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TOXICOLOGICAL EVALUATION OF ACUTE AND SUBCHRONIC ORAL ADMINISTRATION OF ETHYL ACETATE EXTRACT OF *Ziziphus mauritiana LEAF*

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Abstract

This study aimed at evaluating the effect of ethyl acetate extract of *Ziziphus mauritiana* leaves on biochemical parameters in Wistar rats. Twenty-five albino rats equally divided into five experimental groups were used. One group served as control and received the carrier solvent treatment. Four test groups were treated with *Z. mauritiana* extract at 200, 400, 600 and 1000 mg/kg body weight respectively. The experiment lasted for 21 days after which the rats were sacrificed and blood collected for biochemical and haematological evaluation. Liver and Kidney-body weight ratio was computed. histoarchitecture of the Liver and Kidney were also investigated. The results showed no death at 5000mg/kg body weight while some haematological parameters were significantly ($P<0.05$) affected at 400, 600 and 1000mg/kg body weight for haemoglobin, red blood cell, parked cell volume, white blood cell and platelet concentrations. There were also significant ($P<0.05$) alterations in activities of gamma-glutamyl transferase, alanine aminotransferase, and aspartate aminotransferase, as well as the levels of total protein, albumin and globulin in the serum. Significant ($P<0.05$) increase were observed in the computed liver-body weight ratio with marked alterations in histoarchitecture of the liver cells. Significant ($P<0.05$) alterations were observed at all doses administered for creatinine, urea, sodium, chloride and biocarbonate ions with marked difference in kidney-body weight ratio and kidney cell architecture. These alterations in haematological parameters, liver function enzymes, kidney function indices and histological evaluation suggest toxicity of the extract on the animals at 400, 600 and 1000mg/kg body weight despite its non-toxic classification of acute administration of the extract.

INTRODUCTION

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins and other secondary metabolites that possess bioactivity which may be toxic upon its ingestion [1, 2]. medicinal plant extracts from various solvents are used by traditional medicine practitioners in Nigeria for the management or amelioration of various diseases and condition [3, 4] *Ziziphus mauritiana* Lam. belongs to the family *Ramnaceae* [5], known as jujube tree or Indian jujube [6, 7] and as Magarya in Hausa and Whuya in Kilba (Nigeria) and Chinese apple or Indian Jujube in English [8, 9]. The leaves are alternate, entire, with three prominent basal veins, and 2 to 7cm long [10]. The leaves of the plant are used in the treatment of diarrhoea, wounds, abscesses, swelling and gonorrhoea [7]. The fruit is an edible drupe, yellow-brown, red, or black, globose or oblong, 1 to 5 cm (0.39 to 2.0 in) long, sweet and sugary with a date in texture and flavour. the species are *Z. mauritiana*, *Z. lotus* and *Z. mucronata* found

generally in nearly every continent. *Z. mauritiana* and *Z. spina-christi* are the most common with nutritious fruits and are usually eaten fresh [11. 9]. Different solvent extracts of *Z. mauritiana* plant parts have been reported for various bioactivity but their toxic effect is scarcely reported, also the acute and sub-chronic toxicity of these leaf extracts is still scarcely reported. In keeping the above precedence in view this study aimed at bringing fort the knowledge of toxic effects of ethyl acetate solvent extract of the leaf.

MATERIALS AND METHODS

Plant Material, Authentication and Ethical Clearance

Fresh leaves of *Z. mauritiana* were collected from a single population within the premises of Nigeria Police Academy, Wudil Local Government Area, Kano State, Nigeria and was identified and authenticated at the Department of Plant Biology Herbarium, Bayero University, Kano with voucher number BUKHAN 0233 and the plant specimen was deposited. Ethical approval was obtained from the research ethical committee of Bayero University Kano with approval number BUK/CHS/REC/68.

Assay Kits and Enzyme Substrates

The assay kit for gamma-glutamyl transferase (GGT) was a product of Agape Diagnostics, Switzerland. Albumin and total protein kits were products of Randox Laboratories Ltd, U.K., while assay kits for alanine and aspartate aminotransferases (ALT and AST, respectively) were products of TECO diagnostics U.S.A. All other reagents used were of analytical grade.

Preparation of Extract

The fresh leaves were dried in the shade for two (2) weeks, pulverized into powder and stored in a plastic container. The pulverized leaves of *Z. mauritiana* were weighed and 500 g of the powdered material were macerated and extracted in 2000 cm^3 of ethyl acetate for 72 h. The extracted sample was sieved and filtered using sieve cloth and Whatman filter paper (Number 1). The filtrate was concentrated with rotary evaporator and used for the study.

Animal Grouping and Extract Administration

A total of twenty healthy adult Wistar rats weighing 160-180 g were acclimatised to the laboratory conditions for 7 days prior to the experiment. The animals were fed rat pellet diet and layers mesh, exposed to approximately 12 h light: 12 h dark cycle and water was provided *ad libitum*. Animals were treated humanely; veterinary care and supervision were provided throughout study. Group 1 was administered distilled water orally by orogastric cannula. Groups 2, 3, 4 and 5 were administered 200, 400, 600 and 1000 mg/kg body weight, respectively, of the extract of *Z. mauritiana* leaves.

Haematological Analysis

Blood sample was collected from all the test rats and control rats according to the methods described by [8]. The jugular veins were exposed and cut with a surgical blade under mild chloroform anaesthesia. Blood samples were collected into two (2) different sample heparinized test tubes for each rat. The non-coagulated blood samples were used for haematological analysis, i.e. haemoglobin concentration (Hb), red blood cells (RBC), total white blood cell (WBC) count, and packed cell volume (PCV) using an automated haematological analyzer (Mindray KX-21, Japan). Sterile test tubes were used to collect blood samples for serum biochemical analysis, preceded by centrifuging at 300 rpm for 10 min using Bench-top Laboratory Centrifuge and subsequently separated using Pasteur pipette.

Determination of Biochemical Parameters

The activity of serum GGT was assayed according to the method of [9]. ALT and AST were determined in the serum according to the method described by [10]. Total protein concentration was determined as described by [11] and albumin concentration was determined by the method of [12]. Globulin concentration was determined by subtraction of albumin from total protein concentration. The liver and kidney-body weight ratio and histochemistry were determined according to the protocol described by [13, 14].

RESULTS AND DISCUSSION

Acute Toxicity Test

The ethyl acetate extract of *Ziziphus mauritiana* leaf showed symptoms of acute toxicity in both the male and female Wistar rats. There were behavioural alterations such as reduction in spontaneous locomotion activity (corner sitting) at 5000mg/kg body weight. The extract at a single oral dose of 10, 100 and 1000mg/kg body weight showed no sign of toxicity and mortality was not recorded while at 1500, 3500 and 5000 mg/kg showed behavioural changes but no mortality was recorded.

Acute toxicity assay is an important evaluation to determine the lethal dose of a bioactive agent and also aid the determination of dose range for subchronic and chronic administration [15]. No death were observed upon the acute oral administration of ethyl acetate extract of *Z. mauritiana* leaves showed that the extract is not toxic based on the toxicity scale (Hodge and Sterner, Gosselin, Smith and Hodge scale) however the sign of toxicity observed may be

Table 2. Effect of Ethyl acetate Leaf Extract of *Ziziphus mauritiana* on Some Haematological Parameters of Wistar Rats

Parameters	*Ziziphus mauritiana* ethyl acetate leaf extract (mg/kg body weight)				
	Control	200	400	600	1000
Haemoglobin (g/L)	13.64 ± 0.19[a]	13.92 ±0.38[a]	13.11±0.11[a]	13.00±0.59[b]	13.70 ± 0.16 [a]
Red blood cell ($\times10^{12}$/L)	8.15±0.11 [a]	8.03 ± 0.14 [a]	7.28 ± 0.10 [b]	7.66 ± 0.26 [ab]	8.09 ± 0.13[a]
Packed cell volume (L/L)	49.26 ± 1.06 [a]	52.54± 1.49 [a]	45.42 ± 1.18 [ab]	53.60 ± 1.93 [ac]	52.40 ± 0.79 [ac]
Mean corpuscular haemoglobin (pg)	17.20 ± 0.20 [a]	16.76 ± 0.32 [a]	17.34 ± 0.25 [a]	17.70 ± 0.16 [a]	16.72± 0.20[a]
Mean corpuscular haemoglobin concentration (%)	27.74± 0.21 [a]	26.64 ± 0.18 [a]	26.48 ± 0.18 [a]	24.96 ± 0.76 [b]	25.20 ± 0.60[b]
Mean corpuscular volume(fl)	62.52 ± 1.04 [a]	63.14 ± 1.59 [a]	64.16 ± 0.03 [a]	71.58 ± 1.79 [b]	63.92 ± 0.72 [a]
White blood cell ($\times10^9$ /L)	15.38 ± 1.35[a]	15.96 ± 0.56 [a]	15.50 ± 0.54 [a]	15.22 ± 1.26 [a]	18.54 ± 0.76[b]
Lymphocytes ($\times10^9$/L)	80.16 ± 0.84 [a]	82.98 ± 0.78 [a]	84.24 ± 0.47 [a]	67.28 + 1.89 [b]	82.62 ± 0.49 [a]
Neutrophils ($\times10^9$/L)	26.00 ± 2.47[a]	24.24 ± 0.10 [a]	28.26 ± 0.82[a]	40.56 ± 1.91[b]	26.80 ± 3.34[a]
MID (%)	13.00 ±1.70[a]	13.12 ± 0.42[a]	12.90 ±3.02[a]	15.12 ±2.50[a]	14.40 ±1.50[a]
Platelets ($\times10^9$/L)	863.20±45.71[a]	687.20±27.83 [b]	823.40 ±69.53[ab]	488.20 ±16.71[c]	534.60 ±19.84[c]

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 within row are significantly different at P > 0.05

an indication of adverse effect potentials of the extract upon sub-chronic and chronic administration of the extract [16].

Effect of Subchronic Oral Administration of Ethyl acetate Extract on Hematological Parameters

The effect of ethyl acetate leaf extract of *Z. mauritiana* on the heamatological parameters is presented in group 1, it shows no significant difference (P > 0.05) for heamoglobin concentration in all groups treated with ethyl acetate leaf extract of *Z. mauritiana* when compared with the control group except group 4 administered with 600mg/kg which exhibit a decrease in heamoglobin concentration. Red blood cell (RBC) concentration shows no statistical difference among
the groups except in groups 3 and 4 which revealed a reduction in concentration when compared with the control group. The parked cell volume (PCV) values show no significant difference (P>0.05) in all groups treated with ethyl acetate leaf extract of *Z. mauritiana* apart from group 3 (400mg/kg) which reveals a decrease (P>0.05) when compared with the control group. Mean Corpuscular Heamoglobin (MCH) shows there was no significant difference (P>0.05) among the groups administered with ethyl acetate leaf extract of *Z. mauritiana* while Mean Corpuscular Heamoglobin Concentration (MCHC) shows a decrease in group 4 and 5 (600 and 1000mg/kg) when compared with the control. Mean Corpuscular Volume (MCV) shows there was no significant difference in all the values of treated groups when compared with the control group except in group 4 administered with 600mg/kg in which a decrease was observed. The WBC concentration showed no difference in all treated groups except group 5 (1000mg/kg), while Lymphocyte concentration and neutrophils concentration showed a decrease in concentration for group 4 only. MID concentration (average of monocytes, eosinophils, basophils) showed no significant difference in all groups when compared with the control group. Platelet concentration showed a decrease in all groups when compared with the control and these decreases are not dose-dependent with the highest value observed in group 3 administered with 400mg/kg B. W. and are significantly different from the control group.

Administration of ethyl acetate leaf extract of *Z.mauritiana* to the rat model produced a dose – Specific reduction in PCV, Hb, RBC, MCHC (Table 1), this may be due to hemolytic activity of the extract leading to the observed reduction or suppressive action of the extract on erythropoiesis [17]. The observed decrease in MCHC has been reported to be a cause of normocytic, hypochronic anaemia [18] and the reduction of these parameters may be an indication of these conditions mentioned. Reduced blood platelets have been reported to increase the viscosity of the blood and also correlated with positive blood pressure [19, 20], decreased platelets count at 200, 600 and 1000mg/kg B. W. may be a pointer to the adverse effect of administration of *Z. mauritiana* ethyl acetate leaf extract to the rat model. Information about infections, toxicity, allergy, immune-suppression and poisoning may be obtained from the differential WBC count [21] the Lymphocytes counts also a primary indicator of immunological functions [22]. Reduced

Table 2. Effect of Ethyl acetate Leaf Extract of *Ziziphus mauritiana* on Some Liver Function Indices of Wistar Rats

Parameters	*Ziziphus mauritiana* ethyl acetate leaf extract (mg/kg body weight)				
	Control	200	400	600	1000
Alanine aminotransaminase (U/L)	2.93± 0.02[a]	5.65 ± 0.21 [b]	1.43 ± 0.07 [c]	2.46 ± 0.11 [ad]	2.12 ± 0.06 [ad]
Aspartate aminotransaminase (U/L)	22.38 ± 0.26 [a]	29.46 ± 0.26 [b]	13.82 ± 0.91 [c]	17.40 ± 1.23 [d]	21.06 ± 0.72 [a]
Gamma Glutamyl transferase (U/L)	3.03± 0.34 [a]	3.38 ± 0.09 [a]	3.53 ± 0.10 [a]	7.74 ± 0.07 [b]	2.27 ± 0.13 [c]
MDA (mmol/ml)	8.07 ± 0.34 [a]	8.23 ± 0.36 [a]	8.47 ± 0.98 [a]	8.43 ± 0.45 [a]	9.06 ± 0.13 [a]
Total Protein (g/L)	14.48 ± 0.54 [a]	9.31± 0.14 [b]	6.96 ± 0.33 [bc]	8.55 ± 0.08 [d]	8.69 ± 0.88 [d]
Albumin (mmol/L)	2.10 ± 0.17 [a]	3.21 ± 0.25 [b]	3.96 ± 0.11 [b]	3.67 ± 0.29 [b]	3.44 ± 0.27 [b]
Globulin (mmol/L)	12.38 ± 0.37 [a]	6.09 ± 0.89 [b]	3.00 ± 0.22 [c]	4.97 ± 0.79 [b d]	5.25 ± 0.61 [b d]
Liver-body weight ratio (%)	2.65 ± 0.06 [a]	2.87 ±0.13[a]	3.73 ± 0.14 [b]	3.33 ± 0.03 [b]	3.85± 0.18 [c]

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

WBC at 1000mg/kg and reduced lymphocytes at 600mg/kg B.W. may be an indication of immunosuppression ability of the extract which may lower the ability of the animal defence.

Effect of Subchronic Oral Administration of Ethyl acetate Extract on Liver and Kidney Function Biomarkers

The effect of the ethyl acetate leaf extract of *Z mauritiana* on liver and kidney function parameters is presented in Tables 2 and 3. It shows that there was a significant increase in group 2 (200mg/kg) but a decrease was observed in other groups treated with ethyl acetate leaf extract of *Z. mauritiana,* for Aspartate aminotransferase concentration in the serum, while there were differences in the concentration of Alanine aminotransferase in groups administered with the extract, with an increase observed in group 2 and a decrease in groups 3 and 4. The Gamma-glutamyl transferase concentration shows no significant difference in all the groups treated with ethyl acetate leaf extract of *Z. mauritiana* when compared with the control group except groups 4 and 5 (600mg/kg and 1000mg/kg). There is no significant difference in MDA concentration in all treated groups, an indication of no peroxidation taking place within the membrane of the cell involved metabolism. Serum total protein, Albumin and Globulin reveals a significant decrease in all ethyl acetate leaf extract of *Z. mauritiana* treated groups.

Table 3. Effect of Ethyl acetate Leaf Extract of *Ziziphus Mauritiana* on Some Kidney Function Indices of Wistar Rats

Parameters	*Ziziphus mauritiana* ethyl acetate leaf extract(mg/kg body weight)				
	Control	200	400	600	1000
Creatinine (umol/L)	1.31± 0.12 [a]	2.61± 0.09 [b]	3.15 ± 0.05 [c]	5.28 ± 0.09 [d]	5.70 ± 0.08 [d]
Urea(mmol/L)	23.59 ± 1.11 [a]	35.45 ± 1.67 [b]	34.95 ± 0.36[b]	37.63±1.09 [b]	22.69 ± 0.73 [a]
Sodium (mEq/L)	96.17 ± 0.07[a]	67.43 ± 0.03 [b]	40.75 ± 2.91 [c]	95.57 ± 10.78 [d]	99.49 ± 13.50 [d]
Calcium (mg/dL)	0.82± 0.06[a]	0.79± 0.04[a]	0.79± 0.03[a]	0.88± 0.09[a]	0.91± 0.05[a]
Potassium(mEq/L)	14.69 ± 0.09 [a]	13.45 ± 0.89 [a]	5.24 ± 0.23 [b]	6.51 ± 0.38 [b]	12.81 ± 0.67 [a]
Chloride (mEq/L)	76.89± 8.35[a]	97.83± 3.74[b]	78.92± 6.84[a]	71.87± 8.23[a]	68.33± 5.85[c]
Biocarbonate (mmol/L)	650.00 ±16.05 [a]	1240.0 ±60.00[b]	870.00±25.50[b]	896.00 ± 71.88 [ab]	840.00± 73.14[ab]
Kidney-body weight ratio (%)	0.57 ± 0.039[a]	0.71 ± 0.071 [b]	0.75 ± 0.023 [b]	0.80 ± 0.025 [b]	0.77 ± 0.044 [b]

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

The organ-body weight ratio of the liver shows an increase in groups 3 4 and 5 administered with 400mg/kg, 600mg/kg, 1000mg/kg respectively. While kidney function parameter shows that there was a significant difference in creatinine concentration between the control group and the groups treated with Ethyl acetate leaf extract of *Z. mauritiana* which are dose-dependent, while serum urea concentration showed a significant increase in groups 2,3,4 administered with 200mg/kg,400mg/kg, 600mg/kg respectively except group 5 administered with 1000mg/kg body weight of ethyl acetate leaf extracts. The serum sodium ion concentration shows significant decrease between groups 2 and 3 (200 and 400mg/kg b.w.) treated with Ethyl acetate leaf extract of *Z. mauritiana* and the control group while an increase was observed in groups 4 and 5. It was observed that there was no significant difference between all groups administered with Ethyl acetate leaf extract of *Z. mauritiana* and the control for calcium concentration in the serum. The potassium concentration reveals decreased concentration in 400 and 600mg/kg groups while there was no significant difference in 200mg/kg (group 2) and group 5 (1000mg/kg). For chloride concentration, it was observed that significant increase in group 2 (200mg/kg) when compared with the control group while a decrease was observed in group 5 (1000mg/kg) but groups 3 and 4 (400 and 600mg/kg) reveal no significant difference when compared with the control. Bicarbonate ion concentration in the serum showed a significant increase in all groups treated with Ethyl acetate leaf extract of *Z. mauritiana* when compared with control. There was also a significant difference in the values of the treated groups and the control group for kidney body weight ratio. The biochemistry of the serum enzymes reflects the functional status of the internal organs and the increase in the activities of AST, ALT, and GGT in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream [23, 24] because they are cell-derived enzymes [25]. The elevated concentration of AST, ALT, and GGT may be an indication of compromised hepatic cell membrane but the non-significant increase in MDA concentrations when compared with the control group may suggest a mild adverse effect on the cell membrane integrity. Assessing the levels of excretory metabolites of the kidney can be an indicative biomarker for the functionality of the kidney [26, 27]. Urea a product of the urea cycle and creatinine a product of creatine catabolism in the muscle are metabolites use for evaluation of kidney dysfunction [28]. Urea concentration in the serum varies directly with protein intake and inversely with the rate of excretion and an impaired kidney function exhibits low glomerular filtration which leads to an increase in urea retention and thus an increased concentration is observed in the plasma [29, 30] while high creatinine concentration in the serum is a signal of kidney dysfunction [31].

In this study, the observed significant increase ($P< 0.05$) in creatinine in all treated groups (Table 3) when compared with control and high concentration of urea in groups administered with 200, 400 and 600mg/kg B.W. of the extract may be an indication of low glomerular filtration of the renal tissue and a dysfunction of the kidney. Reduction in sodium and potassium serum concentrations have been reported by [32, 33] with increased concentration of biocarbonate could present risk for arrhythmia and muscle weakness [33]. Histological and histomorphometric evaluation of the kidney reveals the late effect of the plant extract on the functionality of the kidney [34], increased organ/body weight observed in this study may be an indication of dysfunction of the kidney and this was corroborated by inflammation observed in the morphology of the organ cell structure evaluation [35].

Present results from this research work suggest mild toxic effects of administration of ethyl acetate extract of *Z. mauritiana* and application of its folklore activities should be carried out with extreme caution.

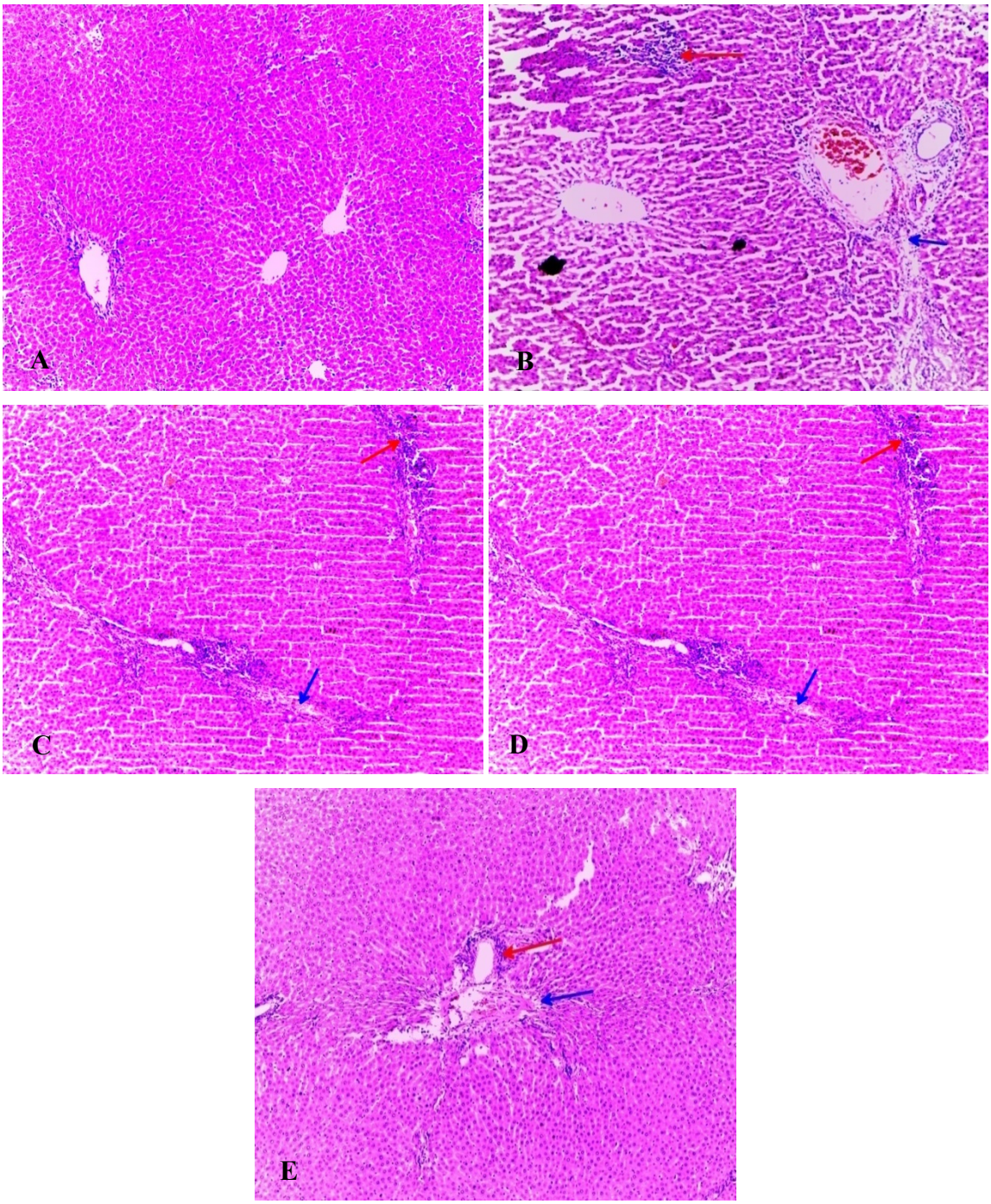

Plate 1. Photomicrographs Cross Section of Liver from Wistar Rats Administered With Distilled Water (A), 200mg/Kg (B), 400mg/Kg (C), 600mg/Kg (D), 1000mg/Kg (E) Ethyl acetate Leaf Extract of *Z. mauritiana* Orally for 21 Day (X 100) Haematoxylin and Eosin.

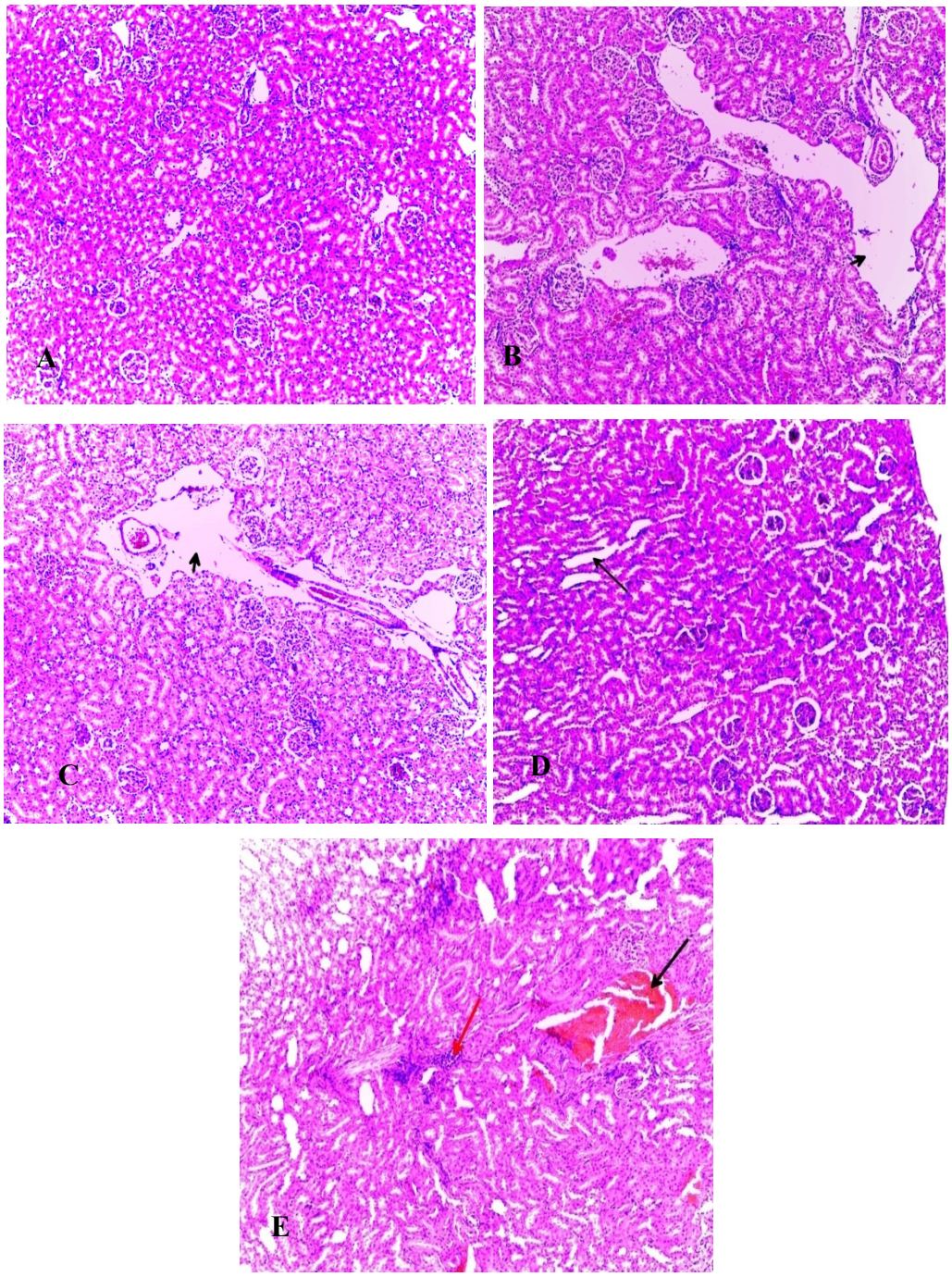

Plate 2. Photomicrographs Cross Section of Kidney from Wistar Rats Administered with Distilled Water(A), 200mg/Kg(B), 400mg/Kg (C), 600mg/Kg(D) And 1000mg/Kg (E) Body Weight of Ethyl acetate Leaf Extract of *Z.Mauritiana* Orally For 21 Days (X 100) Haematoxylin and Eosin

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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