*Special Conference Edition, November, 2019*

**http://dx.doi.org/10.4314/bajopas.v12i1.83S**

***Bayero Journal of Pure and Applied Sciences, 12(1): 545 - 550***
**ISSN 2006 – 6996**

# TOXICITY STUDIES ON THE METHANOL LEAF EXTRACT OF *Ziziphus mucronata* WILLD

***[1]Idris Z. Y., [1]Danmalam U. H. and [2]A. B. Sallau, [1]Jeremiah C., and Galadanchi, F. A.**

[1] Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria- Nigeria
[2]Department of Biochemistry, Ahmadu Bello University Zaria- Nigeria
*Correspondent author: idrisbella@gmail.com Tel: +2347032737309

***ABSTRACT***

***Zizphus mucronata is a specie from the genus Ziziphus which is a member of the Rhamnaceae family. It is commonly known as buffalo thorn and locally as Magaryar kuura in Hausa language. It is being used traditionally in the treatment of various ailments including urinary, metabolic and infectious diseases. This study is aimed at evaluating the effect of methanol extract of Ziziphus mucronata on toxicity studies in Wister rats. The effect of methanol extract on percentage change in body weight and some biochemical parameters on wistar rats was carried out using the OECD guideline. The acute oral toxicity of the extracts on the wistar rats showed no sign of toxicity and mortality at 5000 mg/kg after being administered and observed for 24 hours and subsequently for 48 hours. The effect of subchronic administration of methanol extract on the body weight of the wistar rats showed significant increase (p<0.01) at week 1, 2 and 4 when compared with the control (1ml/kg normal saline). The effect of oral daily administration of the methanol extract on liver function tests produced a significant decrease (p<0.01) in ALT level at the dose of 250, 500 and 1000mg/kg also in the level of AST at the dose of 1000mg/kg (p< 0.01) when compared to control. However, the alkaline phosphatase (ALP), total bilirubin (TB) conjugated bilirubin (CB) showed no significance difference (p>0.05). The kidney function tests for urea and creatinine showed no significant difference (p>0.05) when compared to the control group. The findings from this studies can be used to as a scientific basis for the traditional use as well as safety evaluation of the leaves of this plant.***

***Keywords: Ziziphus mucronata, Percentage change in body weight, Biochemical studies***

## INTRODUCTION

*Ziziphus mucronata* is a small to medium-sized tree, 10-20 m high; with a spreading canopy. The main stem is green and hairy when young; year old branches often zigzag. Leaves are ovate to broadly ovate, shiny, densely hairy the strong thorns are in pairs from where the leaves arise between the two thorns. Flowers are small, inconspicious and yellow. Fruits are reddish-brown drupe (Orwa et al., 2009).

A decoction of the glutinous roots is commonly administered as a painkiller for all sorts of pains as well as dysentery. A concoction of the bark and the leaves is used for respiratory ailments and other septic swellings of the skin. Pastes of the root and leaves can be applied to treat boils, swollen glands, wounds and sores. Steam baths from the bark are used to purify and improve the complexion (Palmer and Pitman 1972). In East Africa, roots are used for treating snake bites. All of the above can be attributed to the peptide alkaloids and antifungal properties isolated from the bark and leaves.(Hutchings *et al.*, 1996)Its leaves and roots have been used to treat diarrhea, tumor, cough, chest complaints (Muhammed *et al.*, 2012). They are also used for treatment of dysentery, sores, glandular swellings, skin diseases, open and swollen wounds, ear inflammation, asthma, syphilis, gonorrhea, lumbago, measles, as well as rheumatic pains and fever (Vhutshilo and Peter, 2015). Other reports include the use of the roots by the Bambara and Malinke tribes in Tanzania for the psychiatric treatment, and the use of the leaf juice to prevent abortion. The plant also has analgesic and anti-inflammatory activity and antibiotic activites (Abdullahi, 2008).

## MATERIALS AND METHODS

Plant materials

The fresh leaves and fruits of *Z. mucronata* was collected from the Kudingi forest in Sabon Gari Local Government Area of Kaduna State and was taken to the herbarium unit of Botany Department of Ahmadu Bello University Zaria, Kaduna State, Nigeria for identification by the taxonomist. The plant was identified and a voucher number of 1054 was deposited for future reference.

**Preparation of Plant Material**

A sufficient quantity of the fresh leaf was collected, washed and cleaned with water, air dried to remove all foreign matter removed. Fresh *Z. mucronata* leaves were used for macroscopic and microscopic evaluation. A portion of the plant collected was shade dried for a few days at room temperature. When the leaves were completely dry, it was pulverized then kept in an air-tight container for subsequent use.

**3.9.1 Experimental Animals**

Healthy adult wistar rats of both sexes were obtained from the Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were kept under well–ventilated conditions, fed on standard laboratory animal feeds and had access to water *ad libitum*. The animals weighed before commencement of the experiment and at the end.

Acute safety studies

This study was carried out to determine the $LD_{50}$ of the extracts using Organization for Economic Cooperation and Development (OECD) method Guideline 425 (OECD, 2001). Three mice were used to determine the $LD_{50}$ of the methanol extract. All three mice were administered the extract at dose of 5000 mg/kg body weight one after the other. The animals were observed after at intervals for 14 days for the short-term outcome.

**Sub Chronic Toxicity**

A 28-days repeated-dose toxicity study of *Z. mucronata* methanol leaves extract was given to 20 rats randomly grouped into four groups containing five rats of both sexes orally based on OECD Test Guideline 425. The methanol leaf extract of *Z. mucronata* was tested at the selected oral doses of 250, 500 and 1000 mg/kg body weight. The selections of doses were based on 20% of $LD_{50}$ of the oral acute toxicity study. The wistar rats were divided into four groups of five animals each. The experimental animals were housed in a cage, made of aluminum with the top covered iron mesh. The bedding was made of saw dust, maintained at room temperature and natural daylight/night conditions. The animals weighed at on the first day before administration of the extract and weekly with the 28 days.

Group I: Received 1 ml/kg of normal saline water which served as the control group.

Group II: Received 250 mg/kg of methanol extract dissolved in distilled water.

Group III: Received 500 mg/kg of methanol extract dissolved in distilled water.

Group IV: Received 1000 mg/kg of methanol extract dissolved in distilled water.

**Biochemical Studies**

At the end of the treatment period (28 days), the animals were euthanized using chloroform and blood samples were collected by puncturing the prominent jugular vein with syringe into anti-coagulated and ethylene-diamine-tetra-acetic acid (EDTA) anti-coagulated tubes. The anti-coagulated blood was centrifuged at 3,000 rpm for 10 min and serum were collected. The level of biochemical indices such as blood urea nitrogen (BUN), Creatinine and bilirubin were determined for the control group and the treated groups using an automated hematological machine (Jaijoy *et al*., 2011).

**Statistical Analysis**

The results were expressed as Mean ± Standard Error of Mean (SEM) and percentages except where otherwise stated. The data obtained were analyzed using Statistical Package for Social Sciences (SPSS), IBM version 20 and Microsoft Excel 2010. The data was statistically analyzed using One Way Analysis of Variance (ANOVA) followed by Tukey post hoc test for multiple comparisons. Values of $p \leq 0.05$ were considered statistically significant (Duncan, 1955; Okasha *et al*, 2012).

## RESULTS

**Acute safety margin of *Z .mucronata***

**Methanol leaves extract**

The acute fixed oral dose toxicity ($LD_{50}$) of the methanol extract of *Z. mucronata* leaves was determined using (OECD, 2001) method via oral route on three (3) healthy Wistar rats for 14 days and showed that the $LD_{50}$ is greater than 5000 mg/kg for the extract. There was no mortality or any toxicity signs in the animals after the treatment.

**Subchronic Toxicity**

**Effect of Weekly Administration of the Extract on the Body Weight of Wister Rats**

The effects of oral daily administration of methanol extract on the body weight of Wistar rats showed significant increase ($p<0.01$) at 250mg/kg in week 1, week 2 and week 4 respectively when compared with the control group (1 mg/kg normal saline) (Fig 1).

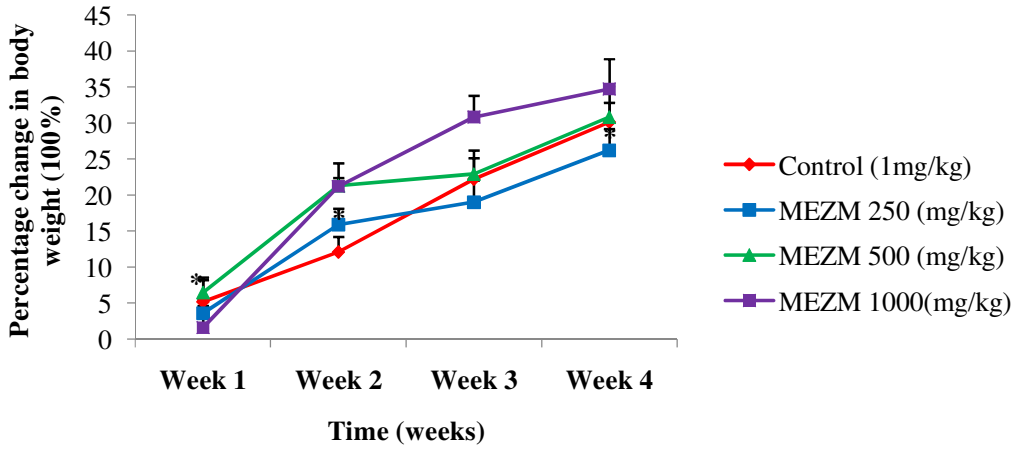


**Fig 1: Effect of sub chronic administration of methanol leaves extract of *ziziphus mucronata* on body weights of wistar rats**

Values are Mean ± S.E.M., $^{**}$= $p$<0.01, $^{***}$= $p$<0.001 compared control group (Normal saline); n=6, MEZM = Methanol extract of *Ziziphus mucronata*–Repeated measure ANOVA followed by Bonferroni post hoc test.

**Table 1: Effect of 28 days Administration of Methanol Leaf Extract of *Ziziphus mucronata* on Relative organ body Weights of Wistar Rats**

| Treatment group | Relative organ-body weight (%) | |
|---|---|---|
| | Liver | Kidney |
| Control 1 ml/kg | 3.04 ± 0.53 | 0.70 ± 0.05 |
| MEZM 250 mg/kg | 5.42 ± 0.53 | 1.21 ± 0.10 |
| MEZM 500 mg/kg | 4.57 ± 0.43 | 0.87 ± 0.07 |
| MEZM 1000 mg/kg | 4.91 ± 0.75 | 1.01 ± 0.21 |

Values are Mean ± S.E.M., No significant difference between the groups - One way ANOVA, n=6, MEZM = Methanol extract of *Ziziphus mucronata*

**Table 2: Effect of 28 days Administration of Methanol Leaf Extract of *Ziziphus mucronata* on Biochemical Parameters in Wistar Rats**

| Treatment groups | ALT (U/L) | AST (U/L) | ALP (U/L) | TB (U/L) | CB (µmol/L) | Urea (mmol/L) | Creatinine (µmol/L) |
|---|---|---|---|---|---|---|---|
| N/S 1 ml/kg | 96.33±6.84 | 60.23±8.66 | 38.80±11.89 | 12.83±2.62 | 6.63±1.27 | 52.00±4.62 | 0.73±0.09 |
| MEZM 250 mg/kg | 63.00±3.21[b] | 39.23±0.82 | 16.10±2.93 | 8.83±1.30 | 4.67±0.72 | 50.67±5.81 | 1.20±0.12 |
| MEZM 500 mg/kg | 60.00±7.02[b] | 50.20±4.95 | 12.23±3.24 | 6.97±0.97 | 4.17±0.17 | 64.00±10.07 | 1.00±0.21 |
| MEZM 1000 mg/kg | 56.67±2.03[b] | 26.10±3.67[b] | 10.63±3.01 | 10.00±0.78 | 5.53±0.87 | 85.33±21.83 | 1.40±0.23 |

Values are Mean ± S.E.M., [b] = $p<0.01$ compared normal group – One way ANOVA followed by Tukey post hoc test, n=6, ALT= Alanine amino transferase, AST = Aspartate amino transferase, ALP = Alkaline phosphatase, TB = Total bilirubin, CB= Conjugated bilirubin, D/W = Distilled water, MEZM = Methanol extract of *Ziziphus mucronata*

## DISCUSSION

The evaluation of herbal remedies for safety and efficacy is very necessary due to their growing use all over the world. Determination of median lethal dose value of traditional medicine using acute toxicity study is of paramount importance as it provides information regarding the safety margin of the plant. The median lethal dosage was estimated to be greater than 5000mg/kg indicating that the extract did not produce any sign of toxicity and could be assumed practically safe and non-toxic which the study of Zezi *et al.,* (2012) who carried out an acute toxicity on the liver and kidney and reported no sign of toxicity on both the liver and kidney function tests. This result is in agreement with Olorunisola *et al.* (2012); Parasuraman *et al*. (2014); Ugbogu *et al*. (2016) and Adesegun *et al.* (2016) that reported acute toxicity of plants could be considered practically nontoxic and safe above administration of 5000 mg/kg especially when administered orally. However, Oslon (2000); Pak *et al*. (2011); Maikai *et al.* (2008); Obidike and Salawu (2013) stated that, further toxicity assay should be carried out in order to revealed possible longer term toxicity effect on the physiology and organs for proper recommendation on its utilization.

Changes in the body weight have been used as an indicator of adverse effects of drugs and chemicals (Nandy, and Datta 2012, Hayelom *et al.,* 2012). Sub chronic toxicity studies on the extract on the mean body weight of the rats showed significant difference only in 250 mg/kg showed in week 1, 2, and 4 when compared to the control. From the result obtained from the percentage body weights calculated, there was no adverse effect on the body weight of the animals with an increasing time (Fig 1). This implies that the methanol extract may have no chemical effect on the appetite and food consumption of the Wistar rats (Grance *et al*, 2008; Nandy and Datta 2012). A similar result was reported by Sireeratawong *et al.,* (2012) who conducted a chronic toxicity studies on toxicities of the water extract from *Ziziphus attopensis* pierre and found that there was no adverse effect on the weight of the animals used over the stipulated period of time and there was also no gross morphological change in the weight of the animals.

The relative organ weight is fundamental to establish whether or not the organ was exposed to the injury. The administration of the methanol extract showed no significant change in the relative organ body weight on either the liver or kidney at all the doses administered to the animals (Table 1), this result means that there was no damage to the organs (liver and kidney).

The result of the biochemical studies of the methanol extract of *Ziziphus mucronata* at all the doses tested showed that there was a significance decrease ($p$<0.01) in the level of alkaline amino(ALT) at 250, 500 and 1000 mg/kg body weight and aspartate aminotransferase (AST) at 1000mg/kg body weight (Table 2) when compared to the control group. Nevertheless, only a significant increase in the level of these enzymes is considered a sign of toxicity. Urea and creatinine usually determine the general function of the kidney while the electrolytes are determinants of the tubular function. These parameters: urea and creatinine which were analyzed in these rats were not statistically different from the control groups and the values are within the standard, suggesting that the methanol extract of *Ziziphus mucronata* is not nephron toxic in rats within the period of the study. A decrease in glomerular filtration rate as in renal dysfunction would result in an increase serum creatinine concentration. Therefore serum creatinine is a reliable index of renal dysfunction. The serum urea level follows similar pattern, but may be affected by some factors, such as increase protein catabolism and hydration status. The Kidney plays a vital role in detoxification of drugs and xenobiotics (Harizal *et al.,* 2010). The kidney as the main excretory organ could be assessed for safety a drug (Gupta *et al*., 1994). The complexity of the liver structure and frequency of its exposure to drugs and foods that might cause harm therefore, make it susceptible to many kinds of diseases, including hepatitis, cirrhosis, fatty liver, liver cancers and genetic diseases. The liver has a unique ability to regenerate itself. Liver diseases are among the most serious ailments (Samir, 2001) and have become some of the major causes of morbidity and mortality in man and animals all over the globe and hepatotoxicity due to drugs appears to be the most common contributing factor (Nadeem *et al*., 1997).

## CONCLUSION

The study has established:

The acute toxicity profile $LD_{50}$ of the methanol extract revealed that the median lethal of the extract was greater than 5000 mg/kg which shows that the extract was practically non-toxic.

The 28 days oral administration of the methanol leaf extract at the dose of 250, 500 and 1000 mg/kg produced no significant changes in the body weight, relative organ weight

## REFERENCES


Adesegun, S.A., Celestine, I O., and Coker, H.A. (2016). Analgesic and Antioxidant Activities of Stem Bark Extract and Fractions of Petersianthus maccrocarpus. *Pharmaconosy research* 8(1): 181-185.

Duncan, D. B. (1955). Multiple ranges and multiple F- test. *Biometrics,* 11, 1–42.

Grance, S. R. M., Teixeira, M. A., Leite, R. S. and Veira, M. C. (2008). Baccharis trimera: Effect on haematological and biochemical parameters and hepatorenal evaluation in pregnant rats. *Journal of Ethnopharmacology,* 117: 28-33.

Gupta, M., Mazumder, U.K and Das, S. (1994). Effect of Alkaloidal Extract from *Clerodendron clebrookianum* on Hematological parameters and Hepatorenal function in mice. Indian Journal of Experimental Biology. 32(3): 189-191.

Jaijoy, K., Soonthornchareonnon, N., Lertprasertsuke, N., Panthong, A. and Sireeratawong, S. (2011). Acute and Chronic Oral Toxicity of Standardized Water Extract from the fruit of *Phyllanthus emblica* Linn. *International Journal of Applied Research Natural. Prod.* 3:48–58.

Muhammad, N., M. Saeed and H. Khan, (2012). Antipyretic, Anti-analgesic and Anti-inflammatory activities of Viola Betonicifolia Whole Plant. *BMC Complementary and Alternative Medicine,* 12: 59.

Nandy, S. and Datta, R. (2012). Acute and Sub-acute Toxicity Studies of Methanol Leaves of *Pterospermium acerifolium* L. Willd in Rodents. *International Journal of Pharmaceutical Life Science*.3 (3): 1519-1529.

Obidike, I. and Salawu, O. (2013). *Screening of herbal medicines for potential toxicities;* Pharmacology, Toxicology and Pharmaceutical Science; New insight in Toxicity and Drug testing. *Pub. Intech;* Pp. 63-67.

OECD. (2007). OECD Guideline for Testing of Chemicals No. 440: Uterotrophic Bioassay in Rodents: A short-term screening test for oestrogenic properties.

Olorunsola, O.S., Bradley, G. and afolayn, A. J. (2012). Acute and Subchronic toxicity of the methanol extract of Tulbaghia Violace rhizomes in Wistar rats, African Journal of Biotechnology. 11 (83):14934-14940.

Orwa C, Mutua A, Kind T R, Jamnadass R. (2009); *Agroforestry database Kenya trees, shrubs and lianas. Regional Soil Conservation Unit* (RSCU); Nairobi, Kenya.

Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., Deun, K.V., Smith, P., Berger, B., and Heller, A. (2000). Concordance of Toxicity of Pharmaceuticals in Humans and in Animals. *Regulatory Toxicology and Pharmacology*, 32:56-67.

Pak, E., Esrason, K. T. and Wu, V. A. (2004). Hepatotoxicity of Herbal remedies: An Emerging dilemma. *Progress in Transplantation,* 14:91-96.

Palmer, E. & Pitman, N. (1972). *Trees of southern Africa,* vol. 2.Balkema, Amsterdam, Cape Town.

Parasuraman S. (2011). Toxicological screening. *Journal of Pharmacol Pharmacothertherapeutics* 2 (2): 74-79.

Parasuraman, S., Raveendran, R., Rajesh, N.G. and Nandhakumar, S. (2014). Sub-chronic toxicological evaluation of cleistanthin A and cleistanthin B from the leaves of *Cleistanthus collinus* (Roxb.). *Toxicol. Rep*. [In press] (DOI: 10.1016/j.toxrep.2014.08.6).

Ugbogu, E. A., Emmanuel, O., Iheanyichukwu E, Friday U., Emmanuel A., Chinyere G. C, Faith, E. and Chizoba J. U. (2016). Toxicological assessment of the aqueous dried leaf extracts of Senna alata L. in wistar rats. *African Journal of Pharmacy and Pharmacology* 10(34): 709-717.

Vhutshilo N, Peter M. (2008) Antioxidant and antibacterial properties of *Ziziphus mucronata* and *Ricinus communis* leaves extracts. African *Journal of Traditional, Complement Alternernative Medicine.*12:81-89

Zezi A.U, Abdoulaye B.B., Danjuma N.M, Aliyu I.S, Yaro A.H and Musa K.Y. (2012), *International Journal of Pharmaceutical Research and Innovation,* 5: 1-6.