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ORIGINAL RESEARCH

Toxicological Investigation of Aqueous Extract of *Ziziphus mauritiana* Leaves on Wistar Rats

Tajudeen Owolarafe[1], Godwin Ihegboro[1], Kailani Salawu[1], Chimaobi Ononamadu[1], Mujiburrahman Fadilu[1], Bashir Musa[2]

[1]Department of Biochemistry and Forensic science, Nigeria Police Academy Wudil, Kano.
[2]Center for Dryland Agriculture, Bayero University Kano.

* Corresponding Author: Tajudeen Owolarafe, e-mail: taowolarafe@gmail.com

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Abstract

Objective: Plant parts have been useful for food and remedies to various disease conditions for man long ago, but few have been studied for their toxicological effects. The aim of this study was to evaluate the phytochemical constituents and toxicological impacts of aqueous extract (AqE) *Ziziphus mauritiana* leaves on Wistar rats.

Material-Method: The plant material was identified and authenticated at the harberium of Bayero University Kano and extraction were carried out by maceration. Phytochemical screening was carried-out using standard methods while administration of the extract was orally. Liver and kidney functional parameters were evaluated using standard kits and the histopathological evaluation were carried out according to the standard method.

Results: Qualitative phytoconstituents screening revealed the presence of all Alkaloids, Saponin. Glycosides, tannin, flavonoids and others except anthraquinones while the quantitative screen showed phenol having the highest concentration while alkaloids have the lowest concentration. Acute toxicity revealed that the extract is non-toxic with LD50 above 5000 mg/kg body weight (BW), while subchronic toxicological evaluation revealed no significant adverse effect on all haematological parameters except WBC while the liver function parameters revealed an increase in serum GGT activity at 400 mg/kg body weight and the kidney function parameters showed alteration in serum creatinine, sodium, potassium, and bicarbonate concentrations. Significant effects on liver/body weight ratio at 400 and 1000 mg/kg BW was observed. Histoarchitectural alteration was observed in liver and kidney histopathological evaluation.

Conclusion: The observation from this research indicates that prolonged administration of this extract may lead the severe adverse effects on the biological system.

Keywords: *Ziziphus* , Toxicological , Extract Phytoconstituents.

INTRODUCTION

Plant parts have been useful for food and remedies to various disease conditions for man long ago, but few have been studied for their toxicological effects [1]. These effects might be caused by secondary metabolites generated by these plants, which are usually by-products that have been reported for various bioactivities [2] such as anti-cardiovascular diseases [3]and a host of others [2]. Traditional herbal remedies have popular usage in developing countries of the world. Interestingly, the World Health Organisation in 1976 recommended its inclusion into national health care programmes with guidelines to that effect released in 1991 and 2000 [4]. Traditional medicine, according to a WHO report in 2008[5], is used for basic healthcare by over 80% of the global population [6]. *Z. mauritiana* fruits from time immemorial have been widely eaten in the northern part of Nigeria for nutrition while extracts of different parts (especially the leaves) are employed in folklore and ethno-medicine as anti-asthmatic, wound healing and aphrodisiac agents [7,8,9]. The family *Rhamnaceae* consists of about 50 - 60 genera and approximately 890 – 900 species. They are flowering plants that are generally trees, shrubs, and vines that are found all over the world, but are more frequent in subtropical and tropical climates[10]. *Ziziphus* is a genus in the *Rhamnaceae* family that contains over 40 different species

ranging from spiny shrubs to small trees [11]; It also has over 100 different tree and shrub species, both deciduous and evergreen[7]. In Nigeria, *Ziziphus species* are found in northern states such as Kano, Katsina, Bauchi, Borno and Adamawa States [12]. The species of *Ziziphus* genus that are common are *Z. mucronata* (English-Buffalo, Hausa-*Mágáryár kúúráá*), *Z. spina-christi* (English-Christs thorn, Hausa-*Kurna*), *Z. abyssinica (*English-Large Jujube, Hausa-*Samo*, *Yabo* or *Babbagi*) and *Z. mauritiana* (English-Chinese date or Indian jujube, Hausa-*Mágáryá*)[12, 13]. The specie *Z. mauritiana* has many common names such as jujube, Indian cherry, geb, ber, Chinese apple, bear tree and desert apple, amongst others. [10] It is also known in different parts of the world with different names such as *manzanita* (Filipinos), *baer*, *badari* (Hindi), *ber* (Urdu) *bidara*, *jujub* (Malaysian) *Indischer Jujubenstrauch* (German), to mention a few [10]. It is generally known as *magarya* (Hausa) in northern Nigeria [14]. It is the most popular specie of the genus and is distributed in the Sahelian region of Africa, warm-temperate and subtropical areas worldwide. According to agroforest database version 4, the documented specie is found in Asian countries (such as Afghanistan, Bangladesh, India, Indonesia Malaysia, China etc), African countries (Algeria, Kenya, Libya, Uganda, Egypt, Tunisia, etc) and Australia. Those are the native ecosystem of the plant. Furthermore, the non-native ecosystem of the plant includes countries like Angola, Burkina Faso, Cameroon, Chad, Nigeria, Philippines, and Zimbabwe, amongst many others[10].

It is a shrub or small tree bearing spines that are evergreen throughout the year. It usually grows up to 7-15 m tall, with a trunk radius of 15-20 cm or more. It has many drooping branches, paired brown spines and a spreading crown with irregularly fissured dark grey or drab black bark. In severe climatic conditions it becomes compacted and barely grows to 3-4 m tall [10,15]. The tree grows rapidly with a 25-year average bearing life [14]. The leaves are variably alternating, length up to 25-60 by 15-50 mm, with round-tip and marginally rough base. There is a delicately wavy tooth on the edges, bright green and hairless above; underneath are thick, white hairs that are soft. It consists of an inflorescence axillary cyme, with 7-20 flowers having 5 petals[10,16]. It has a globose or ovoid drupe fruit, up to 6 by 4 cm in cultivation. It also has smooth or rough skin, but tough and could be yellow, red or black in colour. Its flesh is whitish, crispy, and succulent, with a subacid to sweet flavour that changes to mealy when fully ripe [10, 15, 16].

Also, parts of this plant have been reported for various biological activities such as antinutrient, [17] antioxidant, [14, 18], hepato-protective [19, 20] anticancer [21], and antidiarrheal activities [14]. AqE of *Z. mauritiana* plant parts are widely used for various herbal preparations in northern Nigeria with little information about it attending toxicity, therefore this research was performed to assess the toxicological effect of the plant leaf AqEt so as to provide information and enlighten the consumers of the danger of it consumption.

MATERIALS AND METHODS

Plant material

Z. mauritiana leaves were collected from the premises of Nigeria Police Academy, Wudil, Nigeria. Authentication was performed at the Department of Plant Biology herbarium at Bayero University Kano (BUK), Nigeria, where a voucher specimen (BUKHAN 0233) was issued, then the sample plant was deposited.

Experimental animals

The Department of Physiology, BUK, provided 25 healthy albino rats of both sexes (*Rattus norvegicus*), weighing between 150 and 180 g, and aged 10 weeks. They were housed in a well-ventilated environment having temperature of 28-31℃; photoperiod of 12/12 hours light/dark and humidity of 50-55 percent. They were fed and watered as needed.

Assay kits and other reagents

Kits for assaying ALP, AST and ALT were produced by Randox Laboratories Ltd, U.K., while the kits for albumin and total protein assays were manufactured by TECO Diagnostics Anaheim, U.S.A. Randox Laboratories Limited, Co-Antrim, U.K produced the urea and creatinine assay kits. Agape Diagnostic was the manufacturer of the serum electrolyte assay kits. The rest of the reagents were of analytical grade.

Preparation of the leaf extract

The leaf extract was prepared in accordance with the methodology outlined by Owolarafe *et al* [21]. The leaves were washed, dried in the shade, then powdered with a blender. The powdered leaves measuring 400 g was then extracted in 1.5 L of distilled water by maceration for 3 days with occasional shaking. Following that, it was filtered And the filtrate concentrated on rotary evaporator to obtain the aqueous extract (AqE).

Phytochemical screening

Qualitative and quantitative phytochemical

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screening were carried out according to the procedures described by Owolarafe *et al*, Dyana and Kanchana, Gupta *et al*. and Somit *et al*. [21,22, 23, 24,] for the analysis and quantification of the phytochemicals in the AqE of *Z. mauritiana* leaves.

Grouping and administration of extract to the rats

Twenty-five rats were randomly divided into five groups, and each group contains five rats. The rats were daily given treatments orally for 28 days. The test groups were administered 0.5 ml of AqE of *Z. mauritiana* leaves at a dosage of 200, 400, 600 and 1000 mg/kg BW for groups 1, 2, 3, and 4, respectively. The first group, which is the control, was administered 0.5 ml of distilled water. All of the animals were sacrificed 24 hours following the 21st dosage. The Experimental Animals Ethics Committee of the university approved this protocol, with reference number PEC/HS01/000112.

The doses were obtained from the acute toxicity studies of the extracts and were calculated using the formula:

$$D = \frac{d \times w}{C}$$

where:
D: dosage volume to be administered (0.5 ml)
d: standard dose (in mg/kg)
C: concentration of the extract (mg/ml)
w: weight of the rat to be treated in kg

Tissue samples preparation and serum collection

The weight of each rat was taken and then sacrificed as described by Owolarafe *et al* [25] under chloroform anaesthesia. The blood samples were taken with anticoagulant (for haematological investigations) and with no anticoagulant (for serum analysis) for each animal in separate bottles. After the clotting of the blood, the samples were centrifuged for 5 min at 4000 rpm. The serum was carefully removed and put into sample bottles and stored frozen for further biochemical analysis. The kidneys and the liver were removed, and tissue paper was used to blot them. They were then weighed and maintained in 10% formalin prior to histological examination after the kidney capsule was removed.

Haematological, biochemical and histological analyses

All haematological analyses were performed on a haematological auto analyser (BC 2600). Kochmar and Moss [26] described the method used for the determining ALP activity, while Henry [27] described the method for determining ALT and AST. Tietz [28] method for determining total protein concentration was used. Grant *et al.* [29] method was used to determine albumin concentration. Tietz [28] described the protocol for determining the electrolytes; Fossati *et al*. [30] method was used for determining urea concentration. The procedure of Newman and Prince[31] was employed for determining creatinine concentration (1999) while the procedure of Esterbauer *et al* [32] was adopted for MDA determination (1991). The liver and kidney were histopathologically examined using the Haematoxylin and Eosin staining technique as described by Owolarafe *et al.* [25], assessed with a Leica DM750 microscope (x100 magnification), and snapshots taken with a Leica ICCSOHD camera.

Statistical analysis

The data was presented as a mean ± standard error of mean. One-way ANOVA (analysis of variance) was used to analyse the data, and a $P < 0.05$ value was considered statistically significant. For statistical analysis and table creation, Microsoft Excel 2007 and Graphpad Instat version 3.05 were used.

RESULTS

The phytochemical constituents of leaf extract of Z. *mauritiana* plant is presented in Table 1. The AqE of the leaves showed the existence of alkaloids, saponins, tannins, glycosides, triterpenes, flavonoids, phenols, and steroids, while anthraquinone is absent. The quantification of phytochemicals present in the AqE of Z. *mauritiana* leaves is presented in Table 1 below. It revealed that tannins was the highest followed by phenol content while alkaloids were found to be the lowest (2.095 ug/ml).

Determination of yield and acute toxicity of crude extract of *Z. mauritiana* leaves

The percentage yield for the aqueous extract is 8.5%, while acute toxicity of the AqE of *Z. mauritiana* leaves is presented in Table 2. It shows that the extract is not toxic at 5000 mg/kg body weight with little or no sign of toxicity in behaviours exhibited after administration. The effect of the crude aqueous extract of *Z. mauritiana* leaves on haematological parameters is presented in Table 3. It indicates that there is no statistical difference in all the treated groups (TGs) for red blood cell, packed cell volume, haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume in comparison to the control group (CG).

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Table 1. Qualitative and quantitative phytochemical composition of aqueous extract of *Z. mauritiana* leaves

S/N	Phytochemicals	Qualitative	Quantitative	
1	Alkaloids	√	Alkaloids (µg/ml antropine equivalent)	2.095± 0.01
2	Saponins	√	Saponins (µg/ml diosgenin equivalent)	78.29 ± 3.38
3	Glycosides	√		
4	Triterpenes	√		
5	Phenols	√	Phenols (mg/ml gallic acid equivalent)	2.20 ± 0.03
6	Tannins	√	Tannins (mg/ml tannic acid equivalent)	2.47 ± 0.03
7	Flavonoids	√	Flavonoids (mg/ml rutin equivalent)	2.095 ± 0.01
8	Steroids	√		
9	Anthraquinones	*		

Note: √ indicates presence while * indicates absence. N= 3, X ± SEM

Table 2. Acute Lethal effect of aqueous leaf extract of *Z. mauritiana*

Experiment	Dose (mg/kg body weight)	Number of dead rats after 24 hours	Number of rats after 24 hours	Symptoms
Phase I control	0	0/3	0/3	Nil
	10	0/3	0/3	Nil
	100	0/3	0/3	Nil
	1000	0/3	0/3	Corner sitting
Phase II Control	0	0/1	0/1	Nil
	1500	0/1	0/1	Nil
	3000	0/1	0/1	Nil
	5000	0/1	0/1	Corner sitting

(*Experiment was conducted in two phases; each dose group of phase-1 made up of 3 rats while those in phase 2 have 1 rat per group)

The WBC concentration revealed no difference in all the TGs except group 3 administered with 400 mg/kg which exhibited an increase in concentration which is statistically significant, there were no significant differences in lymphocytes, neutrophils, platelets and average of monocytes, eosinophils and basophils (MID) concentrations in comparison to the CG.The effect of the crude AqE of Z. *mauritiana* leaves on liver function parameters as presented in Table 4 shows that there is no statistical change in all the treated groups for AST, ALT and GGT activities in the serum when compared with the CG, except serum GGT in group 3 (400 mg/kg) which shows an increase and statistically different.

MDA, total protein, albumin, and globulin concentrations reveal no significant difference while the organ body weight ratio for the liver indicates that groups 2,3,4 are statistically different from the control group and group 5 (1000 mg/kg).

The effect of the crude aqueous leaf extract of *Z. mauritiana* on kidney function parameters, as presented in Table 5, shows that there is a statistically significant decrease in creatinine levels between the CG and the TGs while a statistically significant increase was detected in group 3 that were given 400 mg/kg body weight for serum urea concentration. The serum Na^+ levels show a significant difference between the administered groups with a statistically significant decrease was observed in groups 4 and 5 while no difference was detected for Ca^{2+} levels in the serum. The K^+ levels reveal significant decreased concentration in all administered groups at $P < 0.05$ also there were statistically a significant difference between group 4 and 5, while there were no considerable difference in chloride concentration between the CG and the TGs. The levels of HCO_3^- in the serum revealed a statistically significant rise in all TGs when compared with control while there is no difference in the values of the administered groups and the control group in the kidney BW ratio for all TGs and control.

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Table 3. Effect of Aqueous leaf extract of *Ziziphus mauritiana* on some haematological parameters of Wistar rats

Parameters	*Ziziphus mauritiana* Aqueous leaf extract (mg/kg body weight)				
	Control	200	400	600	1000
Hemoglobin (g/L)	14.20 ± 0.61 [a]	12.62 ± 0.91 [a]	13.22 ± 0.11 [a]	15.38 ± 1.33 [a]	14.38 ± 0.28 [a]
Red blood cell ($\times10^{12}$/L)	6.69 ± 0.23 [a]	6.27 ± 0.37 [a]	6.58 ± 0.10 [a]	7.16 ± 0.57 [a]	7.17 ± 0.13 [a]
Packed cell volume (%)	40.24 ± 1.40 [a]	36.00 ± 2.03 [a]	37.94 ± 0.49 [a]	43.72 ± 2.86 [a]	40.66 ± 0.24 [a]
Mean Corpuscular Hemoglobin (pg)	21.20 ± 0.35 [a]	20.04 ± 0.29 [a]	20.38 ± 0.39 [a]	21.38 ± 0.13 [a]	19.84 ± 0.15 [ab]
Mean Corpuscular Hemoglobin Concentration(%)	35.24 ± 0.26 [a]	34.88 ± 0.53[a]	35.34 ± 0.29 [a]	34.96 ± 0.0.68 [a]	35.30 ± 0.48 [a]
Mean Corpuscular Volume(fl)	60.24 ± 0.88 [a]	57.60 ± 0.52 [a]	57.80 ± 1.45 [a]	61.38 ± 0.87 [a]	57.58 ± 0.69 [a]
White Blood Cell ($\times10^{9}$ /L)	11.22 ± 0.20 [a]	11.50 ± 2.48 [a]	22.24 ± 1.31 [b]	12.12 ± 2.04 [a]	11.18 ± 0.37 [a]
Lymphocytes ($\times10^{9}$/L)	68.40 ± 2.75 [a]	58.60 ± 0.68 [b]	61.20 ± 3.43 [a]	65.20 ± 3.14 [a]	64.52 ± 1.84 [a]
Neutrophils ($\times10^{9}$/L)	23.60 ± 2.21 [a]	33.00 ± 0.89 [b]	28.60 ± 3.04 [a]	28.40 ± 5.97 [a]	28.44 ± 1.14 [a]
MID (%)	8.00 ± 0.55 [a]	8.40 ± 0.25 [a]	10.20 ± 0.49 [a]	10.40 ± 0.93 [a]	10.02 ± 0.53 [a]
Platelets ($\times10^{9}$/L)	507.20 ± 55.59[a]	548.00 ± 6.95[a]	416.20 ± 19.71 [a]	370.60 ± 42.85 [a]	444.40 ± 9.57 [a]

Note: MID is the average of monocytes, eosinophils, basophils. N=5, X±SEM.[a-c] test values carrying superscripts different from the control across each parameter are significantly different at $P < 0.05$.

Table 4. Effect of aqueous leaf extract of *Ziziphus mauritiana* on some Liver Function Indices of Wistar rats

Parameters	*Ziziphus mauritiana* aqueous leaf extract (mg/kg body weight)				
	Control	200	400	600	1000
Aspartate aminotransferase(U/L)	31.48 ± 1.02 [a]	32.64 ± 2.14 [a]	35.52 ± 1.91 [a]	32.06 ± 4.18 [a]	22.52 ± .91 [a]
Alanine aminotransferase (U/L)	12.49 ± 0.37 [a]	12.072 ± 0.51 [a]	13.89 ± 0.758 [a]	12.55 ± 1.52 [a]	11.97 ± 0.69 [a]
Gamma Glutamyl transferase (U/L)	3.47 ± 1. 50 [a]	2.55 ± 1.39 [ab]	10.42 ± 3.07 [b]	4.01 ± 1.67 [ab]	1.27 ± 0.12 [a]
Malondialdehyde (nmol/ml) X 10^{-7}	7.67 ± 0.96 [a]	10.83 ± 3.28 [a]	6.68 ± 1.34 [a]	5.37 ± 0.79 [a]	4.87 ± 0.99 [a]
Total Protein (g/dL)	7.58 ± 0.66[a]	7.603 ± 0.78 [a]	8.04± 0.39 [a]	8.65 ± 0.71 [a]	6.879 ± 0.08 [a]
Albumin (g/dL)	2.18 ± 0.26 [a]	1.76 ± 0.12 [a]	1.21± 0.04 [ab]	1.515 ± 0.04 [a]	1.61 ± 0.22 [a]
Globulin (g/dL)	5.40 ± 0.45 [a]	5.84 ± 0.77 [a]	6.83 ± 0.38 [a]	7.14 ± 0.68 [a]	5.26 ± 0.23 [a]
Liver-body weight ratio (%)	6.10 ± 0.27 [a]	5.32 ± 0.15 [b]	4.86 ± 0.19 [b]	5.18 ± 0.13 [b]	3.71 ± 0.08 [c]

N = 5, X ± SEM.[a-c] test values carrying superscripts different from the control across each parameter are significantly different at $P< 0.05$

Table 5. Effect of aqueous leaf extract of *Ziziphus mauritiana* on some Kidney Function Indices of Wistar rats

Parameters	*Ziziphus mauritiana* aqueous leaf extract (mg/kg body weight)				
	Control	200	400	600	1000
Creatinine (umol/L)	2.53 ± 0.37 [a]	0.18 ± 0.08 [b]	0.74 ± 0.25 [b]	0.41 ± 0.14 [b]	1.03 ± 0.38 [b]
Urea (mmol/L)	9.72 ± 1.79 [a]	7.55 ± 0.44 [a]	13.91 ± 1.00 [b]	12.15 ± 1.15 [a]	10.17 ± 1.62 [a]
Sodium (mEq/L)	509.06 ± 31.73 [a]	480.30 ± 21.44 [a]	431.74 ± 18.48 [a]	358.19 ± 6.42 [b]	347. 06 ± 8.55 [b]
Calcium (mg/dL)	4.52 ± 1.22[a]	2.06 ± 0.42 [a]	3.80 ± 0.22 [a]	3.28 ± 0.59 [a]	3.67 ± 0.41 [a]
Potassium(mEq/L)	3.43 ± 0.30 [a]	2.02 ± 0.14 [b]	1.85 ± 0.27 [b]	0.99 ± 0.07 [bc]	2.18 ± 0.27 [bd]
Chloride (mEq/L)	130.35 ± 11.85 [a]	121.19 ± 11.33 [a]	104.89 ± 3.29 [a]	91.85 ± 3.01 [ab]	102.26 ± 3.80 [a]
Bicarbonate (mmol/L)	10.51 ± 0.49 [a]	11.99 ± 0.28 [b]	12.32 ± 0.39 [b]	13.544 ± 0.08 [b]	12.526 ± 0.30 [b]
Kidney-body weight ratio (%)	0.69 ± 0.01 [a]	0.73 ± 0.03 [a]	0.61 ± 0.02 [a]	0.72 ± 0.03 [ab]	0.72 ± 0.02 [ab]

N = 5, X ± SEM.[a-c] test values carrying superscripts different from the control across each parameter are significantly different at $P < 0.05$

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The histopathological evaluation of the liver cell architecture revealed a normal cellular architecture for Figure 1a (control) with all the portal triad in position while Figure 1b (200 mg/kg) is showing mild degeneration of liver tissue structure. Figure 1c (400 mg/kg) reveals a mild inflammation of liver tissue which was not severe while Figures 1d and 1e (600 and 1000 mg/kg) exhibit no significant pathology of the liver tissue.

The kidney cell architecture revealed a normal cellular architecture for Figure 2a (control) with all showing renal tubules intact while Figures 2b and 2c (200 mg/kg and 400 mg/kg) are showing mild dilation of the renal tubules. Figures 2d and 2e (600 and 1000 mg/kg) exhibit no significant dilation.

DISCUSSION

Plants synthesize a wide range of chemical substances, some of which have been described to be very important in treatment of several diseases. The extraction of these secondary metabolites is based on their ability to dissolve in various solvents [33]. The therapeutic benefit of plants lies in their phytoconstituents that have a definite physiological action on human beings [34]. Over the years, several researches have shown that these phytoconstituents exhibit biological actions for example antimicrobial [35], antifungal [36], antioxidant, anticancer [11] and hepatoprotective [37], while other researchers have reported that these bioactive principles exhibit toxic effects such as hepatotoxicity [38] and nephrotoxicity [39]. Some of the active phytochemicals that have been identified in this study include alkaloids, glycosides, flavonoids, terpenoids, saponins, steroids and phenols (Table 1). The saponins concentration is the highest with alkaloids as the lowest (Table 1). Saponins have been reported to exhibit some physiological actions which are both beneficial and detrimental; these activities are exhibited in various biological systems such as microbes, molluscs, herbivores, and humans [40]. These effects include abortifacient, antizygotic, anti-implantation [41], haemolytic [42], hypoglycaemic, cholesterol-lowering, [43, 41] and respiratory epithelia-damaging effects [44].

Acute toxicity is used to calculate the LD_{50}, which is the dose that has been shown to cause death (lethal) in 50% of the animals tested. In determining acute oral toxicity, usually the first step is assessing and evaluating the toxic characteristics of all compounds[45,46]. Currently, there has been a rise in public responsiveness and awareness in therapeutic plants and their preparations, also called herbal medicines. [47] However, the lack of scientific and clinical data to back up traditional healers' claims of efficacy and safety is a major roadblock [45]. *Ziziphus mauritiana* Leaf aqueous extracts are considered practically nontoxic.

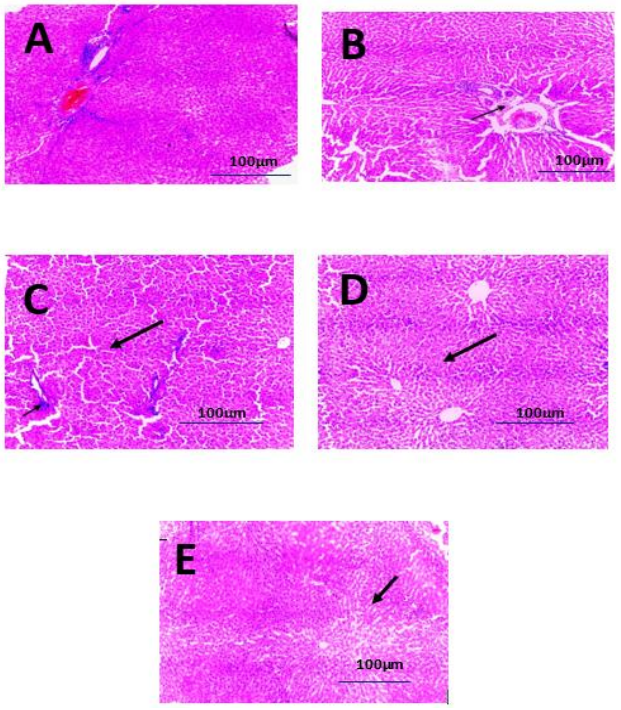

Figure 1. Photomicrographs cross section of Liver of Wistar rats administered with distilled water (A), 200 mg/kg(B) 400 mg/kg (C) 600 mg/kg (D) and 1000 mg/kg €body weight of Aqueous leaf extract of *Ziziphus mauritiana* orally for 21days (X 100) haematoxylin and eosin.

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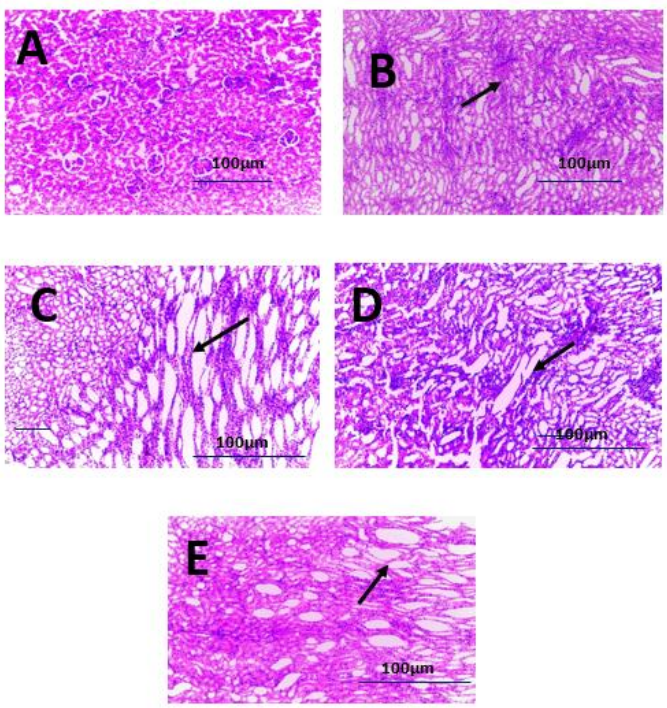

Figure 2. Photomicrographs cross section of Kidney of Wistar rats administered with distilled water (A) 200 mg/kg (B), 400 mg/kg (C), 600 mg/kg (D), 1000 mg/kg (E) body weight of Aqueous leaf extract of *Ziziphus mauritiana* orally with for 21 days (X 100) haematoxylin and eosin.

Subchronic administration of AqE of *Ziziphus mauritiana* leaves on wistar rats

Subchronic toxicity testing of plant extracts by determining their effect on specific blood, biochemical, and morphological composition of major or precise tissues, particularly the liver and the kidneys, can offer beneficial information about the toxicity mechanisms of an extract that is otherwise thought to be a safe medicinal agent[48].

Haematological analysis in animal toxicity studies is important to understand the condition and any pathology as a result of ingestion of chemicals or infection and assess the danger to the hematopoietic system in order to extrapolate these findings when considering the use of these extracts as a therapeutic agent for humans [49,50,] The increase in some haematological parameters upon administration of aqueous extracts of *Z. mauritiana* leaf (PCV, MCV and lymphocytes) which were not statistically different maybe an indication corroborating non-toxic observation made in the acute toxicity evaluation (Table 3**)** [51] but Statistically significant increase in WBC concentration give an opposing suggestion which may be due to toxicity of certain phytoconstituents within the AqE which maybe effecting an inflammatory response in the biological system[52] . The liver function indices are parameters for measuring the functional status of the liver [53]. The elevation of transaminases characterises liver diseases and dysfunction due to toxic compounds. GGT, a cholestatic enzyme, is mainly an affirmation parameter for liver dysfunction because it is found predominantly in the liver and its elevated activity in the serum is a clear sign of damage of the hepatocyte cell membrane [54, 55]. No considerable difference ($P<0.05$) in these parameters between the CG and the TGs indicating that the AqE of *Z. mauritiana* leaves may not be toxic and its therapeutic importance may be explored advantageously because these parameters were affected significantly. The glomeruli in the kidney filter a wide variety of substances from the plasma endogenously, including electrolytes, urea, creatinine, and proteins, etc. and its inability to perform this important function leads to elevation of these metabolites in the system[56, 57, 39]. The alteration in the kidney function parameters could be a sign of the adversity of the AqE administration[56]. The reduction in serum Na^+ concentration could be due to an extreme loss of body fluid (sweat) from the body fluid or decreased production of aldosterone which aids membrane aldosterone involved in the stimulation of Na^+ /H^-

exchanger to other mineral corticoids responsible for sodium ion reabsorption [58]. This is corroborated by decreased serum potassium concentration which is an observation inimical to the sodium pump that controls the extracellular potassium concentration[59]. A rise in serum bicarbonate ions could be useful in evaluating renal function; thus, the significant rise in serum bicarbonate ions at all the dosages studied could be a sign of tubular glomerular dysfunction[57]. Protein catabolism produces urea, which is the primary nitrogen-containing metabolic product and its measurement along with creatinine in the plasma will also indicate renal dysfunction [60]. The significant reduction in serum creatinine concentration and an increase in urea concentration which are not statistically significant different from control except 400 mg/kg bd wt after the administration of leave extract of *Z. mauritiana* at doses administered may be attributed to the inability of the kidney to take care of the by-product of the urea cycle at the observed dose [61]. This may indicate that the aqueous extract may contain nephrotoxic phytoconstituents. Alteration in the morphology of the hepatocytes and cells of the kidney in terms of size and component structure is usually confirmatory biomarkers for dysfunction of the organs [38, 62]. The changes observed in the microscopic presentation of liver and kidney in all groups when compared with the control which presented all portal triad in their position(Liver) and intact renal tubules (Kidney) while the treated groups exhibited mild vascular congestion and inflammation (Liver) and mild dilation of the renal tubules maybe a confirmatory parameter especially in the liver and kidney functionality.

CONCLUSION

Based on the observed result we may conclude that this extract may be regarded as mildly toxic over the period of administration and prolonged administration of this extract may lead the severe adverse effects on the biological system.

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