the abdominal aorta for biochemical and haematological assays, while the vital organs of the heart, right kidney, liver and spleens were isolated and weighed and further processed for histopathological analysis.

Haematological assays: The haematological parameters were analyzed using auto haematology analyzer (Model BC-2800).

Biochemical assays: The blood sample were place in lithium heparin sample bottles and centrifuged at 3000 revolutions per minute (rpm) and plasma was separated using Pasteur pipettes into clear labeled bottles. The samples were stored in deep freezer at -20°C until analyses were carry out. Total protein was assayed by Biuret method (Doumas *et al*., 1981), albumin was assayed by the measurement of serum albumin (Doumas *et al*., 1971). Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were quantified by the method described by International Federation of Clinical Chemistry and Laboratory Medicine (Schumann *et al*., 2002). Alkaline phosphate was assayed using the Principle of Analysis of Alkaline Phosphatase method (Raymond-Habecker and Lott, 1995). Bilirubin was assayed by Jendrassik Grof method (Doumas and Wu, 1991) and GGT (Gamma Glutamyltransferase) was analysed by kinetic photometric method (Szasz, 1969).

Histopathological study: The isolated vital tissues of the liver, kidney, heart and spleen were fixed in 10% formol saline for 18-24 h, dehydrated in an alcohol-xylene series and embedded in paraffin. Sections were stained with haematoxylin and eosin for histological examination.

Statistical analysis: Results were expressed as SEM Mean SEM (Standard error of mean). Data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by newman-keuls multiple comparison test. All data were analyzed using Graph pad prism software (UK). All the statistical analyses and graphs were done using Graphpad prism, Version 6.0 and Graphpad Instat dataset 1.ISD, $p<0.05$ indicates statistically significant difference.

RESULTS

The toxicity signs (Table 1) shown after the extract had been administered to the groups of mice and rats for both oral and intraperitoneal were writhing, calmness, piloerection, sedation, irregular breathing, reduced motor activity and death in mice, no death was recorded in rats even at the highest concentration of 20,00 mg kg^{-1}.

The acute toxicity study (Table 1) shows 16.67% death after the extract was administered to the group of mice of 20,000 mg kg^{-1} through the oral route. But death was not shown in mice administered with 100, 1,000, 5,000 and 10,000 mg kg^{-1}, respectively.

Figure 1 revealed the estimated LD_{50} (Median Lethal Dose) of *M. acuminata* to be 56.23 mg kg^{-1} in mice following intraperitoneal administration.

Percentage change in body weight: Following sub-acute administration of the varying doses of the extract of *M. acuminata* on albino rats Fig. 2a shows a graphical presentation of percentage change in body weight for all the doses after 28 days of administration. The percentage change was not significantly different ($p>0.05$) even between the highest dose and control. Though a change in the percentage body weight was observed in all three medication groups after six weeks, they were not significantly different with respect to control as shown in Fig. 2a.

Weight of vital organs of the body (heart, spleen, liver, right kidney and left kidney): Figures 2b-d and 3a, b graphically present percentage weight changes of vital organs compare with controls, generally there were no significant changes in the weight of vital organs of the treated albino rats as compared with the untreated ones (controls). But a significant change in heart/body weight (Fig. 2b), Liver/body weight (Fig. 2d), right kidney/body

Table 1: Probit value with signs of toxicity administered through oral route on mice

Dose (mg kg^{-1})	Total death/Total animal used	Mortality (%)	Signs of toxicity
20000	1/6	16.67	Writhing after 30 min of administration, calmness, piloerection, sedation, irregular breathing, reduced motor activity and death after 48 h of administration
10000	0/6	-	Writhing after 30 min of administration, calmness, piloerection, sedation, irregular breathing and reduced motor activity
5000	0/6	-	Calmness, sedation and writhing after 40 min of administration
1000	0/6	-	Writhing after 40 min of administration, calmness and sedation
100	0/6	-	Piloerection, writhing after 50 min of administration, calmness and sedation
(Control)	0/6	-	-

Animals were first observed for 72 h and then for 14 days after extract administration

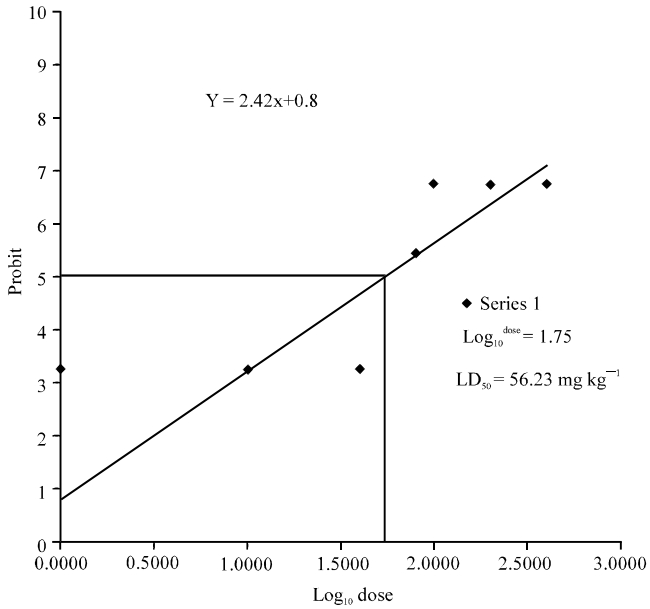

Fig. 1: Graphical estimation of intraperitoneal LD_{50} of the aqueous stem extract of *M. acuminata* in mice, LD_{50} = 56.23 mg kg^{-1}, n = 6 mice per group

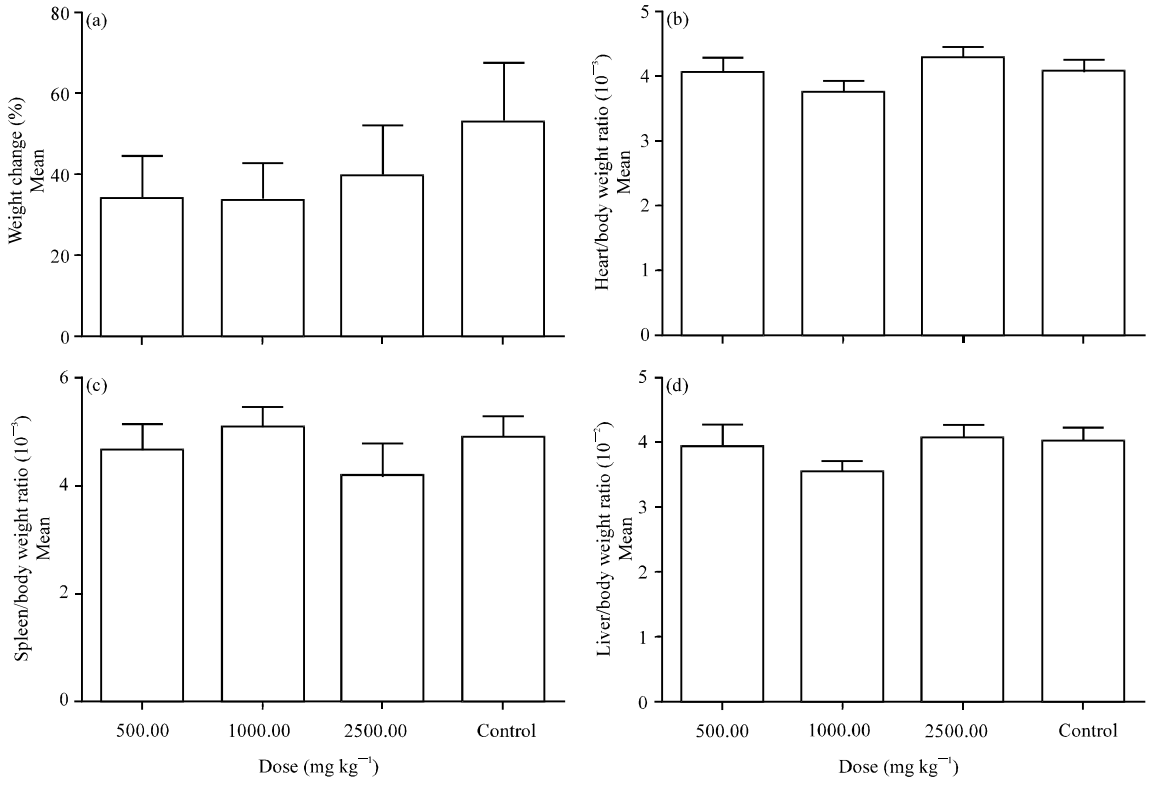

Fig. 2(a-d): Bar charts of (a) Percent weight change, (b) Heart/body weight ratio, (c) Spleen/body weight ratio and (d) Liver/body weight ratio against dose, non-significant with ($p>0.05$)

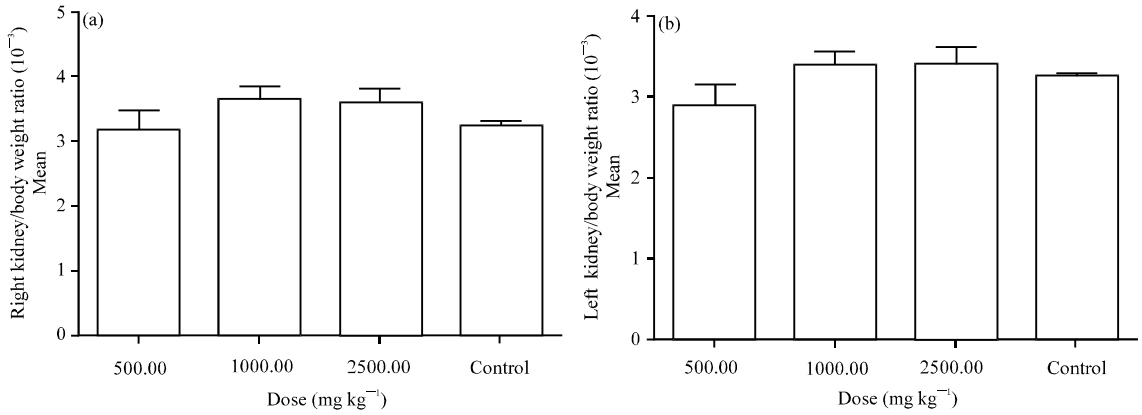

Fig. 3(a-b): Bar charts of (a) Right and (b) Left kidney/body weight ratio against dose, non-significant with ($p>0.05$)

Table 2: Haematological analysis of rats administered with various concentration of aqueous extract of the stem of *M. acuminata*

Parameters	Control	500 (mg kg^{-1})	1000 (mg kg^{-1})	2500 (mg kg^{-1})
WBC ($\times10^3$ UL^{-1})	14.950±1.148	16.876±2.173	11.000±1.452	12.040±0.708
LYP (%)	76.500±4.880	61.526±5.196	59.326±3.940	60.700±3.298
MO (%)	11.017±0.979	23.500±12.505	12.200±0.964	9.300±1.048
GR (%)	21.483±4.264	26.966±4.585	28.476±3.662	30.000±3.540
RBC ($\times10^6$ UL^{-1})	6.640±0.183	6.486±0.276	6.232±0.458	6.434±0.345
Hgb (g dL^{-1})	13.000±0.601	12.976±0.742	12.400±0.765	12.520±0.822
PCV (%)	39.683±1.774	40.150±2.310	37.560±2.213	38.820±2.333
PLT ($\times10^3$ UL^{-1})	527.22±131.73	614.80±89.034	496.200±37.126	493.16±132.50

Values are expressed as Mean±SEM, $p>0.05$, WBC: White blood cell count, LYP: Lymphocytes, PVC: Packed cell volume, Hgb: Haemoglobin, PC: Platelet count, RBC: Red blood cell, MO: Monocytes, GR: Granulocytes n = 6 control or treated

Table 3: Biochemical analysis of rats administered with various concentration of aqueous extract of the stem of *M. acuminata*

Parameters	Control	500 (mg kg^{-1})	1000 (mg kg^{-1})	2500 (mg kg^{-1})
ALT (U L^{-1})	18.200±1.960	19.000±3.592	15.000±0.000	14.800±1.281
AST (U L^{-1})	44.800±3.707	53.000±6.058	37.800±2.709	38.600±4.179
ALP(U L^{-1})	10.600±5.085	23.800±6.037	15.600±5.988	21.600±5.144
DB (mg dL^{-1})	6.916±1.167	11.978±1.480	8.958±0.384	7.08±1.3250
TB (mg dL^{-1})	6.466±1.661	12.086±0.853	8.540±0.610	10.334±1.481
TP (g dL^{-1})	10.104±2.709	7.120±0.843	5.622±0.462	6.320±1.683
ALB (g dL^{-1})	7.762±0.915	6.720±1.908	8.276±0.929	7.498±0.404
GGT (U L^{-1})	83.150±49.494	82.218±50.071	48.056±25.236	62.532±29.945
Cl^- (mmol L^{-1})	93.200±2.728	96.000±1.897	94.000±2.280	89.600±2.227
HCO_3 (mmol L^{-1})	22.400±0.980	24.000±0.316	26.200±0.970	24.400±1.208

Values are expressed as Mean±SEM $p>0.05$, GGT: Gamma glutamyl transferase; AST: Aspartase aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline phosphatase, DB: Direct bilirubin, TB: Total bilirubin, TP: Total protein, ALB: Albumin, Cl^-: Chloride, HCO_3: Bicarbonate, n = 6 control or treated

weight (Fig. 3a) and left kidney/body weight (Fig. 3b) were observed in the overdose of 2500 mg kg^{-1} administration.

Haematological analysis: Heamatological indices in the rats after sub-acute oral treatment with 500, 1000, 2500 mg kg^{-1} and untreated set (control) body weight of the extracts are presented in Table 2. White Blood Cell count (WBC), Lymphocytes (LYM), the haematocrit (Packed Cell Volume or PCV), Haemoglobin (HGB) and Platelet Count (PC) were all not significantly altered by the treatment when compared untreated (controls).

Serum analysis: The results of serum Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP) of animals treated with doses of the crude aqueous extract of the plant material were presented in Table 3. The results indicated significant difference ($p<0.05$) for ALT and AST and ALP. Further analysis of the results indicated that animals that receives 500 mg kg^{-1} had significant ($p<0.05$) higher serum ALT, AST and ALP than the animals that received 1000 and 2500 mg kg^{-1} when compare with individual control. Administration at 1000 and 2500 mg kg^{-1} ($p<0.05$) had significantly lower AST and ALT than their

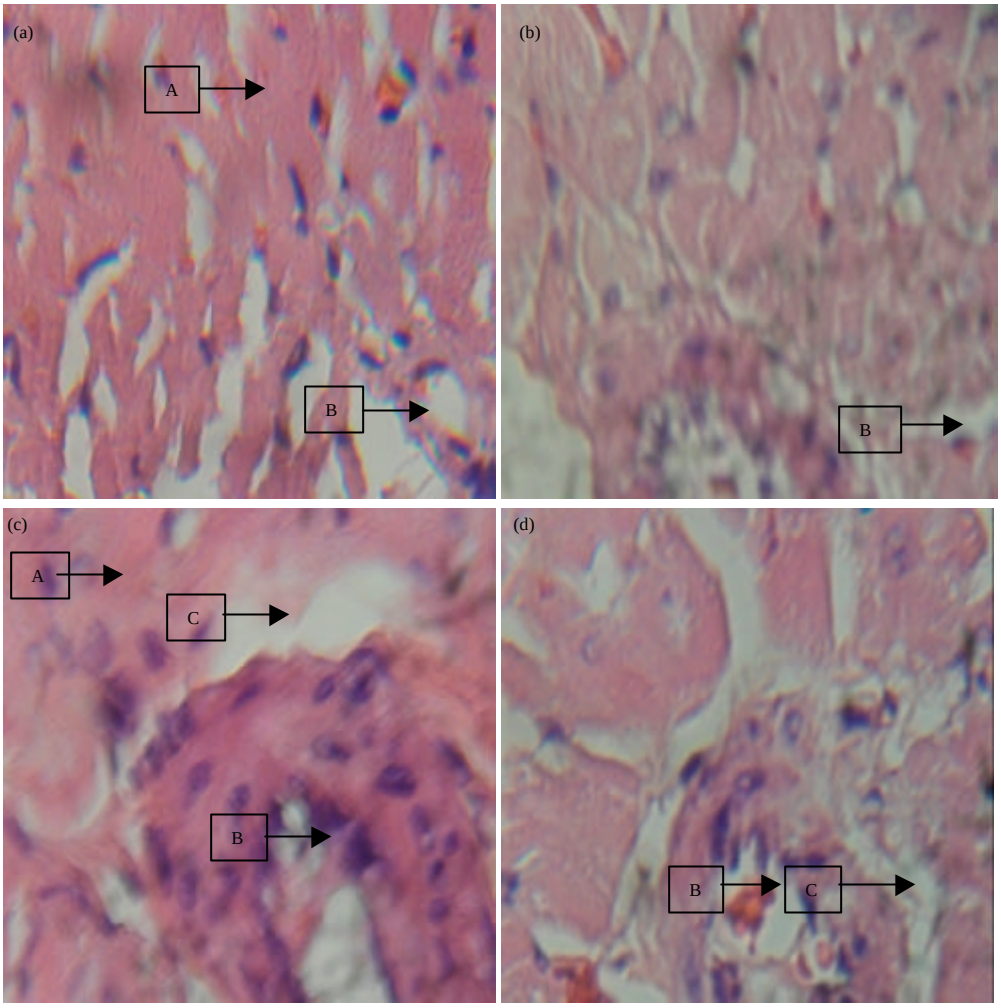

Fig 4 (a-c): Photomicrograph of (H and E×40) stained section of albino rat heart, (a) Control treated with (b) 500 mg kg^{-1} *M. acuminata* (c) 1000 mg kg^{-1} *M. acuminata* and (d) 2500 mg kg^{-1} *M. acuminata* (H and E×40) stained section

respective control values, while they had higher ALP than their controls. Changes in the level of other parameters are not too significantly different from their controls as shown in Table 3.

Histopathology of the vital organs: Figure 4a-d shows the effect of various concentrations of *M. acuminata* on the heart of albino rats after 6 weeks of administration. Figure 4a is the untreated rat heart showing bundles of myocytes A, pierced by coronary vessel B. Figure 4b shows rat heart treated with 500 mg kg^{-1} *M. acuminata* with unremarkable myocytes A and mild vessel wall thickening B. while Fig. 4c,d revealed rat hearts treated with 1000 and 2500 mg kg^{-1} *M. acuminata* with unremarkable myocytes A with mild vessel wall thickening B and mild tissue separation C, respectively. Figure 5a-d showed the effect of various concentrations of *M. acuminata* on the spleen of albino rats after 6 weeks of administration. Figure 5a is the untreated rat spleen showing white pulp A (lymphoid follicle) and red pulp B., Fig. 5b and c were rat spleen treated with 500 and 1000 mg kg^{-1} of *M. acuminata* with mild activation of the white pulp A. while Fig. 5d was rat spleen treated with 2500 mg kg^{-1} of *M. acuminata*, it revealed moderate activation of the white pulp A and mild activation of sinus histiocytes B.

Figure 6a-d showed the effect of various concentrations of *M. acuminata* on the liver of albino rats

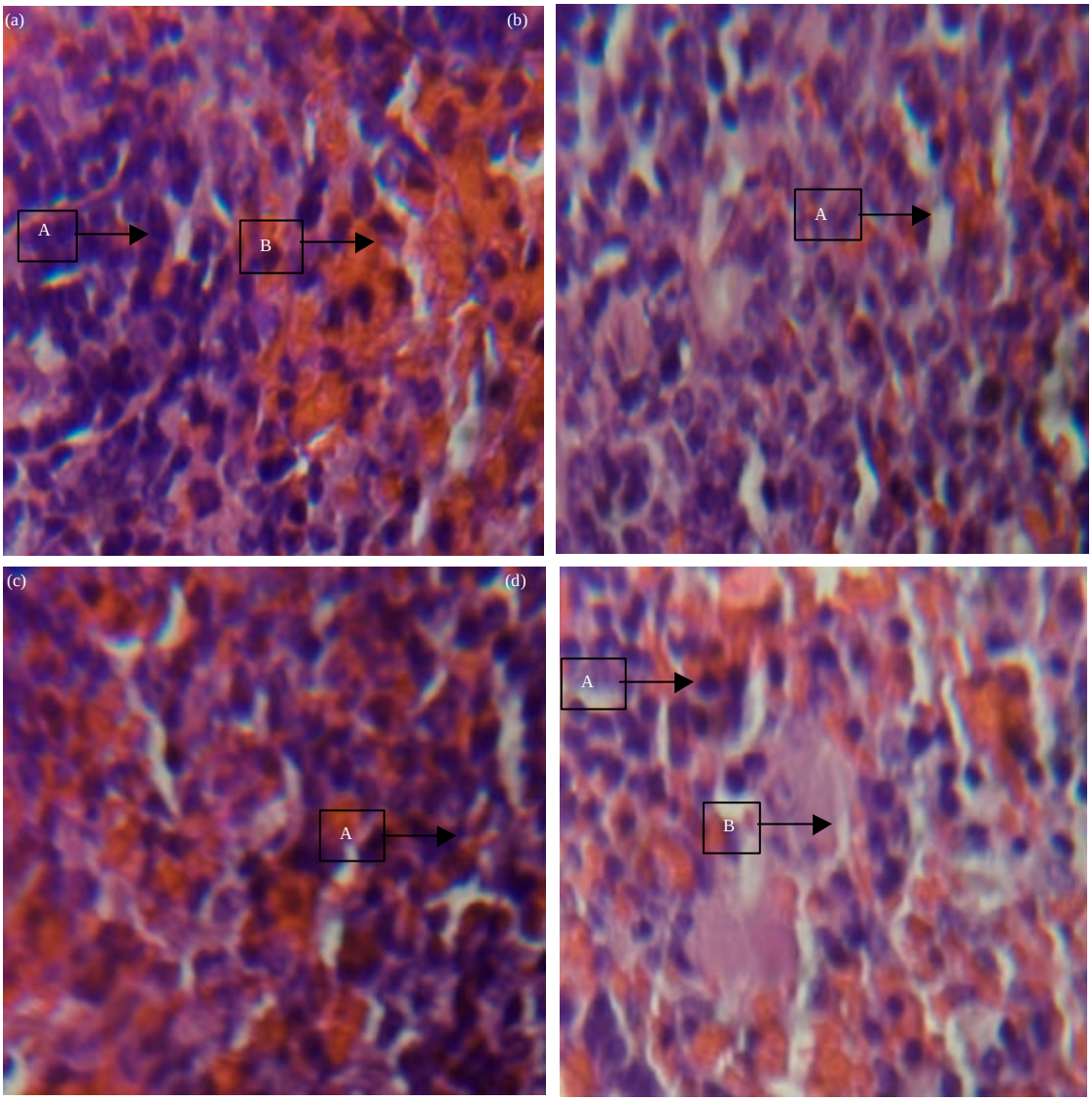

Fig 5 (a-d): Photomicrograph of (H and E×40) stained section of albino rat heart, (a) Control treated with (b) 500 mg kg^{-1} *M. acuminata* (c) 1000 mg kg^{-1} *M. acuminata* and (d) 2500 mg kg^{-1} *M. acuminata* (H and E×40) stained section

after 6 weeks of administration. Figure 6a was the untreated rat liver showing portal triad A, hepatocytes B separated by sinusoids C, Fig. 6b was rat liver treated with 500 mg kg^{-1} of *M. acuminata* with mild tissue separation A and Fig. 6c showed the rat liver treated with 1000 mg kg^{-1} of *M.acuminata* showing mild vascular congestion A and kupffer cell activation B, while fig.6d showed rat liver treated with 2500 mg kg^{-1} of *M. acuminata* showing mild vascular congestion and thickening A and mild kupffer cell activation B.

Figure 7a was the untreated rat kidney showing cortical glomerulus A, tubules B separated by interstitial space C, Fig. 7b, c were rat kidneys treated with 500 and 1000 mg kg^{-1} of *M. acuminata* with mild interstitial vascular congestion A at both concentrations, Fig. 7d indicated rat kidney treated with 2500 mg kg^{-1}

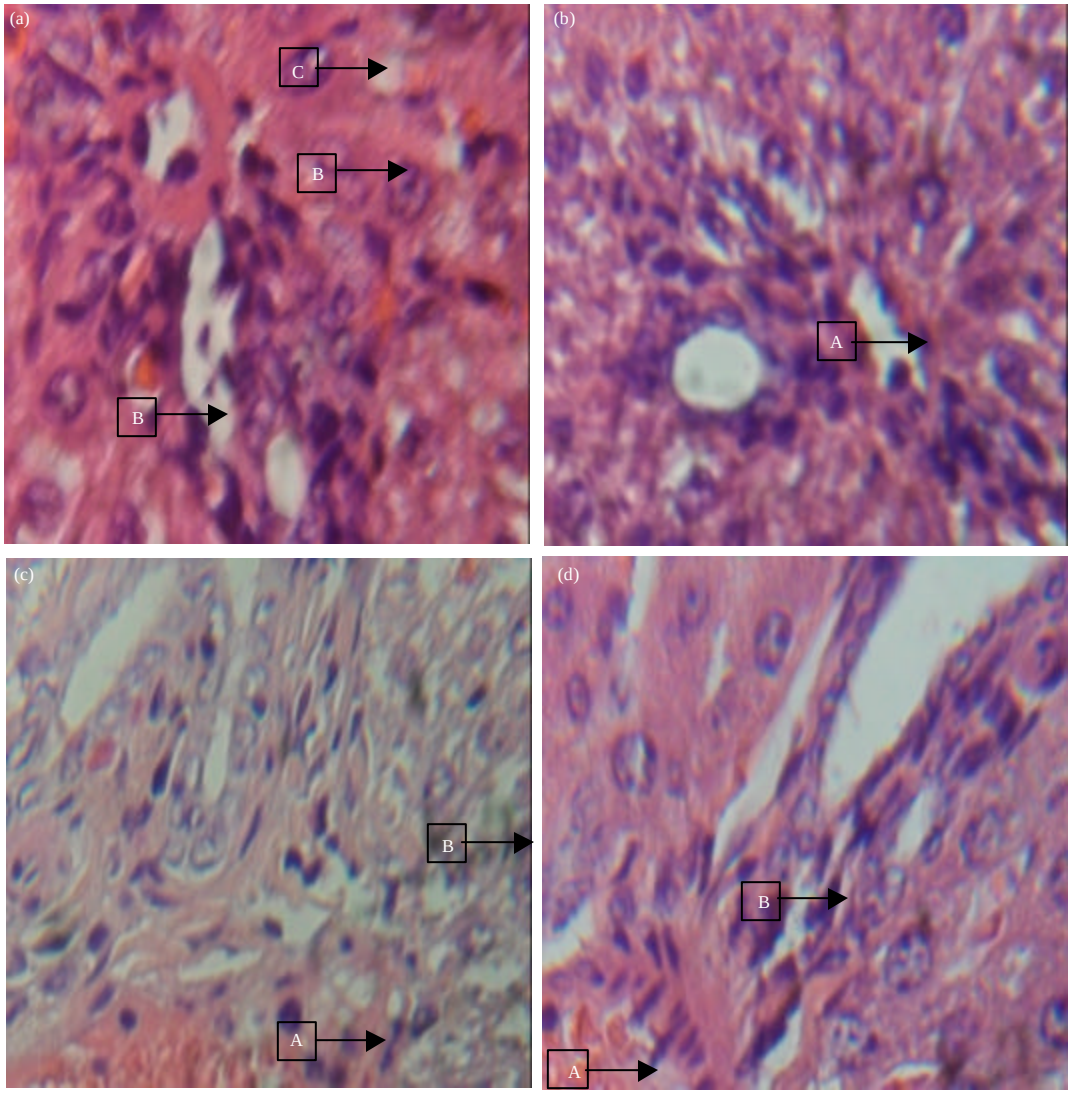

Fig 6 (a-d): Photomicrograph of (H and E×40) stained section of albino rat heart, (a) Control treated with (b) 500 mg kg^{-1} *M. acuminata* (c) 1000 mg kg^{-1} *M. acuminata* and (d) 2500 mg kg^{-1} *M. acuminata* (H and E×40) stained section

M. acuminata with mild interstitial vascular congestion A and mild infiltrates of chronic inflammatory cells B.

DISCUSSION

The main hindrance for the use of traditional herbal preparation is lack of scientific and clinical data to support better understanding of the efficacy and safety of herbal preparation. This is due largely to negligence of the evolution of the toxicity and adverse drug reaction of herbal medicines, as they are considered natural and thus, erroneously, safe. Some plant extract could be inherently dangerous, containing naturally occurring toxins, which may be cytotoxic or carcinogenic (Humphrey and McKenna, 1997). Accordingly most of the herbal preparations do not have drugs regulatory approval to demonstrate their safety and efficacy (Seth and Sharma, 2004). It is therefore pertinent to establish the safety of this preparation through toxicological assessments. Liver, being the primary organ for the detoxification and distribution of drugs and the kidney, the major excretory organ, could be assessed to establish

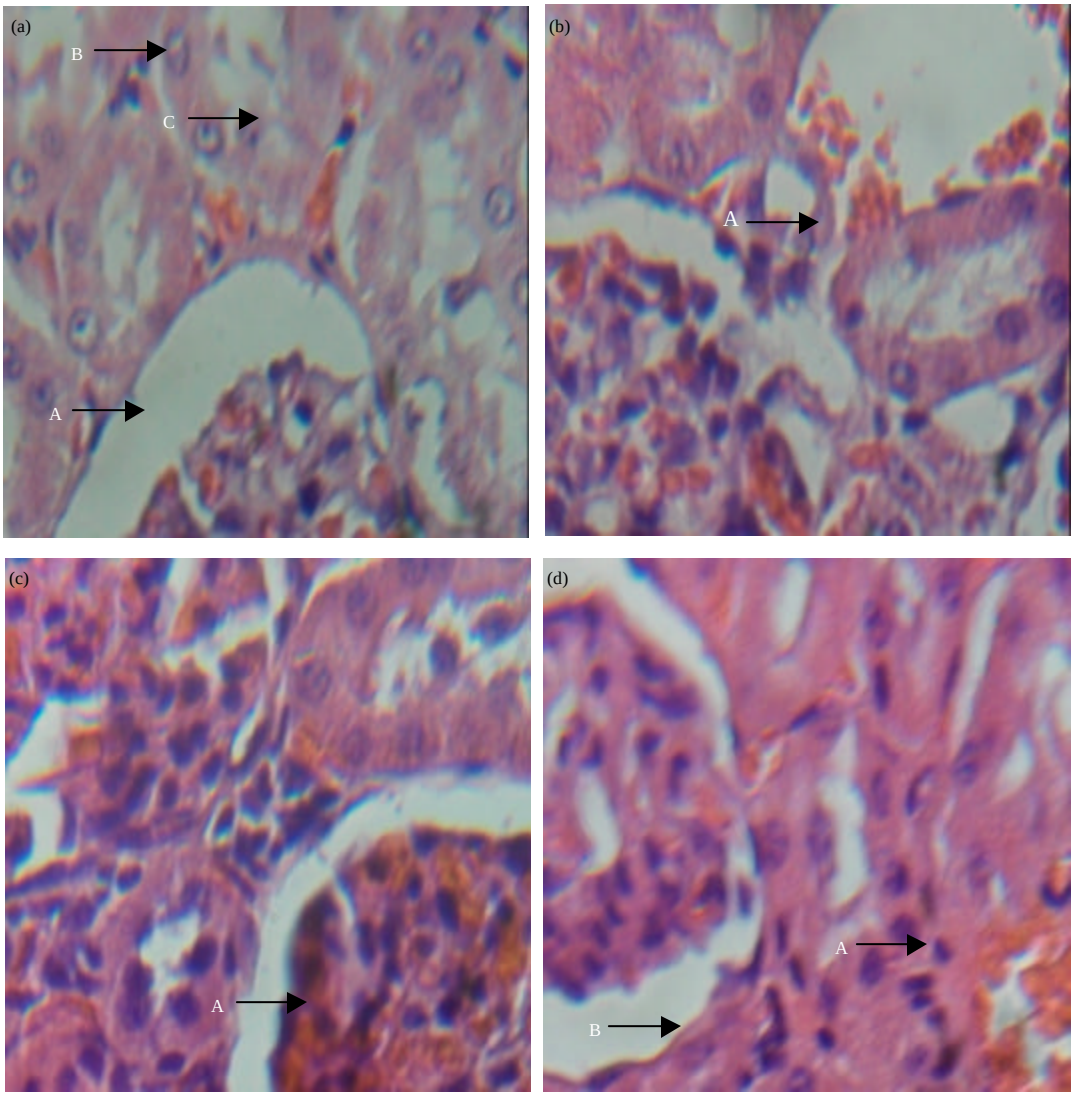

Fig 7 (a-d): Photomicrograph of (H and E×40) stained section of albino rat heart, (a) Control treated with (b) 500 mg kg^{-1} *M. acuminata* (c) 1000 mg kg^{-1} *M. acuminata* and (d) 2500 mg kg^{-1} *M. acuminata* (H and E×40) stained section

safety of a substance (Gupta *et al*., 1994). In the current study therefore, liver and kidney play vital role in the assessment of animals treated with both acute and sub-chronic doses of the crude aqueous preparation of *M. acuminata*. One of the toxicological indices used for the assessment of the safety of drug is lethal dose 50% (LD_{50}), the amount of acute dose of the drug required to kill half of the test population.

Results obtained from the acute toxicity study, shown on (Fig. 1) indicated that *M. acuminata* administered via intraperitoneal route produced an LD_{50} of 56.23 mg kg^{-1} while through the oral route LD_{50} is above 1,000 mg kg^{-1}. Thus, referring to the Hodge and Stemer scale, the orally administered *M. acuminata* could be considered practically non-toxic (CCOHS, 2005).

Repeated dose studies were conducted to evaluate the adverse effects of a test substance after prolonged use and are carried out to provide information about the possible health hazards likely to arise from repeated exposure over a relatively limited period of time including information about target organs, the possibilities of cumulative effects, and an estimate of the

dose at which there is no observed adverse effect. The sub-acute treatment indicated that *M. acuminata* in doses of 500, 1000 and 2500 mg kg^{-1} per day orally during a 6 weeks consecutive period did not produce any death or clinical signs of toxicity. A decrease in body weight was observed to be an indicator of adverse effects but there were no significant changes in animal behaviour, food and water consumptions in *M. acuminata* treated group at any dosage.

Analysis of blood parameters is relevant to risk evaluation of alterations of the haematological system in humans. No significant alterations of the haematological and biochemical parameters of both male and female-treated rats can be attributed to the plant extract. However, the White Blood Cells (WBC), lymphocytes (LYP), monocytes (MO), granulocytes (GR), Red Blood Cells (RBC), Haemoglobin (HGB), Packed Cell Volume (PCV) and Platelets (PLT) with mean values of 16.876±2.173, 61,526±5.196, 23.500±12.505, 26.966±4.585,6.486±0.276,12.976±0.742,40.150±2.310, 614.80±89.034 at the dosage of 500 mg kg^{-1}, respectively when compared with the mean values of their individual controls of 14.950±1.148, 76.500±4.880, 11.017±0.979, 21.483±4.264,6.640±0.183,13.000±0.601,39.683±1.774, 527.22±131.73 were not significant ($p>0.05$), while the decreased lymphocytes and monocytes (60.700±1.048 and 9.300±1.048) at a higher dose of 2500 mg kg^{-1} when compared to the controls (76.500±4.880 and 11.017±0.979) were not significant. All concentrations used were tolerable to the animal (Table 2).

Table 3 shows the various biochemical parameters, amount of ALT, AST and ALP increases with dose of 500 mg kg^{-1} when compared with control, while ALT and AST decreases below their control at higher doses of 1000 and 2500 mg kg^{-1}, ALP at higher doses (15.600±5.988 and 21.600±5.144) increases more than control (10.600±5.085) but lower than the minimal dose of 500 mg kg^{-1} (23.800±6.037), respectively. Elevated levels of bilirubin, AST, ALT and ALP are often diagnostic of underlying cellular injuries (Wittekind, 1995). In the present study, these parameters are comparable between the control and treated groups.

The histological analysis was carried out on the following vital organs kidney, heart, spleen and liver. Kidney is a sensitive organ, whose function is known to be affected by a number of factors such as drugs including phytochemicals of plant origin that ultimately lead to renal failure (Saidu *et al*., 2007). There were minimal changes in the kidney mainly mild interstitial vascular congestion, tissue separation across the dosages and focal (isolated) infiltrates of lymphocytes in the cortex at 2500 mg kg^{-1} dose. There were minimal changes in the heart, ranging from mild vascular wall thickening (hypertrophy) at 500, 1000 and 2500 mg kg^{-1} to mild tissue separation (oedema) at the two higher doses. In the spleen we observed mild activation of the white pulp (lymphoid follicles) at both 500 and 1000 mg kg^{-1}, as well as mild activation of sinus histiocytes (cells of the mononuclear phagocytes system). While at 2500 mg kg^{-1}, the lymphoid follicles and the histiocytes were slightly more activated than the two lower doses. The liver plays a key role in many metabolic processes of not only itself but of other tissues as well. This fact demonstrates the biochemical altruistic nature of this organ. It showed mild tissue separation (oedema) at 500-2500 mg kg^{-1}. There was increased vascular congestion as well as mild activation of the kuffer cells (cells of the mononuclear phagocytes system). In addition, it was observed across board increased prominence of the nucleoli.

The results of the weight changes (Fig. 2a) in the current study indicated no significant ($p>0.05$) effects in both acute and sub-chronic toxicity tests. This may be an indication that the drug does not affect the feed utilization ratio of the animals. In all the groups and both test models, the animals fed well.

CONCLUSION

In acute studies *M. acuminata* through intraperitoneal route produced an LD_{50} of 56.23mg/kg. The sub-acute toxicity studies carried out could be considered with a wide margin of safety for oral use at doses below 1000mg/kg as there were no significant alterations in the haematological and biochemical parameters.

The extract administered causes no damage to the tissue analyzed, however it produced mild inflammation in all the tissues as well as activating the local immune system, with changes been most apparent at a very high dose (2500 mg kg^{-1}). Since toxicity in humans cannot always be entirely extrapolated from animal studies, clinical evaluation should be performed to precisely define the safe dosage to advice in humans.

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