Safety Evaluation of Crude Ethanol Extract of *Waltheria Indica* Linn. Root In Male Wistar Rats

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ABSTRACT:
Safety evaluation of *Waltheria indica* Linn. root was carried out in Wistar rats. Twenty adult male Wistar rats were randomly divided into four groups (n=5); A–D. Group A served as control group while groups B, C and D were administered with 200, 400 and 800 mg/Kg of crude ethanol extract of *Waltheria indica* Linn. root. After 28 days of administration, the rats were sacrificed and blood samples were collected and serum prepared. Haematological and biochemical parameters, as well as serum electrolyte were determined according to standard procedures. The 200 mg/Kg of *Waltheria indica* Linn. root caused the highest percentage increase in body weight. The kidney was also significantly increased at 400 mg/kg. The total WBC and lymphocytes counts were significantly increased at 400 mg/kg. The serum levels of ALT and chloride were significantly increased at 800 mg/kg. *Waltheria indica* Linn. root also caused hepatic necrosis and renal tubular degeneration in Wistar rats. Therefore, *Waltheria indica* Linn. root should be used with caution especially at higher dose despite its therapeutic potentials.

Keywords: *Waltheria indica* Linn. root, haematology, Serum chemistry, electrolytes, Wistar rats

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INTRODUCTION

*Waltheria indica* Linn. (Sleepy morning) belongs to the family *Sterculiaceae* [1]. It is widespread in West Africa [2]. In Nigeria the plant is locally known as 'hankufah' or 'hankubah' in Hausa, 'kafafi' in Fulfulde, 'korikodi' in Yoruba and 'efu-abe in Nupe [3].

The uses of the plant are diverse. The antioxidant effects [4], antibacterial effects [5], antimalarial [6], anti-inflammatory effects [7], anticonvulsants effects [8], antidiabetic effect [9] and trypanocidal effects [10, 11] of *Waltheria indica* have been reported.

Despite the numerous uses of this plant, there is dearth of information on the safety of this plant. Therefore, we evaluated the safety of *Waltheria indica* Linn. root in Wistar rats.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The *Waltheria indica* Linn. plants were obtained from a farm land in Moniya in Akinyele area Council of Ibadan, Oyo state, Nigeria. The plant was identified at the University of Ibadan Herbarium and a specimen was deposited and assigned a voucher number UIH-22371. The *Waltheria indica* plants were separated into the leave, stem and root. The root was air-dried at room temperature (25 °C) under the shade in a room for three weeks. The *Waltheria indica* Linn. root was sorted to eliminate any dead matter and other unwanted particles and then pulverized into fine powder using a mechanical blender (Henry West®, China). The grounded *Waltheria indica* Linn. root was then used for the extraction of crude extract.

Preparation of the crude extract of *Waltheria indica* Linn. root

The extraction was done as previously described by [12]. One kilogram of powdered *Waltheria indica* Linn. root was soaked in five Litres of ethanol for 24 hours at ambient temperature (25 °C). The mixture was filtered and the filtrate concentrated using a rotary evaporator at 40 °C (Jinotech instruments, China) and then evaporated to produce a brown powdery dry extract.

Experimental Animal

Twenty adult male Wistar rats obtained from the Experimental Animal House, Faculty of Veterinary Medicine, University of Ibadan, Ibadan were used for the study. The rats were fed with pelletised Grower poultry feeds (Vital feeds® produced by Grand Cereals Limited, Jos, Nigeria) and water provided *ad libitum*. The rats were kept at the Experimental Animal House of the Faculty of Veterinary Medicine, University of Ilorin, Ilorin. They were acclimated to their new environment for two weeks before the commencement of the experiment. All experimental protocols were in compliance with the University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care. University of Ibadan Animal Care and Use Research Ethics Committee gave approval for this research. The reference number for the approval is UI-ACUREC/APP/2016/002.

Acute toxicity studies

Acute toxicity studies of ethanol extract of *Waltheria indica* root was carried out in male Wistar rats according to Organisation for Economic Co-operation and Development (OECD) guideline 425 [13]. Nine rats were randomly divided into three groups (n=3). The first group was administered with distilled water and served as control while the second and third groups were administered with 2000 and 3000 mg/kg of ethanol extract of *Waltheria indica* Linn. root respectively using distilled water as a vehicle. Distilled water and *Waltheria indica* Linn. root extract were given to rats using oral gavage. The experimental animals were monitored for behavioural changes, toxicity signs and death of the rats after treatment for 2-3 hours, then over a period of a day and thereafter daily for two weeks.

Dosing protocol

One-fifteenth of (3000 mg/Kg) LD<sub>50</sub> was used as the minimum dose for the sub-acute toxicity study. Twenty adult male rats were divided into four groups of five animals per group. The first
group was administered with distilled water (3 mL/Kg) and served as control. While the treatment groups were administered with 200, 400 and 800 mg/kg of crude ethanol extract of *Waltheria indica* Linn. root. The administration was done orally using oral gavage daily for 28 days. The rats were thereafter sacrificed and organ and blood samples collected.

**Weighing of rats and their organs**
All experimental rats were weighed before the start of the experiment and thereafter on weekly basis until the last day of the experiment. The organs were weighed with electronic balance (Golden Metler®) and relative organ weights calculated [14].

Relative organ weight (%) = \( \frac{\text{Weight of the organ}}{\text{Body weight}} \times 100 \)

**Collection of blood samples and serum preparation**
Blood samples (about 2 mL) were collected through the orbital sinus from diethyl ether anaesthetized rats into heparinised bottles for haematological studies. Blood samples (about 3 mL) collected in plain tubes were allowed to clot. The serum was separated from the clot and centrifuged (3000 rpm for 15 minutes) into Eppendorf tubes for biochemical.

**Determination of haematological parameters**
Haematological parameters such as Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell count (RBC), White Blood Cell (WBC) count, platelet count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined using an automatic analyzer (HA6000 Auto Hematology Analyzer, China).

**Differential Leucocyte Count**
A fresh smear of each blood sample was prepared, fixed with methanol and then stained with Giemsa stain. One hundred cells were identified morphologically, counted and the number of each leukocyte type was calculated as a percentage of the total white blood cells from which the absolute lymphocyte, neutrophil, eosinophil, basophil and monocyte counts were determined.

**Determination of serum biochemical parameters**

**Determination of Serum AST, ALT and ALP Activities**
Serum levels of AST, ALT and ALP activities were determined using Randox diagnostic kits (UK). Briefly, AST and ALT activities were based on the principle described by [15]. AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546 nm, and ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546 nm. ALP was determined in accordance with the principles of [16]. The p-nitrophenol formed by the hydrolysis of p-nitrophenyl phosphate confers yellowish colour on the reaction mixture and its intensity was monitored at 405 nm to give a measure of enzyme activity.

**Estimation of serum proteins**
Total serum protein, albumin were estimated by the method of [17] using Randox assay Kits, UK. Blood Urea Nitrogen (BUN) and Creatinine were assayed using Randox assay kits, (UK) following the manufacturer's instructions.

**Estimation of serum level of electrolyte**
Serum levels of Sodium and potassium ion were determined by flame photometric method using a flame photometer (Jenway Limited, Donmow Essex, UK) at a wavelength of 598 nm and 769 nm for sodium and potassium ions respectively [18]. Chloride ion in serum was determined by end point colourimetric titration method [19]. Serum bicarbonate was estimated by titrimetric method [18].

**Histopathology**
Small pieces of liver, kidneys, spleen, seminal vesicle and testes were collected from each rat in 10% formaldehyde solution for histology.
The tissues were dehydrated by passing them through different concentration of ethanol (70%, 95% and 100%) for 2 hours at each concentration. The tissues were then cleared to remove the alcohol (ethanol) that the tissues had bathed in and to initiate and complete a process that will make cells transparent at microscopic level. The clearing was done with xylene for 6 hrs. The tissues were then infiltrated by placing them in molten paraffin wax which serves as support to the tissues for subsequent stage of sectioning. Thereafter the tissues were embedded, which is the positioning of the processed, infiltrated tissues in molten paraffin wax within an enclosure called a mould. The embedded tissues were left until the wax solidified. Thereafter, the tissues were cut into blocks (little chunks) and they are held in position by paraffin wax. The blocks were then clamped and positioned for sectioning. Sectioning was done with a Semi-automatic microtome (Kedee®, China) at a preset thickness of 4 µm. The satisfactory sections were picked up with microscope glass slides that had been coated on one side with glycerin egg albumin. The slides carrying the sections were then labelled with a diamond pencil. They were then arranged in a slide carrier and then put in an oven (DHG-9023A oven, Labscience, England) to dry.

The slides were then stained with Haematoxyline and Eosin (H&E) stain as follows: the slides were de-waxed with xylene three times for 3 minutes each, they were then re-hydrated in 100%, 96% and 75% ethanol for 3 minutes each and then in water for 3 minutes. The slides were thereafter stained in Haematoxylin for 15 minutes; the excess stain was washed off. The slides were then placed in 1% acetic acid for 5 seconds, which was then washed off. The slides were then counterstained in Eosin for 3 seconds. The slides were then dehydrated in serial ethanol solutions (50%, 70%, 95%, and 100%). The slides that have been stained were then cleared in xylene. After staining with Haematoxylin and Eosin (H&E), slides were examined under microscope (Olympus®, Germany) for histopathological changes and photographed with camera AmScope®, Japan mounted on the microscope [20].

Statistical analysis
The data obtained were expressed as Mean ± Standard Deviation (Mean ± SD). The data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Dunnett’s Multiple Comparison Test using GraphPad Prism® (Version 5.0, San Diego, CA). P values less than or equal to 5% were regarded as significant.

RESULTS

Result of extraction of Waltheria indica Linn. root
About 3.31Kg of Waltheria indica Linn. root yielded 221.8 g of the crude ethanol extract (6.7%).

LD₅₀ and acute toxicity study of Waltheria indica Linn. root in male rats
Acute toxicity study of Waltheria indica Linn. root in male rats is shown in Table 1. The highest dose of Waltheria indica Linn. root (3000 mg/kg) did not cause death. Therefore, the LD₅₀ of crude ethanol extract of Waltheria indica Linn. root in male Wistar rats is more than 3000 mg/kg.

Result of sub-acute toxicity study

Effect of Waltheria indica Linn. root on Body weight male Wistar rats
The changes in body weight of rats after exposure to crude ethanol extract of Waltheria indica Linn. root is shown in Table 2. The treatment groups showed higher increase in percentage body weight than the group orally administered with distilled water. Highest increase in body weight was observed with the group given 200 mg/kg (group B) while the 800 mg/kg caused the least increase in body weight.

Effect of Waltheria indica Linn. root on relative organ weight of rats
The 400 mg/kg dose of ethanol extract of Waltheria indica Linn. root significantly increased (p<0.05) the weight of the kidneys. There was no significant change in the weight of other organs measured (Table 3).
Haematological parameters of male Wistar rats after the administration of crude ethanol extract of *Waltheria indica* Linn. root.
The effect of crude ethanol extract of *Waltheria indica* Linn. root on haematological parameters of male rats is shown in Table 4. The 400 mg/kg dose caused a significant increase (p<0.05) in total white blood cell counts. More so, lymphocyte count also showed significant increase (p<0.01) at 400 mg/kg. There was no significant effect (p>0.05) on PCV, RBC count, haemoglobin concentration, platelets and red cell indices (MCH, MCV, MCHC) of male Wistar rats at the three doses of the extract administered.

Effects of crude extract of *Waltheria indica* Linn. root on serum biochemistry and electrolytes parameters of male Wistar rats.
The 400 and 800 mg/kg caused significant increase (p<0.001) in level of Alkaline phosphatase. The serum level of Alanine Amino Transferase (ALT) showed significant increase (p<0.05) at the highest dose (800 mg/kg) administered. More so, blood urea level showed significant increase (p<0.001) for the 200 and 800 mg/kg group compared to the control group. There were no significant effects on other serum biochemical parameters analysed (Table 5).

The changes in serum electrolyte of male rats after exposure to crude ethanol extract of *Waltheria indica* Linn. root is shown in Table 6. The 200 mg/kg dose of *Waltheria indica* Linn. root significantly increased (p<0.05) levels of sodium and chloride ions. More so, chloride ion showed significant increase (p<0.001) at 800 mg/kg dose when compared with the control.

Result of the histopathology of vital organs
The result of the effect of *Waltheria indica* Linn. root on histopathology of liver, kidney, spleen, seminal vesicle and testis of rats is shown in Figures 1 to 5 respectively. The crude ethanol extract of *Waltheria indica* Linn. root caused hepatic necrosis at 200 and 800 mg/kg. There was also interstitial congestion (200 mg/kg), renal tubular degeneration (400 mg/kg) in the kidney. All doses of crude ethanol extract of *Waltheria indica* Linn. root caused lymphoid hyperplasia in the spleen. The 400 and 800 mg/kg of crude ethanol extract of *Waltheria indica* Linn. root caused germ cell depletion and loss of interstitium in the testis respectively.

DISCUSSION
The LD₅₀ of crude ethanol extract of *Waltheria indica* Linn. root in male Wistar rats is greater than 3000 mg/kg. This shows that *Waltheria indica* Linn. root has wide safety margin and can be classified as “Class IV” compound. This LD₅₀ is similar to the one obtained by [21] for *Waltheria indica* Linn. leafy stem. This similarity in the LD₅₀ is due to similar phytochemical components of the *Waltheria indica* Linn. root and stem [22].

The 200 mg/kg of crude ethanol extract of *Waltheria indica* Linn. root caused the highest percentage increase in body weight while the percentage change in body weight decreases as the dose increases (Table 2). This is in agreement with other plants in the family sterculiaceae [23]. This observation indicates that the 200 mg/kg is the optimal dose for use of *Waltheria indica* Linn. root as feed supplement in male Wistar rats. This may be associated with nutritional composition of *Waltheria indica* Linn. root. More so, the decrease body weight with increasing dose of *Waltheria indica* Linn. root indicates that *Waltheria indica* Linn. root may be toxic at higher doses.

The effect of crude extract of *Waltheria indica* Linn. root on the relative weight of most of the organs evaluated showed low toxicity of *Waltheria indica* Linn. root [24]. Organ-somatic index is used to assess toxicity of medicinal plants. The non-significant effect of crude ethanol extract of *Waltheria indica* Linn. root on haematological parameters disagrees with the report of haematinic potential of *Waltheria indica* Linn. root [25]. The haematinic effect may be due to iron content of *Waltheria indica* Linn. root. The non-significant increase in haematological parameters may be due to the fact that red blood cell cannot concentrate haemoglobin
beyond its normal level [26]. Hence, the haematological parameters were not significantly increased despite the iron content of *Waltheria indica* Linn. root. The non-significant effect on haematological parameters also indicates the non-toxic effect of *Waltheria indica* Linn. root on haemopoietic organs.

The increase in white blood cell count shows that the principal function of phagocytes which is to defend against microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes. This leukocytosis corroborates the use of *Waltheria indica* Linn. as immune booster [27]. This is supported by the lymphoid hyperplasia caused by *Waltheria indica* Linn. root in the spleen observed in the present study (plate 3). Lymphocytes accounted for the observed increases in white blood cell. Lymphocytes are the most active immune cell during trypanosomosis [28].

The assessment of serum levels of biochemical metabolites provides useful information on the toxicity or safety of plant extract and or therapeutic agent [29]. Aminotransferases (ALT and AST) served as markers of damage to liver cells. ALT is more specific to determine liver damage than AST due to its distinctive abundance in the hepatocytes [30]. While the AST is found in other organs such as the heart, brain, skeletal muscle etc.

The significant increase in ALT and ALP at 800 mg/kg as well as histological changes in the liver indicates hepatotoxic effect of crude ethanol extract of *Waltheria indica* Linn. root at this dose. This finding contrasts the report by [31] who reported the hepatoprotective effect of methanol extract of *Waltheria indica* Linn. against various NSAID-induced hepatic damage in rats. This can be attributed to the lower dose (400 mg/kg) of *Waltheria indica* Linn. and the solvent used as well as the experimental design used. The significant increase in blood urea nitrogen at 200 and 800 mg/kg indicate renotoxic effect of *Waltheria indica* Linn. root. This is corroborated by the histopathological changes in the kidneys [32].

**Conclusion**

*Waltheria indica* Linn. root caused hepatic and renal damage at 800 mg/Kg dose in male Wistar rats. *Waltheria indica* Linn. root should therefore be used with cautions despite its numerous therapeutic potentials.

**Acknowledgement**

The authors acknowledge the support of Mr. Adegboyega Afolabi of the histopathology unit and Mr. Bolaji Moshood of the clinical biochemistry unit of the Department of Veterinary Pathology, University of Ilorin for the support during the histopathology and serum biochemical assay of our samples.

**REFERENCES**


Table 1: Acute toxicity study of crude ethanol extract of *Waltheria indica* Linn. root in male Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of death recorded</th>
<th>Signs of toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0</td>
<td>No toxicity signs observed</td>
</tr>
<tr>
<td>2000 mg/kg</td>
<td>0</td>
<td>Weakness, dullness, reduced feed intake</td>
</tr>
<tr>
<td>3000 mg/kg</td>
<td>0</td>
<td>Weakness, dullness, reduced feed intake</td>
</tr>
</tbody>
</table>

Table 2: Percentage change in body weight of male Wistar rats during the sub-acute toxicity study

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight after the extract administration (g)</th>
<th>Weight before the extract administration (g)</th>
<th>Percentage difference in weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water)</td>
<td>273.6±12.22</td>
<td>268.4±11.61</td>
<td>1.90</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>242.6±13.85</td>
<td>230.6±13.50</td>
<td>4.95</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>211.6±28.26</td>
<td>206.5±35.91</td>
<td>2.41</td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>238.8±24.50</td>
<td>233.8±32.76</td>
<td>2.09</td>
</tr>
</tbody>
</table>

Results are shown as mean ± standard deviation. (n=5)

The percentage difference in weight between the two groups were not significantly different.
Table 3: Effect of ethanol extract of *Waltheria indica* Linn. root on relative organ weight of male Wistar rats

<table>
<thead>
<tr>
<th>Parameters (%)</th>
<th>Control</th>
<th>200 mg/kg</th>
<th>400 mg/kg</th>
<th>800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>1.03±0.06</td>
<td>1.17±0.09</td>
<td>1.15±0.12</td>
<td>1.12±0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>2.98±0.05</td>
<td>2.96±0.10</td>
<td>2.83±0.13</td>
<td>2.85±0.09</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>0.33±0.07</td>
<td>0.35±0.06</td>
<td>0.47±0.07</td>
<td>0.40±0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>0.36±0.03</td>
<td>0.33±0.01</td>
<td>0.36±0.02</td>
<td>0.38±0.02</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.60±0.03</td>
<td>0.71±0.04</td>
<td>0.74±0.04a</td>
<td>0.59±0.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.45±0.05</td>
<td>0.46±0.06</td>
<td>0.37±0.04</td>
<td>0.35±0.03</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SD. Values significantly different from control values

^a - p < 0.05, n=5 for each group

Table 4: Effects of crude ethanol extract of *Waltheria indica* Linn. root on the haematological parameters of male Wistar rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>200 mg/kg</th>
<th>400 mg/kg</th>
<th>800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>30.50±4.68</td>
<td>31.17±3.06</td>
<td>30.83±1.72</td>
<td>31.67±1.75</td>
</tr>
<tr>
<td>RBC (×10^{12}/L)</td>
<td>5.49±0.51</td>
<td>5.78±0.48</td>
<td>5.34±0.15</td>
<td>5.63±0.29</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>15.40±1.85</td>
<td>16.68±2.39</td>
<td>15.23±1.48</td>
<td>15.85±1.55</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>56.50±2.95</td>
<td>54.50±1.05</td>
<td>56.00±1.26</td>
<td>55.33±1.37</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>27.70±2.99</td>
<td>29.37±2.18</td>
<td>26.68±0.98</td>
<td>26.78±2.16</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>48.87±7.95</td>
<td>54.08±5.36</td>
<td>48.80±2.55</td>
<td>51.82±5.30</td>
</tr>
<tr>
<td>Platelet (×10^9/L)</td>
<td>313.20±90.29</td>
<td>383.50±91.32</td>
<td>318.20±55.28</td>
<td>314.00±86.93</td>
</tr>
<tr>
<td>WBC (×10^9/L)</td>
<td>8.88±1.34</td>
<td>10.93±1.46a</td>
<td>9.87±0.27</td>
<td>7.60±0.46</td>
</tr>
<tr>
<td>Neutrophil (×10^9/L)</td>
<td>5.10±0.78</td>
<td>6.10±1.23</td>
<td>4.39±0.40</td>
<td>4.47±0.33</td>
</tr>
<tr>
<td>Lymphocyte (×10^9/L)</td>
<td>3.58±0.74</td>
<td>4.99±1.39a</td>
<td>5.49±0.38b</td>
<td>3.30±0.16</td>
</tr>
<tr>
<td>Monocyte (×10^9/L)</td>
<td>0.20±0.08</td>
<td>0.22±0.10</td>
<td>0.22±0.10</td>
<td>0.18±0.02</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD. n=5 for each group. Significantly different values from control values

^a - p < 0.05, ^b - p < 0.01
Table 5: Result of the serum biochemical parameters of male Wistar rats after administration of crude ethanol extract of *Waltheria indica* Linn. root

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>200 mg/kg</th>
<th>400 mg/kg</th>
<th>800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Urea (mmol/L)</td>
<td>3.10±0.60</td>
<td>4.55±0.47*</td>
<td>3.15±0.71</td>
<td>4.90±0.47*</td>
</tr>
<tr>
<td>ALT (iu/L)</td>
<td>83.33±6.12</td>
<td>71.33±4.46</td>
<td>76.17±18.13</td>
<td>94.17±9.30*</td>
</tr>
<tr>
<td>ALP (iu/L)</td>
<td>46.50±3.51</td>
<td>52.67±4.63</td>
<td>64.67±5.75*</td>
<td>71.67±2.34*</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>41.00±8.51</td>
<td>35.83±8.45</td>
<td>35.00±4.56</td>
<td>44.17±6.85</td>
</tr>
<tr>
<td>AST (iu/L)</td>
<td>162.30±11.96</td>
<td>153.80±8.79</td>
<td>171.20±5.19</td>
<td>166.00±5.52</td>
</tr>
<tr>
<td>Total Protein (g/L)</td>
<td>79.00±5.93</td>
<td>85.67±7.87</td>
<td>80.33±7.53</td>
<td>76.00±4.05</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>47.00±9.53</td>
<td>48.83±4.45</td>
<td>45.17±5.57</td>
<td>39.33±18.96</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>28.83±12.98</td>
<td>30.17±3.25</td>
<td>33.33±6.62</td>
<td>26.0±5.18</td>
</tr>
</tbody>
</table>

Results are Mean±SD. n=5. a - p < 0.05, c - p < 0.001.

Table 6: Effects of ethanol extract of *Waltheria indica* Linn. root on serum electrolytes of male Wistar rats

<table>
<thead>
<tr>
<th>Parameters (mmol/L)</th>
<th>Control</th>
<th>200 mg/kg</th>
<th>400 mg/kg</th>
<th>800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>139.30±7.58</td>
<td>142.50±2.51</td>
<td>136.20±6.31</td>
<td>151.00±9.21a</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.85±0.84</td>
<td>5.15±0.71</td>
<td>4.90±0.36</td>
<td>4.98±0.16</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>26.00±3.29</td>
<td>26.17±4.88</td>
<td>26.67±2.81</td>
<td>24.83±0.75</td>
</tr>
<tr>
<td>Chloride</td>
<td>76.00±5.18</td>
<td>63.33±4.84a</td>
<td>81.00±12.33</td>
<td>60.83±2.86b</td>
</tr>
</tbody>
</table>

Mean±SD. n=5 for each group. a - p < 0.05, b - p < 0.01
Figure 1: Photomicrographs of the liver of male Wistar rats exposed to different doses of crude ethanol extract of *Waltheria indica* Linn. root. Control (A): normal liver with intact hepatocytes (white arrow). 200 mg/kg (B): mild hepatic necrosis (yellow arrows). 400 mg/kg (C): severe portal congestion and periportal cellular infiltration (oval outline). 800 mg/kg (D): severe hepatocellular necrosis (black arrow). H&E. X400

Figure 2: Photomicrographs of the kidneys of male Wistar rats exposed to different doses of crude ethanol extract of *Waltheria indica* Linn. root. Control (A): normal kidney tissue with intact glomerulus (star) and renal tubules (white arrow). 200 mg/kg (B): mild renal interstitial congestion (white arrow). 400 mg/kg (C): severe renal tubular degeneration (white arrow). 800 mg/kg (D): severe renal interstitial congestion with cellular infiltration (oval outline) as well renal tubular necrosis (white arrow). H&E. X400
Figure 3: Photomicrographs of the spleen of male Wistar rats exposed to different doses of crude ethanol extract of *Waltheria indica* Linn. root. Control (A): normal spleen showing the white pulp (WP) and red pulp (RP). 200 mg/kg (B): lymphoid hyperplasia (white arrows). 400 mg/kg (D): lymphoid hyperplasia (white arrows). 800 mg/kg (D): generalised lymphoid hyperplasia (white arrows). H&E. X400

Figure 4: Photomicrographs of the Seminal Vesicle (SV) of male Wistar rats exposed to different doses of crude ethanol extract of *Waltheria indica* Linn. root. Control (A): normal Seminal Vesicle showing the epithelial cells (arrow) and lumen of the SV (square) 200 mg/kg (B): epithelial cell hyperplasia (black arrow). 400 mg/kg (C): epithelial cell hyperplasia (black arrow). 800 mg/kg (D): epithelial cell hyperplasia (black arrow). H&E. X400
Figure 5: Photomicrographs of the testes of male Wistar rats exposed to different doses of crude ethanol extract of *Waltheria indica* Linn. root. Control (A): normal testicular tissue with intact seminiferous (germinal) epithelium (square outline) and interstitium (white star) as well as evidence of spermatozoa within the seminiferous tubular lumen (oval outline). 200 mg/kg (B): moderate depletion of germinal epithelium (square outline) and interstitial oedema (white star). 400 mg/kg (C): germ cell depletion (black arrow) and interstitial oedema (white star). 800 mg/kg (D): germ cell depletion (black arrow), reduced luminal spermatozoa (black star) and interstitium (white star). H&E. X400.