Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2022, Vol 3, Issue 2: 66-74

Original Research Article

# Toxicological evaluation of different solvent fractions of *Waltheria indica* linn. root in male wistar rats

**[*1]Basiru, A., [1]Sanusi, F., [2]Olatunji, A.O., [1]Suleiman, K.Y., [2]Aremu, A. and [2]Akorede, G.J**

*[1]Department of Veterinary Physiology and Biochemistry, [2]Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin, Nigeria.*

*Corresponding author: basiru.a@unilorin.edu.ng.com +234

## ABSTRACT

*Waltheria indica* Linn. root (WILR) is a multipurpose medicinal plant with abundance of phytochemical compounds. The optimal solvents for extraction of a particular bioactive compound depends not only on the yield of the compound but also on the toxic effect of the solvent used. Therefore, this study was designed to evaluate the toxic effects of hexane, dichloromethane and ethyl acetate solvent fractions of WILR in male Wistar rats. Three doses (200, 500, or 1000 µg/kg BW) of hexane, dichlorometh ane and ethyl acetate solvent fractions of WILR were used. Male Wistar rats (n=5) were administered with 200, 500, or 1000 µg/kg of hexane, dichloromethane, and ethyl acetate soluble extracts of WILR, while control received distilled water, daily for 15 days. The rats were thereafter sacrificed, blood samples were collected and serum separated. Haematological serum biochemical parameters were determined according to standard procedure. The result showed non-significant effect on relative organ weight and haematological parameters while the serum ALT was significantly increased by administration of hexane, dichloromethane, and ethyl acetate soluble extracts of WILR. Blood Urea Nitrogen was also increased significantly ($p < 0.05$) at 200 and 500 µg/kg of hexane and dichloromethane respectively. The ethyl acetate and hexane fraction also caused severe periportal cellular infiltration in the liver while the hexane fraction caused glomerular necrosis. The hexane, dichloromethane and ethyl acetate soluble extracts of (WILR) have adverse effect on the integrity liver and kidney. Hence, these extracts should be used with caution in patient with hepatic and renal impairment.

KeyWords: *Waltheria indica* root, Wistar rats, Liver, Kidney, Toxicity

## INTRODUCTION

Drug discovery from medicinal plants involves extraction, purification and characterization of active compound from medicinal plant (Sasidharan *et al*., 2011). Solvent extraction is the most common method and involves adding the solvent into the solid matrix; the solute dissolves in the solvents; the solute is diffused out of the solid matrix; the extracted solutes are then collected and concentrated. For the extraction procedures, solvents such as water, ethanol, chloroform, dichloromethane, hexane, ethyl acetate, and methanol are most commonly used (Mohammed, 2018). *Waltheria indica* Linn. plant (Sleeping morning) belongs to the family *Sterculiaceae* (Cacao family). It is a perennial shrub that grows in the tropical and subtropical environment. Waltheria indica Linn. plant has been reported for use in the management of many diseases in traditional medicine. Some of these diseases are animal and human trypanosomosis, malaria, bacterial infection, anemia, and fungal infection. These diseases are of economic and public health significance in sub-Sahara Africa. This makes Waltheria indica Linn. plant an essential ethnomedicinal plant in Africa (Basiru *et al*., 2021).

Different phytochemical compounds have been isolated from *Waltheria indica* Linn. roots (Termer *et al*., 2021). These compounds include flavonoids, sterols, alkaloids, terpenes, anthraquinones and phenols (Basiru *et al*., 2016). These compounds are responsible for the pharmacological properties of *Waltheria indica* plant that include anti-trypanosomal, anti-inflammatory, antimicrobial, antifungal and contraceptive effects (Zongo *et al*., 2013). To isolate the bioactive compound responsible for each of these properties from plant material, solvents such as methanol, ethanol, hexane, dichloromethane, acetone, and water, have been used. These bioactive compounds have differing solubility



http://doi.org/10.54328/covm.josvas.2022.067a

tendencies in different solvents (Truong *et al*., 2019). Therefore, this study was designed to evaluate the toxic effects of hexane, dichloromethane and ethyl acetate solvent fractions of *Waltheria indica* Linn. root in male 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 extraction of crude extract.

**PREPARATION OF THE CRUDE EXTRACT OF *WALTHERIA INDICA* LINN. ROOT**

The extraction was done as previously described by Basiru and Olayemi, (2014). Briefly, 1 Kg of powdered *Waltheria indica* Linn. root was soaked in five Litres of ethanol for a day at ambient temperature. 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 powdry dry extract.

**FRACTIONATION OF CRUDE ETHANOLIC EXTRACT OF *WALTHERIA INDICA* LINN. ROOT**

The crude ethanol extract of *Waltheria indica* Linn. root (100 g) was sequentially fractionated with hexane, dichloromethane and ethyl acetate in order of increasing polarity according to standard techniques.

**EXPERIMENTAL ANIMALS AND DOSING PROTOCOL**

Fifty healthy adult male Wistar rats were used in this experiment. The rats were obtained from the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The rats were fed with pelletized grower poultry feeds (Vital feeds®, Grand cereals Limited, Jos, Nigeria) and water was provided *ad libitum*. All experimental protocols were carried out according to internationally approved principles for the handling of experimental animal, use, and care. The ethical approval was obtained from University of Ibadan Animal Care and Use Research Ethics Committee with reference number UIACUREC/APP/2016/002. Fifty rats were randomly divided into ten groups (n = 5). Three doses (200, 500, and 1000 μg/Kg BW) each of the solvent fractions (hexane, dichloromethane, and ethyl acetate) were administered to each group, while the control was administered with distilled water. The administration was done using oral gavage for 15 consecutive days. The dosage was chosen based on the $LD_{50}$ of *Waltheria indica* Linn. root (Basiru *et al*., 2019) and the trypanocidal dose of *Waltheria indica* Linn. root (Sylvian *et al*., 2014).

**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.

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

**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 revolution per minutes (rpm) for 15 minutes) into Eppendorf tubes for biochemical and hormonal assay.

**DETERMINATION OF HAEMATOLOGICAL PARAMETERS**

Haematological parameters such as Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cells counts (RBC), White Blood Cell (WBC) counts, platelets 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 (Reltman and Frankel, 1957). 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 (Tietz *et al*., 1994**)**. 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 and globulin were estimated by the method of (Dumas *et al*., 1971) using Randox assay Kits, UK.

Blood Urea Nitrogen (BUN) and Creatinine were assayed using Randox assay kits, (UK) following the manufacturer's instructions.

**HISTOPATHOLOGY**

Small pieces of spleen, kidneys and liver were collected from each rat in 10% formaldehyde solution for histology as described by Musumeci (2014).

**STATISTICAL ANALYSIS**

The relative organ weights, haematology and serum biochemical parameters 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**

**TOXIC EFFECTS OF HEXANE FRACTION OF WALTHERIA INDICA LINN. ROOT IN MALE WISTAR RATS**

The relative weights of the testis, liver, seminal vesicles, heart, kidneys and epididymis of rats were not significantly different when treated with hexane fraction of *Waltheria indica* Linn. root (Figure 1).

There was significant increase in serum levels of ALT for the groups administered with 500 µg/Kg ($p<0.01$) and 1000 µg/Kg ($p<0.001$) of hexane fraction of *Waltheria indica* Linn. root while the serum level of AST was significantly decreased for groups administered with 200 µg/Kg ($p<0.05$) and 1000 µg/Kg ($p<0.001$) doses (Table 1). The serum level of total proteins was significant increase for all the treated groups. The group HA which was administered with the lowest dose had the most significant increase ($p<0.001$). In addition to the significant increase in total proteins, serum globulins showed significant ($p<0.001$) increase when compared with the control (Table II). More so, the blood urea showed significant ($p<0.001$) increase at the lowest dose administered.

Table III shows the haematological parameters of rats after exposure to hexane fraction of *Waltheria indica Linn.* root. There was no significant effect on haematological parameters of male rats when the treatment groups were compared with the group administered with distilled water (control).

***TOXIC EFFECT OF DICHLOROMETHANE FRACTION OF WALTHERIA INDICA LINN. ROOT IN MALE WISTAR RATS***

The result of the effect of dichloromethane fraction of *Waltheria indica* Linn. root on weight and organ weight of the male Wistar rats is presented in Figure II. The relative weight of organs of rat (testis, liver, seminal vesicles, kidney, heart and epididymis) were not significantly ($p>0.05$) affected. The administration of 500 and 1000 µg/Kg BW of dichloromethane fraction of *Waltheria indica* Linn. root significantly increased ($p<0.001$) the blood level of ALT. In addition, the group administered with the lowest dose (200 µg/Kg) also showed significant increase ($p<0.05$) in ALT level. The serum level of ALP also showed significant ($p<0.05$) increase for the 1000 µg/Kg BW group (Table 4). The blood urea level showed significantly ($p<0.05$) increased at the 500 µg/Kg BW dose (Table V). The effect of dichloromethane fraction of *Waltheria indica* Linn. root on haematology is presented in Table VI. Dichloromethane fraction of *Waltheria indica* Linn. root did not have significant effect on red blood cell count, haemoglobin value

**Table I: Effect of hexane fraction of *Waltheria indica* Linn. root on Liver enzymes of male Wistar rats**

| Parameters (IU/L) | Control | 200 µg/Kg BW | 500 µg/Kg BW | 1000 µg/Kg BW |
|---|---|---|---|---|
| ALT | 17.80±1.92 | 27.60±5.41 | 32.60±4.51[b] | 40.40±0.89[c] |
| AST | 64.60±5.73 | 47.60±13.83[a] | 65.60±3.05 | 39.60±3.78[c] |
| ALP | 41.40±6.54 | 37.60±7.30 | 44.60±3.21 | 35.20±5.81 |

Results are presented as Mean±SD. n=5 for each group. [a] - $p < 0.05$, [b] - $p < 0.01$, [c] - $p < 0.001$

**Table II: Effect of hexane fraction of *Waltheria indica* Linn. root on serum proteins, urea and Creatinine of male Wistar rats**

| Parameters | Control | 200 µg/Kg | 500 µg/Kg | 1000 µg/Kg |
|---|---|---|---|---|
| Total protein (g/L) | 47.80±2.77 | 72.80±6.02[c] | 71.00±9.82[b] | 66.60±11.72[a] |
| Albumin (g/L) | 27.40±1.67 | 33.80±7.26 | 34.80±6.76 | 36.80±9.76 |
| Globulin (g/L) | 15.60±3.65 | 30.20±2.28[c] | 23.80±2.49 | 24.00±3.39 |
| Urea (mmol/L) | 3.18±0.26 | 5.82±0.87[c] | 3.46±0.71 | 4.40±0.79 |
| Creatinine (mmol/L) | 49.00±2.74 | 47.40±2.30 | 49.60±5.13 | 42.20±6.49 |

Results are presented as Mean±SD. n=5 for each group.
[a] - $p < 0.05$, [b] - $p < 0.01$, [c] - $p < 0.001$

and red cell indices of male rats in comparison to the control (distilled water).

**Table III: Effect of hexane fraction of *Waltheria indica* Linn. root on haematological parameters of male Wistar rats**

| Parameters | Control | 200 µg/Kg | 500 µg/Kg | 1000 µg/Kg |
|---|---|---|---|---|
| RBC ($\times10^{12}$/L) | 5.67±0.39 | 5.59±0.23 | 5.40±0.05 | 5.53±0.31 |
| HB (g/dL) | 8.83±1.42 | 6.63±3.35 | 10.57±3.09 | 10.67±0.81 |
| PCV (%) | 32.33±2.52 | 32.00±1.73 | 33.33±4.93 | 30.33±2.31 |
| MCV (fL) | 57.00±2.00 | 57.00±1.00 | 55.00±1.73 | 54.33±2.08 |
| MCH (pg) | 15.70±3.47 | 11.73±5.41 | 19.63±5.84 | 19.30±1.35 |
| MCHC (g/dL) | 27.60±6.22 | 20.50±9.20 | 35.83±11.20 | 35.33±1.62 |
| WBC ($\times10^{9}$/L) | 17.60±8.43 | 17.33±3.650 | 11.83±1.31 | 8.17±2.87 |
| Neutrophil ($\times10^{9}$/L) | 8.37±4.87 | 8.73±1.44 | 5.80±0.69 | 3.85±1.59 |
| Lymphocytes ($\times10^{9}$/L) | 8.75±3.46 | 8.11±1.91 | 5.88±0.71 | 4.31±1.45 |
| Monocytes ($\times10^{9}$/L) | 0.49±0.22 | 0.43±0.19 | 0.22±0.13 | 0.18±0.12 |

Results are presented as Mean±SD. n=5 for each group. WBC = White blood Cell count

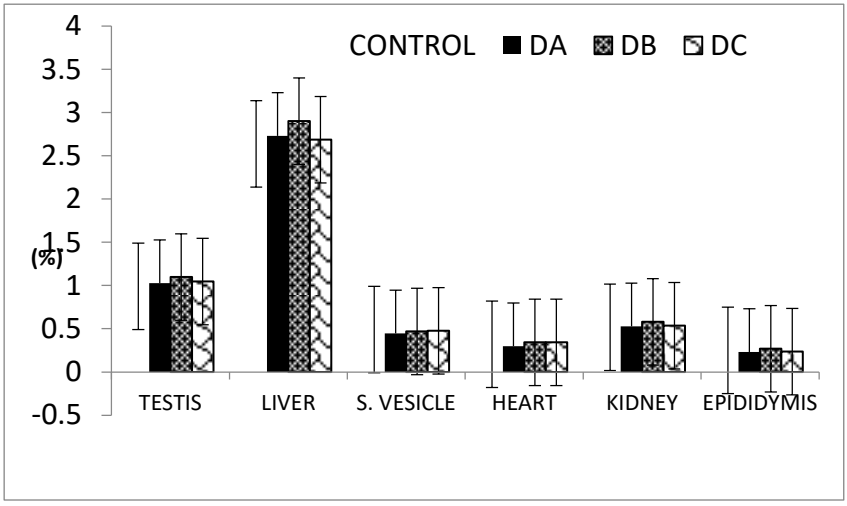


**Figure I: Effect of hexane fraction of *Waltheria indica* Linn. root on relative organ weight of male Wistar rats. n=5 for each group. S. Seminar vesicle.** HA = 200 µg/Kg, HB = 500 µg/Kg, HC = 1000 µg/Kg

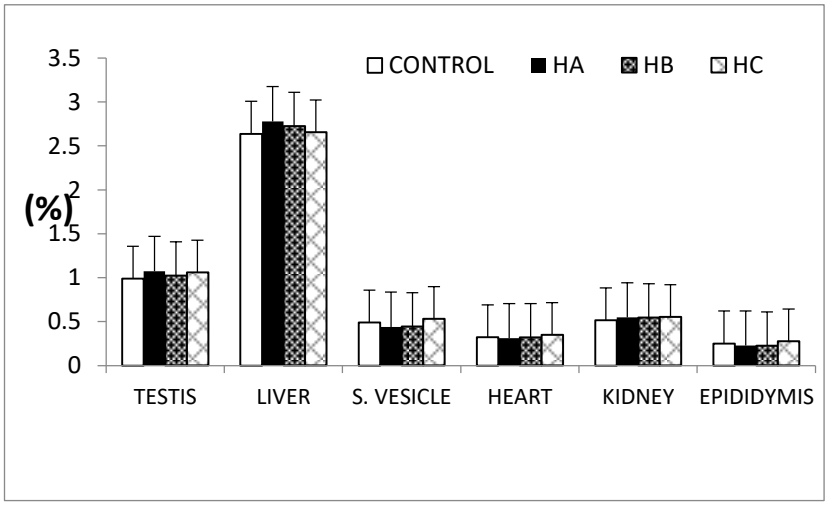


**Figure II: Effect of dichloromethane fraction of *Waltheria indica Linn.* root on organ weight of male Wistar rats. n=5 for each group.**
DA = 200 µg/Kg, DB = 500 µg/Kg, DC = 1000 µg/Kg

**TOXIC EFFECT OF ETHYL ACETATE FRACTION OF WALTHERIA INDICA LINN. ROOT IN MALE WISTAR RATS**

The administration of ethyl acetate fraction of *Waltheria indica* Linn. root to male rats did not have any significant ($p>0.05$) changes on weight and relative organ weight of rats (Figure III). There was significant increase in serum level of ALT for all the treated groups (Table 7). Groups administered with 200 and 1000 µg/Kg of ethyl acetate fraction of *Waltheria indica* Linn. root showed significant increase in ALT ($p<0.001$). The globulin also showed significant increase for the groups administered with 200 µg/Kg ($p<0.01$) and 500 µg/Kg ($p<0.001$) of ethyl acetate fraction of *Waltheria indica* Linn. root. The blood urea significantly increases following oral administration of 500 µg/Kg ($p<0.001$) and 1000 µg/Kg ($p<0.05$) of ethyl acetate fraction of *Waltheria indica* Linn. root. The effect of ethyl acetate fraction of *Waltheria indica* Linn. root on haematology of male Wistar rats is presented in Table IX. Haematological parameters of rats did not change significantly after the administration of ethyl acetate fraction of *Waltheria indica Linn.* root in comparison to the control (distilled water).

Result of histopathology of liver and kidney following the administration of hexane, dichloromethane and ethyl acetate fractions of *Waltheria indica* root

The result of the effect of hexane, dichloromethane and ethyl acetate fractions of *Waltheria indica* root on histopathology of liver and kidney of rats is shown in figure 4 to 9. The dichloromethane fraction of *Waltheria indica* root caused severe hepatocellular necrosis and renal tubular necrosis on the liver and kidney respectively. The ethyl acetate and hexane fraction also caused severe periportal cellular infiltration in the liver while the hexane fraction caused glomerular necrosis.

**Table IV: Effect of dichloromethane fraction of *Waltheria indica* Linn. root on Liver enzymes of male Wistar rats**

| Parameters (IU/L) | Control | 200 µg/Kg BW | 500 µg/Kg BW | 1000 µg/Kg BW |
|---|---|---|---|---|
| ALT | 17.80±1.92 | 33.20±5.63[b] | 39.80±3.56[z] | 38.20±8.53[z] |
| AST | 64.60±5.73 | 57.80±6.18 | 61.20±4.76 | 57.20±14.48 |
| ALP | 41.40±6.54 | 49.40±7.23 | 41.20±4.38 | 50.60±1.95[a] |

Values are expressed as Mean±SD. n=5 for each group. [a] - $p < 0.05$, [b] - $p < 0.01$, [z] - $p < 0.001$

**Table V: Effect of dichloromethane fraction of *Waltheria indica* Linn. root on serum proteins, urea and creatinine of male Wistar rats**

| Parameters | Control | 200 µg/Kg BW | 500 µg/Kg BW | 1000 µg/Kg BW |
|---|---|---|---|---|
| Total protein (g/L) | 47.80±2.77 | 50.80±8.70 | 47.60±4.16 | 36.40±2.41 |
| Albumin (g/L) | 27.40±1.67 | 33.00±6.21 | 27.20±5.22 | 23.00±3.32 |
| Globulin (g/L) | 15.60±3.65 | 17.00±6.71 | 15.40±3.65 | 14.00±3.46 |
| Urea (mmol/L) | 3.18±0.26 | 3.98±0.68 | 4.64±0.59[a] | 4.52±0.83 |
| Creatinine (mmol/L) | 49.00±2.74 | 45.80±3.70 | 46.60±4.51 | 44.20±2.58 |

Values are expressed as Mean±SD. n=5 for each group. [a] - $p < 0.05$

**Table VI: Effect of dichloromethane fraction of *Waltheria indica* Linn. root on haematology male Wistar rats**

| Parameters | Control | 200 µg/Kg | 500 µg/Kg | 1000 µg/Kg |
|---|---|---|---|---|
| RBC counts ($\times 10^{12}$/L) | 5.67±0.39 | 5.72±0.18 | 5.74±0.29 | 6.06±0.13 |
| HB (g/dL) | 8.83±1.42 | 9.97±4.35 | 8.00±2.43 | 8.07±3.40 |
| PCV (%) | 32.33±2.52 | 33.00±1.73 | 33.67±3.79 | 36.00±1.00 |
| MCV (fL) | 57.00±2.00 | 57.67±0.57 | 57.00±1.73 | 59.00±1.73 |
| MCH (pg) | 15.70±3.47 | 17.57±8.29 | 14.00±4.52 | 13.20±5.35 |
| MCHC (g/dL) | 27.60±6.22 | 37.23±15.35 | 24.53±8.35 | 22.37±9.17 |
| WBC ($\times 10^9$/L) | 17.60±8.43 | 13.30±12.92 | 23.30±9.40 | 30.73±8.62 |
| Neutrophil ($\times 10^9$/L) | 8.37±4.87 | 5.10±4.36 | 10.31±3.89 | 15.98±4.17 |
| Lymphocytes ($\times 10^9$/L) | 8.75±3.46 | 7.54±7.64 | 12.29±5.41 | 13.67±4.03 |
| Monocytes ($\times 10^9$/L) | 0.49±0.22 | 0.26±0.26 | 0.70±0.49 | 1.08±0.58 |

Results are shown as Mean±SD. n=5 for each group. No significant changes between the control and the treatment groups

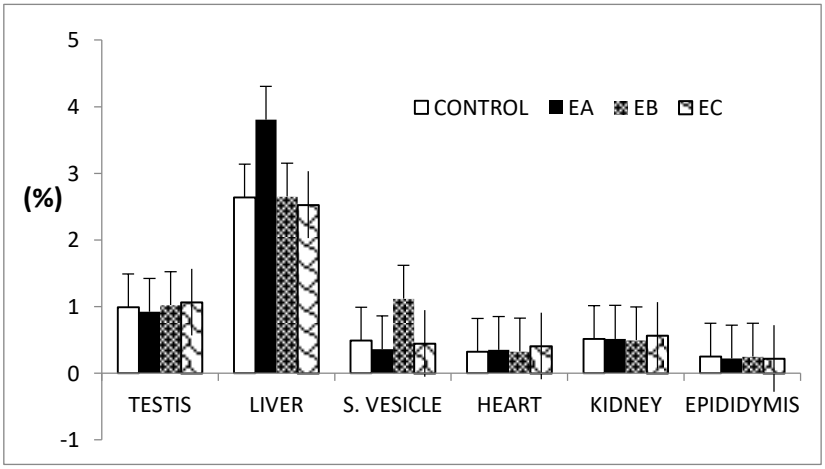


Figure III: effect of ethyl acetate fraction of *Waltheria indica* Linn. root on relative organ weight of male Wistar rats. n=5 for each group. **EA =** 200 µg/Kg, **EB =** 500 µg/Kg, **EC** = 1000 µg/Kg

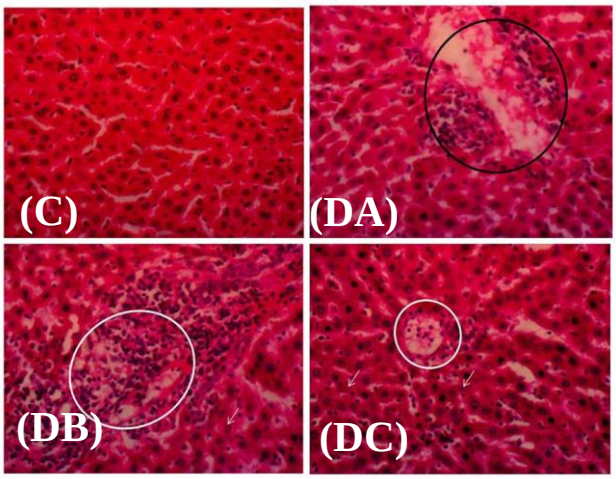


Figure IV: **Photomicrographs of the liver of male Wistar rats exposed to different doses of dichloromethane fraction of *Waltheria indica* Linn. root.** Control (C): normal liver with intact hepatocytes (white arrow). 200 µg/kg (DA): mild peri-portal cellular infiltration (oval outline). 500 µg/kg (DB): severe periportal cellular infiltration (oval outline) and hepatocellular necrosis (white arrow). 1000 µg/kg (DC): severe hepatocellular necrosis (white arrow) and moderate cellular infiltration (oval outline). H&E. ×100.

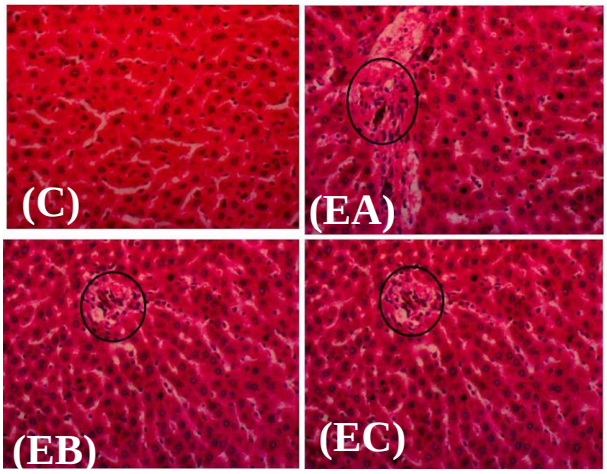


**Figure V: Photomicrographs of the liver of male Wistar rats exposed to different doses of ethyl acetate fraction of *Waltheria indica* Linn. root**. Control (C): normal liver with intact hepatocytes (white arrow). 200 µg/kg (EA): severe peri-portal cellular infiltration (oval outline). 500 µg/kg (EB): severe periportal cellular infiltration (oval outline) and hepatocellular necrosis (white arrow). 1000 µg/kg (EC): severe periportal cellular infiltration (oval outline) and hepatocellular necrosis (white arrow). H&E × 100.

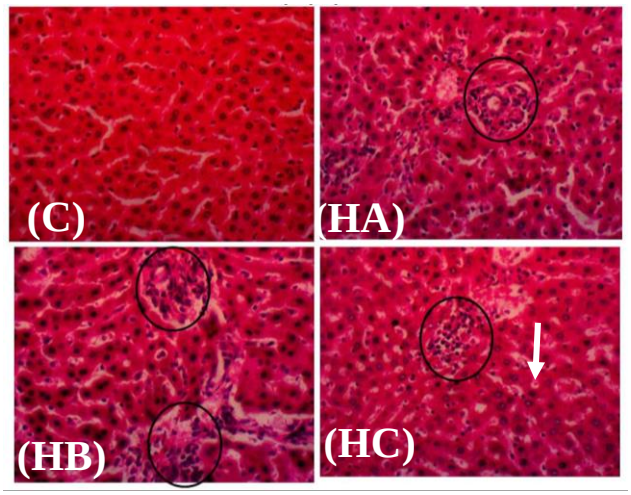


**Figure VI: Photomicrographs of the liver of male Wistar male Wistar rats exposed to different doses of hexane fraction of *Waltheria indica* Linn. root.** Control (C): normal liver with intact hepatocytes (white arrow). 200 µg/kg (HA): severe peri-portal cellular infiltration (oval outline). 500 µg/kg (HB): severe periportal cellular infiltration (oval outline) 1000 µg/kg (HC): severe periportal cellular infiltration (oval outline) and hepatocellular necrosis (white arrow). H&E. × 100.

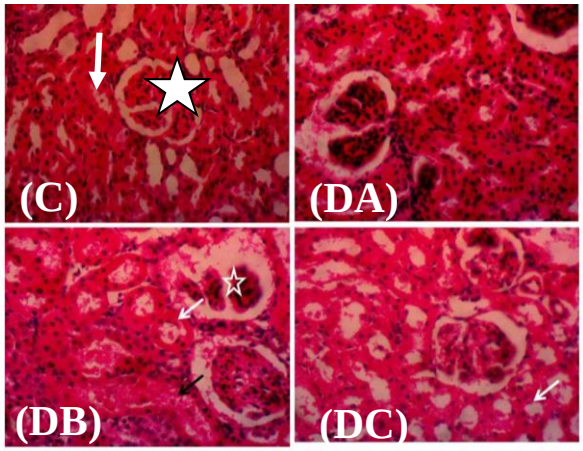


**Figure VII: Photomicrographs of the kidneys of male Wistar rats exposed to different doses of dichloromethane fraction of *Waltheria indica* Linn. root.** Control (C): normal kidney tissue with intact glomerulus (star) and renal tubules (white arrow). 200 µg/kg (DA): No visible renal lesion. 500 µg/kg (DB): severe glomerular necrosis (star), tubular necrosis (white arrow) and renal interstitial congestion (black arrow) 1000 µg/kg (DC): severe renal tubular necrosis (white arrow). H&E. × 400

## DISCUSSION

The non-significant changes on the relative weight of the organs evaluated following the administration of hexane, dichloromethane and ethyl acetate fractions of *Waltheria indica* Linn. root showed low toxicity (Zongo *et al*., 2013). This corroborates the pevious reports of high $LD_{50}$ of *Waltheria indica* root (Basiru *et al*., 2019). Organ-somatic index is used to assess toxicity of medicinal plants.

Alanine aminotransferase (ALT) is an enzyme that is aggregated within the cytosol of the hepatocyte. This enzyme consists of 496 amino acids, and is coded by the ALT gene, which is on the long arm of chromosome 8. The Alanine aminotransferase catalyzes the transfer of amino groups from L-alanine to α-ketoglutarate, and the converted products are

Alanine aminotransferase (ALT) is an enzyme that is aggregated within the cytosol of the hepatocyte. This enzyme consists of 496 amino acids, and is coded by the ALT gene, which is on the long arm of chromosome 8. The Alanine aminotransferase catalyzes the transfer of amino groups from L-alanine to α-ketoglutarate, and the converted products are L-glutamate and pyruvate in the liver, which is a critical process of the tricarboxylic acid (TCA) cycle. ALT activity in hepatic cells is approximately 3000 times higher than serum ALT activity. When liver injury occurs, ALT is released from injured liver cells and causes a significant elevation in serum ALT activity (Liu *et al*., 2014). Significant increase in Alanine aminotransferase in this study compared with treatment group showed the interference of these extracts with hepatocyte integrity. This is corroborated by the histopathological changes such as peri-portal cellular infilteration, hepatocellular necrosis observed in the liver. ALT catabolizes amino acids, permitting them to enter the citric acid cycle. Elevated ALT values are signs of hepatic injury caused by toxic substance (Howida, 2016). Although, the hexane, dichloromethane and ethyl acetate fractions caused elevation of serum ALT, all the treatments groups administered with dichloromethane showed elevated ALT values. This points to the fact that dichloromethane fraction of WILR has

**Table VII: Effect of ethyl acetate fraction of *Waltheria indica* Linn. root on Liver enzymes of male Wistar rats**

| Parameters (iu/L) | Control | 200 µg/Kg BW | 500 µg/Kg BW | 1000 µg/Kg BW |
|---|---|---|---|---|
| ALT | 17.80±1.92 | 35.20±5.07[c] | 28.80±6.54[a] | 35.80±10.33[c] |
| AST | 64.60±5.73 | 69.20±7.49 | 63.40±9.58 | 50.40±11.91 |
| ALP | 41.40±6.54 | 47.40±2.30 | 40.40±2.07 | 39.20±4.60 |

Results are expressed as Mean±SD. n=5 for each group. [a] - $p < 0.05$, [c] - $p < 0.001$

**Table VIII: Effect of ethyl acetate fraction of *Waltheria indica* Linn. root on serum proteins, urea and Creatinine of male Wistar rats**

| Parameters | Control | 200 µg/Kg BW | 500 µg/Kg BW | 1000 µg/Kg BW |
|---|---|---|---|---|
| Total protein (g/L) | 47.80±2.77 | 60.60±10.95 | 63.00±16.78 | 58.20±6.54 |
| Albumin (g/L) | 27.40±1.67 | 31.00±4.06 | 31.00±7.58 | 31.40±4.34 |
| Globulin (g/L) | 15.60±3.65 | 26.80±6.30[b] | 30.60±9.37[z] | 22.00±1.58 |
| Urea (mmol/L) | 3.18±0.26 | 4.22±0.69 | 5.56±1.35[z] | 4.86±0.63[a] |
| Creatinine (mmol/L) | 49.00±2.74 | 47.00±4.58 | 51.80±16.07 | 42.40±10.24 |

Results are shown as mean ±standard deviation. n=5 for each group. [a] - $p < 0.05$, [b] - $p < 0.01$, [z] - $p < 0.001$.

**Table IX: Effect of dichloromethane fraction of *Waltheria indica* Linn. root on haematological parameters of male Wistar rats**

| Parameters | Control | 200 µg/Kg | 500 µg/Kg | 1000 µg/Kg |
|---|---|---|---|---|
| RBC ($\times 10^{12}$/L) | 5.67±0.39 | 5.71±0.25 | 5.72±0.14 | 5.90±0.60 |
| HB (g/dL) | 8.83±1.42 | 9.57±1.79 | 6.60±0.10 | 8.07±1.71 |
| PCV (%) | 32.33±2.52 | 32.67±1.53 | 32.67±0.58 | 34.67±4.93 |
| MCV (fL) | 57.00±2.00 | 57.00±1.00 | 57.00±1.00 | 58.67±2.31 |
| MCH (pg) | 15.70±3.47 | 16.77±3.23 | 11.53±0.46 | 13.53±1.68 |
| MCHC (g/dL) | 27.60±6.22 | 29.47±5.99 | 20.27±0.51 | 23.03±2.11 |
| WBC ($\times 10^9$/L) | 17.60±8.43 | 26.20±8.22 | 24.90±5.80 | 15.40±3.28 |
| Neutrophil ($\times 10^9$/L) | 8.37±4.87 | 10.70±4.97 | 11.34±1.15 | 6.79±1.32 |
| Lymphocyte ($\times 10^9$/L) | 8.75±3.46 | 14.69±3.35 | 12.68±4.20 | 8.25±1.98 |
| Monocyte ($\times 10^9$/L) | 0.49±0.22 | 0.60±0.16 | 0.87±0.53 | 0.40±0.07 |

Results are presented as mean±SD. n=5 for each group. No significant difference between the control and treatment groups.

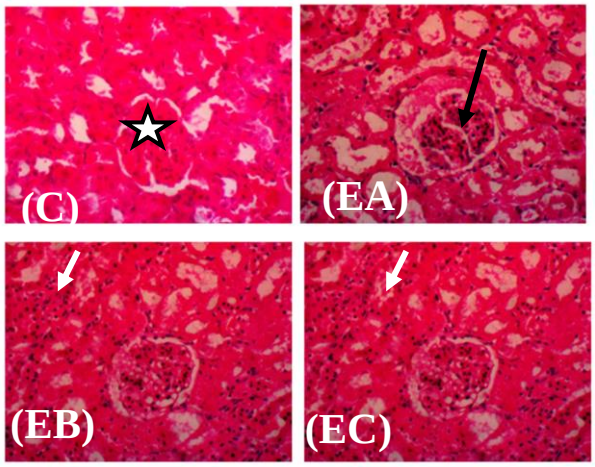


**Figure VIII: Photomicrographs of the kidneys of male Wistar rats exposed to different doses of ethyl acetate fraction of *Waltheria indica* Linn. root. A.** Control (C): normal kidney tissue with intact glomerulus (star) and renal tubules (arrow). 200 µg/kg (EA): severe glomerular necrosis (arrow). 500 µg/kg (EB): tubular necrosis (arrow). 1000 µg/kg (EC): severe renal tubular necrosis (white arrow). H&E. × 400.

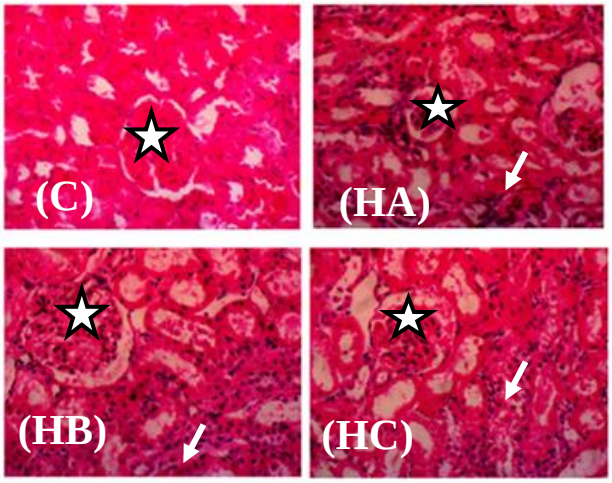


**Figure IX: Photomicrographs of the kidneys of male Wistar rats exposed to different doses of hexane fraction of *Waltheria indica* Linn. root.** A. Control (C): normal kidney tissue with intact glomerulus (star). 200 µg/kg (HA): severe glomerular necrosis (star) and tubular necrosis (white arrow) 500 and1000 µg/kg (HB and HC): severe renal tubular necrosis (white arrow). H&E. × 400.

the most toxic effect on the liver.

Blood Urea Nitrogen (BUN) when measured in the blood, is a product of protein metabolism. BUN is a non-protein nitrogenous (NPN) waste product. Amino acids derived from the breakdown of protein are deaminated to produce ammonia. Ammonia is then converted to urea through liver enzymes. Therefore, the concentration of urea is dependent on protein intake, the body's capacity to catabolize protein and adequate excretion of urea by the renal system. The body's dependency on the renal system to excrete urea makes it a useful analyte to evaluate renal function. An increase in BUN can be the result of a diet that is high in protein content or decreased renal excretion.

The significant increase in blood urea nitrogen as observed in the present study indicate renotoxic effect of *Waltheria indica* root. This is corroborated by the histopathological changes in the kidneys. The significant increase in BUN can also be attributed to high protein content of *Waltheria indica* (Basiru *et al*., 2016; Lan *et al*., 2021).

The increase serum level of globulin by the dichloromethane and ethyl acetate fractions indicates immunity enhancing effect of *Waltheria indica* root and support the traditional use of this plant as immune booster (Mohammed *et al*., 2007).

The non-significant effect of crude ethanolic extract of *Waltheria indica* root on haematological parameters disagrees with the report of haematinic potential of *Waltheria indica* root (Oladiji *et al*., 2005). The haematinic effect may be due to iron content of *Waltheria indica* root. The non-significant increase in haematological parameters was due to the fact that red blood cell cannot concentrate haemoglobin beyond its normal level (Navya *et al*., 2022). Hence, the haematological parameters were not significantly increase despite the iron content of *Waltheria indica* root. The non-significant effect on haematological parameters also indicates the non-toxic effect of *Waltheria indica* root on haemopoietic organs.

## CONCLUSION

The hexane, dichloromethane, and ethyl acetate soluble extracts of *Waltheria indica* root have adverse effect on the integrity of liver and kidney. Hence, these extracts should be used with caution in patient with hepatic and renal impairment.

## ACKNOWLEDGEMENTS

The authors wish to thank Mr. Bolaji and Adegboye of the Department of Veterinary pathology, University of Ilorin, Nigeria for his assistance.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict interests regarding this research.

## REFERENCES

Basiru, A. & Olayemi, F.O. (2014). Effects of aqueous extract of *Waltheria indica* leaves on reproductive indices of male albino rats. *African Journal of Biotechnology*, 13, 3307–3312.

Basiru, A., Akorede, G.J., Soetan, K. & Olayemi, F.O. (2019). Adverse reproductive effects of ethanolic root extract of *Waltheria indica* in male Wistar rats. *Journal of Complementary and Integrative Medicine*, 16(4), 1–6.

Basiru, A., Soetan, K.O. & Olayemi, F.O. (2016). Comparative proximate, minerals composition and antinutritional factors of *Waltheria indica* leave, root and stem. *Annals Food Science and Technology*, 17, 478–84.

Dumas, B.T., Watson, W.A. & Bis, H.G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chemica Acta*, 31, 87-96.

Howida S.A.S. (2016). Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. *Beni-suef university journal of basic and applied sciences*, 5, 134 – 146

Lan, Q., Zheng, L., Zhou, X., Wu, H., Buys, N., Liu, Z., Sun, J. & Fan H. (2021). The Value of Blood Urea Nitrogen in the Prediction of Risks of Cardiovascular Disease in an Older Population. *Frontiers in Cardiovascular Medicine*, 20, 8:614117.

Liu, Z., Que, S., Xu, J. &Peng, T. (2014). Alanine aminotransferase-old biomarker and new concept: a review. *International journal of medical sciences*, 11(9), 925–935.

Mohammed, Z., Shok, M., IIyas, N., Musa, K.Y. &Yaro, A.H. (2007). Analgesic activity of *Waltheria indica* Linn.. *European Journal of Scientific Research*, 16 (1), 1-6.

Musumeci, G. (2014). Past, present and future: overview on histology and histopathology. *Journal of Histology and Histopathology*, 1, 1–5.

Navya, K.T., Prasad, K. & Singh, B.M.K. (2022). Analysis of red blood cells from peripheral blood smear images for anemia detection: a methodological review. *Medical & Biological Engineering and Computing*, 60, 2445–2462.

Oladiji, A.T., Abdullahi, S.A. & Yakubu M.T. (2005). Evaluation of haematinic potentials of aqueous extract of *Waltheria indica Linn.* (L) root on rats reared on iron sufficient and iron deficient diets feeds. *Nigerian Journal of Biochemistry and Molecular Biology*, 20(2), 115 – 122.

Reltman, S. & Frankel, S.A. (1975). Colorimetric method for the determination of serum ALT and AST. *American Journal of Clinical Pathology*, 28, 56–63.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., & Yoga L.L. (2011). Extraction, isolation and characterization of bioactive compounds from Plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicine*, 8(1), 1-10.

Sylvian, C., Lise, B., Lucie, P., Chiara, A., Laurence, M., Samad, N.E., Matthias, H., Remo, P., Soumana, K., Marcel, K., Muriel, C. & Philippe, C. (2014). Antitrypanosomal quinoline alkaloids from the roots of *Waltheria indica* Linn. *Journal of Natural Product*,

Termer, M., Carola, C., Salazar, A., Keck, C.M., Hemberger, J. & von Hagen, J. (2021). Identification of plant metabolite classes from *Waltheria indica* L. extracts regulating inflammatory immune responses via COX-2 inhibition. *Journal of Ethnopharmacology*, 270, 113741.

Tietz, N.W., Pruden, E.L. & Siggaard-Andersen, O. (1994). Liver Function. In Tietz Textbook of Clinical Chemistry; Burtis, A.C., Ashwood, E.R., (Eds., pp. 1354–1374). London, UK: WB Saunders.

Truong, D.H., Nguyen, D.H., Ta, NTA., Bui, A.V., Do, T.H & Nguyen, H.C. (2019). Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of *Severinia buxifolia*. *Journal of Food Quality*, 2019, 8178294.

Zongo, F., Ribuot, C., Boumendjel, A. & Guissou, I.J. (2013). Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica* Linn. L. (syn. *Waltheria americana*). *Journal of Ethnopharmacology*, 148, 14–26.