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Nephrotoxicity assessment of the hydroethanolic leaf extract of *Ziziphus mauritiana* LAM (Rhamnaceae) in mammalians.

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Abstract

Ziziphus mauritiana LAM is traditionally used in the treatment of various ailments. The purpose of this study was to check out the effect of the hydroethanolic leaf extract of *Ziziphus mauritiana* LAM on Wistar rats kidney functions. Determination of LD50 and the evaluation of the haematological, biochemical parameters followed by the histopathological assessment of kidney tissues in treated rats was carried out. The acute toxicity study showed a low toxicity with LD50 > 20000 mg /Kg. However, chronic treatment of rats resulted in a significant rise of platelet counts in both sexes of animals at experimental doses. Moreover, a significant increase of neutrophils in male was recorded but decreased in female rats at high doses. Biochemical analysis revealed significant increase of Urea and Creatinine in both sexes of rats followed by a decrease of uric acid in male rats at a dose of 1200mg / Kg. there was no significant difference in blood electrolytes except for Ca2+ at experimental doses in female rats compared to the control. Histopathological examination confirmed biochemical tests with presence of tissue damages. This study demonstrates the ability of the extract to cause thrombocytosis and induce kidney tissue damages in rats.

Keywords: *Ziziphus mauritiana* LAM, Kidney, Hematological and Biochemical parameters, Histopathological examination

Introduction

Kidneys play a key role in body functions by helping to maintain fluid, electrolytes and acid-base balance (Koeppen, 2009). They are particularly sensitive to xenobiotics actions and constitute theirs main route of excretion, be it drugs, toxins or toxic substances (Beneditti et al., 2003). Metabolites derived drugs excreted by kidney can cause cellular damage by disrupting its functioning (Sands and Verlande., 2010). The proximal tubule and renal interstitium are the major targets of nephrotic substances (Lee and al.,2013). In advanced renal degradation, disturbances appear in mineral metabolism and bone structure (Muschio and Oldri., 2000). *Ziziphus mauritiana* LAM belongs to Rhamnaceae family, traditionally the fruit is used as an analgesic and anti-cancer. It heals ulcers and is also used against asthma (Morton,1987; Verhei and Calabura., 1991). The leaves of the plant are used in the treatment of diarrhea, abscesses and gonorrhea (Michel, 2002). The plant is also used in the treatment of high blood pressure, liver disorders and diabetes (Morton, 1987; Diallo et al., 2004). However traditional use of plant can induce lethal risk, because doses administered are not well controlled. This present study was therefore undertaken in order to ascertain the dose dependent effect of the hydroethanolic leaf extract of *Ziziphus mauritiana* on kidney functions and structure in Wistar rats.

1. Materials and Methods

1-1-Materiel

1-1-1-Plant material

Leaves of *Ziziphus mauritiana* LAM were collected in Abidjan, Côte d'Ivoire. The leaves were carefully washed with tap water, air dried in shade for 2 weeks and grounded into powder. The plant sample was authenticated at the National Floristic Center of Felix Houphouet Boigny University, (Abidjan, Côte d'Ivoire).

1-1-2-Experimental animals

10 weeks aged Swiss mice of both sexes with body weight ranging from 20g to 23g and 12 weeks Wistar rats of both sexes weighting between 115g and 122g used for this study were obtained from the animal husbandry of the department of Nutrition and Pharmacology (Faculty of Biosciences, University of Felix Houphouet Boigny ,Abidjan-Côte d'Ivoire). The animals were housed in plastic cages in a temperature and light controlled room with 12h dark and 12h light. All experiments in this study were conducted in accordance with the international standards of animal welfare as recommended by the European Union on animal care.

1-2-Methods

1-2-1-Preparation of extract

The hydroethanolic leaf extract of *Ziziphus mauritiana* LAM (HEZm) was carried out according to the method described by Zirihi et al(2003).

100g of *Ziziphus mauritiana* LAM bark powder were macerated with 70% ethanol (1.5 L) at room temperature and frequently shook for 72 h and filtered through cotton sieve then on whattman filter paper for 24 h. The filtrate was evaporated through rotary vacuum evaporator and dried in, drying oven at 45°C for 48 h to obtain a dry extract which was stored at 4°C for further use.

1-2-2-Toxicological study

1-2-2-1-Acute toxicity study

The acute toxicity study of the leaves of *Ziziphus mauritiana* LAM was performed on Swiss mice by using OECD guidelines (2000).

Sixty (60) Swiss mice of both sexes were used for the test. Mice were grouped in 6 groups of 10 animals each (5males and 5 females) and were kept fasting prior to extract administration. A single dose of the extract ranged from 2 to 20g/Kg body weight was administered by oral route to each group of mice.

After administration of extract animal were observed individually for signs of toxicity such as behavioral changes including convulsion, tremor, salivation, writhing and death every 1 h for 4h the first day and every day for two (2) weeks**.**

LD_{50} of the extract was determined by using the graphic Method of Miller and Tainter (1944).

1-2-2-2- Chronic toxicity study

The chronic toxicity study was performed according to OECD (2009) modified method. 50 Wistar rats of both sexes divided into five (5) groups of 10 animals each (5 males and 5 females) were used for this study, male and female rats were separated during experimentation

and were orally administered a single dose of extract daily for 180 days. Group 1 received 1 ml of distilled water, Group 2 received 400mg/Kg body weight of HEZm, Group 3 received 800mg /Kg body weight of HEZm, Group 4 received 1200mg/Kg body weight of HEZm and Group 5 (Satellite) received 1200mg/Kg body weight of HEZm. On completion of the experimental periods blood was collected from every group of rats for hematological and biochemical parameters analyses.

1-2-2-2-1-Body weight and relative organ weight measurements

Individual body weight of each group of rats was recorded prior to the administration of extract and every month throughout the experimental periods. At the end of the experiment the body weight gain of each group of rats was calculated.

1-2-2-2-2-Blood sampling

At the end of the experimental period, the rats of each group were anesthetized using diethyl ether and blood was collected by tail incision according to the modified method described byFlutter et *al*(2000)**.**

1-2-2-2-2-1-Hematological studies

A wide range of hematological parameters including hematocrit (Hct), hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH) , mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), total leukocyte count (WBC) lymphocyte, eosinophil, neutrophil, monocytes and platelet count were determined using an automated biochemistry analyzer at the Hematology Department of Cocody Teaching Hospital (Abidjan, Côte d'Ivoire).

1-2-2-2-2-2-Biochemical analyses

Blood samples were collected in 4cc dry tubes without anticoagulant and were centrifugated at 3000rpm for 5 minutes. Serum samples were removed and kept in Eppendorf tubes and stored at -20°c. The serums were further analysed using automated biochemistry analyzer at the Hematology Department of Cocody Teaching Hospital (Abidjan, Côte d'Ivoire) to determine the level of blood urea nitrogen (BUN), Creatinine (Crea), Uric acid and electrolytes.

1-2-3-Statistical analyses

Graphics were performed using Graphpad prism 5 software (Microsoft, San Diego California, USA).

All values were expressed as mean ±SD. Data analysis were analyzed using one way analysis of variance (ANOVA), followed by Tukey-kramer multiple comparisms test using graphpadinstat® software. Values were statistically significant at $P< 0.05$.

2. Results and Discussion

2-1-Resultats

2-1-1-Acute toxicity

After the oral administration of HEZm, several clinical signs were recorded in experimental animals at higher doses such as difficulties of movement, sleepiness, water and food refusal. However no death was observed. The LD_{50} of HEZm was estimated to be above 20000mg/Kg body weight.

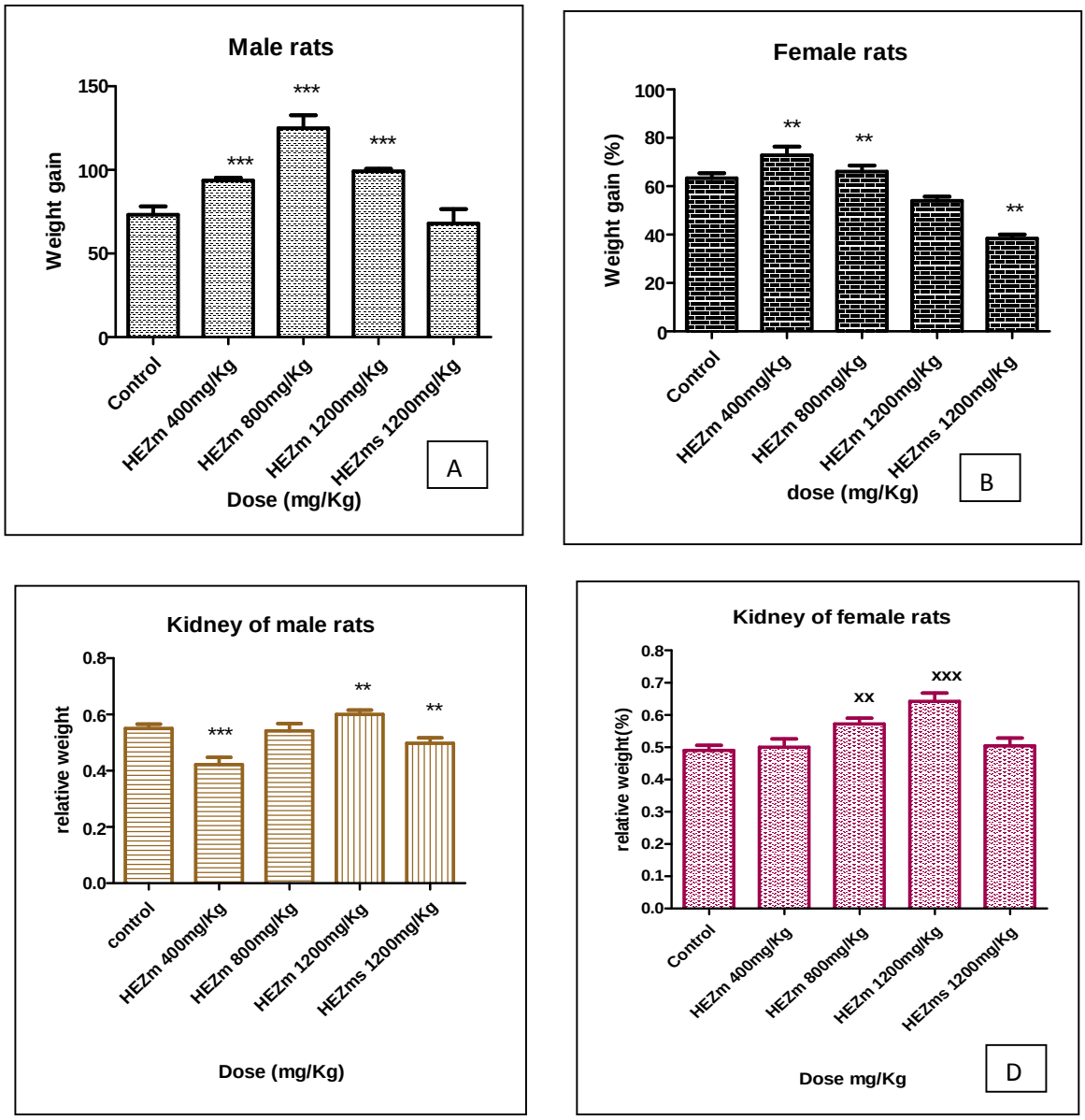
2-1-2-Chronic toxicity assessment

2-1-2-1-Evolution of body weight

Body weight of rats treated with HEZm was followed before and during experiment. A weight gain in all groups of animals including both controls and tested groups was observed regardless of sex. In Female rats treated with HEZm, a high significant ($P<0.01$) increase of body weight gain was observed as compared to the control at doses of 400mg/Kg and 800mg/Kg body weight (Figure1B). Thus, in male rats a highly significant ($P <0.001$) increase of body weight gain was noticed in all treated groups as compared to the control except for the satellite group (figure 1A).

2-1-2-2- Evaluation of the relative organ weight

As far as the kidney relative organ weight is concerned, a higher significant increase ($P> 0.01$) was noticed at doses of 800 mg/Kg and 1200mg/Kg body weight in female (Figure 1D). However a higher significant decrease ($P> 0.01$) of this organ was recorded at a dose of 400 mg/Kg and increase at a dose of 1200mg/Kg in male rats (Figure 1C).



Each value represents the mean ± Standard deviation; (n = 5); values are statiscally different from control at *p<0, 05, **p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisms test

A: Body weight gain of male rats treated with HEZm B: Body weight gain of female rats treated with HEZm

C: Relative organ weight of male rats treated with HEZm D: Relative organ weight of female rats treated with HEZm

HEZms: satellite rats treated at a dose of 1200mg/Kg body weight

Figure 1: Effect of chronic oral administration of HEZm for 180 days on body gain weight and relative organ weight in Wistar rats

2-1-2-3-Haematological parameters

The hematological parameters of experimental animals were assessed 180 days after the oral administration of HEZm. The hematological indices observed were illustrated in tables (1 and 2). Although no significant (P> 0.05) changes of WBC, lymphocytes, monocytes, eosinophils, total erythrocyte counts, Hemoglobin, Hematocrit and MCHC were recorded in all groups of experimental animals compared to the control group. A highly significant rise (P< 0.001) of platelet counts was noticed in both sexes of rats at doses of 400mg / kg, 800mg / kg, 1200mg / kg and in satellite group (tables 1and 2). Moreover a significant increase of neutrophils in male was recorded at the doses of 800mg / kg and 1200mg/Kg body weight. However a decrease of this parameter was noticed in female rats (table 2) at the same doses.

Table 1: Effect of chronic oral administration of HEZm for 180 days on the haematological parameters in male wistar rats

		***Ziziphus mauritiana* LAM (HEZm)**			
Parameters	**Control**	**400mg/Kg**	**800mg/Kg**	**1200mg/Kga**	**1200mg/Kb**
Hemoglobin (g/dl)	17,03±1,6	15,92±0,28	15,46±1,59	15,4±0,6	16,17±0,77
Redbloodcells (106/µl)	7,15±0,7	6,45±0,41	6,56±0,62	6±0,56	6,75±0,8
Hematocrit (%)	41,33±2,3	50,75±3,59	46,33±1,52	46±2,82	48±1,41
MCV (fl)	57±2	55,5±6,45	58±3,5	57,5±4,25	58,5±2,84
MCHC (g/dl)	34,33±1,5	35±2.1	34,66±1,5	34,5±2,12	35,75±1,41
MCH (pg)	20±1,2	20,25±0,5	20,33±0,57	20,5±0,7	20,5±0,7
Platelets (103/µl)	664,33±5,13	1002±26,87***	1028±24,42***	1162,33±18,33***	792,25±10,6*
Leucocytes (103/µl)	13,3±3,49	15,18±3,2	12,59±3,83	12,28±0,74	13,83±2,02
Neutrophils (103/µl)	14,33±2,08	16,33±1,52	19±2,58**	22,5±0,7**	21±1,41**
Eosinophils (103/µl)	2,33±0,57	3,5±1	3±0,86	2,5±0,7	2,5±0,7
Lymphocytes (103/µl)	76,66±2,88	69,75±4,5	75±3,6	69±1,41	70±1,19
Monocytes (103/µl)	6,66±1,88	7,75±0,95	5,66±1,52	7,5±0,7	6±0,57

Each value represents the mean ± Standard deviation; (n = 5); values are statiscally different from control at *p<0, 05, **p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisms test.
(1200mg/Kga): Treated group of rats at a dose of 1200mg/Kg.
(1200mg/Kgb) : Treated satellite group of rats at a dose of 1200mg/Kg followed by 28 days without treatment.

Table 2: Effect of chronic oral administration of HEZm for 180 days on the haematological parameters in female wistar rats

		Ziziphus mauritiana LAM (HEZm)			
Parameters	**Control**	**400mg/Kg**	**800mg/Kg**	**1200mg/Kga**	**1200mg/Kb**
Hemoglobin (g/dl)	14,33±0,76	16±0,66	14,88±1,59	15,95±0,6	15,32±1,58
Redbloodcells (106/µl)	5,7±0,1	6,63±0,5	6,26±0,62	6,47±0,4	6,16±0,99
Hematocrit (%)	44±1	47,75±1,89	45,25±2,5	46,75±1,5	45,25±3,5
MCV (fl)	70±1,63	65±2,5	63±2,44	64,75±1,25	66,5±2,64
MCHC (g/dl)	34±1	34,5±0.57	35,75±1,57	34,25±0,95	36,75±1,7
MCH (pg)	24±2	22,5±1,29	22,5±1	22,5±0,57	24,75±1,5
Platelets (103/µl)	714,33±6,73	1026±48,54***	1058±24,42***	1098,5±81,94***	995,25±83,17**
Leucocytes (103/µl)	10,5±0,98	11,34±1,92	14,37±2,28	11,54±2,51	9,72±1,70
Neutrophils (103/µl)	25±1	24±1,41	19±2,58**	17,25±0,5**	20±0,81**
Eosinophils (103/µl)	4,25±0,92	3,25±1,25	3,75±0,95	3,5±0,57	3,25±0,5
Lymphocytes (103/µl)	67±2	67,5±4,72	71,5±5,56	74,5±5,31	72,5±1,29
Monocytes (103/µl)	4±1	5,25±0,57	5,75±1,5	4,75±0,5	4,25±0,57

Each value represents the mean ± Standard deviation; (n = 5); values are statiscally different from control at *p<0, 05, **p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisms test.
(1200mg/Kga): Treated group of rats at a dose of 1200mg/Kg.
(1200mg/Kgb) : Treated satellite group of rats at a dose of 1200mg/Kg followed by 28 days without treatment.

2-1-2-4-Biochemical parameters

The chronic effect of HEZm on the biochemical blood parameters was recorded in (tables 3 and4). A Significant (P< 0.05) rise in creatinine level was recorded at doses of 800mg/Kg and 1200mg/Kg body weight in both male and female rats (table 3 and 4). Moreover, at a dose of 1200mg/Kg body weight a significant (P< 0.05) increase of blood urea and a significant (P< 0.05) was noticed in both sexes of rats. However a decrease of uric acid were recorded in male rats as compared to the control (table 3).There was no significant difference in blood electrolytes such as Na^{+}, K^{+}, Cl^{-}, Mg^{2+}, except for Ca^{2+} where a highly significant (P< 0.001) increase was observed at the doses of 400mg/Kg, 800mg/Kg and 1200mg/Kg body weight in female rats compared to the control (Table 4).

Table 3: Effect of chronic oral administration of HEZm for 180 days on the biochemical parameters of male Wistar rats

		Ziziphus mauritiana Lam			
Parameters	**Control**	**400mg/Kg**	**800mg/Kg**	**1200mg/Kga**	**1200mg/Kgb**
Creatinine (mg/L)	5,45±1	5,75±0,95	7,5±0,89*	8,2±0,7*	6,5±0,70
Urea (g/L)	0,07±0,005	0,1±0,03	0,12±0,01	0,16±0,014**	0,075±0,007
Uricacid (mg/L)	35,33±1,52	37,5±4,12	36±4,58	19,5±0,70**	24,5±2,12*
Na+ (mEq/L)	146±1	143,5±2,08	143±2,64	144,5±2,12	141,5±2,12
K+ (mEq/L)	5,43±0,49	5,25±0,40	5,33±0,33	5,45±0,91	5,15±0,07
Cl- (mEq/L)	103,33±3,51	108,5±3,69	105±4,58	109±1,41	100,5±3,53
Ca2+ (mg/L)	90±4,58	79±6,05	86±6,08	89,5±4,95	84±4,24
Mg2+ (mg/L)	22,56±1,25	20,95±1,57	20,63±2,24	19,15±0,63	22,5±0,77

Each value represents the mean ± Standard deviation; (n = 5); values are statiscally different from control at *p<0, 05, **p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisms test.
(1200mg/Kga): Treated group of rats at a dose of 1200mg/Kg.
(1200mg/Kgb) : Treated satellite group of rats at a dose of 1200mg/Kg followed by 28 days without treatment.

Table 4: Effect of chronic oral administration of HEZm for 180 days on the biochemical parameters of female Wistar rats

		*Ziziphusmauritiana*Lam			
Parameters	**Control**	**400mg/Kg**	**800mg/Kg**	**1200mg/Kga**	**1200mg/Kgb**
Creatinine (mg/L)	5,13 ± 0,81	5,5±0,57	7,85±0,81*	8,84±0,75*	7±0,70
Urea (g/L)	0,11±0,005	0,1±0,008	0,15±0,02	0,21±0,03**	0,11±0,03
Uricacid (mg/L)	34±0,81	36±2,94	36,25±3,4	33,5±1	34±0,81
Na+ (mEq/L)	146,75±0,95	143,5±3,69	142,75±4,57	144,25 ±3,59	144±2,94
K+ (mEq/L)	5,6±0,081	5,35±0,46	5,3±0,4	4,93±0,82	5,41±0,28
Cl- (mEq/L)	107,75±1,70	108,5±2,38	103,75±3,59	105,25±3,3	105,75±5,31
Ca2+ (mg/L)	70±1	88,25±2,63***	89,5±4,23***	91,75±2,36***	79,25±7,18
Mg2+ (mg/L)	22,5±0,7	21,12±1,14	20,67±1,67	22,22±0,71	22,45±1,47

Each value represents the mean ± Standard deviation; (n = 5); values are statiscally different from control at *p<0, 05, **p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisms test.
(1200mg/Kga): Treated group of rats at a dose of 1200mg/Kg.
(1200mg/Kgb) : Treated satellite group of rats at a dose of 1200mg/Kg followed by 28 days without treatment.

2-1-3- Histopathological examination

Histopathological evaluation of Control group of rats treated with distilled water showed normal structure of glomerulus (Figures 2A and 3A). Kidneys of rats treated with EHEZm at 400mg/Kg showed glomeruli and renal tubes sometimes atrophic, sometimes normal associated with vascular congestion and edema(Figure B). At a dose of 800mg/Kg interstitial edema with lympho-plasmocyte infiltration and presence of leukocytes in the tubular lumen was found in female rats (Figure 3C). Likewise a tubular cystic degeneration followed by a lympho-plasmocytic infiltrate and vascular congestion were observed in male rats (Figure 2C). As for the rats treated at the dose of 1200mg/Kg a congestion and an edema followed by a tubular atrophy (Figure 3D) and a deposit of amorphous substances (Figure 2D) were noticed respectively. As for the satellite groups no reversibility of lesions were shown (Figures 2E and 3E).

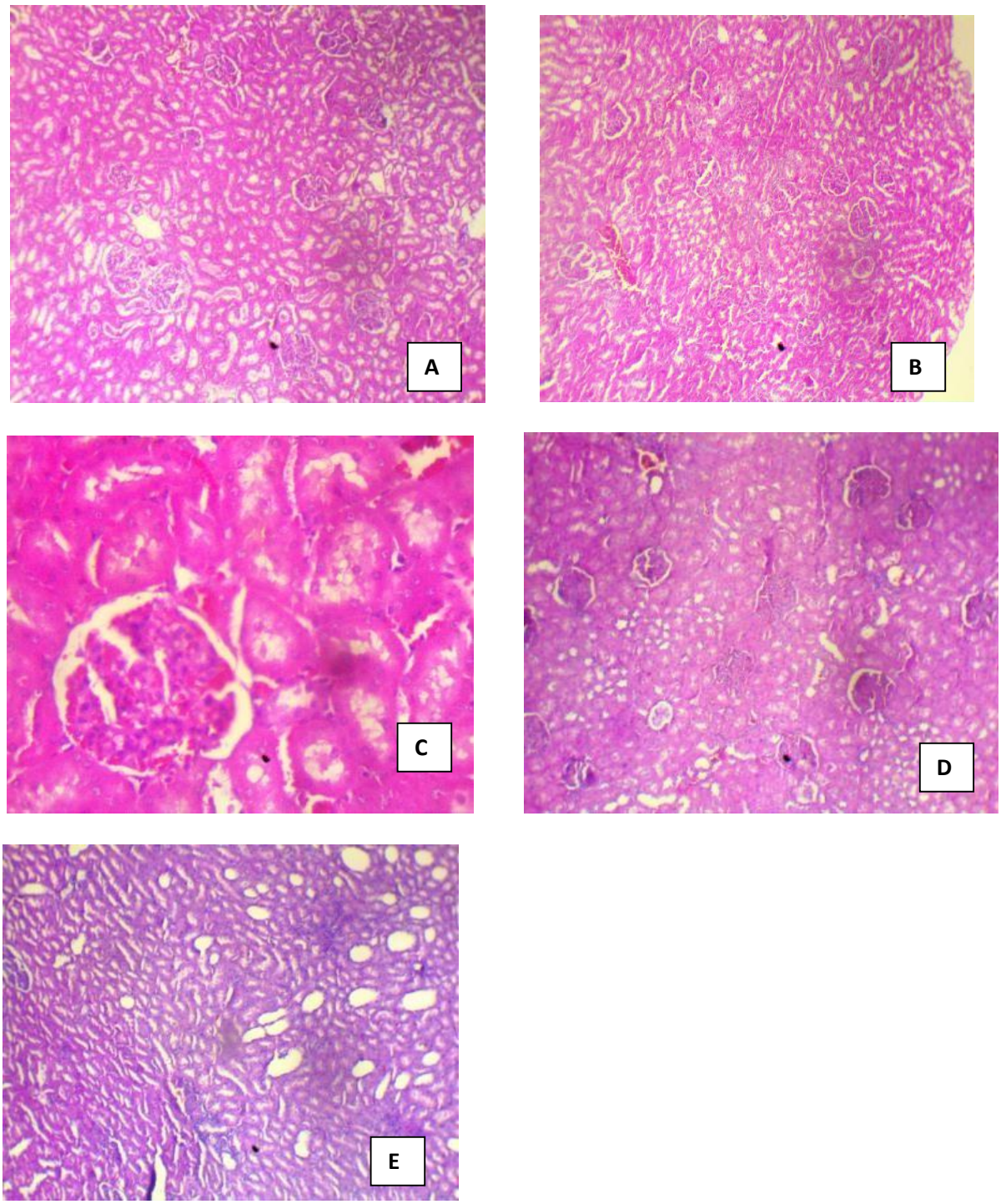


Figure 2: Histological sections of Wistar male rat kidney stained with hematoxylin and eosin (under × 100 magnification power) showing the effect of the hydroethanolic leaf extract of *Ziziphus mauritiana Lam* in a 180 days chronic toxicity study.

A: kidney of control male rat treated with distilled water, showing renal glomeruli and tubes without histological abnormalities. **B:** male rat kidney stained with hematoxylin-eosin treated with 400 mg / Kg of EHEZm Showing Glomeruli and renal tubes sometimes atrophic, sometimes normal associating vascular congestion and edema**. C:** Male rat kidney colored with haemato-eosin treated with 800 mg / Kg of EHEZm Tubular cystic degeneration followed by lympho-plasmocytic infiltrate and vascular congestion. **D:** kidney of Male rats treated with 1200 mg / Kg of EHEZm showing deposits of amorphous substances. **E:** Male rat kidney of the satellite group treated with 1200 mg / Kg showing tubular cystic degeneration followed by lympho-plasmocytic infiltration and vascular congestion.

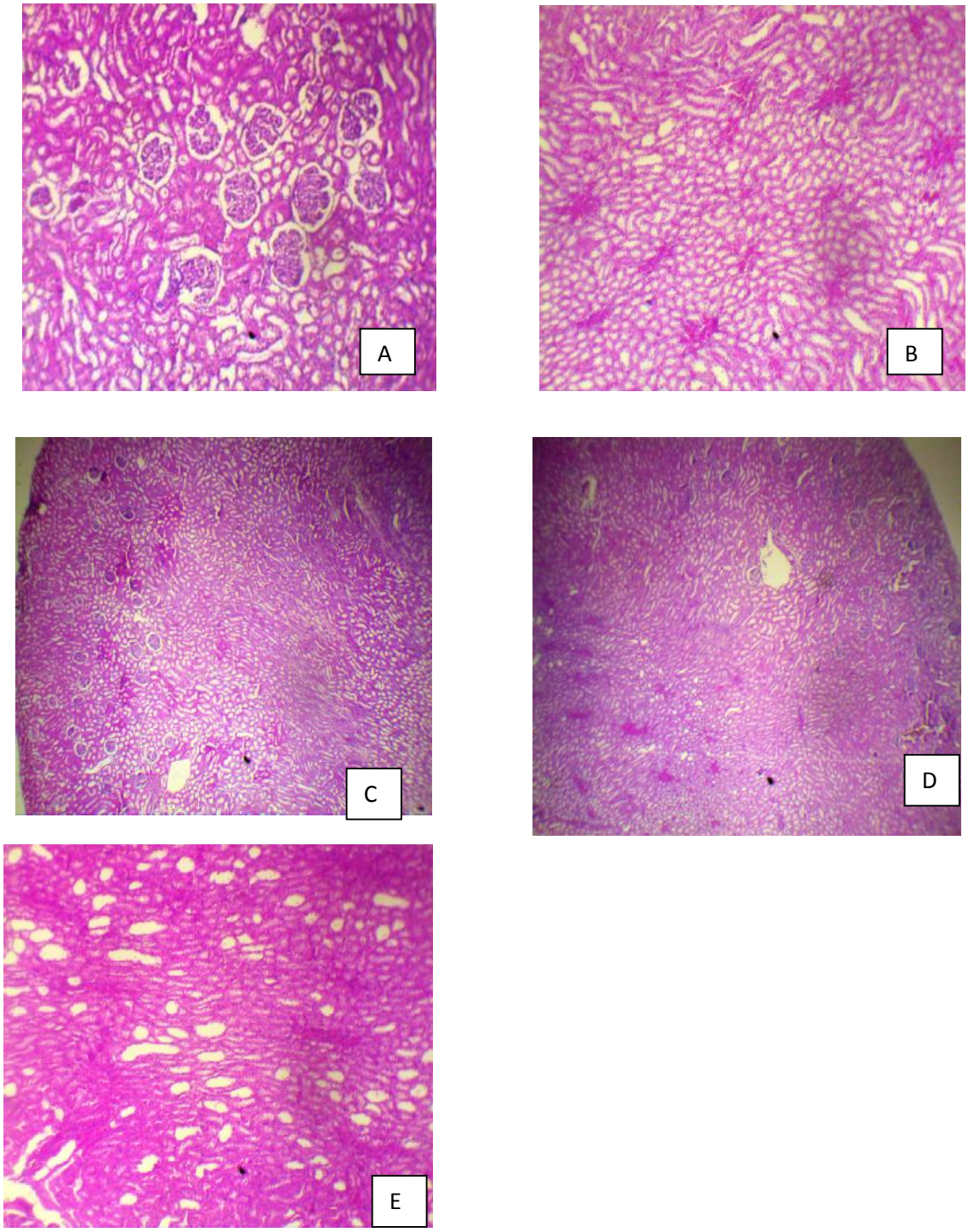


Figure 3: Histological sections of Wistar female rat kidney stained with hematoxylin and eosin (under × 100 magnification power) showing the effect of the hydroethanolic leaf extract of *Ziziphus mauritiana Lam* in a 180 days chronic toxicity study.

A: Control Female rat treated with distilled water showing Renal Glomeruli and tubule tissues without abnormalities.**B :** : Female rat kidney stained with hematoxylin-eosin treated with 400 mg / Kg of EHEZM Showing Glomeruli and renal tubes sometimes atrophic, sometimes normal associating vascular congestion and edema**. C:** Female rat kidney treated with 800 mg / Kg of EHEZM showing interstitial edema With lympho-plasmocyte infiltration and presence of leukocytes in the lumen tubular **D:** Female rat kidney stained with hematoxylin-eosin treated with 1200 mg / Kg of EHEZM showing congestion and edema followed by tubular atrophy. **E:** Satellite female rat kidney treated with 1200 mg / kg EHEZM Showing atrophied, bloated renal tubules and glomerular atrophy.

2-2-Discussion

Toxicity studies in animals help to investigate the potential health risk in humans caused by the effects of bioactive compounds found in plant extracts (Asiimwe et al., 2014). The acute toxicity assessment of HEZm revealed that the DL_{50} of this plant extract was above 20 g/Kg body weight in mice. According to the chemical labeling and classification defined by Gosslin et *al* (1984), Natural extract with LD_{50} greater than 15g/Kg of body weight are considered non-toxic. According to those authors HEZm could be safe for oral route.

Changes in body and organ weight are as well key indicators for predicting the toxicity of a chemical compound or plant extract (Grance et al., 2008). In this study, a body weight gain was observed in both male and female rats at low doses but a decrease of body weight gain was recorded at the highest dose. This increase could be due to the growth inducing effect of the extract at low doses in experimental animals through appetite stimulation.

An increase in organ weight may be a sign of inflammation while a reduction of this parameter could be due to cellular constriction (Moore and Dalley., 1999). In this study a decrease and an increase in relative kidney weight was noticed in Wistar rats treated with HEZm and this could indicate that the extract may be toxic for this organ.

This study showed as well that, HEZm did not produce any significant change in the hematological parameters of male and female rats except for blood platelet counts and circulating neutrophils. Indeed, a significant increase was observed at experimental doses for the rate of platelets in both sexes of rats. The increase circulating platelets, called thrombocytosis could be due to thrombopoetin production and secretion (Kaushansky, 1995; Ajibade et al., 2012). The observed effect of the extract may be due to the alkaloids it contains for they are believed to increased megakaryocytic precursors leading to the production of platelets (Ukwuani et al., 2012). The observed effect of HEZm on blood platelets is similar to the results obtained with *Carica papaya* (Arollado et al., 2013). The extract might have a stimulatory effect on thrombopoeitin in the bone marrow by inducing overproduction of circulating platelets in Wistar rats at studied doses and might predispose its users to hypercoagulable state and spontaneous intravascular clotting (Chang-Gue et al., 2003).

As for the circulating neutrophils, a significant rise of this parameter was observed in male rats at doses of 800mg/Kg and 1200mg/Kg. Whereas, a significant decrease was observed at the same doses in female rats. The rise of circulating neutrophils in male rats observed with HEZm could be due to the stimulation of bone marrow through myeloid procursors controlled by granulocyte colony stimulating factor (Ley et al., 2007), participating in the communication networks that form the foundations of immunity, issuing instructions to practically all other immune cells (Borko et al., 2012). Therefore, neutrophils are the body first line defense against infection and any decrease of this parameter makes the individual vulnerable to infections (Gupta et al., 2007). However, a decrease of neutrophils observed in female rats may suggest that the extract could induce immunodeficiency by weakening female rats body defense. The extract may have an immune boosting effect on male rats and an inhibiting stimulatory effect on the effectors cells of the immune system in female rats. The difference observed between both sexes of rats may rest on gonadal hormones, including estrogen suppressing neutrophil functions (Miller et al., 2004; Speyer et al., 2005).

In this study, the kidney functioning ability was investigated through the evaluation of creatinine, urea, uric acid and the levels of electrolytes in the serum of Wistar rats. This study showed a rise in the levels of creatinine and urea at doses of 800mg/Kg and 1200mg/Kg in both male and female rats for the former and at a dose of 1200mg/Kg for the latter. Blood Urea Nitrogen is the major indicator of renal functions (Vidal et al., 2003; Oduolo et al., 2010) and is used as a strong indicator of renal impairment (Nduka, 1999). As for Creatinine, it is an endogenous indicator of the glomerular filtration (Tsinalis and Binet, 2006). So an increase of both urea and creatinine in blood is observed in case of nephron's functions affection (Frank, 1992; Borges et al., 2005; Lameira et al., 2005). At high doses HEZm could alter the disfunctioning of the glomerular filtration by causing impairment in kidney functions in Wistar rats. Therefore, the effects of HEZm on rat kidney is similar to the aqueous extract of *Hippobromus pauciflorus* and *Alstonia boonei* (De Wild) which caused renal impairment in rats (Oze et al., 2006; Pendota et al., 2009).

A decrease in uric acid was observed in male rats treated with HEZM at experimental doses. This decrease could be due to a synthesis failure of xanthine oxidase, in a case of severe hepatocellular

failure, isolated failure of urates absorption or to an excessive elimination of urates through urine (Kamoum and Frejaville, 1993). HEZm at experimental doses could prompt elimination of urates by the kidney.

Plasma electrolytes can be also used to assess the nephron functioning (Yakubu et al., 2003).In this study, the effect of the extract on blood electrolytes after administration revealed no marked differences. From this observation, it can be suggested that the extract has no adverse effect on some blood electrolytes and could not affect the kidneys in terms of water balance maintenance and plasma electrolytes regulation.

As for calcium ions an increase of its level was noticed at experimental doses in female rats. This ion like the other electrolytes are involved in many physiological processes such as nerve impulses transmission and is crucial for body homeostasis regulation (Agbafor et al., 2015). This study showed that HEZm at experimental doses may have an adverse effect on female rat's bones. A rise of calcium ion in plasma is promoted by Para Thyroid Hormone through the transfer of this ion from the bones to the plasma and this may lead to osteoporosis causing bones to become porous and brittle (Metheny,2011). This study showed that HEZm at experimental doses may have an adverse effect on female rat's bones.

The histopathological examination of kidney tissues showed morphological changes in treated rats such as tubular cystic degeneration followed by a lympho-plasmocytic infiltrate and vascular congestion, tubular atrophy, deposit of amorphous substances and interstitial edema with lympho-plasmocyte infiltration and presence of leukocytes in the tubular lumen were noticed notwithstanding the dose administered and endure 28 days after discontinuation of treatment. These observations were backed up by the renal biochemical markers. Thus, results of this study demonstrated HEZm at a mild dose could entail kidney cellular distortions in Wistar rats.

Conclusion

This study provides valuable information on chronic oral toxicity of the hydroethanolic leaf extract of *Ziziphus mauritiana* LAM in the kidney of Wistar rats. According to the findings of this study, HEZm may cause thrombocytosis in both sex of rats at experimental doses, stimulate the bone marrow in male rats but may weaken body defense in female rats. Moreover the extract may induce as well kidney tissue damages. The hydroethanolic leaves extract of *Ziziphus mauritiana* LAM may be safe for oral route, however it should be taken with care in a case of a prolonged use.

Conflict of interest

The authors declare no competing conflict.

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