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

Angela Nnenna Ukwuani-Kwaja, Ibrahim Sani, Abdulhamid Zubairu

**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* stem bark extract was subjected to qualitative phytochemical screening, acute oral toxicity (LD50) and subchronic 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 subchronic 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 basophil 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 widespread 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 synthesized thus make more compatible with human system (Paarakh, 2010).

Herbal medicines and their derivatives have been used 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°C. The dried extract was stored in an air tight container and kept in refrigerator at 4°C. Percentage yield was calculated using the formula.

\[
\text{Percentage yield} = \left( \frac{\text{weight of extract}}{\text{weight of ground plant material}} \right) \times 100
\]

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 (LD₅₀)
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.
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 bodyweight.
- **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 Jaffé’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 bicarbonate 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, eosinophils, 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 separated 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.
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.](image-url)

(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)).
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, HCO₃⁻, Na⁺, K⁺, and Cl⁻), biomarkers are not significantly different (>0.05) compared to normal control group, except for groups treated with 500mg/kg and 2000mg/kg of 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 1: Effect of MSBEVD on Biomarkers of Liver Function in Female Albino Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>(250mg/kg)</th>
<th>(500mg/kg)</th>
<th>(1000 mg/kg)</th>
<th>(2000 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L)</td>
<td>22.00±5.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.33±4.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.00±2.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.33±7.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.00±3.00&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>AST (U/L)</td>
<td>137.00±12.49&lt;sup&gt;d&lt;/sup&gt;</td>
<td>111.00±5.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>124.67±9.71&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>93.67±22.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.33±10.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>39.67±3.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.00±3.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.00±2.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.0000±4.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.33±9.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALB (G/l)</td>
<td>52.67±4.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.33±6.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.67±6.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.67±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.33±8.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TP (G/l)</td>
<td>84.33±3.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.67±3.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.33±4.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.67±3.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.33±3.06&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>TB (mg/dL)</td>
<td>0.67±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DB (mg/dL)</td>
<td>0.35±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>(250mg/kg)</th>
<th>(500mg/kg)</th>
<th>(1000 mg/kg)</th>
<th>(2000 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mmol/l)</td>
<td>7.10±0.403&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.97±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.84±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.43±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.47±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.37±0.065&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.45±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.45±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.69±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.86±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.20±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4633±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.73±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na⁺ (mmol/l)</td>
<td>138.26±1.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>135.79±1.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>143.10±0.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>141.65±2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>130.13±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>K⁺ (mmol/l)</td>
<td>5.56±1.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.42±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.12±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.79±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.47±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO₃⁻(mmol/l)</td>
<td>21.43±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.39±0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.52±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.22±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.11±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cl⁻ (mmol/l)</td>
<td>92.11±6.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.58±3.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.08±3.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.85±1.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.98±3.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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 (HCO₃⁻).
Table 3: Effect of MSBEVD on Haematological Indices in Female Albino Rats

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>WBC (x10^9/L)</th>
<th>HGB (g/dL)</th>
<th>PCV (%)</th>
<th>LYM (%)</th>
<th>NEU (%)</th>
<th>MON (%)</th>
<th>EOS (%)</th>
<th>BAS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.73±0.67a</td>
<td>14.53±0.41a</td>
<td>44.67±0.88a</td>
<td>29.33±4.18a</td>
<td>48.33±5.24a</td>
<td>5.33±0.33a</td>
<td>2.67±0.33a</td>
<td>0.67±.33a</td>
</tr>
<tr>
<td>250</td>
<td>6.67±0.34a</td>
<td>16.57±0.67a</td>
<td>44.00±1.15a</td>
<td>33.33±2.33a</td>
<td>52.33±2.03a</td>
<td>6.67±0.67ab</td>
<td>4.00±0.58ab</td>
<td>2.00±0.00c</td>
</tr>
<tr>
<td>500</td>
<td>8.93±0.97a</td>
<td>16.63±1.45a</td>
<td>42.33±1.20a</td>
<td>36.00±3.61ab</td>
<td>47.67±4.98a</td>
<td>7.00±0.58ab</td>
<td>3.33±0.67a</td>
<td>1.00±0.00ab</td>
</tr>
<tr>
<td>1000</td>
<td>7.50±0.61a</td>
<td>16.37±1.23a</td>
<td>41.33±1.86a</td>
<td>47.67±2.33bc</td>
<td>53.33±4.33a</td>
<td>6.67±0.33ab</td>
<td>4.33±0.33ab</td>
<td>1.67±0.33bc</td>
</tr>
<tr>
<td>2000</td>
<td>8.60±1.10a</td>
<td>16.33±0.69a</td>
<td>42.67±1.45a</td>
<td>45.33±3.18bc</td>
<td>57.00±0.57ab</td>
<td>8.33±0.67b</td>
<td>5.33±0.67b</td>
<td>1.33±0.33abc</td>
</tr>
</tbody>
</table>

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-Neutrophils, 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)
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)
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)
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 LD50 in rats falls between 50-500 mg/kg should be regarded as very toxic, while substances with LD50 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, LD50 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, loss 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 consequence 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, cytoxicity 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 extract 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, polypegia and brain
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 stem bark 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


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