

Effect of ethanolic leaf extract of *Macaranga spinosa* Muell-Arg on selected biomarkers of toxicity in Wistar rats

Joshua David * and Peters Dikioye Emmanuel

Department of Biochemistry, Faculty of Science University of Port Harcourt, Nigeria.

World Journal of Advanced Research and Reviews, 2022, 14(03), 696–705

Publication history: Received on 27 April 2022; revised on 23 June 2022; accepted on 26 June 2022

Article DOI: <https://doi.org/10.30574/wjarr.2022.14.3.0662>

Abstract

In Nigeria, plant-based herbal medicines are vital component of traditional medicine (TM) and their use for the maintenance of health and wellbeing has been a common practice. These TMs are freely used without mandatory safety or toxicological evaluation. Thus, it is necessary to identify plants with toxicity potentials among the plants used for therapeutic purposes in the country. This study investigated effect of ethanolic leaf extract of *Macaranga spinosa* Muell-Arg on selected biomarkers of toxicity in Wistar rats. Fifteen Wistar rats of both sexes weighing 96.8 -250 g were divided into three groups comprising of group 1 (control 0.5ml distilled water); group 2 (500 mg/kg b.w *Macaranga spinosa* Muell-Arg leaf extract) and group 3 (1000 mg/kg b.w *Macaranga spinosa* Muell-Arg leaf extract). At the end of one week oral administration, animals were sacrificed; blood and tissues samples were taken for biochemical and histopathological investigations respectively. Phytochemicals present were alkaloids, flavonoids, tannins, triterpeneoid/steroids, carbohydrates, cardenolide, and saponins. Urea, sodium ion, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentration were significantly elevated in group 3; non-significant increase in creatinine, potassium, bicarbonate, chloride ion and alkaline phosphatase (ALP) concentration in group 2 and 3 compared to group 1. Total protein and triglyceride were significantly decreased in group 3 compared to group 1. Albumin, total and conjugated bilirubin were non-significantly decreased in group 2 and 3 when compared to control group 1. Histological examination of kidney tissue showed no change in kidney histology; liver tissue indicated vacuolar and cytoplasm degeneration, and fatty change. Heart tissues showed normal cardiac myocytes. This study has established that ethanolic leaf extract of *Macaranga spinosa* Muell-Arg can cause hepatotoxicity, mild nephrotoxicity and no significant alteration of lipid profile in Wistar rats. Therefore, users of this plant in TM should avoid excessive and prolong use, as it can be toxic to the liver.

Keywords: *Macaranga spinosa* Muell-Arg; Kidney; Liver; Lipid profile; Toxicity

1. Introduction

Plant-derived medicines have been part of traditional health care in most parts of the world for thousands of years. They form a vital component of traditional medicine (TM) and their use for the maintenance of health and wellbeing is a common practice in most African societies [1-2]. They are used as remedies for the prevention and treatment or management of a plethora of disease conditions [1]. Over the past three decades, the use of herbal medicine has increased tremendously with not less than 80% of people globally relying on them for some part of primary healthcare [2-3]. There is now increasing interest in these plants based products as sources of drugs in the treatment of diseases [4]. Although therapies involving compounds isolated from some of these plants have shown promising potential with the efficacy of a good number of them clearly established [5]. However, in developing countries where testing, monitoring and regulatory policies are poor, many of the herbal medicine products remains untested and their uses are either poorly monitored or not even monitored at all [4]. The consequence of this is an inadequate knowledge of their

* Corresponding author: David Joshua

Department of Biochemistry, Faculty of Science University of Port Harcourt, Nigeria.

mode of action, potential adverse reactions, contraindications, and interactions with existing orthodox medicines and functional foods to promote both safety and rational use [4,6]. Generally, medicinal plants contain bioactive compounds which demonstrate both intra- and inter-species variation in type and constituents [5]. Some of the phytochemicals constituents of some plants are potentially toxic; thus, some plants used in TM are intrinsically toxic [1].

The plant *Macaranga spinosa* Muell-Arg is a dioecious shrub that grows up to 10m, with a spiny bole directed downwards, simple or forked, woody; twigs often spiny, and young shoots densely softly hairy [7]. The leaf is alternate, simple and entire; stipules linear-lanceolate, 5–7 mm long [7]. Ecologically, *Macaranga spinosa* Muell-Arg occurs along edges of primary forest and in secondary forest, often on soils with a high groundwater table, from sea-level up to 1200m altitude. It is native mainly to the tropics of Africa, South-East Asia, Australia and the South Pacific region [8-10].

Several *Macaranga* species are used as part of TM [9]. *Macaranga spinosa* is used in Côte d'Ivoire in the treatment of dysentery and cough [12]. In Congo, leaf sap or bark sap is drunk, rubbed or used in a vapour bath to treat lung complaints (including bronchitis, cough and asthma), headache, feverish stiffness, rheumatism, liver complaints and stomach-ache [12]. A bark decoction is gargled or used as a mouth wash to treat toothache, stomatitis and aphthae. A maceration of the crushed leaves is taken by women to treat amenorrhoea [13-14]. The root ash is inhaled to treat haemorrhoids [14]. Ethno botanical information gathered during this research indicated that in Nigeria, the leaf of *Macaranga spinosa* Muell-Arg locally called "Owiliwa" are used by the Ehuaji people of Ahoada-East local government area (LGA) of Rivers state to induce menstruation in women who are unable to naturally menstruate, while in Abua/Odual LGA which is a neighbouring LGA, the plant (locally known as "Oo-ka") roots are used to treat stomach ache and the mature stem used as fire wood for cooking.

In most African cultural settings, the local people believed that plants used in TM are safe due to their long history of use in the treatment of diseases based on knowledge passed to them by their ancestors or passed from parents to children over several centuries [1, 15]. However, safety of the different decoctions consumed from various part of *Macaranga spinosa* Muell-Arg is a major problem. These preparations are consumed without recourse to dosage, even perceived safe herbal medicine in overdose could cause pose safety problems. Also, dose-toxicity relationship of *Macaranga spinosa* Muell-Arg has not been previously documented in Nigeria. There is no data on toxicity-related issues about *Macaranga spinosa* Muell-Arg such as cytotoxicity, carcinogenicity, hepatotoxicity, nephrotoxicity and cardiotoxicity. Safety consideration of this plant cannot be disregarded, as knowledge is key in preventing overdoses, abuse or toxicity related problem. Therefore, to bridge the toxicity knowledge gap, this study investigated the effect of ethanolic leaf extract of *Macaranga spinosa* Muell-Arg on selected biomarkers of toxicity in Wistar Rats.

2. Material and methods

2.1. Experimental Animals

A total of 15 Wistar albino rats of both sexes weighing 96.8-250 g were purchased from an animal breeding facility in Choba community of Obio/Akpor LGA of Rivers state and kept in the Animal Research Unit of the Department of Biochemistry, University of Port Harcourt, Nigeria. The rats were acclimatized for 2 weeks with unrestricted access to rat chow and water.

2.2. Plant Collection and Authentication

Based on ethno botanical information obtained from a local Herbalist that *Macaranga spinosa* Muell-Arg leaf is used for treatment amenorrhoea, fresh leaf were collected in Ehuaji community in Ahoada-East LGA of Rivers state, Nigeria. The plant was authenticated by a Botanist Dr. N. L. Edwin-Wosu. A voucher specimen with the number UPH-NO.V-1136 was deposited at the Herbarium unit of Department of Plant Science and Biotechnology, University of Port, Nigeria.

2.3. Extract Preparation

Extract was prepared according to the method described by Handa et al. [16] with slight modification. The fresh leaf of *Macaranga spinosa* Muell Arg. were washed with running tap water and air dried for 2 weeks before blending into powdered form using a Qlink grinder. The coarsely powdered material was macerated with ethanol (99.5%) in a ratio of 1:4 respectively and then left inside a sealed 5000 ml conical flask for 72 hours. The mixture was filtered first using a muslin cloth and thereafter with a filter paper. The filtrate was concentrated using a soxhlet extractor to recover the ethanol from the solution. A gel-like extract which was oven dried at 41°C was obtained. The crude extract was preserved in a refrigerator.

2.4. Phytochemical Screening

Powered leaf (4 grams) material was used for phytochemical tests. The leaf was tested for alkaloids, flavonoids, tannins, anthraquinone, triterpeneoid/steroids, carbohydrates, cardenolide, cyanogenic glycosides and saponins according to the protocol described by Trease and Evans [17].

2.5. Lethal Dose (LD₅₀) Determination

The LD₅₀ was done according to the method describes by Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals [18]. Three doses of 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg were orally administered to the rats according to their weight. The rats were observed for 24 hours and 1 week, and there was no death recorded. Therefore, safe doses of 500 and 1000 mg/kg were selected for the research.

2.6. Experimental Design

The rats were divided into 3 groups of 5 rats per cage. Group 1(Control) animal received 0.5ml distilled water, group 2 were given 500 mg/kg *Macaranga Spinosa* Muell-Arg leaf extract, while group 3 animals received of 1000 mg/kg of *Macaranga spinosa* Muell-Arg extract. Oral administration was done daily as a single dose for 1 week.

2.7. Biochemical Test

At the end of one week of daily oral administration of extract, animals were anaesthetized with chloroform, and blood samples collected into lithium heparin specimen bottles for biochemical analysis. Selected biomarkers of kidney and liver toxicity, and lipid profile were performed on the blood samples using standard techniques according to Burtis *et al.* [19].

2.8. Tissue Processing and Histopathological Examination

Tissues processing were performed according to the method described by Windsor [20]. Immediately after blood sample collection, the kidney, liver and heart were collected for tissue section (5-micron thickness). These tissues were further fixed in 10% formalin and then, embedded in paraffin wax for histopathological examination. They were routinely stained with haematoxylin and eosin (H & E), and examined under a light microscope. Any alterations compared to the normal structures were registered.

2.9. Data Analysis

Data were analyzed using Statistical Package for Social Science (SPSS) version 20.0 (IBM, U.S.A). Results were expressed as mean \pm standard error of mean (M \pm SEM.). Within group, comparisons were performed by analysis of variance (ANOVA) test. Significant difference between control and experimental groups were assessed by least significant difference (LSD) for Post Hoc test of multiple comparisons at $p \leq 0.05$.

3. Results

Result of phytochemical screening (Table 1) revealed the presence of alkaloids, flavonoids, tannins, triterpeneoid/steroids, carbohydrates, cardenolide, and saponins.

In Table 2, plasma urea and sodium ion concentration were significantly elevated in the 1000mg/kg b.w *Macaranga spinosa* Muell-Arg leaf extract treated group (group 3) compared to group 1(control group); non-significant increase in creatinine, potassium, bicarbonate and chloride ion concentration in group 2 and 3 compared to control group.

Table 3 revealed significant increase in AST and ALT concentration in group 3; non-significant increase in ALP concentration in group 2 and 3 when compared to group 1. Total protein was significantly decreased in group 3 compared to control group. Albumin, total and conjugated bilirubin were non-significantly decreased in group 2 and 3 when compared to control group.

Lipid profile result in Table 4 showed non-significant elevation of total cholesterol in groups 2 and 3 compared to control group; significant decreased in triglyceride in group 3 compared to control and group 2; non-significance difference in HDL-cholesterol in group 2 and 3 compared to control. LDL-cholesterol was significantly decreased in groups 2 and 3 compared to control group.

Table 1 Qualitative phytochemical screening of *Macaranga spinosa* Muell-Arg Leaf

Secondary metabolite	Test	Result
Alkaloids	Drangedorff	+ve
	Mayer	-ve
	Hager	-ve
Flavonoids	Shinoda	ND
	Lead acetate	ND
	AlCl ₃	ND
	NaOH	+Ve
Tannins	FeCl ₃	+Ve
	Phlobatannins	-Ve
	Gelatin	ND
	Albumin	ND
Anthraquinone	Free Anthraquinone	-Ve
	Combined Anthraquinone	-Ve
Triterpenoid/Steroids	Liebermann-Buchard	+Ve
	Salwoski	+Ve
Carbohydrates	Molisch	+Ve
	Fehlings	+Ve
Cardenolide	Keller Killani	+Ve
	Kedde	ND
Cyanogenic Glycosides		-Ve
Saponins	Frothing	+Ve
	Haemolysis	ND
	Emulsion	ND

Note: +Ve = present, -Ve = absent, while ND = Not Determined

Table 2 Mean urea, creatinine, sodium, potassium, bicarbonate and chloride ion concentration in Wistar rats treated with ethanolic leaf extract of *Macaranga spinosa* Muell-Arg after one week

Group	Treatment	Urea (mmol/L)	Creatinine (mmol/L)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	HCO ₃ ²⁻ (mmol/L)	Cl ⁻ (mmol/L)
1	Control (0.5 ml distilled water)	3.55±0.48 ^a	2.08±0.55	38.00±6.20 ^a	3.78±0.26	12.50±1.02	69.75±7.31
2	500 mg/kg <i>Macaranga Spinosa</i>	4.13±0.43	2.60±0.55	48.50±5.91	4.08±0.19	13.00±1.22	73.00±4.49
3	1000 mg/kg <i>Macaranga Spinosa</i>	4.80±0.12 ^a	2.70±0.41	64.00±2.65 ^a	4.25±0.10	13.75±1.93	75.00±3.63

Values are presented as Mean± Standard Error of Mean (M±SEM) (n =5). Values with similar superscripts indicate statistical significance difference (p≤ 0.05) down the column while those without superscripts shows non-significance difference (p≥ 0.05) down the column when compared with the control and between groups

Table 3 Mean plasma AST, ALT, ALP, total protein, albumin, total and conjugated bilirubin concentration in Wistar rats treated with ethanolic leaf extract of *Macaranga spinosa* Muell-Arg after one week

Group	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Protein (g/L)	Albumin (g/L)	Total Bilirubin (mmol/L)	Conjugated Bilirubin (mmol/L)
1	Control (0.5 ml distilled water)	22.00±1.00 ^a	48.25±4.42 ^a	47.75±2.39	66.25±2.53 ^a	40.00±2.86	6.05±0.88	3.60±0.49
2	500 mg/kg <i>Macaranga Spinosa</i>	26.00±1.00	54.75±5.51	52.00±0.82	62.00±2.35	38.25±2.29	6.50±0.37	3.60±0.69
3	1000 mg/kg <i>Macaranga Spinosa</i>	29.50±1.84 ^a	67.00±0.00 ^a	53.50±3.59	57.00±2.45 ^a	31.75±2.84	6.95±1.17	4.20±0.60

Values are presented as Mean± Standard Error of Mean (M±SEM) (n =5). Values with similar superscripts indicate statistical significance difference (P≤ 0.05) down the column while those without superscripts shows non-significance difference (p≥ 0.05) down the column when compared with the control and between groups

Table 4 Lipid profile of Wistar rats treated with ethanolic leaf extract of *Macaranga spinosa* Muell-Arg after one week

Group	Treatment	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-Cholesterol (mmol/L)	LDL-Cholesterol (mmol/L)
1	Control (0.5 ml distilled water)	2.15±0.39	1.75±0.14 ^a	2.10±0.12	0.58±0.12 ^a
2	500 mg/kg <i>Macaranga Spinosa</i>	2.43±0.05	1.38±0.14 ^b	2.33±0.19	0.29±0.04 ^a
3	1000 mg/kg <i>Macaranga Spinosa</i>	2.35±0.19	0.95±0.09 ^{a,b}	1.88±0.26	0.29±0.09 ^a

Values are presented as Mean± Standard Error of Mean (M±SEM) (n =5). Values with similar superscripts indicate statistical significance difference (p≤ 0.05) down the column while those without superscripts shows non-significance difference (p≥ 0.05) down the column when compared with the control and between groups

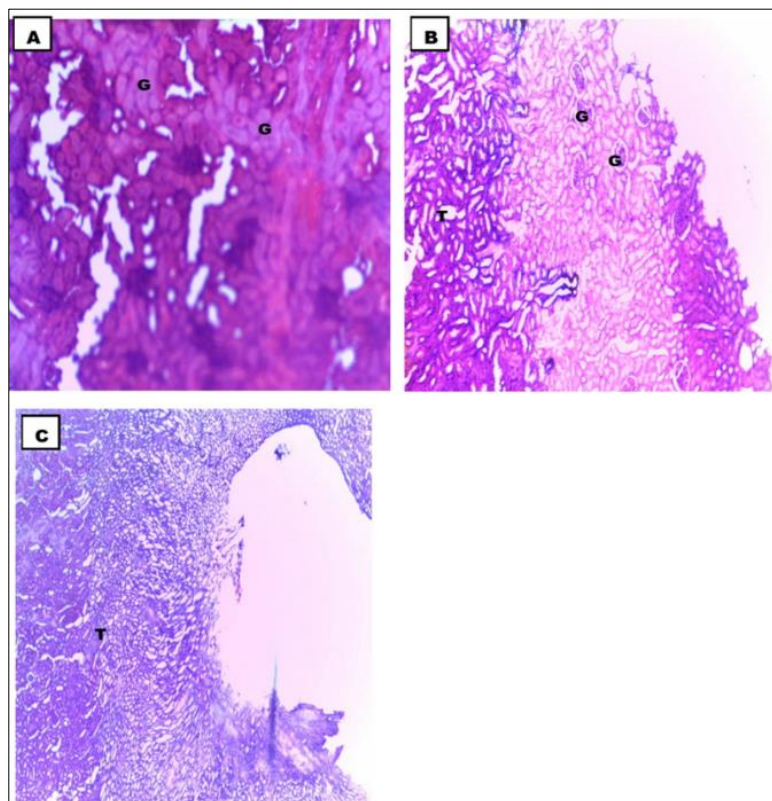


Figure 1 A, B and C are photomicrograph of kidney tissue section of control, 500 and 1000 mg/kg *Macaranga Spinosa* extract treated groups (H&E X200) respectively

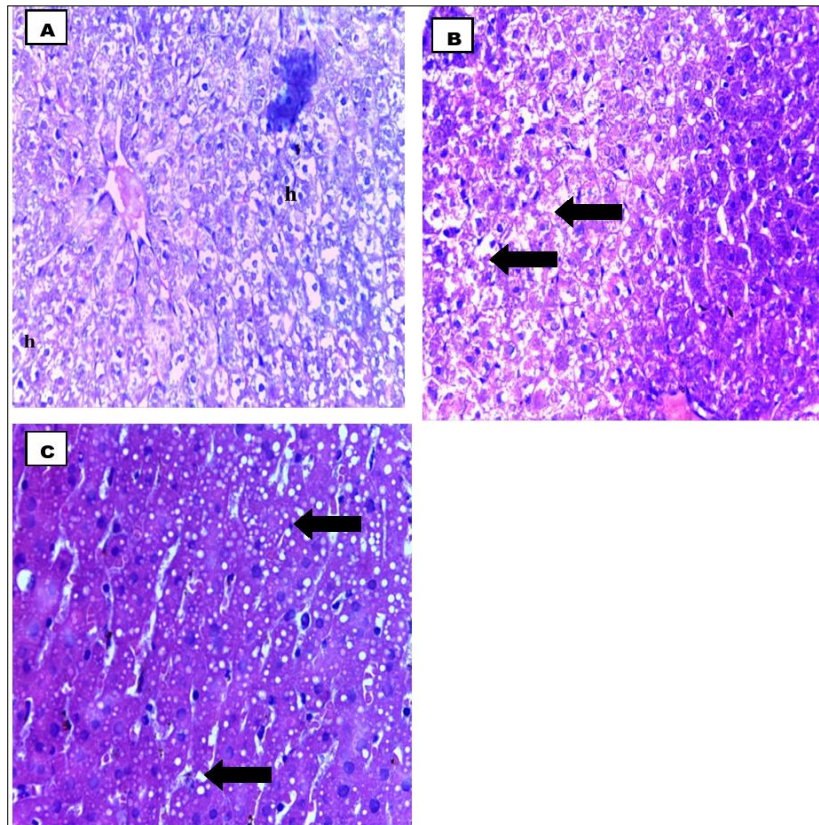


Figure 2 A, B and C are photomicrograph of liver tissue section of control, 500 and 1000mg/kg *Macaranga Spinosa* extract treated groups (H&E X400) respectively

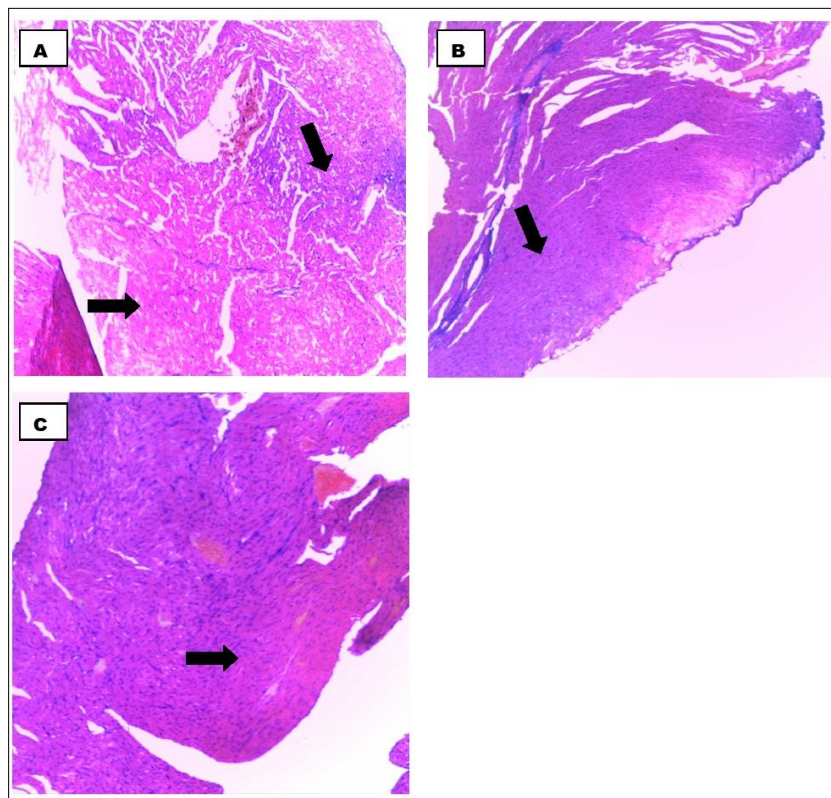


Figure 3 A, B and C are photomicrograph of heart tissue section of control, 500 and 1000mg/kg *Macaranga Spinosa* extract treated groups (H&E X200) respectively

Histological investigations in figure 1A showed glomeruli (G) indicating no change in kidney histology, figure 1B and 1C showed tubules (T) and glomeruli (G) indicating normal renal histology. Figure 2A showed hepatocytes (h), 2B and 2C arrows indicates vacuolar degeneration of cytoplasm and micro-vesicular fat droplets respectively, indicating cytoplasmic degeneration and fatty change in liver. Figure 3A arrows indicates normal Cardiac myocytes, Plate 3B and 3C showed no histologic change.

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Figure 3 A arrows indicates normal Cardiac myocytes, Figure 3B and 3C showed no histologic change.



Figure 4 (A) Showed *Macaranga spinosa* stalk (4B) *Macaranga spinosa* leaf attached to stalk

4. Discussion

In Nigeria, plant based herbs are freely used without mandatory safety or toxicological evaluation. Although there are government regulatory agencies like the National Agency for Food and Drug Administration and Control (NAFDAC). Yet, the country lack effective machinery to ensure that only tested and safe plant based ethno medicine are used especially among rural dwellers with low literacy level [21]. These herbal preparations are consumed without prescription in most cases and the potential hazards in consuming plants with toxic secondary metabolites are hardly recognized. There are plethora of studies [22-26] that have demonstrated the toxicity of plants used in ethno medicines. Thus, it is necessary to identify plants with toxicity potentials among the plants used for therapeutic purposes in Ehuaji community in Ahoada-East LGA of Rivers State, Nigeria. This will help in reducing the rate of plant-associated toxicity. This present study evaluated the effect of ethanolic leaf extract of *Macaranga spinosa* on selected biomarkers of toxicity in Wistar rats. Result of phytochemical screening of *Macaranga spinosa* leaf revealed the presence of alkaloids, flavonoids, tannins, triterpeneoid/steroids, carbohydrates, cardenolide, and saponins. Similar result has been report in Tanzania for the *Macaranga* genus [27]. An increasing number of phytochemical screening are being done on plants belonging to the *Macaranga* genus due to their various reported ethno medicinal uses [27]. Thus, secondary metabolites from the *Macaranga* genus have been reported to display array of biological activities including antioxidant [28-29], antifungal [30], antitumor [31-32], and anti-inflammatory [33].

Biochemical analysis of nephrotoxicity biomarkers showed significant elevation of blood urea nitrogen and sodium in the 1000mg/kg b.w *Macaranga spinosa* Muell-Arg; non-significant increase in creatinine, potassium, bicarbonate and chloride ion concentration in both the 500 and 1000mg/kg b.w *Macaranga spinosa* Muell-Arg leaf extract treated groups compared to control group. However, these increases were not corroborated by the histology of the kidney (Plate 1B and 1C) which showed normal renal histology. Thus, the changes in the biomarkers observed were only physiological and not structural; suggesting that *Macaranga spinosa* Muell-Arg leaf extract at 500 and 1000mg/kg b.w may not induced acute nephrotoxicity.

The enzymes AST, ALT, ALP and GGT, are helpful in the assessment of the proper functioning and inflammatory status of the liver [35]. Because the liver is the site for metabolism of carbohydrate, protein, and lipids, as well as for the synthesis of many proteins, the conjugation of bilirubin, and detoxification of drugs and other substances, the liver may be assessed by measurement of total and direct bilirubin, total protein and albumin [35-36]. Therefore, the significant elevation of AST, ALT and ALP concentration, accompanied by decreased in the total protein. This result conform to Saravanapriya and Devi [37] report on plant extracts with putative hepatotoxicity activity, and Chukwudoruo et al. [38] who asserted that total protein are synthesised in by the liver and in liver damage, total protein production is reduced or completely ceased, which is suggestive of damaged hepatocytes and impaired ability to synthesis protein which may be due to bridge in hepatocytes membrane integrity leading to expression of these enzymes in the general circulation. This finding is also corroborated by the histological investigation (Plate 2B-2C) which showed vacuolar degeneration of cytoplasm and micro-vesicular fat droplets, indicating cytoplasmic degeneration and fatty change in liver tissues, imply that prolong intake of the extract at 500 and 1000 mg/kg b.w can cause hepatotoxicity.

Our study further found significant decreased in triglyceride in the extract treated groups, in a pattern similar to Ighodaro and Omole [39] study. However, other lipids were not significantly altered compared to control group, in agreement with Thrinitha et al. [40]. This finding is further corroborated by the normal histology of heart tissue in Plate 3B-3C, implying that the extract may not be cardiotoxic.

5. Conclusion

This study has established that ethanolic leaf extract of *Macaranga spinosa* Muell-Arg can cause physiological and structural hepatotoxicity, mild nephrotoxicity and no significant alteration of lipid profile in Wistar rats. Therefore, users of this plant in ethno medicine should avoid excessive and prolong use, as it can be toxic to the liver.

Compliance with ethical standards

Acknowledgments

The authors acknowledge the Department of Anatomical Pathology Faculty of Basic Medical and Department of Pharmacognosy Faculty of Pharmaceutical Science, University of Port Harcourt Nigeria for making available their laboratories for the histopathological examination of tissues and qualitative phytochemical screening of plant sample respectively.

Disclosure of conflict of interest

We declare that there is no conflict of interest.

Statement of ethical approval

This study design was approved by the Department of Biochemistry Faculty of Science, University of Port Harcourt Research Ethics Committee (Approval number UPH/BCHREC/2022/002).

Funding

This study did not receive any grant from any organization.

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