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Toxicological evaluation of *Vitex doniana* stem bark methanol extract in female albino rats

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ABSTRACT

Modern therapeutic drugs are not generally acceptable, their accessibility are limited and also have more side effects hence there is need to search for safe natural alternatives from medicinal plants. This research was aimed at evaluating the toxicological effect of methanol stem bark extract of Vitex doniana in female albino rats. Vitex doniana stembark extract was subjected to qualitative phytochemical screening, acute oral toxicity (LD50) and sub-chronic toxicity studies were carried out adopting standard procedure. Phytochemical screening showed that alkaloids, flavonoids, tannins, steroids, glycoside, balsam and volatile oil are present. The LD50 of the extract is estimated to be greater than 5000 mg/kg and no mortality or any sign of toxicity recorded within 14 days. Sub chronic toxicity studies showed a significant ($P<0.05$) progressive increase between weeks 2 - 4. In the sub-chronic toxicity study serum, AST and ALT significantly decrease ($P<0.05$) in most extract treatment groups compared to the control group. There was no significant differences ($P>0.05$) in ALB, TB and DB when compared to control group. However, ALP and TP levels where showed significantly altered ($P<0.05$) in the treated groups compared to control. Serum creatinine, urea, HCO-3 and Cl- levels of treated groups were not significantly different ($P>0.05$) when compared to control. Although K,+ uric acid and Na+ showed significant increase ($P<0.05$) in several treatment groups when compared to control group. Heamatological indices such as in WBC, haemoglobin, neutrophils and PCV of the treatment groups were not significant different ($P>0.05$) compared to control. However, lymphocytes, monocytes, eosinophil and basophils showed a significant increase ($P<0.05$) in some of the extract treatment groups compared to control group. Histopathological examination on liver and uterus of rats showed normally distributed portal triad, central vein and hepatocytes and regular endometrial gland and stroma respectively. The uterus also exhibit a dose dependent decrease in endometrial thickness. In conclusion, Vitex doniana methanol stem bark extract is relatively nontoxic at both acute and subchronic treatment.

Keywords: Toxicity, Acute, Subchronic, Vitex doniana, liver, kidney, histology.

1. INTRODUCTION

The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that synthetic products have contraindication and side effects. Now-a-days, there is rapid increase in the use of medicinal plant in industries for medicinal purposes globally, with a speed of 7-15% yearly (Paarakh, 2010). amidst recent advances in modern medicine, the synthesis of medicine from plant products is still considered more important; this is simply because they are not artificially synthetized thus make more compatible with human system (Paarakh, 2010).

Herbal medicines and their derivatives have been use for disease therapy in traditional medicine virtually since the beginning of recorded history, but it is only in recent times that the wider use of medicinal plants is beginning to widely draw attention and interest of people globally (Daniyal and Akram, 2015). There are certain challenges in the process, including inadequate quality control and toxicity study, the idea to manage expiring duration, and adherence to global regulatory protocols that need to be pass before subjecting them to marketing stage (Daniyal and Akram, 2015).

Alam *et al*., (2006) defined toxicity as an aspect of pharmacology which deals with the adverse effects of bioactive substances on living organisms. Toxicological studies are required to generate the safety, efficiency of any plant and to make a decision whether it should be adopted for clinical use or not (Anisuzzaman *et al*., 2001). Information on the toxicity both acute and chronic toxicity are important since science requires the validation of drugs by medicinal practitioners and drug regulatory authorities demand that all potential drugs should pass through a rigorous series of study and scrutiny (Abdulrahman, 2004).

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Sample

The plant sample was collected in July 2019 from Zuru town, Zuru Local Government Area of Kebbi State. It was authenticated by a Taxonomist from Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aleiro, with a voucher specimen (V.N. 307) deposited in the herbarium of the same Department.

2.2 Plant Preparation and Extraction

The stem bark of *V. doniana* were washed with clean water and allowed to dry under shade for two weeks. It was then grinded to coarse powder using grinding machine. One thousand five hundred grams (1500g) of the powdered stem bark was soaked in 4000mls of methanol for 72 hrs (Dupont *et al.,* 2002). Subsequently it was filtered using muslin cloth and the filtrate was evaporated using an oven set at 45^{o}C.The dried extract was stored in an air tight container and kept in refrigerator at 4 ^{o}C. Percentage yield was calculated using the formula.

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of ground plant material}} \times \frac{100}{1}$$

2.3 Experimental Animals

The albino (Wistar) rats used in this study are purchased from Animal House, Usmanu Danfodiyo University, Sokoto in August, 2019. They were transported in plastic ventilated cages to Animal House, Faculty of Science, Kebbi State University of Science and Technology, Aleiro. The rats were allowed to acclimatise for two (2) weeks before the commencement of the experiment. The rats are fed with standard rodent pellets and have freedom to water *ad libitum.*

2.4 Acute Oral Toxicity Studies (LD_{50})

The acute oral toxicity study was conducted according to Organization for Economic and Cultural Development for testing of chemicals (OECD, 2001) guideline and up and down method was used for the study. A total of Fifteen (15) animals were divided into five (5) groups of three (3) rats each and used for the experiment. A single oral limit test dose of 1000, 2000, 3000, 4000 and 5000 mg/kg bodyweight was administered to group I, II, III, IV and V respectively. After the administration, food was withheld for a further 3-4 hrs. The animals were observed individually at least once during the first 30min after dosing, periodically at 8hrs, 14hrs, 24hrs, and 48hrs intervals. The animals are observed for signs of drowsiness, hair loss, loss of appetite, salivation, tremors, convulsion and bulging of the eyes. The animals were thereafter observed for a period of 14 days for any signs of delayed toxicity and mortality.

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2.5 Sub-chronic Oral Toxicity Study

Sub-chronic oral toxicity study was carried out in compliance to Organization for Economic and Cultural Development (OECD 407, 2008) guideline. Fifteen female albino rats were grouped into five groups containing three animals each. The rats were treated as follows:

Group I	Served as the normal control. No extract treatment.
Group II	Administered with 250 mg/kg bodyweight.
Group III	Administered with 500 mg/kg bodyweight.
Group IV	Administered with 1000 mg/kg body weight.
Group V	Administered with 2000 mg/kg body weight.

The methanol extract is orally administered to the animals daily for 28 days. Bodyweight changes were also monitored weekly throughout the period of experiment. The rats were sacrificed on the twenty-ninth day of the experiment. Blood samples are collected in heparinized bottles for biochemical analysis while organs (livers and uterus) were collected for histopathology evaluation.

2.5.1 Measurement of Liver Function Test

Alkaline phosphatase activity was estimated using the method of Sood, (1999).Aspartate aminotransferase catalytic activity and alanine aminotransferase activity were determined by the method of Reitman and Frankel, (1957). Albumin was determined using bromocresol green method as modified by Doumas *et al*., (1971). Total protein was estimated using the Biuret reaction method by Lowry *et al*., (1951). Total and Direct Bilirubin were determined by the calorimetric method of Jendrassik and Grof, (1938).

2.5.2 Measurement of Renal Function Markers

Serum urea was determined using the Berthelot colorimetric method of Young, (1937). Serum creatinine was determined using Jaffe's method as described by Bartels and Bohmer, (1971). Serum uric acid concentration was determined using the method of Henry *et al*., (1957). Serum sodium and potassium ions were measured using flame photometry (Cheesbrough, 1991). Serum bicaronate and chloride ions were measured using titration/volumetric method (Chapman, 1961).

2.5.3 Haematological Analysis

Hematological parameters via, white blood cells count (WBC), hemoglobin concentration, packed cell volume (PCV), lymphocytes, neutrophils, esosinophils, monocytes and basophiles, , were analysed using an automated hematological analyzer Sysmex XS800i (Sysmex corporation, USA) (Theml *et al*., 2004).

2.5.4 Histopathological Examination

Histopathology was done according to the method of Drury *et al*., (1967). Liver and uterus of the rats were harvested and preserved in 10 % formalin. The organs were fixed in 10 % buffered formalin for 72 hours. The tissues are then dehydrated in alcohol of graded concentrations and embedded in paraffin. Embedded tissues were cut into sections of 5 μm thick and these are stained with eosin and hematoxylin for photo microscopic assessment and placed on a clean labelled microscope glass slide. The slide was mounted on an electric light microscope for examination of any possible histopathological features. Photomicrographs of the samples were then taken.

2.6 Data Analysis

The data generated from the study are presented as mean ± Standard Deviation (SD), followed by one-way analysis of variance (ANOVA) and statistical difference between means were seperated using Duncan multiple comparison test using (SPSS) version 20. Values are considered statistically significant at $P<0.05$. Graphs are plotted using Microsoft excel and Prism software, micrographs and diagrams were presented where necessary using digital camera.

3. RESULTS

3.1. Acute Toxicity (LD_{50}) Profile

The testing was terminated when the upper limit (5000 mg/kg) was reached without mortality and the LD_{50} was considered to be greater than 5000mg/kg b.w.

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3.2. Sub-chronic Effect of *V. doniana* Stem Bark Extract

3.2.1 Effect of *V. doniana* Stem Bark Extract on Body Weight (g) of Rats

The body weight of albino rats treated with methanol stem bark extract of *V. doniana* (MSBEVD) for 28 days showed no significant reduction ($P>0.05$) in bodyweight across all treated groups between week 0 to week 1 of administration. Subsequently, progressive increase in bodyweight of rats was observed over the remaining period of extract treatment week 2 to week 4. However in control group, the bodyweight increases consistently from week 0 to week 4 (Figure 1).

3.2.2 Effect of MSBEVD on Biomarkers of Liver Function

The liver biomarkers of toxicity assayed revealed a significant reduction ($P<0.05$) in AST in all the treated groups compared to normal control group except for the group treated with 500mg/kg which it's reduction is not significant ($P>0.05$), ALT shows a significant reduction ($P<0.05$) in all the treatment groups compared to normal control group except for the group treated with 500mg/kg which it's reduction is not significant ($P>0.05$). ALP biomarkers on the other hand increases across all groups however the increase are not significantly different ($P>0.05$) in all the treated groups as compared to normal control group except for the group treated with 1000mg/kg which showed a significant increase ($P<0.05$) compared to control group (Table 1). However total protein, total bilirubin, direct bilirubin, and ALB decreased in value, these decreases were not significant ($P>0.05$), except the group treated with 1000mg/kg of total protein which significantly reduced compared to control group ($P<0.05$).

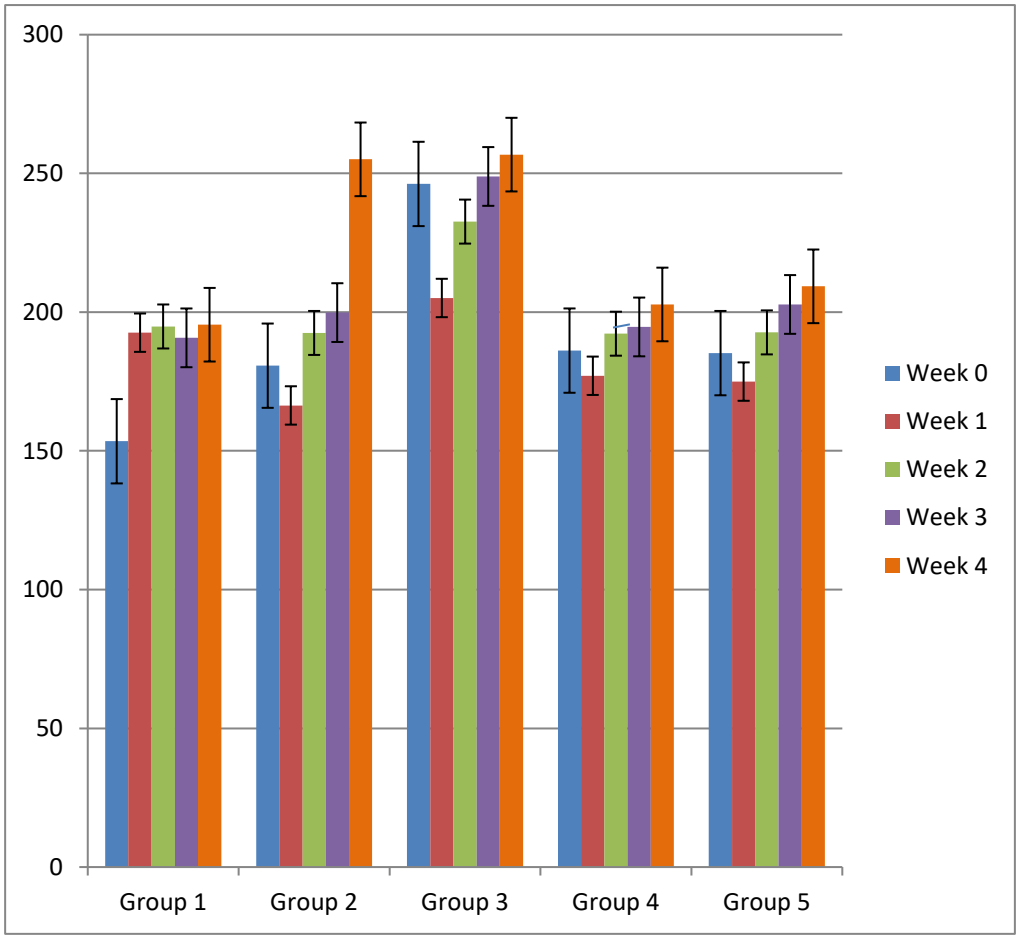

Figure 1: weight of animals administered with MSBEVD for four weeks.

{Group 1(normal control), group 2 (250mg/kg b.w), group 3 (500mg/kg b.w), group 4 (1000mg/kg b.w) and group 5 (2000mg/kg b.w)}.

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Table 1: Effect of MSBEVD on Biomarkers of Liver Function in Female Albino Rats

Parameter	Control	(250mg/kg)	(500mg/kg)	(1000 mg/kg)	(2000 mg/kg)
ALP (U/L)	22.00±5.57^{a}	23.33±4.93^{a}	28.00±2.00^{ab}	35.33±7.77^{b}	28.00±3.00^{ab}
AST (U/L)	137.00±12.49^{d}	111.00±5.57^{bc}	124.67±9.71^{cd}	93.67±22.19^{b}	58.33±10.60^{a}
ALT (U/L)	39.67±3.22^{b}	24.00±3.61^{a}	38.00±2.65^{b}	28.0000±4.36^{a}	22.33±9.07^{a}
ALB (G/l)	52.67±4.73^{a}	47.33±6.11^{a}	47.67±6.43^{a}	49.67±1.53^{a}	48.33±8.33^{a}
TP (G/l)	84.33±3.22^{b}	80.67±3.06^{ab}	84.33±4.93^{b}	76.67±3.51^{a}	78.33±3.06^{ab}
TB (mg/dL)	0.67±0.12^{a}	0.67±0.15^{a}	0.60±0.27^{a}	0.50±0.10^{a}	0.47±0.06^{a}
DB (mg/dL)	0.35±0.12^{a}	0.20±0.09^{a}	0. .21±0.03^{a}	0.28±0.04^{a}	0.32±0.03^{a}

Values are presented as mean ± SD (n = 3) value having similar superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. AST-Aspartate Amino Transferase, ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, ALB- Albumin, TP- Total Protein, TB- Total Bilirubin and DB- Direct Bilirubin

3.2.3 Effect of MSBEVD on Biomarkers of Kidney Function

The results of renal function biomarkers are present in Table 2 (Creatinine, Urea, Uric Acid, HCO_3^-, Na^+, K^+, and Cl^-), biomarkers are not significantly different (P>0.05) compared to normal control group, except for groups treated with 500mg/kg and 2000mg/kg 0f uric acid and Na^+ respectively which significantly increases when compared with control groups (P<0.05). 500, 1000 and 2000mg/kg body weight of K^+ parameter significantly increase compared to control group, while group treated with 250mg/kg is not significantly different with control group (P>0.05).

3.2.4 Effect of MSBEVD on Haematological indices

The extract (MSBEVD) did not cause significant changes in all the treated groups compared to control (P>0.05) in white blood cells, Haemoglobin, neutrophils and Packed Cell Volume as showed in (Table 3). Also, eosinophils and monocytes did not show any significant different (P>0.05) compared to normal control group except for the groups treated with 2000mg/kg which significant increase (P<0.05) from the control groups respectively. There was significant increase (P<0.05) in basophils in all the treated groups as compared to normal control groups except the group treated with 500mg/kg b.w which it's increase is not significant when compared with control group (P>0.05) . Also, there is no significant difference in groups treated with 250mg/kg and 500mg/kg at (P>0.05) in lymphocytes, While there is significant increase in groups treated with 1000mg/kg and 2000mg/kg of lymphocytes when compared to control groups (P<0.05).

Table 2: Effect of MSBEVD on Biomarkers of Kidney Function in Female Albino Rats

Parameter	Control	(250mg/kg)	(500mg/kg)	(1000 mg/kg)	(2000 mg/kg)
Urea (mmol/l)	7.10±0.403^{a}	6.97±0.39^{a}	7.84±0.03^{a}	7.43 ±0.02^{a}	7.47±0.24^{a}
Creatinine (mg/dl)	1.37±0.065^{a}	1.45±0.03^{a}	1.45±0.05^{a}	1.34±0.04^{a}	1.30±0.00^{a}
Uric acid (mg/dl)	2.69±0. 21^{a}	2.86±0. .21^{ab}	3.20±0.06^{b}	2.4633±0.04^{a}	2.73±0.04^{a}
Na^+ (mmol/l)	138.26±1.35^{bc}	135.79±1.82^{b}	143.10±0.90^{c}	141.65±2.33^{c}	130.13±0.15^{a}
K^+ (mmol/l)	5.56±.19^{a}	5.42±0.27^{a}	6.12±0.12^{b}	6.79±0.12^{c}	6.47±0.10^{bc}
HCO_3^-(mmol/l)	21.43±0.26^{ab}	21.39±0.61^{ab}	21.52±0.42^{ab}	21.22±0.51^{a}	23.11±0.68^{b}
Cl^- (mmol/l)	92.11±6.32^{a}	90.58±3.59^{a}	104.08±3.65^{a}	92.85±1.89^{a}	96.98±3.63^{a}

Values are presented as mean ± SD (n = 3) value having similar superscript are not significantly different at (P>0.05) using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. Potassium (K^+), Sodium (Na^+), Bicarbonate (HCO_3^-).

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Table 3: Effect of MSBEVD on Haematological Indices in Female Albino Rats

Doses (mg/kg)	WBC (x10⁹/L)	HGB (g/dL)	PCV (%)	LYM (%)	NEU (%)	MON (%)	EOS (%)	BAS (%)
Control	6.73±0.67[a]	14.53±0.41[a]	44.67±0.88[a]	29.33±4.18[a]	48.33±5.24[a]	5.33±0.33[a]	2.67±0.33[a]	0.67±.33[a]
250	6.67±0.34[a]	16.57±0.67[a]	44.00±1.15[a]	33.33±2.33[a]	52.33±2.03[a]	6.67±0.67[ab]	4.00±0.58[ab]	2.00±0.00[c]
500	8.93±0.97[a]	16.63±1.45[a]	42.33±1.20[a]	36.00±3.61[ab]	47.67±4.98[a]	7.00±0.58[ab]	3.33±0. 67[a]	1.00±0.00[ab]
1000	7.50±0.61[a]	16.37±1.23[a]	41.33±1.86[a]	47.67±2.33[c]	53.33±4.33[a]	6.67±0.33[ab]	4.33±0.33[ab]	1.67±0.33[bc]
2000	8.60±1.10[a]	16.33±0.69[a]	42.67±1.45[a]	45.33±3.18[bc]	57.00±0.57[a]	8.33±0.67[b]	5.33±0. 67[b]	1.33±0.33a[bc]

Values are presented as mean ± SD (n = 3) value having similar superscript are not significantly different at (P>0.05) using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. EOS- Eosinophils, MON- Monocytes, NEU- Neeutrophils, LYM- Lymphocytes, HGB- Haemoglobin, PCV- Packed Cell Volume, BAS- Basophils, WBC- White Blood Count.

3.4 Histopathological Examination of Liver and Uterus Tissues

Histopathological examination results show that the Liver of rats in control group and groups treated with 250, 500, 1000, 2000 mg/kg of (MSBEVD) exhibited normally distributed portal triad, central vein and hepatocytes (Plate1-5) respectively. Also uterus of rats in control group and groups treated with 250, 500, 1000, 2000 mg/kg of MSBEVD showed regular endometrial gland and stroma (Plate 6-10) respectively. However there was decrease in endometrial thickness in all extract treated groups compared to control. There was no obvious light microscopic evidence of cellular injury.

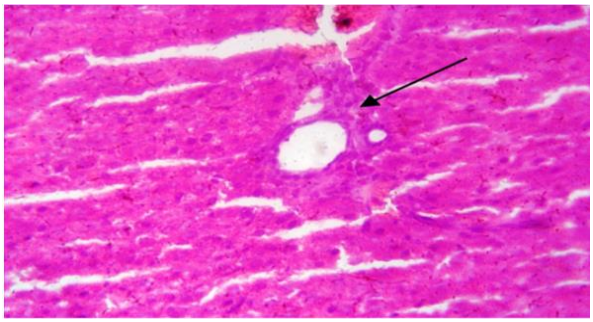

Plate 1: Photomicrograph of rat's liver obtained from control
(H and E stain, x 100 magnification). Showing normal portal triad and hepatocyte (Arrow)

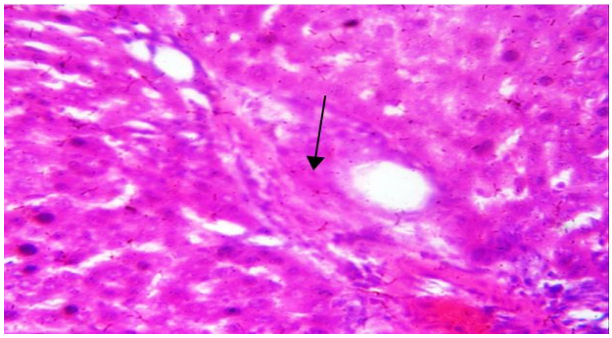

Plate 2: Photomicrograph of rat's liver obtained from group administered with 250 mg/kg of extract
(H and E stain, x 100 magnification). Showing normal portal triad and hepatocyte (Arrow)

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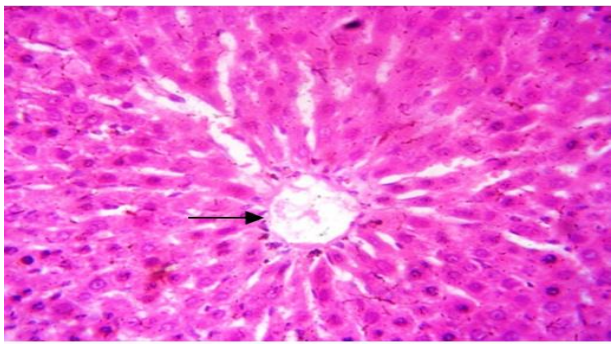

Plate 3: Photomicrograph of rat's liver obtained from group administered with 500 mg/kg of extract
(H and E stain, x 100 magnification). Showing normal portal triad and hepatocyte (Arrow)

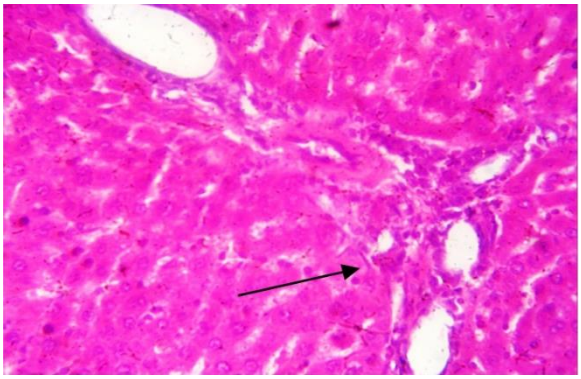

Plate 4: Photomicrograph of rat's liver obtained from group administered with 1000 mg/kg of extract
(H and E stain, x 100 magnification). Showing normal portal triad and hepatocyte (Arrow)

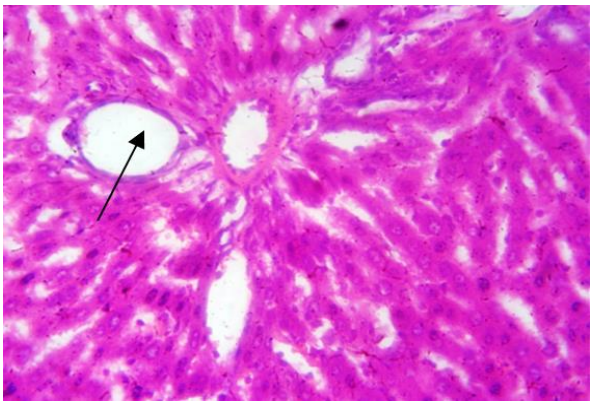

Plate 5: Photomicrograph of rat's liver obtained from group administered with 2000 mg/kg extract
(H and E stain, x 100 magnification). Showing normal portal triad and hepatocyte (Arrow)

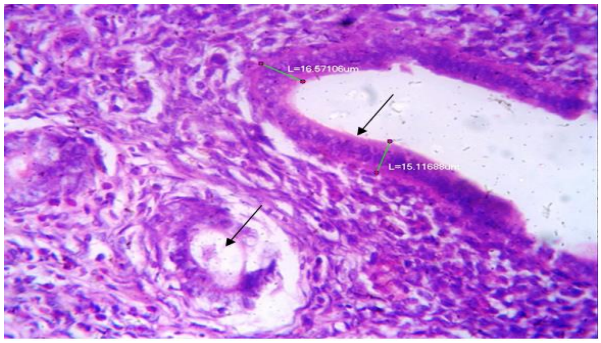

Plate 6: (control): Photomicrograph of rat's uterus obtained from control
(H and E stain, x 100 magnification). Showing normal uterus structure Regular endometrial gland and stroma (Arrow)

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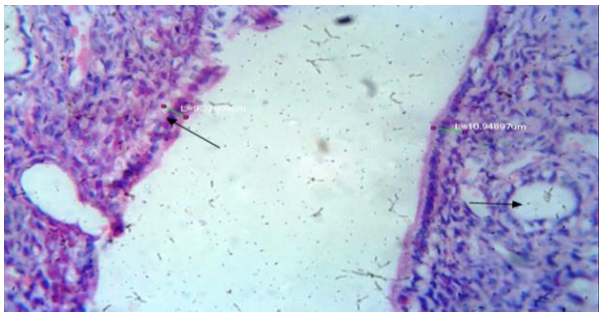

Plate 7: Photomicrograph of rat's uterus obtained from group administered with 250 mg/kg of m extract

(H and E stain, x 100 magnification). Showing normal uterus structure Regular endometrial gland and stroma (Arrow)

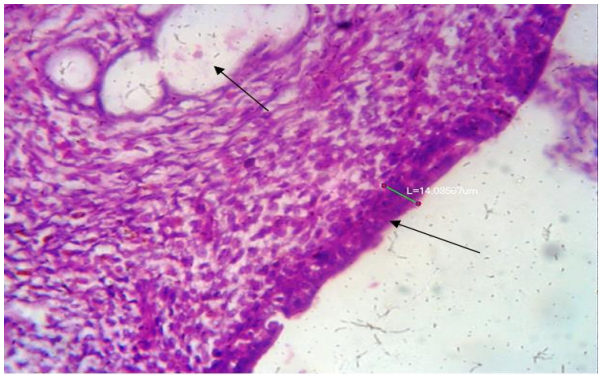

Plate 8: Photomicrograph of rat's uterus obtained from group administered with 500 mg/kg of extract

(H and E stain, x 100 magnification). Showing normal uterus structure Regular endometrial gland and stroma (Arrow)

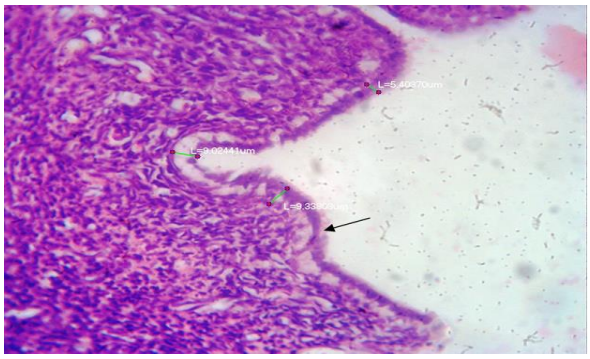

Plate 9: Photomicrograph of rat's uterus administered with 1000 mg/kg of extract

(H and E stain, x 100 magnification). Showing normal uterus structure Regular endometrial gland and stroma (Arrow)

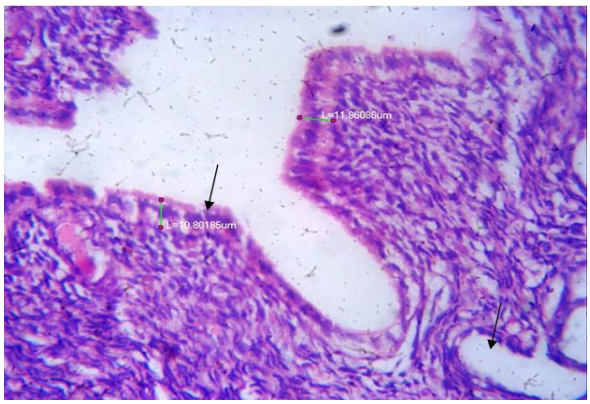

Plate 10: Photomicrograph of rat's uterus administered with 2000 mg/kg of extract

(H and E stain, x 100 magnification). Showing normal uterus structure Regular endometrial gland and stroma (Arrow)

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4. DISCUSSION

Toxicological evaluation is done to determine the safety of drugs and plant products for human use (Gautam *et al*., 2012). Determination of LD50 (lethal dose that would kill 50% of the tested population) is usually the first step in the evaluation of the toxic characteristics of a substance (Ogbuehi *et al*., 2015). According to Akhila *et al*. (2007), it provides information on health consequences likely to arise from short-term exposure to drugs. Clark and Clark, (1997) were of the opinion that any substance who's LD_{50} in rats falls between 50-500 mg/kg should be regarded as very toxic, while substances with LD_{50} above 500 mg/kg but below 1000 mg/kg are classified as being moderately toxic and those with 5000mg/kg are nontoxic. The present study showed that, LD_{50} of MSBEVD was greater than 5000 mg/kg indicating that it is nontoxic at "acute dose.

Weight loss or gain in animals has major financial and medical implications, as witnessed by the plethora of popular diets and the relationship of weight change to health and disease (Wing and Phelan 2005). Body weight loss is one of the dramatic and consistent changes resulting from chronic social stress and considerably more indicative for underlying toxicity than is weight gain (Wing and Phelan 2005). The decreased body weight gain in extract treated rats in the first week is not extract related but may be attributed to stress, lost of appetite, increase muscle waste, loss of tissue proteins and decreased feed consumption (De *et al*., 2011). After the first week of treatment, the animals might have acclimatized and adapt to the environmental conditions leading to efficient feeding and storage of excess macro-nutrients as reported by Ryosuke *et al.,* (2012).

Alkaline phosphatase (ALP) catalyses phosphorylation and dephosphorylation of DNA, RNA and proteins (Adebayo *et al*., 2007)] Serum amino transferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicate the concentration of hepatic intracellular enzymes that leaked into the circulation. These are the markers for hepatocellular injury (Hyder *et al*., 2013). An increase in ALP level may be due to membrane damage, because ALP is a membrane bound enzyme (Jaroslaw *et al*., 2009). High levels of serum ALP activity is usually detected in patient with liver damage, cancer and heart infections (Ophardt, 2003). ALT and AST are clinically significant aminotransferases both are markers of liver disease; however. They are predominantly present in the liver elevated levels of one or more liver enzyme is an indication for hepatotoxicity (Weibrecht *et al*., 2010). Lack of alteration in serum ALP, AST and ALT suggest that *V. doniana* stem bark did not cause hepatocellular injury.

Albumin (ALB) and total proteins (TP) are globular proteins found in the serum and they are synthesized by the liver (Thapa and Walia, 2007). Elevation in serum total protein is as a result of tissue injury while a significant decrease in total protein contents of the liver is a consequences resulting from hepatic toxicity (Gatsing *et al*., 2005). Decrease in albumin and total protein is a sign of reduced synthetic ability of the liver or might be due to impaired hepatocellular function (Thapa and Walia, 2007). Serum TP and ALB concentration of the present study were not altered indicating that the synthetic capacity of the liver was not affected.

Bilirubin is an essential biomarker of excretory function of the liver and in the diagnoses of haemolytic anaemia (Thapa and Walia, 2007). Oboh, (2005) stated that catabolized product of haemoglobin and is also use to estimate the binding, excreting and conjugating ability of a hepatocyte. Bilirubin is produced mainly from the haem moiety of haemoglobin molecules and synthesized in the liver. Persistence increase in serum bilirubin (hyperbilirubinaemia) is as a result of abnormal bilirubin metabolism, which leads to chemical condition called jaundice (Garba *et al*., 2014). High concentration of serum bilirubin may be as results of consequences from excessive haemolysis, cytotoxicity to the liver or from blockage into the bile ducts (Garba *et al*., 2014). In this study, serum level of bilirubin was not altered indicating that the binding, excreting and conjugating ability of the liver was not affected by MSBEVD.

The kidneys play a vital role in the excretion of waste products and toxins such as urea, creatinine and uric acid, regulation of extracellular fluid volume, serum osmolality and electrolyte concentrations, Creatinine values are illustrators of renal functions; increased serum creatinine concentration are associated with significant renal impairment (Aliyu *et al*., 2007). Urea and uric acid are the major nitrogen containing metabolic end products of protein and purine catabolism respectively (Wright, 1995). The function of a healthy kidney is to remove these compounds from the blood to be excreted in the urine; elevation of these metabolites in the blood is an indication of renal dysfunction (Cotran *et al*., 2005). In the present study, *V. doniana* stem bark extact did not affect the serum concentration of creatinine, urea, and uric acid indicating that the excretory functions of the kidney are not affected.

Electrolyte panels are consistently used to examine for an electrolyte or acid-base imbalance and to check the effect of therapeutics on a known imbalance that is affecting tissue function (Gowda *et al*., 2010). Testing for electrolytes includes the measurement of Na^+, K^+, bicarbonate and chloride for both diagnosis and treatment of renal, endocrine, acid-base, water balance, and several conditions (Gowda *et al*., 2010). The complication of both decreased filtration and decreased secretion of potassium in distal tubule during renal failure leads to increased plasma potassium (Gowda *et al*., 2010). High potassium level (hyperkalemia) is among the most significant and life-threatening complication of renal failure (James *et al*., 2006). Low sodium level (hyponatremia) has many implications, including consumption of too many fluids, kidney failure, heart failure, cirrhosis, polyuria and brain

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dysfunction (James *et al*., 2006). Although increase and decrease in the level of potassium and sodium respectively was observed in the present studies, but clinical implications such as renal failure, heart failure, polyuria or brain dysfunction were not noticed. However clinical implication of hyperkalemia and or hyponatremia may be persistence at higher dose of MSBEVD.

Haematology is the specialty responsible for the detection and control of a wide range of benign and malignant disorders of the red and white blood cells, platelets and the coagulation system in animals (Akpamu *et al*., 2011). WBC are important components of the blood, their function is to fight against foreign substances, and they are also substantial part of immune system, high concentration of WBC count is an indication of immune response, fighting against foreign bodies (Akpamu *et al*., 2011). Administration of *V. doniana* stem bark extract stimulated increased production of WBC even thus the increase is not significant from day 28 of treatment. This could be as a result of possible stimulation of immune system as reported by Kashinath, (1990). Furthermore, reports have shown that persistent antigen load in the body results in lymphocytosis (Schalm, *et al*., 1975). Lymphocytosis may be primarily responsible for the increases in WBC count in the present study since MSBEVD showed effect on serum concentration of lymphocytes.

A mild elevation of monocytes is relatively common and does not usually cause any clinical problem (Nelson *et al*., 2008). Neutrophils are the primary white blood cells that respond to a bacterial infection (Nelson *et al*., 2008). The most common cause of marked neutrophilia is a bacterial infection Persistent elevation of neutrophils may be a sign of acute myeloid leukaemia, and elevation of basophils may indicate a myelo proliferative disorder (Nelson *et al*., 2008). Although the extract does not cause any change in serum neutrophils, however the effect seen in monocyte, eosinophils and basophils may not be associated with any disease but may be due immune response provoked by the extract (Kashinath, 1990).

Haemoglobin (Hb) and packed cell volume (PCV) are among the most useful indicators in the diagnosis of anaemia in humans and animals (Akpamu *et al*., 2011). Destruction of (Hb) or (PCV) or their decreased production, may lead to anaemia (Adedapo *et al*., 2007). In the present study, (Hb) and (PCV) did not show any alteration and suggest that there is no lysis of blood cells and anaemia.

5. CONCLUSION

Vitex doniana is one of the plant used for several medicinal purposes, several species of Vitex genus have been reported with medicinal potential. The result from the present study concludes that oral administration of methanol stembark extract of *Vitex doniana* did not cause any toxic effect at both acute and subchronic treatment, suggesting that the extract is relatively nontoxic at both acute and subchronic treatment.

Ethical approval

The Animal ethical guidelines are followed in the study for experimentation. The ethical guidelines for plants & plant materials are followed in the study for experimentation.

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Abdulrahman, F. I. (2004) Studies on the chemical and pharmacological activities of the root back extract of Vitex doniana Guill and pers. (black plum). Ph.D Thesis, Department of Chemistry, University of Maiduguri, Maiduguri, Nigeria. 1-4.
2. Adebayo HA, Aliyu R, Gatsing D, Garba HI. The effects of ethanolic leaf extract of Commiphora africana (Burseraceae) on rat liver and kidney functions. Journal of Pharmacology and toxicology. 2007;2(4):373-9.
3. Adedapo AA, Abatan MO, Olorunsogo OO. Effects of some plants of the spurge family on haematological and biochemical parameters in rats. Vet Arhiv. 2007 (1):29-38.

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

4. Akhila JS, Shyamjith D, Alwar MC. Acute toxicity studies and determination of median lethal dose. Current science. 2007 Oct 10:917-20.
5. Akpamu U, Nwaopara AO, Izunya AM, Oaikhena GA, Okhiai O, Idonije BO, Osifo UC. A comparative study on the acute and chronic effect of oral administration of Yaji (a complex Nigerian meat sauce) on some hematological parameters. British Journal of Pharmacology and Toxicology. 2011 Aug 5;2(3):108-12.
6. Alam AH, Islam R, Salam KA, Manir MM, Baki MA, Hossain MA, Sadik G. Toxicological studies of N-trans-ferloyl-4methyldopamine isolated from Achranthes ferruginea. Pak J Biol Sci. 2006;9:1052-5.
7. Aliyu R, Adebayo HA, Gatsing D, Garba HI. The effects of ethanolic leaf extract of Commiphora africana (Burseraceae) on rat liver and kidney functions. Journal of Pharmacology and toxicology. 2007;2(4):373-9.
8. Bartels H, Bohmer M. Microestimation of creatinine. Clinica Chimica Acta. 1971;32:81-5.
9. Chapman, D. H., and F. Parker. "Determination of NPK; Methods of Analysis for Soil, Plants and Water, pp: 150–179." *Pub. Div. Agri. Univ. California, USA* (1961).
10. Cheesbrough M. Medical laboratory manual for tropical countries. M. Cheesbrough, 14 Bevills Close, Doddington, Cambridgeshire, PE15 OTT.; 1991.
11. Clark E. GC., and Clark M.L. *Veterinary toxicology*. 2nd ed, 1977; Nailliere Tindall, New York.; pp10
12. Cotran RS, Robbins SL, Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease. InRobbins and Cotran pathologic basis of disease 2010 (pp. 1450-1450).
13. Daniyal, Muhammad, and Muhammad Akram. "Antifertility activity of medicinal plants." *Journal of the Chinese Medical Association* 78, no. 7 2015: 382-388.
14. De, D., Chatterjee, K., Ali, K. M., Bera, T. K., & Ghosh, D. Antidiabetic potentiality of the aqueous-methanolic extract of seed of Swietenia mahagoni (L.) Jacq. in streptozotocin-induced diabetic male albino rat: a correlative and evidence-based approach with antioxidative and antihyperlipidemic activities. *Evidence-Based Complementary and Alternative Medicine*, *2011*.
15. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta. 1971 1;31(1):87-96.
16. Drury, Roger Anderson Brownsword, Eric Alfred Wallington, and Sir Roy Cameron. *Carleton's histological technique*. London, 1967.
17. Dupont E, Falardeau P, Mousa SA, Dimitriadou V, Pepin MC, Wang T, Alaoui-Jamali MA. Antiangiogenic and antimetastatic properties of Neovastat (AE-941), an orally active extract derived from cartilage tissue. Clinical & experimental metastasis. 2002;19(2):145-53.
18. Garba A, Mada SB, Ibrahim G, Abarshi MM. Hepatoprotective effect of ethyl acetate extract of vitex doniana stem bark on Carbon Tetrachloride (CCl4) induced liver damage in Wistar rats. Am J Biochem and Molecul Biol. 2014;4(1):35-41.
19. Gatsing D, Aliyu R. Kuiat, e JR, Garba IH, Jaryum KH, Tedongma N, Tchouanguep MF, Adoga GI. Toxicological evaluation of the aqueous extract of Allium sativum bulbs on laboratory mice and rats. Cameroon Journal of Experimental Biology. 2005;1:39-45.
20. Gautam MK, Singh A, Rao CV, Goel RK. Toxicological evaluation of Murraya paniculata (L.) leaves extract on rodents. Am J Pharmacol Toxicol. 2012;7(2):62-7.
21. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AA, Vernekar SN. Markers of renal function tests. North American journal of medical sciences. 2010 Apr;2(4):170.
22. Henry RJ, Sobel C, Kim J. A modified carbonate-phosphotungstate method for the determination of uric acid and comparison with the spectrophotometric uricase method. American journal of clinical pathology. 1957 Aug 1;28(2):152-60.
23. Hyder MA, Hasan M, Mohieldein AH. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. European journal of experimental biology. 2013;3(2):280-4.
24. James, S. and Mitchell, G. (2006). Physiology and disorder of water, electrolytes and acid-base metabolism. In: Carl, A.B., Edward, R., David, E. editors. Tietz textbook of clinical chemistry and molecular diagnostics. 4th Edition. New Delhi: Elsevier inc. 1747-1776.
25. Jaroslaw S, Armand M, Gizowska M, Marcinek M, Sasim E, Szafran M, Wieczorek W. Ceramic-in-polymer versus polymer-in-ceramic polymeric electrolytes—A novel approach. Journal of Power Sources. 2009 Oct 20;194(1):66-72.
26. Jendrassik, L. and Grof, P. Simplified photometric methods for the determination of bilirubin. *Biochemistry*, 1938; 297:81-89
27. Kashinath, R. T. "Hypolipimedic effect of disulphide in rate fed with high lipid and/or ethanol." PhD diss., Ph. D Thesis, University of Bangalore, 1990.
28. Khalil MI, Motallib MA, Anisuzzaman AS, Sathi ZS, Hye MA, Shahjahan M. Biochemical analysis of different brands of unifloral honey available at the northern region of Bangladesh. Sciences. 2001;1(6):385-38.
29. Lowry, O.H., Rosenbrough, N.T., Farr, A.L. and Randall, R.J. Protein measurements with the Folin phenol reagent. *Journal of Biology and Chemistry*, 1951;193:265-275.
30. Nelson M, Horsman DE, Weisenburger DD, Gascoyne RD, Dave BJ, Loberiza FR, Ludkovski O, Savage KJ, Armitage

JO, Sanger WG. Cytogenetic abnormalities and clinical correlations in peripheral T-cell lymphoma. British journal of haematology. 2008 May;141(4):461-9.
31. Oboh G. Hepatoprotective Property of Ethanolic extract of fluted pumpkin leaves against garlic–Induced Oxidative stress. J. Med. Food. 2005;8(4):560-3.
32. OECD,. OECD Guidelines for testing chemicals. Organisation for economic operation and development: Acute toxicity – up and down procedure (2001). No., 425:1-26.
33. Ogbuehi, Ijeoma H., Omotayo O. Ebong, and Atuboyedia W. Obianime. "Oral acute toxicity (LD50) study of different solvent extracts of Abrus precatorius Linn leaves in wistar rats." *European Journal of Experimental Biology* 5, no. 1 (2015): 18-25.
34. Ophardt CE. Diagnostic serum enzymes. Retrieved on December. 2003;6:2018.
35. Organisation for Economic Co-operation and Development. Test No. 407: repeated dose 28-day oral toxicity study in rodents. OECD Publishing; 2008.
36. Paarakh, Padmaa M. "Nigella sativa Linn.–A comprehensive review." (2010).
37. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology. 1957 J 1;28(1):56-63.
38. Ryosuke, F, Oyaizu S, Nukui Y, Ngwe T, Shinkai T, Koike S, Kobayashi Y. Use of bean husk as an easily digestible fiber source for activating the fibrolytic rumen bacterium Fibrobacter succinogenes and rice straw digestion. Animal Science Journal. 2012;83(10):696-703.
39. Schalm, Oscar William, Nemi Chand Jain, and Edward James Carroll. *Veterinary hematology*. No. 3rd edition. Lea & Febiger., 1975.
40. Sood, R. Medical laboratory technology. Method and interpretation. Sed. Jaypee brothers medical publishers Ltd, 1999; 488-490.
41. Thapa BR, Walia A. Liver function tests and their interpretation. The Indian Journal of Pediatrics. 2007;74(7):663-71.
42. Theml, Harald, Heinz Diem, and Torsten Haferlach. *Color atlas of hematology: practical microscopic and clinical diagnosis*. Thieme, 2004.
43. Weibrecht K, Dayno M, Darling C, Bird SB. Liver aminotransferases are elevated with rhabdomyolysis in the absence of significant liver injury. Journal of Medical Toxicology. 2010;6(3):294-300.
44. Wing RR, Phelan S. Long-term weight loss maintenance–. The American journal of clinical nutrition. 2005;82(1):222S-5S.
45. Wright PA. Nitrogen excretion: three end products, many physiological roles. The Journal of experimental biology. 1995; 198(2):273-81.
46. Young DS. Effects of drugs on clinical laboratory tests. Annals of clinical biochemistry. 1997;34(6):579-81.

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