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SUB-CHRONIC TOXICITY STUDIES OF METHANOLIC ROOT EXTRACT OF *Ziziphus mauritiana* (Jujube)

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ABSTRACT
Ziziphus mauritiana commonly called jujube in English is known to be used in traditional medicine to cure various diseases such as fever, abdominal pain, nausea, wound healing, diarrhea, antiseptic, to mention few. Thus, toxicity studies are necessary to establish the safety of the plant. The present study aimed at determining the oral sub-chronic toxic effect of methanolic root extract of Z. mauritiana plant. Adult laboratory rats (Rattus norvegicus) were used for the study. Three different concentrations were administered orally for duration of 28 days. Food and water consumption, toxicity signs and mortality were observed daily and the mortality recorded. The body weight of each animal was monitored on weekly basis. Biochemical parameters and histopathological changes were analyzed to evaluate the liver and kidney functions. There is significant change in the average weight of the animals over the period of time at P-value <0.05. Death was recorded at higher (1000mg/kg) and medium (500mg/kg) concentrations only, with LD_{50} of 375mg/kg. There was no significant alteration in the biochemical parameters, however, there were slight abnormalities including slight vacuolation and hepatic necrosis, and slight tubular necrosis and hyperplasia of inflammatory cells in the histopathology of both liver and kidney respectively, for medium and higher treated group compared to control group. This indicated that daily repeated exposure to Ziziphus mauritiana root extract may be safer only at lower doses.
Keywords: Sub-chronic, Ziziphus mauritiana, Toxicity, Biochemical, Histopathology, Root Extract

INTRODUCTION

Ziziphus mauritiana common name is jujube in English or magarya in Hausa, is a fast growing, spiny thicket-forming evergreen shrub or small tree with spreading crown, stipular spines and many drooping branches which has the ability to resist fire and mechanical treatment (Orwa *et al*., 2009). It grows in warm-temperate region (Siddiqui and Patil, 2015), tropical and sub-tropical regions of the world (Ashraf *et al*., 2015; Chebouat *et al*., 2013).All parts of the plant are used in the treatment of various diseases, particularly in aiding digestion, alleviation of sores and other lesions (CABI, 2019), stomachache, chronic fatigue, loss of appetite, anemia, hysteria, sedative, tonic, insomnia, excessive perspiration, dyspepsia, blood purification (Palejkar *et al*., 2012), pulmonary ailments, laxative, anti-nausea, anti-rheumatic areas, abscesses healer, swelling, gonorrhea (Hussain *et al*., 2011), obesity, skin infections, diabetes, digestive disorder, weakness, bronchitis, pharyngitis, liver complaints (Umar and Babalola, 2019).

Since *Ziziphus mauritiana* plant is used for medicinal purposes, toxicity study is necessary to determine its median lethal dose, its safety and efficacy. Toxicity studies is carried out to determine the toxic effect, lethal dose at 50% concentration (LD_{50}) and to establish safety profile of tested compounds. Many studies have reported the toxicity status of *Ziziphus mauritiana* (acute, sub-acute, sub-chronic and chronic) (Dahiru *et al*., 2006; Mishra and Bhatia, 2014; Sireeratawong *et al*., 2012; Sukirti *et al*., 2013). Hence, this study aimed to evaluate the toxicity of methanol root extract of *Ziziphus mauritiana.*

MATERIALS AND METHODS

Sub-chronic Toxicity: For the Sub-chronic toxicity test, the OECD 452 guidelines was followed with little modifications (Muluye *et al*., 2019) for 28 days.

The test animals were obtained from Animal house, Bayero University Kano and ethical clearance was approved and obtained from the Department of Pharmacology, Bayero University Kano.

Test Animals (*Rattus norvigecus* 80-100kg of age between 6-12 weeks) were collected for the toxicity analysis. The rats were maintained at 22^0C at 50-70% humidity, feed with diet containing P-aminobenzoic acid 45mg/kg and water ad libitum.

A total number of 20 rats were randomly assigned to 4 groups of 5 animal each. The animals were treated with 1000mg/kg, 500mg/kg and 250mg/kg doses of plant extracts and control group treated with distil water use to dissolve the plant extract. The doses were administered orally every day for 28 days (4 weeks). Food and water consumption, toxicity signs and mortality were observed daily and the death recorded. The body weight of each animal was monitored on weekly basis.

Toxicity Analysis: At the end of the experiment, 6 hours post dose administration. All animals from each group were euthanized with ether and sacrificed, blood samples were taken in plain bottles for biochemical analysis (Schnell *et al.,*2002), kidney and liver were then removed for histopathological analysisusing double staining technique (Hematoxylin and Eosin Technique) as described by Verma, (2012).

RESULTS AND DISCUSSION

The body weight of the animals was recorded weekly as indicated in Table 1. There was significant change in the average weight of the animals over the period of time at P-value <0.05. Table 2 showed the effect of sub-chronic toxicity of methanol root extract of *Z. mauritiana.* One (1) mortality was recorded in the first week for group 2 (1000mg/kg), two (2) mortality recorded in the first and second week for group 3 (500mg/kg), while no death was recorded for group 4 (250mg/kg) and group 1 (control).The findings indicated that the toxicity of *Ziziphus mauritiana* methanolic root extract (ZMRm) was detected at higher doses. Accordingly, the lethal dose of ZMRm at 50% (LD_{50}) was determined to be 375mg/kg. Indicating that the lower dose (250 mg/kg) is relatively safe. The weights of the selected organs of the ZMRm group were within the normal range and no significant differences (P-value =0.2804) were observed between the weights of the selected organs of the treated group and those of the normal control group. This study disagrees the work of Sireeratawong *et al*., (2012) where it was reported the chronic toxicity of *Ziziphus attopensis* (ZA), according to the studies, the body weight changes, hematological and biochemical parameters, organ weights and histopathology examination did not show any differences from the control groups. Moreover, LD_{50} of ethanol seed extract of *Z. mauritiana* revealed that the extract was safe up to a dose level of 1000 mg/kg body weight. Although no mortality was recorded at higher doses, the animals showed lethargic behavior, but the body weight and spleen weight did not show any abnormal rise (Mishra and Bhatia, 2014).

The results of biochemical evaluation is shown in Table 3 and 4, there was no significant (P-value = 0.0690) alteration that can be seen in the serum creatinine, urea, sodium, potassium, chlorine, bicarbonate, albumin, total protein, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase of the ZMRm group as compared with those of the normal control group. According to Kaid *et al*., (2019) damage of the hepatic cell membrane released cytosol enzymes into the blood, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), therefore, their determination in serum could be used to assess incidence of organ damage. Hence, a study on haematological amelioration by *Ziziphus mauritiana* in silica induced toxicity in wistar albino rats revealed that animals administered with silica and simultaneous feeding of extracts of root, stem and leaves of *Ziziphus mauritiana* had a significant effect and decreased serum levels of ALT, AST, ALP, urea, uric acid, creatinine and LDH, as well as pro-inflammatory cytokines TNF-α and IL-6 as compared with normal control group (Dutta and Patil, 2018).

Plate 1 showed the micrograph of liver tissue for the tested animals (laboratory rats). The tissue of control group was represented as 1a, 1b showed 250mg/kg, 1c showed 500mg/kg and 1d showed 1000mg/kg. Normal features of hepatocytes (H) and central vein (CV) was showed in 1a and 1b, while 1c showed slight vacuolation and necrosis, and 1d showed slight hepatic necrosis. Plate 2 showed the micrograph of kidney tissue for the tested animals (rats). Normal features of Glomerulus (GM), Bowman Capsule (BC) and Proximal Convulated Tubule (PCT) were shown in 3a and 3b, while 3c showed slight tubular necrosis (TN), and 3d

showed slight hyperplasia of inflammatory cells (LH) and tubular necrosis (TN).

Liver and kidney are important organs responsible for the metabolism, detoxification, storage, excretion of substances and their metabolites, and are therefore susceptible to damage by external substances. The release of AST can be caused by acute liver diseases, while the major elevation of serum ALP is associated with liver, bone dysfunctions, bile impairment and lesions of different types. Furthermore, the elevation of urea, nitrogen, and creatinine may be attributed to kidney failure due to the building-up of end product of nitrogen metabolism or formation of the end product of protein metabolism in the liver from ammonia and its elimination by the kidney; or a product of non-enzymatic cleavage of phosphocreatine in the muscle excreted through the kidney (Kaid *et al.*, 2019). These findings indicated that the biochemical parameters showed non-significant alteration with P-value >0.05. Nonetheless, the prolong exposure caused slight abnormalities to the liver and kidney of the treated groups compared to the control group.

Table 1: Weekly Weight of the Rat (*Rattus norvegicus*) During the Sub-chronic Toxicity

Dose(mg/ml)	D1	D7	D14	D21	D28
1000	108.4±3.66[a]	119.30±04.57[d]	125.75±03.38[d]	127.75±03.97[d]	151.00±06.04[d]
500	119.2±04.67[b]	121.30±03.25[d]	125.00±10.95[a]	138.67±03.33[d]	140.67±03.38[d]
250	144.8±19.68[d]	152.80±18.99[d]	146.20±21.59[d]	143.60±18.17[a]	**153.06±18.33[c]**
Control	111.0±2.88	120.80±01.83	131.20±01.83	137.80±01.88	145.80±02.22

Values are presented as Means±SEM
Key: D = days
a,b, and c were statistically significant compared to control

Table 2: Sub-chronic Toxicity of Methanol Root Extract of *Ziziphus mauritiana* on Rat (*Rattus norvegicus*)

Dose (mg/kg)	Mortality at 24hrs	Mortality at 7 days	Mortality at 14 days	Mortality at 21 days	Mortality at 28 days	Number of survivals
Control	0/5	0/5	0/5	0/5	0/5	5/5
1000	0/5	1/5	1/5	1/5	1/5	4/5
500	0/5	1/5	2/5	2/5	2/5	3/5
250	0/5	0/5	0/5	0/5	0/5	5/5
LD_{50}		**375**				

Table 3: Effect of Methanol Root Extract of *Ziziphus mauritiana* on the Organ Weight of the Rat (*Rattus norvegicus*)

Treatment (mg/kg)	Body weight (g)	Kidney (g)	Liver (g)
control	145.8±2.22	1.9±0.07	11.9±0.31
1000	151.0±6.04	1.7±0.05	11.4±0.28
500	140.7±3.38	1.4±0.03	9.5±0.40
250	153.0±18.33	1.3±0.02	5.6±0.59

P-value = 0.2804

Table 4: Effect of the Sub-Chronic Oral Administration of Methanolic Root Extract of *Ziziphus Mauritiana* on Liver Function in Rat (*Rattus norvegicus*)

Parameters	Control	1000(mg/kg)	500(mg/kg)	250(mg/kg)
ALT (IU/L)	10±0.71	45±3.00	37±2.12	40±2.35
AST (IU/L)	12±1.14	15±1.14	65±1.82	90±1.14
ALP (IU/L)	14.8±1.11	25.8±1.49	22±1.30	31.9±1.50
Total Protein(g/dl)	1.1±0.15	0.3±0.17	1.4±0.27	3.0±0.71
Albumin (g/dl)	0.7±0.13	0.8±0.11	1.2±0.11	1.3±0.11

P-value = 0.0690

Table 5: Effect of The Sub-Chronic Oral Administration of Methanolic Root Extract of *Z Mauritiana* on Kidney Function in Rat (*Rattus norvegicus*)

Parameters	Control	1000(mg/kg)	500(mg/kg)	250(mg/kg)
Urea (mg/dl)	8.0±1.00	6.7±1.43	13.1±0.81	33.2±0.93
Creatine (mg/dl)	0.8±0.08	0.7±0.11	0.9±0.19	1.1±0.08
Na (mmol/l)	34.5±0.74	81.8±0.98	156.8±1.41	68.2±0.72
K (mmol/l)	4.2±0.45	7.1±0.18	10.0±0.55	7.9±0.19
Cl (mg/dl)	28±0.84	23±0.71	33±0.84	19±1.14
$(CO_3)_2$ (mg/dl)	85±2.07	97±1.14	102±4.82	79±0.84

P-value = 0.0690

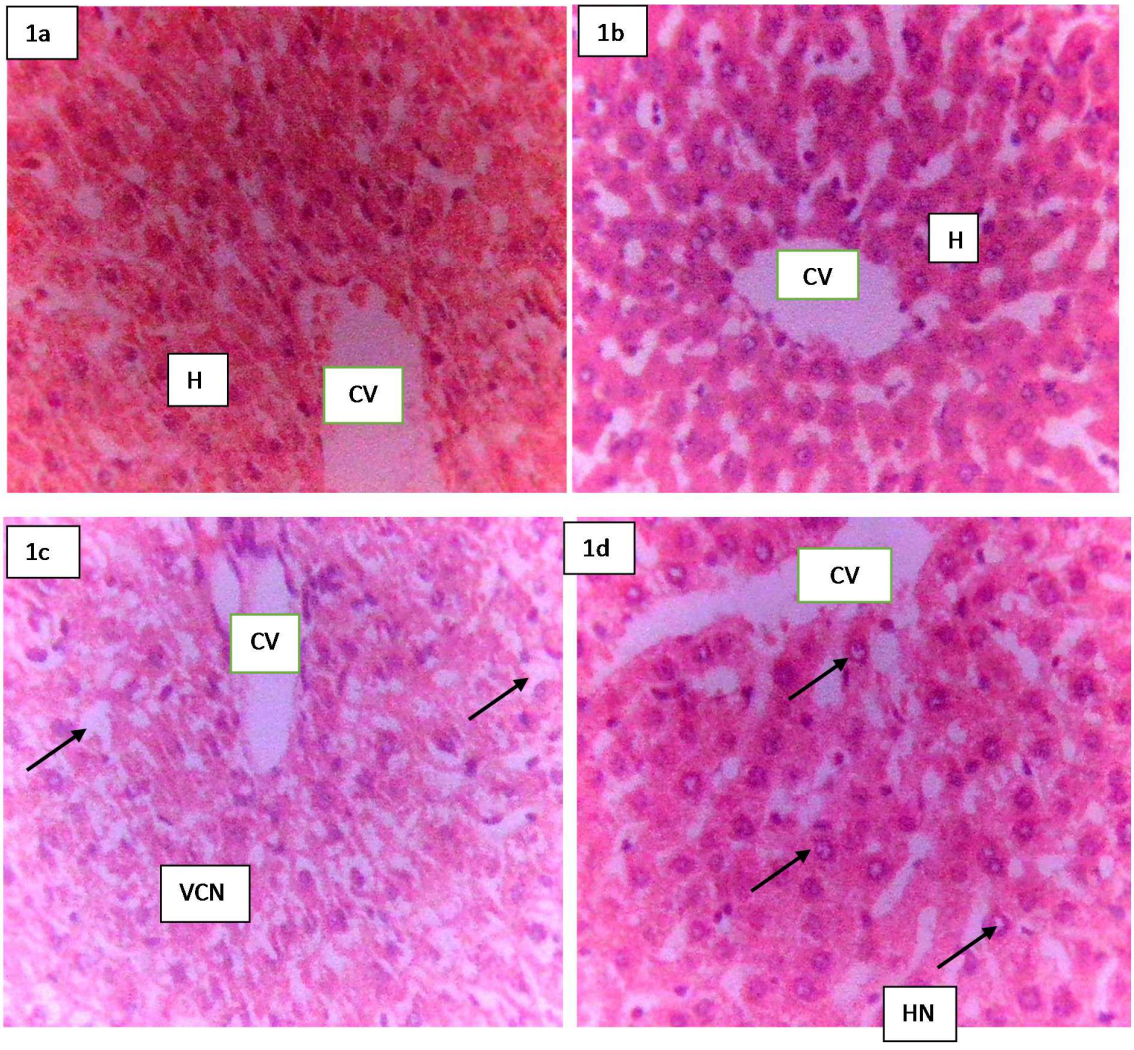

Plate I: Micrograph of Different Liver Tissue of *Rattus norvegicus*.
1a is tissue of control, 1b, 1c,1d are tissues at different concentration
H – Hepatocytes, VCN – Vacuolation and necrosis, HP – Hepatic necrosis

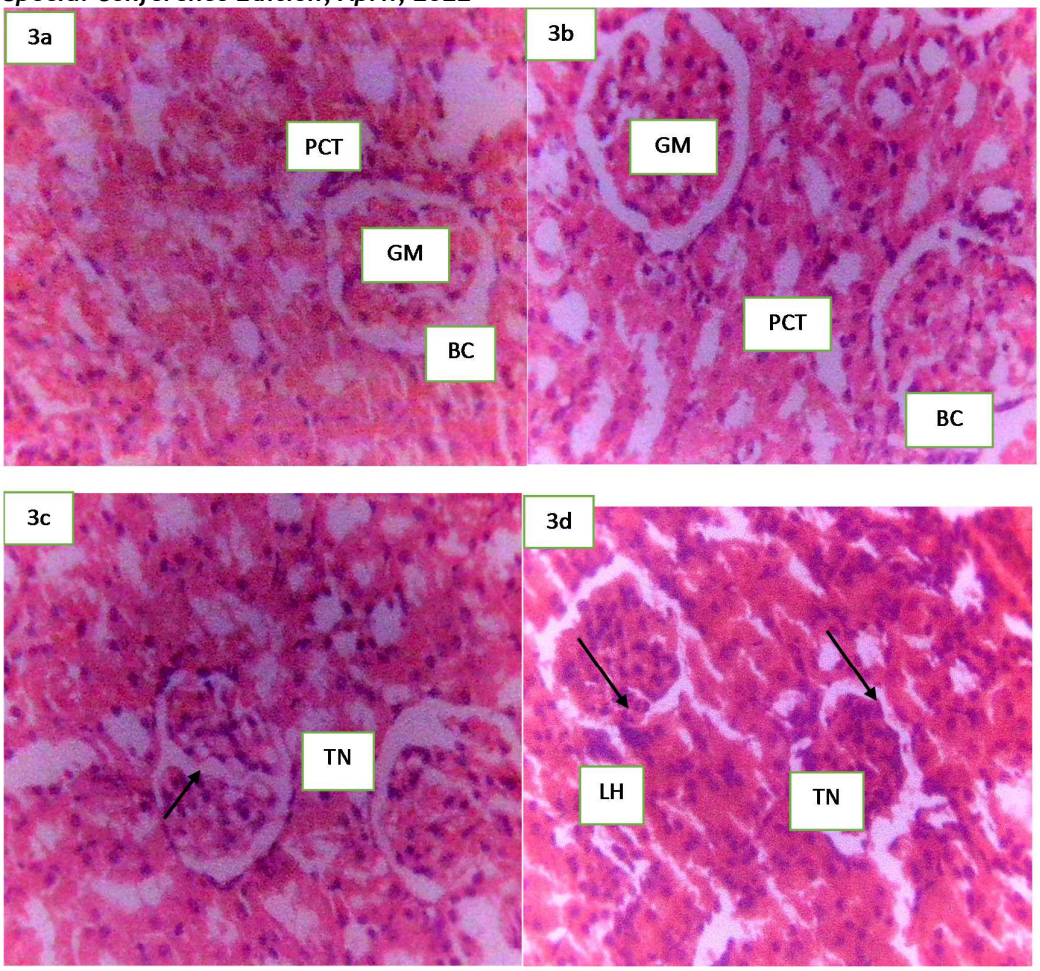

Plate II: Micrograph of Different Kidney Tissues *Rattus norvegicus*
TN – Tubular necrosis, LH – hyperplasia of inflammatory cells, GM – glomerulus, PCT – proximal convulated tubule, BC – bowman capsule

CONCLUSION

The extract showed no toxicity for both the acute and sub-acute tests, where no clinical signs were observed and no death recorded and at lower concentration (250mg/kg) for the sub-chronic test, but death was recorded at medium and higher concentrations (500mg/kg & 1000mg/kg) with LD_{50} of 375mg/kg. There were no significant alterations for both biochemical and hematological indices This indicated that *Z. mauritiana* root extract is relatively safe at lower concentration for prolong usage.

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