

Acute and Sub-Acute Toxicity Study of Ethanolic Crude Extract of *Mitracarpus hirtus* Plant on Wistar Rats

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ABSTRACT

Background: Plants are still considered among the important source of bioactive compound, especially in traditional medicine that has been used for centuries. Ethno botanical survey showed that *Mitracarpus hirtus* is used for the treatment of microbial infections, skin disease and ulcer. However the is hardly a published on safety regarding to this herb, which may cause clinical challenges.

Objective: The aim of this study was to determine the acute and sub-acute toxicity of *Mitracarpus hirtus* plant.

Methods: *Mitracarpus hirtus* was obtained, dried under normal room temperature. Extraction was performed by macerating process. We used Miller and Tainter (1944) method adopted for acute toxicity study. Sub-acute study was carried out according to the OECD 407 guidelines for 28days repeated dose in rat's models. The results were presented as Mean and SD, One Way Analysis Of Variance (ANOVA) was used, followed by Turkey's post-hoc test to determine the final significance ($p \leq 0.05$).

Results: The acute toxicity result from this study showed LD₅₀ greater than 5000mg/kg. For sub-acute, significant increase of ALP, ALT and CREAT, and decreased of AST, LDL and LDH was observed on biochemical parameters. While hematological parameters WBC, PLT, MONO and NEU values showed decrease and others were within normal range. Histopathology of liver and kidney revealed normal structure.

Conclusion: the acute toxicity study showed LD₅₀ greater than 5000mg/kg. Sub-acute toxicity study showed increase of ALT, ALP and CRET in some groups, but decrease of AST, LDH and LDL were observed, while WBC, PLT, MONO, and NEU showed decrease, while others not affected in all the

treated groups. Histology of kidney and liver revealed normal structure. Concluded that the extract is relatively safe at lower concentrations.

Key words: *Mitracarpus hirtus* herbs; lethal dose; Biochemical, hematological and histopathological parameters

INTRODUCTION

Medicinal plants are considered among the important source of bioactive compound, especially in traditional medicine that has been used for centuries by parents, herbalists, healers, spiritualists, hunters and farmers as a primary health care at the local and community level. [1] People use available indigenous plants for treating and prevention of common illnesses, which include both infectious and non-infectious. According to WHO, over 70% of the world populations rely on medicinal plants for primary healthcare. [2] It is a common practice in Africa and other parts of the world to use plants in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. [3]

The therapeutic efficacies of many indigenous plants for various diseases have been described by herbal medicine practitioners. [4] Overtime indigenous plants have offered important raw materials for many industries such as pharmaceutical, cosmetic, perfumery and food. [5] The presence of various life sustaining constituents in plants have stimulate scientists to examine various plants with a view of determining their potential on antimicrobial effects. [6]

Mitracarpus hirtus (L) belongs to the Rubiaceae family, and is commonly distributed throughout gardens, farms and fields in tropical and subtropical regions such as United States of America, India, Malaysia, Myanmar Thailand, west and East African countries. *M. hirtus* has been described as approximately 40 cm tall, opposite leaves with 2-6 cm long, 0.5-2 cm wide with white flower in dense axillary clusters, and pale yellowish brown seeds with ellipsoid-rectangular shape. [7]

Ethno botanical survey showed that *M. hirtus* is used for the treatment of fungal infections, skin disease such as rashes, itching, eczema, ringworm, toothache, wound healing, ulcer and venereal diseases etc., by applying the leaf sap, rubbing leaves on skin or taken orally. [1,8]

Phytochemical analysis of this plant has been carried out and found to contain bioactive compound like Glycosides, Steroids, Tannins, Alkaloids, Saponins, Triterpenes and so on. [9] Scientifically there is hardly sufficient information's published in the literature to support the concept about the safety of this plants extract. Recently there are increased cases of poisoning associated with the use of herbal medicine in many part of the world. [10] Auerbach *et al.* [11] reported liver fibrosis cases due to the use of herbals plants, in some African countries like Uganda. So, continuous use of this plant without safety data can cause serious clinical challenges. Therefore, the purpose of this study was to assess the acute and sub-acute toxicity profile of *Mitracarpus hirtus*, ethanolic crude plant extract in order to ensure its safe use, and protect the public health.

MATERIALS AND METHODS

Plant materials, collection and identification

The whole plant was collected from the bush in Uganda, and was taken to Botanist for identification. The collected samples were washed, stored and dried in a shade to avoid direct sunshine. The dried samples were ground using the mortar and

pestle according to standard procedures described by Paola *et al.* [12] Fine powder was obtained by sieving. After weighing, the powder was packed in clean labeled bottles and stored a 20 °C until use.

Plant Extraction

Extraction was performed by macerating air-dried, powdered of *M. hirtus* (30g) soaked on 70% ethanol (600ml) at room temperature for 24hrs, and was occasionally shaken. The crude hydroalcoholic extract was filtered using, Whatman filter paper NO. 1, concentrated using hot air oven (50°C). The dry residue was stored at 4°C, and at the time of use, was re-suspended in distilled water. [13]

Determination of extract yield (% yield)

The yield (% , w/w) from all the dried extracts was Calculated as: Yield (%) = (W1 x 100)/W2 where W2 is the weight of the dry extract after getting in solvent, and W1 is the weight of the plant powder.

Preparation of stock solution

Preparation of the stock solution was done by dissolving four grams of the extract in ten millilitres of distilled water to give a stock solution of concentration 400 mg/ml. This stock solution was prepared for administering to rats. The volumes of the extract administered to the animals was calculated using the formula by Gosh (1984) as shown below: [14]

$$\text{Volume given to each animal (ml)} = \frac{\text{body weight of the animal (kg)} \times \text{dose (mg/kg)}}{\text{Concentration of the extract (mg/ml)}}$$

Experimental animals

Healthy adult male and female, aged 2 and 3 months, weighing 100–180g and 120–200g, respectively, were obtained and used. The animals were maintained in standard conditions (22–24° C; 12:12 h dark/light cycle), water and industrialized dry food were all available. We followed procedures with modifications, described accordance with the National Institute of Health Guidelines (USA) for the Care and Use of Laboratory Animals, Humane Care and Use of Laboratory Animals published by National Institutes of Health, United States and OECD 2008. [15]

Acute toxicity (LD₅₀)

Lethal dose (LD₅₀) was determined According the Miller and Tainter. [16] Healthy Wistar rats were fasted overnight, with free access to water. Animals were randomly divided in five groups of both sexes (n = 5/group). The first group (control group) were received orally distilled water (10mg/kg). Groups 2, 3, 4 and 5 were orally treated with *M. hirtus* in doses of 0.625, 1.25, 2.5 and 5.0 g/kg, respectively as a single dose. [13] The animals were observed for sign of toxicity continuously for 2h and then occasionally for a further 4h, and finally over night to note mortality. [13]

Sub-acute toxicity test

The healthy male and female rats were randomly divided into four groups by sex (n = 10/group).

Animals were received vehicle orally water (control group) and *M. hirtus* in doses of 0.10, 0.50 and 1g/kg/day for 28 consecutive days. Body weight was weekly recorded and food consumption and water intake were daily monitor. Animals were observed for signs of abnormalities during whole treatment. At the end of the treatment, animals were fasted overnight, but with water. They were anesthetized with pentobarbital sodium 0.035 g/kg (i.p.). The blood samples were obtained by cardiac puncture, for hematological and biochemical studies, with and without anticoagulant ethylenediaminetetraacetic acid (EDTA), respectively. [13]

Hematological evaluation

After 28 days heparinised blood samples collected for Hematological analysis, and were. Red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets count and mean platelet volume (MPV) and The differential leukocyte count Neutrophilic (NEU), Eosonophilic (EOSO), Basophilic (BASO), Lymphocyte (LYM) and Monocyte (MONO) were performed with an optical

microscopy after staining and, in each case, 100 cells were counted. The result obtained were compared with normal control group for the analyses the differences.

Biochemical evaluation

Biochemical analysis, non heparized blood collected from animals after 28days were used, the blood were centrifuged at 1500×g for 10 min. parameters of blood urea nitrogen (URE); creatinine (CRE); aspartate aminotransferase (AST); alanine aminotransferase (ALT); total cholesterol (LDH); triglycerides; alkaline phosphatase (ALP); total protein. Dosages were made using Architect (Abott®) automation with Boehringer Ingelheim® biochemical. At the end the result were compared with the control group to observe the variations.

Histopathological studies

Histological examination was performed in 3 animals per group, randomly selected in the group.

Macroscopic and microscopic features of liver and kidney (fragment of 6–7 cm) were compared in the control and 100, 500, 1000mg/kg treated groups. After the animals were euthanized with an excess of phenobabitone (0.035 g/kg, i.p.), followed by buffered formalin solution (10%) for 10 min and the same organs that are removed and fixed in Bouin solution for 48 h at room temperature. These organs were carefully removed and weighed individually, and organ weights were expressed in absolute and relative terms (g and g/100 g of body weight, respectively).

Data analysis

The results were analyzed using One Way of Variance Analysis (ANOVA), followed by Tukey's post-hoc test using the Past 3 and Graph prism 6, Software, inc., USA. The mean statistical differences tested at 95 % confidence interval (P<0.05). Histopathological data was analyzed by a well-qualified, registered and practicing veterinary pathologist, and significant histopathological changes detected were used to indicate toxicity to either the liver, kidney tissue.

RESULTS

Percentage yield of the plant extract

$$\begin{aligned} & \text{Percentage yield (\% age yield)} \\ &= \frac{\text{Weight of concentrated extract}}{\text{Weight of the plant power soaked}} \times 100 \\ &= \frac{88.8}{500} \times 100 \\ &= 17.76\% \end{aligned}$$

The results of acute toxicity study does not shows any mortality, or serious clinical sign of toxicity (table I), Even at higher dose (5000mg/kg), LD₅₀> 5000mg/kg. But minor changes were observed (table II) like urination, lethargy and increased of respiration rate of some groups (2500mg/kg) and (5000mg/kg

Result for acute toxicity study

Table I: Results of lethal dose of *Mitracarpus hirtus* ethanolic crude extract after 24hrs oral administration in rats both sexes (n=5)

Group	Dose(mg/kg)	Log-dose (mg/kg)	% Dead	*Corrected %	Probits
1	625	2.79	0	5	3.36
2	1250	3.09	0	5	3.36
3	2500	3.39	0	5	3.36
4	5000	3.69	0	5	3.36

*Corrected % were calculated using corrected % formula for 0 and 100% mortality. For 0%dead: 100(0.25/n), 100% dead: 100(n-0.25/n)

Table II: Result of clinical symptom for acute toxicity study of *M. hirtus* plant on Wister rats both sexes after 24hrs oral administration. (n=5)

Observations	Control group	625mg/kg	1500mg/kg	2500mg/kg	5000mg/kg
Food intake	Normal	Normal	Normal	Decreased	Decreased
Digestion	No	No	No	No	No
Urination	Normal	Normal	Increased	Increased	Increased
Sedation	Absent	Absent	Present	Present	Present
Diarrhea	Absent	Absent	Absent	Absent	Absent
Rate of respiration	Normal	Normal	Normal	Normal	Increased
piloerection	Normal	No effect	No effect	Present	Present
Sleeping	Present	Present	Present	present	Present
Drowsiness	Absent	Absent	Absent	Absent	Absent
General Physique	Absent	Absent	Absent	Lethargy	Lethargy
Temperature	Normal	No effect	No effect	No effect	No effect
Eye color	Normal	Normal	Normal	normal	normal
Grooming	Absent	Present	Present	Present	present
Tremors	Absent	Absent	Absent	Absent	Absent
Coma	Absent	Absent	Absent	Absent	Absent
Convulsion	Absent	Absent	Absent	Absent	Absent
Death	Alive	Alive	Alive	Alive	Alive

Result of sub-acute toxicity study

Result of the Body weight

The figure I below, showed *M. hirtus* plant has no effect on body wight, it indicated normal progression of the body weight from day 0 to 28days. The organs wight of both liver and kidney indicated no effect in all the treated groups as compared with control (figure II).

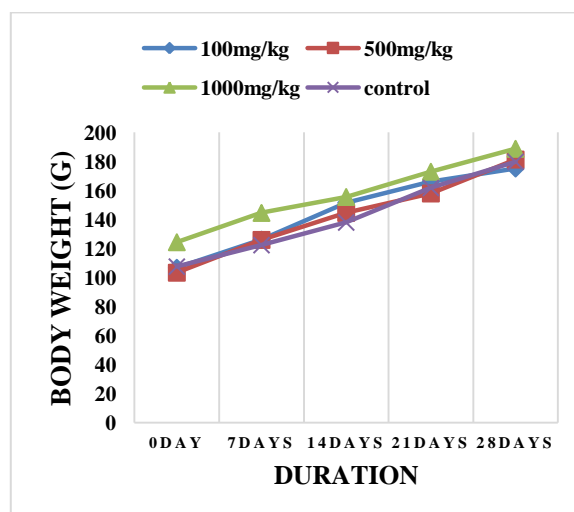


Figure 1: Effect of *M. hirtus* plant on the body weight of rats both sexes after 28 days of oral administration (n=10)

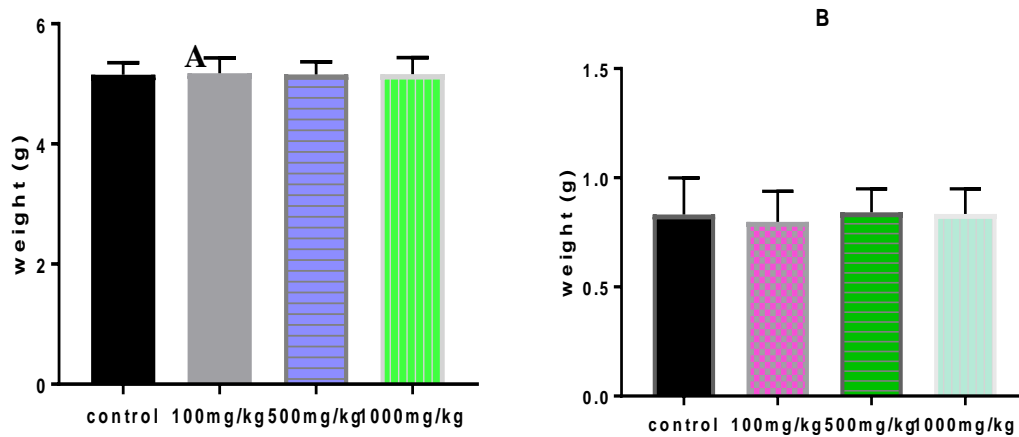


Figure 2: weight (g) of the liver (A) and kidney (B) of the rats after oral administration of the ethanolic crude extract of *M. hirtus* plant for 28days

Hematology/ Biochemical parameters

Hematological parameters (Table III), no significant differences were found on HGB, HCT, RBC, BASO, EOSO and LINO. But significant decrease of WBC, MCV and platelet were obtained at doses (500mg/kg) and (1000mg/kg), when their mean values compared with the mean

control group from all the tested group. The extract showed significant decreases of AST and LDH in all the treated groups (Tablet IV). ALT, ALP and CREAT mean values significantly increase at higher dose (1000mg/kg) and (500mg/kg), when compared with 100mg/kg and the control group.

Table III: Effect of ethanolic crude extracts of *M. hitrus* plant on hematological parameters of Wister rats both sexes, after 28days oral administration (n=10)

Parameters	Reference value	Control group	Dose (mg/kg)		
			100	500	1000
WBC (10 ³ /uL)	5.00-10.50	10.5±1.51	12.6±1.87	8.04±1.25 ^{*a}	9.44±1.43 ^a
RBC (10 ⁶ /uL)	4.00-6.00	6.892±0.69	6.682±0.18	6.792±0.62	6.89±0.77
PLAT (10 ³ /uL)	150.00-450.00	625.42±37.43	614.2±4.14	548.6±14.38 ^{*a}	618.8±59.51 ^b
HGB (g/dL)	11.00-17.00	14.38±1.03	13.96±0.43	14.14±1.88	14.1±1.36
HCT (%)	35.00-50.00	42.1±4.04	42.26±2.30	42.7±5.51	42.82±3.48
MCV (fL)	80.00-99.90	63.28±0.89	62.92±2.62	66.64±8.034	59.64±1.89 [*]
MCH (pg)	27.00-32.00	20.958±0.65	20.66±0.70	23.52±2.74	22.2±2.88
MCHC (g/dL)	32.00-37.00	33.28±0.98	32.96±0.86	32.8±1.04	33.4±0.93
MONO (%)	2.00-10.00	3.068±0.01	2.89±0.07	2.16±0.30 ^{*a}	2.11±0.16 ^{*a}
EOSO (%)	0.50-5.00	0.58±0.004	0.39±0.04	0.56±0.09	0.57±0.12
BASO (%)	0.10-2.00	0.92±0.007	0.688±0.20	0.92±0.08	0.88±0.08
NEUT (%)	50.00-80.00	7.99±1.16	6.96±0.59	6.60±0.46 [*]	8.14±0.81 ^a
LINO (%)	25.00-50.00	57.26±145.40	54.64±6.03	54.66±13.99	53.86±9.04

n=10, X±SD. Values with the symbol * ^a^b are significantly different (p≤ 0.05). Control, 100mg/kg, 500mg/kg respectively.

Table IV: Effect of ethanolic crude extracts of *M. hirtus* plant on Biochemical parameters of Wister rats both sexes, after 28days oral administration (n=10)

Parameters	Reference value	Control Group	Dose (mg/kg)		
			1000	500	100
ALT (U/L)	18-66	97.06±9.82	157.92±27.52	101.62±12.88 [*]	99.38±15.17 ^{*b}
AST(U/L)	74-143	135.60±35.08	146.42±37.04 [*]	95.74±10.11	99.88±8.08 ^{*b}
ALP (U/L)	62-232	371.43±33.80	465.64±29.35	421.92±10.69	391.37±30.04 ^{*b}
ALB (g/L)	3.5-5.00	37.37±2.66	33.72±0.86	33.9±0.52 ^a	35.78±0.93 ^{*ab}
UREA (mg/dL)	15-24.6	9.73±1.27	8.59±1.19 [*]	9.848±1.22 ^{*a}	9.672±1.38 [*]
CRET (mg/dL)	0.7- 1.5	63.68±4.92	80.42±4.92	62.72±4.59 [*]	70.166±1.59
LDH (U/L)	250-1250	1063.3±243.00	696.36±233.74	252.18±80.10 ^a	601.1±107.14 ^b
LDL (mg/dL)	24-73	80.04±2.81	53.12±6.31	61.26±2.70	50.52±6.14 ^b

n=10, X±SD. Values with the symbol * ^a^b are not significantly different (p≤ 0.05). Control, 1000mg/kg, 500mg/kg respectively

Histopathology of the liver and kidney

The figure 3 below indicated normal cell structure of liver, no abnormality was recorded when compared with control group (level A). Similarly, the extract did not show any histopathological abnormalities on the nephron and kidney (figure 4).

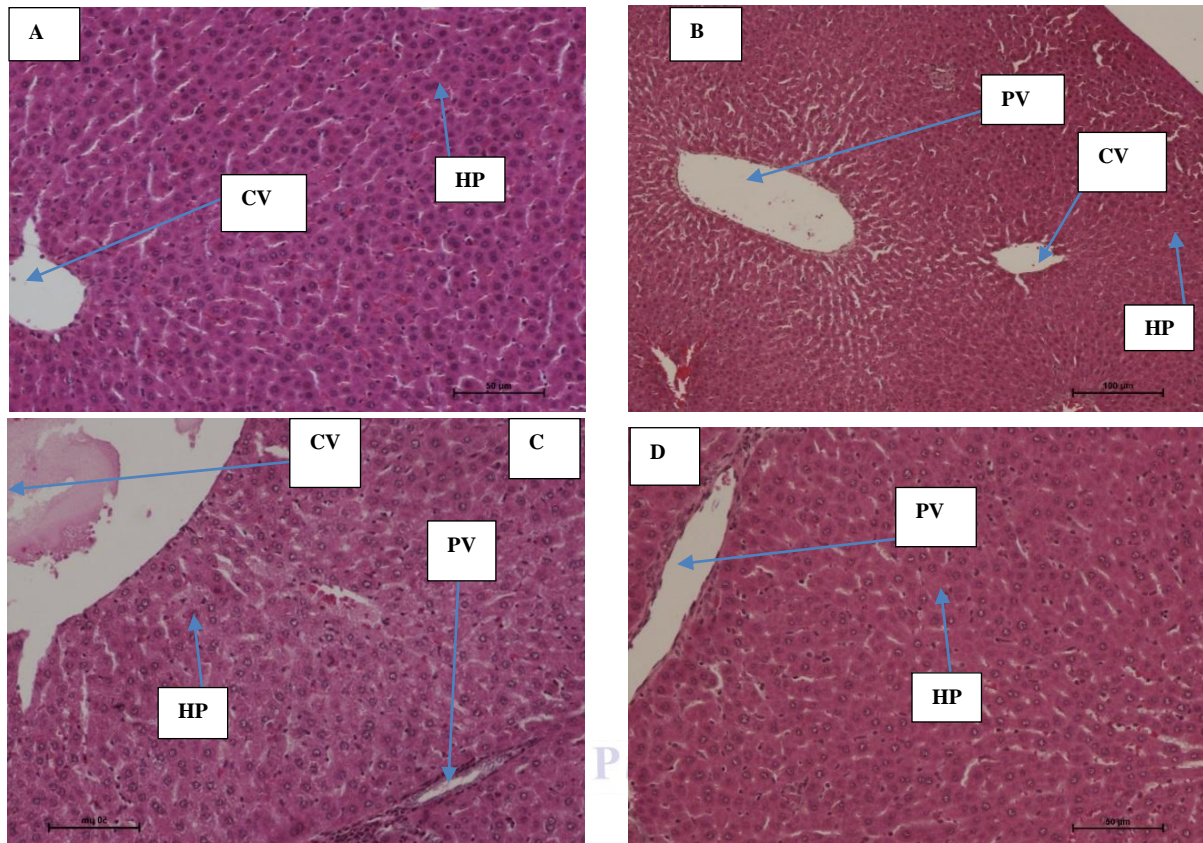


Figure 3: Effect of *M. hirtus* plant extract on liver tissue after 28days of oral administration CV (central vein), PV (portal vein), HP (hepatocyte) A (control group), B (100mg), C (500mg), D(1000mg/kg)

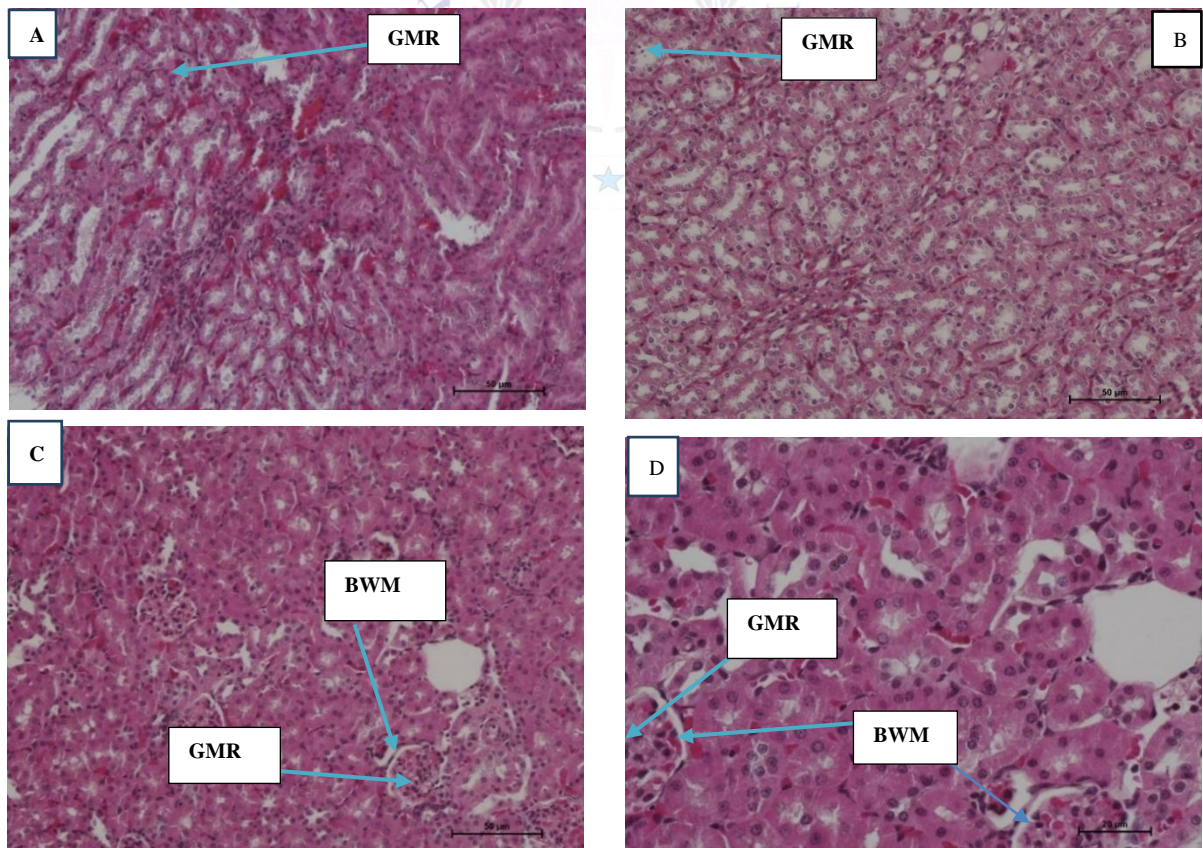


Figure 4: Histopathology of the kidney cell on rats both sexes treated with *M. hirtus* plant for 28days oral administration. DT (Distal tube), BWM (bowman capsule), PT (proximal tube), GMR (glomerular): A (control), B (100mg/kg), D (500mg/kg) and E (1000mg/kg).

DISCUSSION

Mitracarpus hirtus is a plant species that has numerous bioactive compounds with different pharmacological effects, either beneficial and/or toxic on human health. According to Saad *et al.*,^[17] they reported that the apprehension of consumers for lack of scientifically valid evidence has favorite to perform studies concerning the toxicity of plant species which are used by the public as natural drugs.

This study indicated that the ethanolic crude extract of *M. hirtus* plant after 24hrs of oral administration did not cause any mortality on the treated rats table I. The LD₅₀ was obtained from the graph of probit against log-dose, and the dose corresponding to probit 5 (50%), even at higher dose (5000mg/kg) the results showed the probit less 5 (50%). According to World Health Organization (WHO) and OECD guideline, LD₅₀ > 5000mg/kg is considered as not toxic. We thus concluded that the LD₅₀ of *M. hirtus* plant was > 5000mg/kg. The study did not showed any serious clinical symptoms at lower doses 625-1500mg/kg (Table II), but at higher doses (2500-5000mg/kg) some clinical symptoms were observed, like change in breathing and increased in urine out put which resolve within 10hrs. The urination and piloerection could have occurred due to the present of dianthrone, an anthraquinone-derived compound.^[18] Saponin compound reported to have an effect on respiration and mood changes.^[19] The results corresponded with research done by Abere *et al.*,^[20] which reported that the aqueous and ethanolic extract of *M. scaber* did not produce major sign of toxicity, but some behavioural changes were reported in *Tamarindus indica pulp* ethanolic extract.^[21]

The body weight result after 28days showed gradual increased on both control and treated groups. No significant change in mean body weight between the control and the treated groups (figure I), this indicated that *M. hirtus* plant extract has insignificant levels of toxicity on the growth of the rats as also reported by Mir, Sexena, and Malla^[22]

and Rajalakshmi *et al.*,^[23] It showed that there was no disruption in protein, carbohydrate, or fat metabolism.^[24] However on the organs weight of both liver and kidney (figure II), the extract had no significant effect in all the treated rats when their mean weight compared with that of control. The results indicated the extract does not caused any damage to such organs, by either increase or decrease. It has been documented by Kluwe^[25] that the increase in organ weight (kidney or liver) had been observed to be a relative sensitive indicator of nephrotoxicity or hepatotoxicity respectively. Thus, *M. hirtus* did not induce any toxic effect on the kidneys and the liver going by this indicator.

On haematological parametrs (table III), RBC did not show any statistical significance using plant extracts, revealed that there is a stability between production and destruction of RBC. On HGB and HCT mean values, the data showed that the extract does not affect the red heamoglobin, which allows a normal oxygen supply for lung and tissue function, and subsequently cell function. The platelet count decreased (548.6U/L) by using plant extracts at 500 mg/kg when it compared with the control group (625.1U/L), meaning the extract had an effect on platelet production leading an induced thrombocytopenia at this dose (500mg/kg). Adedapo *et al.*;^[26] Adeniyi *et al.*,^[27] pointedly out that reduced blood platelets affect the viscosity of blood, which is connected positively to blood pressure. Decreased count of WBC shows the suppression of leucocytes and their production from bone marrow.^[28-30] WBC differential count, Monocytes and Neutrophilic showed significant variation in the dose of 500mg and 1,000 mg/kg body weight compared to 100 mg/kg body weight and also to control groups, while Eosinophilic count and others showed no significant alterations at all dose levels compared to control.

Onyeyilli, Iwuoha, and Akinniyi,^[31] reported that administration of an agent can result in loss of blood cells and/or inhibition

of blood cell, due to the presence of anthraquinone-derived compound found in the plant as bioactive compound. The results corresponded with the work done by Carlos *et al.*, [32] which indicated that the ethanol extract of dried leaves of *Pseudocalymma alliaceum* (Bignoniaceae) on Wistar rats did not show any variation of RBC, HCT, HGB, but significant decrease of WBC and platelet were recorded when compared with control. *M. hirtus* organic extracts containing bioactive flavonoids, and revealed that plants containing flavonoids revealed platelets and antioxidant action in the prevention of thrombosis, and have capability to lower blood lipid levels. [27] However significant increase of MCV value recorded at dose of 1000mg/kg indicated the rats have no hemolytic anemia.

The biochemical value (table IV), the CRE significantly increased at dose of 100 and 1000mg/kg with extractions employed, CRE being an indicator of renal impairment, [33] which indicates that the extract may be toxic to the kidney, or the alteration occur due to other diseases. The AST values decreased suddenly at 500mg/kg, while others showed normal when it compared with control. The AST is found in almost all the organs including the blood, but when it is present in blood at very high levels, it means there has been cell destruction, but on this study it showed no effect. ALP level increased as the doses increase with the doses 500mg to 1000mg/kg. While ALT values significantly increased at 1000mg/kg as compared with control and within the groups. The ALT is located in the liver and its function is the production of glucose. Transaminases are enzymes that are indicators of liver function and as biomarkers of toxicity and the increase of these enzymes in the blood demonstrates the existence of cell injury in the kidney, liver, muscle and heartm [34] Thus histopathology of both liver and kidney on this study were normal (figure III and IV).

This alteration of ALT, ALP possibly occur due to the presence of flavonoids in the

extracts used, as mentioned by Singab *et al.*, [35] and Wu *et al.*, [36] in studies with *Pachyptera hymenaea*, and *Laggera alata* (Don) which prospered in reducing the levels of AST and provided protection to the liver. The extract had no effect on URE as reported in this results, which showed no significant variation when compared with control, indicate no azotemia on treated rats. Significant decreases of LDL values were observed in all the treated groups, indicating a possible cardioprotective action [37] Decreased level of LDH was observed in all the treated groups, and LDH been the marker of tissue damage when it increase, because of the tumor or diseases [37] This corresponded with the results reported by Verma *et al.*, [37] where they documented that the leaf extract from *Pachyptera hymenaea* (DC.) (syn: *P. alliaceum*) has hypolipidemic activity at low doses and antihyperlipidemic at high doses in normal rats and compared with hypercholesterolemia induced after 28 days.

Histological studies showed no abnormalities in kidney and liver tissue in treated rats. The liver tissue appeared normal hepatocytes without any enlargement in sinusoidal vein, central vein, no necrosis and fibrosis with no cystic formation in all groups treated (B, C and D) with *M. hirtus* plant, when related to control group A (Figure III). Comparable observation was also reported on *Alstonia scholaris* Stem Bark plant by Bello *et al.*, [38] in rat liver. For the kidney, the micrograph exposed normal architecture of glomerulus and Bowman's capsules with no disintegration, necrosis, or inflammation. No inflammatory cell infiltration in the cortex were observed, the medulla showed no significant lesions (Figure IV level B, C and D), in all the treated groups, when compared with control (level A). Similar with the study made by Ping *et al.* [39] and Nabukenya *et al.*, [40] on *Euphorbia hirta* and *Tephrosia vogelii*, *Vernonia amygdalina*, *Senna occidentalis* plants respectively.

CONCLUSION

The single dose of acute toxicity study from *M. hirtus* plant did not produced any serious clinical sign and no mortality was recorded. This suggested that the extract was not toxic at single dose, as recommended by WHO and OECD, meaning the LD₅₀ is greater than 5000mg/kg. However, the reduction ability of WBC, Platelet, Monocyte and Neutrophil count were observed in some groups, on hematological parameters, while others within normal range. The biochemical values of liver and kidney were normal at 100mg/kg, but at 500 and 1000mg/kg, ALT, ALP and CREAT values increased, while AST, LDL and LDH showed decreased. Histological study indicated normality on both liver and kidney. Based on this study, it can be concluded that the extract is safe at lower dose.

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