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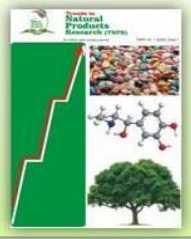

Sub-chronic Safety Assessment of Crude Methanol Leaf Extract of *Terminalia macroptera* (Guill. & Perr.) in Rats

Ibrahim Haruna Sani[1,2*], Abdullahi Rabiu Abubakar[2], Abdullahi Hamza Yaro[2] and Sani Malami[2]

[1]Unit of Pharmacology, College of Health Sciences, Yusuf Maitama Sule University, Kano, Nigeria.
[2]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical sciences, Bayero University Kano, Nigeria

Keywords: *Terminalia macroptera*, toxicity; haematological indices and Herbal medicine

Abstract: Traditionally plant extracts were used in the treatment of many diseases and disorders. However, before usage, it is vital to ensure their safety. Hence, the toxicity assessment of medicinal plants with proven therapeutic use is of utmost importance. The tree *Terminalia macroptera (Combretaceae*) is widespread in Western Africa, with the different parts of the plant having been utilized in the treatment of various diseases. This study evaluated the safety profile of crude methanol leaf extract of *Terminalia macroptera* in rats using acute and sub-chronic toxicity assessments. In the acute study, a single oral dose (5000 mg/kg) of the extract was administered to three rats and observed for 14 days for signs of acute toxicity. In the sub-chronic study, experimental animals were administered with the extract at 250 - 1000 mg/kg for 28 days. Acute toxicity (LD_{50}) was estimated to be > 5000 mg/kg. No mortality or signs of toxicity were observed in the rats following acute and sub-chronic drug administration. In sub-chronic toxicity testing, administration of the extract did not cause any changes in body weight of treated animals when compared to the control group (distilled water treated animals). There was no significant difference (p >0.05) observed in the relative organ's weights, bodyweights, haematological indices, biochemical parameters, and gross abnormalities, compared to the control. Therefore, it is concluded that the oral administration of the crude methanol leaf extract of *Terminalia macroptera* for 28 days is safe with no acute or sub-chronic toxicity

*Corresponding author:
harunaibrahim81@yahoo.com,
ihsani@yumsuk.edu.ng

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INTRODUCTION

Medicinal plants from ancient times play a key role in the search for lead compounds for the drug development (WHO, 2018). This supports the report of the World Health Organization, in 2008 that more than 80 % of the total population of the world especially those of the developing countries depend majorly on medicinal plants for their daily essential medical needs (WHO, 2012). Nowadays, over 20% of the currently available drugs contain phytochemicals as their active components (WHO, 2018). Furthermore, the medicinal plants have very important role in the health of human beings as well as animals. As the World health organization (WHO) estimates about two thirds of the world populations currently use herbs and other traditional medicines to cure various diseases (WHO, 2018). Safety assessment of medicinal plant is vital and necessary during the course of drug development. Establishing the toxicity profile of a medicinal plant approves or disapproves next step during bioassay screening (Abubakar *et al.* 2020). However, at times, medicinal plants were found to cause side effects, toxicity and even drug interactions (Nath and Yadav, 2015).

Terminalia macroptera (Guill & Perr) is a species of flowering plant in the *Combretaceae* family known by the Hausa "Kwandari" or "Baushe". It is native to Africa where it can be found in Benin, Burkina Faso, Ghana, Senegal, Sudan, Uganda and Nigeria. (Yahaya *et al*. 2017). *Terminalia macroptera* is a plant which is widely spread in savanna region which may be recognized by the prominent pale green leaf, and by its large fruits. The tree is about 13m high and 2m in girth, with an open spreading crown (Silva *et al.* 2012). In Guinea-Bissau, it is used by traditional healers for the treatment of hepatitis and venereal diseases frequent use of this plant in healing of microbial infections in Africa and in Asia has led to its choice for studying (Deniz *et al.* 1996). The leaf and roots of the plant are extensively used in ethno medical practices of various cultures. The extracts of *T. macroptera* have given slight activity against Candida albicans and interesting profile of activity against enteropathogenic microorganisms, including Shigella dysenteriae. Most of the species from *Combretaceae* family have been showed to contain antibacterial activities (Silva *et al.* 2012). *T. macroptera* plant is a medicinal plant whose roots, leaf and bark are well known in Northern Nigeria with variety of therapeutics benefits (Silva *et al.* 2012; Yahaya *et al.* 2017). Hence, the toxicity assessment of plants with proven therapeutic use is of ultimate importance. The present study aimed to evaluate the safety profile of crude methanol leaf extract of *T. macroptera* in rats using acute and sub-chronic toxicity assessment.

MATERIALS AND METHODS

Plant collection and extraction

Fresh leaf of *Terminalia macroptera* was collected from Kiru Local Government Area of Kano State, Nigeria. The plant was identified and authenticated in the herbarium of the Department of Biological Sciences, Bayero University, Kano, Nigeria, and a voucher specimen number (BUKHAN 0511**)**.

The plant was dried under shade for three weeks and then pulverized into a fine powder with the aid of mortar and pestle. About 500 g of powdered material was cold macerated with 2.5 L 70 % (v/v) methanol with constant shaking for 5 days and then filtered using Whatman filter paper No 1. The filtrate was then concentrated to dryness in an oven at 50 °C, which was then kept in desiccators for use in the study.

Experimental animal

Wistar strain rats (110-120 g) were obtained from Animal House Facility of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University, Kano. Rats were housed and allowed to acclimatize with free access to food and water in the animal house and maintained under standard laboratory conditions in accordance with the guide for the care and use of laboratory animals. All experimental protocols were as approved by the C.H.S Animal Ethics Committee BUK, with reference number BUK/CHS/REC/VII/53

Drugs and Chemicals

The chemicals used for the experiment included: Methanol (Sigma Aldrich, USA) and distilled water.

Acute Toxicity Study

The oral median lethal dose (LD_{50}) was determined using the Organization for Economic Co-operation and Development (OECD 420) guidelines (OECD, 2001). Three mice were fasted 3 hours before the experiment. The limit test was conducted in two stages. In the first stage, 5000 mg/kg of the extract was used for one mouse and observed for 48 h. On survival, the same doses were administered to two additional mice in the second stage. Animals were observed during the first 30 minutes of treatment and then occasionally within 24 h, and finally daily for 14 days. Animals were monitored for tremors, convulsions, salivation, diarrhoea, sleep, behavioural changes and coma.

Sub-chronic Toxicity Study

This was performed according to the Organization of Economic Co-operation and Development guideline (OECD) for testing of chemicals (OECD, 2008). Twenty-four (24) female Wister rats

weighing 110–120 g was randomly assigned into 4 groups of 6 rats each. Group 1, the control group was given distilled water (1 ml/kg) while groups 2, 3 and 4 were orally administered with 250, 500 and 1000 mg/kg of the extract daily for 28 days. The weight of the rats in each group was determined and documented weekly. Also, signs of toxicity such as body weight, mortality, food and water intake were monitored. After 28 days, the animals were euthanized with chloroform and blood was collected through cardiac puncture. An aliquot (2 mL) of the blood was collected into ethylene diamine tetra-acetic acid (EDTA) bottle and was used for the analysis of haematological parameters. Another 5 mL of the blood was collected in non-heparinized bottles, centrifuged at 1000 r/min for 10 minutes and the resulting serum was aspirated and used for biochemical analysis using auto-analyser. The animals were quickly dissected and the brain, heart, kidney, liver and lungs were excised and weighed to determine the relative organ weights.

Weekly body weight

The body weight of each rat was assessed at different times during the study including; during the acclimatization period, and before the commencement of each dosing, once weekly and finally on the day of sacrifice.

Mortality and clinical signs

During the four-week dosing period, animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing and up to 4 h after dosing.

Relative organ weight

On the 29th day, all the animals were euthanized with chloroform. Organs namely; the brain, heart, kidney, liver and lungs were carefully dissected out and weighed in grams. The relative organ weight of each animal was then calculated as follows:

$$Relative\ organ\ weight = \frac{Absolute\ organ\ weight\ (g)}{Body\ weight\ (g)} \times 100$$

Determination of haematological parameters

Blood samples were collected through cardiac puncture into EDTA containing tubes and analysed using Sysmex SF-XE-21N Automated Haematological Analyzer. The haematological parameters analysed include: Red Blood Cell (RBC), Packed Cell Volume (PCV), White Blood Cell (WBC), Haemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) (Stiene-Martin *et al.* 1999).

Biochemical estimations

Blood collected in non-heparinized tubes were centrifuged at 1000 r/min for 10 min. The serum was separated and analysed for parameters such as serum creatinine, urea, potassium sodium, chloride and bicarbonate. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (ALB), total and direct bilirubin were analysed using BS-200 biochemistry autoanalyzer (Das *et al.* 2015).

Statistical analysis

Results were expressed as Mean ± Standard Error of the Mean (SEM). Statistical analysis for difference between means were carried out using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Values of $p < 0.05$ were considered significant.

RESULTS

Acute toxicity studies

LD_{50} is the dose that will kill fifty per cent of the population (Van Brummelen, 2000). The oral median lethal dose of the extract in mice was estimated to be >5000 mg/kg. The administration of 5000 mg/kg body weight to rats did not result in the death of any of the animals for the 14-days experimental period

Sub-chronic Toxicity Studies

Effect of the extract on body and organ weight following twenty-eight days daily oral treatment

The extract at doses of 250-1000 mg/kg had no significant ($p > 0.05$) effects on body and organ weight of rats following twenty-eight days daily oral treatment (Table 1).

Effect of the extract on haematological indices in Rats after twenty-eight days daily oral treatment

The twenty-eight days daily oral treatment with the extract at doses of 250-1000 mg/kg had no significant effect ($p > 0.05$) on all the haematological indices (Table 2).

Effect of the extract on renal function indices in Rats after twenty-eight days daily oral treatment

The extract did not significantly ($p > 0.05$) effect the serum concentration of urea, creatinine, sodium, chloride, potassium and bicarbonate at tested doses (250-1000 mg/kg) (Table 3). However, a significant ($p < 0.05$) increase in Creatinine level was observed at dose of 250 mg/kg when compared with control rats.

Effect of the extract on liver function indices in Rats after twenty-eight days daily oral treatment

There was no significant ($p > 0.05$) change on serum concentrations of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the treated rats when compared with the control. Similarly, the total protein (TP), Albumin (ALB), total bilirubin (TB) and direct bilirubin (DB) levels were not significantly ($p > 0.05$) altered (Table 4).

Table 1: Effect the Extract on Body and Organ Weight of Rats Following Twenty-Eight Days Daily Oral Administration

Parameters	Treatments (mg/kg)			
	Control	**TMME (250)**	**TMME (500)**	**TMME (1000)**
Initial Weight (g)	114.00±3.76	116.33±4.37	115.17±4.66	116.00±3.61
Final Weight (g)	149.50±4.20	155.00±2.98	146.17±0.91	140.83±1.34
Weight Change (g)	35.50±1.34	38.67±2.25	31.00±4.31	24.83±2.79
Brain (g)	1.43±0.04	1.39±0.08	1.55±0.07	1.51±0.11
Heart (g)	0.67±0.018	0.61±0.04	0.75±0.17	0.62±0.043
Kidneys (g)	1.07±0.43	0.94±0.04	0.92±0.3	1.09±0.08
Liver (g)	6.46±0.16	6.92±0.23	6.16±0.25	6.08±023
Lungs (g)	1.57±0.11	1.58±0.19	1.47±0.18	1.56±0.11
Brain (%)	0.96±0.03	0.90±0.06	1.06±0.05	1.08±0.08
Heart (%)	0.45±0.01	0.39±0.20	0.51±0.12	0.44±0.03
Kidneys (%)	0.72±0.03	0.61±0.20	0.63±0.02	0.78±0.06
Liver (%)	4.33±0.12	4.48±0.18	4.22±0.17	4.32±0.16
Lungs (%)	1.02±0.09	1.02±0.13	1.01±0.12	1.11±0.07

Data presented as Mean ± SEM, analysed using one-way ANOVA followed by Dunnett's post hoc test: n = 6, TMME = *Terminalia macroptera* methanol extract

Table 2: Effect of the Extract on Haematological Indices in Rats after Twenty-Eight Days Daily Oral Administration

Parameter	Treatments (mg/kg)			
	Control	**TMME (250)**	**TMME (500)**	**TMME (1000)**
WBC	5.03±0.26	5.65±0.12	5.48±0.43	4.75±0.22
LYMPH#	5.23±0.71	4.98±0.53	5.28±0.32	6.05±0.27
MID#	0.40±0.04	0.45±0.03	0.68±0.30	0.35±0.06
GRAN#	2.05±0.26	2.95±0.74	3.53±0.31	2.70±0.23
LYMPH%	65.38±1.89	57.73±3.98	57.10±0.80	64.73±2.03
MID%	4.25±0.33	4.70±0.49	4.73±0.78	5.13±0.39
GRAN%	30.08±1.93	37.43±3.94	38.73±1.42	30.35±2.27
RBC (10^6/uL)	5.83±0.14	6.05±0.09	6.05±0.10	6.05±0.09
HGB (g/dL)	13.40±0.50	13.48±0.13	12.95±051	12.68±0.49
HCT (10^3/uL)	40.50±1.32	41.25±1.03	40.50±2.10	37.75±1.25
MCV (fL)	87.48±3.75	91.30±2.36	85.93±2.38	87.55±1.11
MCH (pg)	33.38±2.29	33.80±2.19	29.10±1.14	29.45±0.51
MCHC (g/dL)	32.23±0.75	32.26±0.67	33.95±1.93	33.35±0.41
PLT (10^3/uL)	181.28±5.43	169.50±10.34	183.25±11.20	222.00±29.31

Data presented as Mean ± SEM, analysed using one-way ANOVA followed by Dunnett's post hoc test: n = 6, TMME = *Terminalia macroptera* methanol extract. WBC= White Blood Cell, LYMPH# = Lymphocyte Count, MID# = Monocyte Count, GRAN# = Granulocyte Count, LYMPH%= Percentage Lymphocytes, MID% = Monocyte Percentage, GRAN%= Percentage Granulocyte, RBC= Red Blood Cells, HGB= Hemoglobin, HCT= Hematocript, MCV = Mean Corpuscular Volume, MCH= Mean Corpus Hemoglobin, MCHC=Mean Corpuscular Hemoglobin Concentration, and PLT=Platelets Count

Table 3: Effect of the Extract on Urea, Creatinine and Electrolytes levels after Twenty-Eight Days Daily Oral Administration

Parameters	Treatments (mg/kg)			
	Control	TMME (250)	TMME (500)	TMME (1000)
Urea (mg/dL)	18.45±2.19	20.10±6.44	21.90±3.18	21.88±4.17
Sodium (mmol/L)	141.18±3.44	134.85±9.73	149.28±7.91	150.23±11.83
Potassium (mmol/L)	8.40±1.02	11.80±1.52	8.43±0.76	9.48±1.16
Creatinine (meq/L)	0.70±0.07	1.05±0.09*	0.80±0.07	0.80±0.04
Chloride (mg/dL)	23.00±3.37	21.50±1.55	23.25±3.84	23.25±3.75
Bicarbonate (mg/dL)	75.00±5.23	77.50±3.50	76.50±2.63	77.75±11.27

Data presented as Mean ± SEM, analyzed using one-way ANOVA followed by Dunnett's post hoc test: * = $p < 0.05$ compared control groups. n = 6, TMME = *Terminalia macroptera* methanol extract.

Table 4: Effect of the Extract on Liver Function Indices in Rats after Twenty-Eight Days Daily Oral Administration

Parameter	Treatments (mg/kg)			
	Control	TMME (250)	TMME (500)	TMME (1000)
ALT (iu/L)	18.75±2.78	22.00±5.79	21.75±1.49	19.00±6.36
AST (iu/L)	28.75±7.97	20.25±6.02	25.75±5.59	40.50±12.14
ALP (iu/L)	20.55±2.03	25.05±5.75	27.10±9.19	25.60±5.46
TP (g/dL)	5.88±1.53	5.48±1.34	6.35±0.62	6.05±0.73
ALB (g/dL)	2.68±1.41	2.93±1.10	3.20±1.44	3.08±1.03
TB (mmol/L)	9.65±0.94	8.68±0.58	8.28±0.68	9.20±0.64
DB (mmol/L)	6.38±2.47	5.90±1.94	5.70±1.00	6.65±1.36

Data presented as Mean ± SEM, analysed using one-way ANOVA followed by Dunnett's post hoc test: n = 6, TMME = *Terminalia macroptera* methanol extract, ALT=Alanine Aminotransferase, AST=Aspatate Aminitransferase, ALP=Alkaline Phosphatase, TP=Total Protein, ALB=Albumin, TB=Total Bilirubin, DB=Direct Bilirubin

DISCUSSION

The body weight changes may reflect the general health status of animals (Das *et al.* 2015). In this study, the body and relative organ weights of the rats were not affected by the *T. macroptera* extract which probably suggest that the extracts may not produce any toxic effect in the organs, following twenty-eight (28) days daily oral treatment. Studies showed that toxic substance produced significant effects in the body weight by either an increase or decrease (organomegaly or atrophy) as a result of organs or systems damage (Michael *et al.* 2007; Ebadan *et al.* 2014). Haematological study is one of the important ways for the diagnosis of causes of diseases (Jorum *et al.* 2016). Haematological parameters such as packed cell volume, haemoglobin, red blood cell, white blood cell, neutrophil and lymphocyte are used to provide useful information for diagnosis in routine clinical evaluation of the state of health of a patient (Jorum *et al.* 2016).

In this study there were no significant changes in all the haematological parameters evaluated. The values obtained for MCV were indicative of normocytic red blood cells. Similarly, the MCH and MCHC values also reflected a normochromic red blood cell. However, reduction or decrease in HGB, RBC, PLT, HCT, MCV, MCHC, monocytes or basophils values are indication of anaemia and or bone marrow toxicity (Abubakar *et al.* 2020). Therefore, changes of WBC values may be due to the stimulation of immune defence system or changes in immunological status of the body (Choudhury and Sinha, 2015). However, WBC have been considered a reliable cellular biomarker of inflammation and chronic elevation of WBC has been linked to several chronic conditions such as coronary artery disease, stroke, type 2 diabetes and leukemia (Nwuke *et al.* 2020). Some plant extracts have been reported to cause anaemia which may result from sequestration of red blood cell in the spleen, impaired red cell production or primary bone marrow dysfunction (Choudhury and Sinha, 2015; Putra and Rifa'I, 2019).

In toxicity studies, assessment of renal and liver functions is vital because both organs are essential for the survival of the animals (Olorunnisola *et al.* 2012). Electrolytes such as sodium, potassium, chloride and bicarbonate are vital for the normal body functions and they were excreted through kidney. The urea and creatinine are excreted through the kidney while electrolytes are reabsorbed and excreted in the tubules. Therefore, tubular damage may lead to retention of urea and creatinine in the blood and non-reabsorption of electrolytes. Hence, urea, creatinine and electrolytes (potassium and bicarbonate) are the most sensitive biochemical markers employed in the diagnosis of renal function (Afolabi *et al*. 2014). In liver function test, increase in AST, ALT, ALP and total protein suggests liver damage, AST is also associated with diseases of other organs such as heart and muscle but the most important marker and more specific for liver damage is the elevation of ALT (Ozer *et al.* 2008; Ezeji *et al.* 2014).

ALP is found generally in cells lining the biliary duct of the liver and it aid in the diagnosis of obstruction to the biliary system and its elevation in the blood indicates diseases like gallstone or tumour blocking the bile duct (Ozer *et al.* 2008).

CONCLUSION

The results from this study showed that twenty-eight daily oral administrations of *T. macroptera* extract at doses of 250-1000 mg/kg did not cause signs of toxicity or death in the in rats hence the extract is considered non-toxic within the tested doses.

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CONFLICT OF INTEREST

Authors have declared that there is no conflict of interest reported in this work

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