

Full Length Research Paper

Effects of aqueous extract of *Waltheria indica* leaves on blood profile of male albino rats

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This study was to investigate the effect of oral administration of aqueous extract of *Waltheria indica* leaf on the blood profile of male albino rats. The extract showed significantly ($p < 0.01$) different increase of red blood and white blood cell counts values at 400 and 800 mg/kg doses. However, the extract showed insignificant difference in packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and haemoglobin concentrations between the control and the treated groups. Result also showed significant increase ($p < 0.01$) in the values of alanine and aspartate aminotransferases (ALT and AST), blood urea nitrogen (BUN) and creatinine at 800 and 2000 mg/kg doses. *W. indica* leaf extract also induced periportal cellular infiltration and diffuse hydropic degeneration of the hepatocytes in the liver. The study emphasized the cautious use of *W. indica* leaf as it may be hepatotoxic at high doses.

Key words: *Waltheria indica* leaf, blood profile, liver, rats.

INTRODUCTION

Traditionally, plants are used in treatment of diseases in different parts of the world (Hostettman et al., 2000) and their use contribute significantly to primary health care delivery (Holetz et al., 2002). Herbal medicines still remain the mainstay of about 75 to 80% of the whole population in developing countries, for primary health care because of cultural acceptability (Parekh and Chanda, 2006). Each culture or community within an area, whether large or small, has its own ethnobotanical perspective which differs from one another. Plants are regarded as invaluable sources of pharmaceutical products (Olalde, 2005). While traditional healers are still consulted in Nigeria as a first choice due to the fact that traditional medicine blends readily into the socio-cultural

life of the people (Kela and Kufeji, 1995), healing from diseases are gotten from plants by other countries of black Africa (Grierson and Afolayan, 1999; Anani et al., 2000). Sofowora (1984) defined medicinal plant as any plant in which one or more of its parts contain substance(s) that can be used for therapeutic purpose or as precursors for pharmaceutical synthesis. The use of plant and animal parts in medicine have since been widely documented in the records of ancient China, India and Egypt, and practice was based on series of "trial and error", which could not be substantiated by proven scientific theories. However, these practices have produced results of proven efficacies compared to the conventional modern medicine (Chopra et al., 1956). *Waltheria indica* L.,

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also known as sleepy morning (Burkill, 2000), belongs to the family Sterculiaceae. It is widespread in West Africa (Akobunda and Agyakwa, 1998). Locally, the plant is called 'hankufah' in Hausa and 'korikodi' in Yoruba (Hutchinson and Dalziel, 1958; Irvine, 1961). The uses of the plant are diverse; the plant has been used as an infusion or decoction where febrifugal, purgative, emollient, tonic, analgesic and astringent action is sought (Burkill, 2000). It is used in Northern Nigeria by the Hausas for the treatment of skin diseases, as an aphrodisiac and as children's medicine at birth and during teething (Mohammed et al., 2007). In the Fulani community, the aqueous extract of the root is used in relieving aches and pains during the 'Sharo' festival. Among the Yoruba, the aqueous extract of the root and stem are used in treating syphilis, internal haemorrhage, and as a restorative after the labours of farming activity (Mohammed et al., 2007).

The trypanocidal, antibacterial, anti-inflammatory, analgesic and haematinic properties of *W. indica* plant have been reported (Oladiji et al., 2005; Bala et al., 2010; Olajuyigbe et al., 2011). However, there is the need to ascertain the safety of this plant because of their wide usage. Therefore, the aim of this study is to investigate the effect of aqueous extract of *W. indica* leaves on haematological and serum biochemical parameters of male albino rats.

MATERIALS AND METHODS

Experimental animals

Thirty six healthy white male adult albino rats (100 to 190 g) obtained from the Animal House, Faculty of Veterinary Medicine, University of Ibadan, Nigeria, were used for the study. The rats were fed with rat cubes (Ladokun Feeds Limited, Ibadan, Nigeria) and water *ad libitum*. During the study, the rats were kept at the Experimental Animal House of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan. Animals were acclimated to their new environment for two weeks before the commencement of the experiment. All experimental protocols were in compliance with University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

Plant

The *W. indica* plants were obtained from a farm land at Moniya in Akinyele Area Council of Ibadan, Oyo state, Nigeria and identified at the Herbarium, Department of Botany, University of Ibadan with voucher number UIH-22371.

Extract preparation

The leaves of the plant were separated from the whole plant and air dried at room temperature for two weeks. A total of 200 g of the ground powder was soaked in 1 L of distilled water for 24 h at room temperature. The mixture was filtered into conical flask with Whatman filter paper. The filtrate was concentrated *in vacuo* using a rotary evaporator at 40°C to produce a gel-like extract, which weighed 43 g (21.5% yield). Appropriate concentration of the extract

extract was then subsequently made by dilution with distilled water into graded doses and administered to the rats.

Experimental design

Thirty six male albino rats were randomly divided into six groups (n = 6), labeled A to F where group A served as the control while the animals in the groups B, C, D, E and F served as the treated group. The treated groups were then orally administered with 200, 400, 800, 1600 and 2000 mg/kg body weight of the extract, respectively for 21 days.

Acute toxicity study

The acute toxicity study of aqueous extract of *W. indica* was determined according to the method of Adedapo et al. (2009). Rats that have been fasted for 16 h were randomly divided into six groups of six per group. Graded doses of the extract (200, 400, 800, 1600, 2000 mg/kg p.o) corresponding to groups B, C, D, E and F were separately administered to the rats in each of the 'test' groups by means of bulbed gavage needle. Meanwhile for the control group (group A) was orally administered with distilled water (3 ml/kg) only. All the animals were then allowed free access to food and water and observed for 48 h, for any signs of toxicity. The numbers of deaths within this period were recorded.

Collection of blood and serum samples

Blood samples were collected through the orbital sinus from diethyl ether anaesthetized albino rats into heparinised bottles for haematological studies. Meanwhile, for non-heparinised bottles, the blood samples were allowed to clot. The blood samples were centrifuged at 3000 rpm for 10 min and the serum was separated from the clot and transferred into clean bottles for biochemical analysis.

Haematological and serum biochemical studies

The packed cell volume (PCV) and haemoglobin concentration were determined by conventional method (Duncan et al., 1994). Erythrocyte count was determined by the haematocytometry method as described by Jain (1986). Total white blood cell (WBC) counts were made in a haemocytometer using the WBC diluting fluid and differential leucocytes counts were made by counting the different types of WBC from Giemsa stained slides viewed from each of the 30 fields of oil immersion objective of a microscope (Coles, 1989). Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined from the values obtained from red blood cells (RBC) count, haemoglobin concentration and PCV values (Duncan et al., 1994). Total protein was measured using biuret reaction, while albumin was measured by colorimetric estimation using Sigma diagnostic reagent (Sigma Diagnostic, UK.), which contained bromocresol green (BCG). Meanwhile, globulin was obtained from difference total protein and albumin. AST and ALT were determined using a photoelectric colorimeter as described by Duncan et al. (1994). However, serum urea and creatinine levels were determined using photoelectric colorimeter as described by Coles (1989).

Histopathology

All the animals from each of the treated groups B, C, D, E, F and the control were sacrificed 24 h after their respective daily doses. The rats were thereafter quickly dissected to remove the liver and then

Table 1. Acute toxicity study in rats after 48h of administration with aqueous extract of *W. indica*.

Group	Dose (mg/kg)	No of dead rats	Toxic signs observed
Control	Distilled water	0	No toxic changes observed
B	200	0	No toxic changes observed
C	400	0	No toxic changes observed
D	800	0	No toxic changes observed
E	1600	0	No toxic changes observed
F	2000	0	Slight diarrhoea

Table 2. Effect of graded doses of the aqueous extract of *W. indica* on haematological parameters of rats (n = 6).

Parameter	Control	B (200 m/kg)	C (400 m/kg)	D (800 m/kg)	E (1600 m/kg)	F (2000 m/kg)
RBC ($\times 10^{12}/L$)	7.80 \pm 0.09	8.11 \pm 0.19	8.87 \pm 0.04**	8.90 \pm 0.27**	7.63 \pm 0.11	7.50 \pm 0.14
PCV (%)	47.0 \pm 0.45	48.0 \pm 0.71	49.6 \pm 0.51	48.6 \pm 0.40	46.2 \pm 0.92	45.0 \pm 1.27
Hb (g/L)	15.3 \pm 0.07	15.7 \pm 0.09	16.3 \pm 0.11	15.9 \pm 0.23	15.3 \pm 0.28	15.5 \pm 0.94
MCHC (%)	32.48 \pm 0.27	32.74 \pm 0.35	32.96 \pm 0.31	32.84 \pm 0.24	33.22 \pm 0.95	34.46 \pm 2.09
MCH (pg)	19.58 \pm 0.27	19.40 \pm 0.42	18.42 \pm 0.12	18.00 \pm 0.62	20.10 \pm 0.39	20.70 \pm 1.33
MCV (fl)	60.24 \pm 0.70	59.30 \pm 1.10	55.90 \pm 0.50	54.80 \pm 1.75	60.54 \pm 1.07	59.96 \pm 0.72
WBC($\times 10^9/L$)	9.80 \pm 0.01	9.70 \pm 0.13	10.50 \pm 0.20**	11.00 \pm 0.10**	11.20 \pm 0.15**	9.50 \pm 0.19
Eosinophil (%)	2.2 \pm 0.20 (0.22 \pm 0.019) ^a	2.0 \pm 0.32 (0.19 \pm 0.033) ^a	2.2 \pm 0.49 (0.23 \pm 0.053) ^a	2.2 \pm 0.73 (0.24 \pm 0.081) ^a	2.4 \pm 0.4 (0.27 \pm 0.048) ^a	2.0 \pm 0.55 (0.19 \pm 0.053) ^a
Lymphocytes (%)	65.6 \pm 1.29 (6.4 \pm 0.12) ^a	67.2 \pm 1.07 (6.5 \pm 0.10) ^a	70.0 \pm 0.84 (7.4 \pm 0.21) ^a	71.4 \pm 1.97 (7.8 \pm 0.26) ^a	73.0 \pm 1.70* (8.2 \pm 0.15) ^a	73.2 \pm 2.22* (6.9 \pm 0.21) ^a
Neutrophils (%)	22.8 \pm 2.31 (2.2 \pm 0.23) ^a	30.4 \pm 1.17* (2.9 \pm 0.15) ^a	31.8 \pm 2.42* (3.3 \pm 0.25) ^{a**}	25.8 \pm 1.11 (2.8 \pm 0.11) ^a	25.4 \pm 1.72 (2.8 \pm 0.19) ^a	22.8 \pm 2.18 (2.2 \pm 0.22) ^a
Monocyte (%)	2.6 \pm 0.25 (0.25 \pm 0.019) ^a	1.6 \pm 0.25 (0.15 \pm 0.023) ^a	3.8 \pm 0.37 (0.40 \pm 0.046) ^{a*}	1.2 \pm 0.20 (0.13 \pm 0.022) ^a	2.2 \pm 0.50 (0.25 \pm 0.058) ^a	2.0 \pm 0.32 (0.19 \pm 0.033) ^a
Platelets (μ l)	69000 \pm 8983	111400 \pm 20951	103600 \pm 10414	119200 \pm 8291	111600 \pm 15095	109800 \pm 13800

Results are reported as mean \pm standard error of mean (S.E.M) and analyzed using the one-way analysis of variance (one-way ANOVA) and Duncan Multiple range Tests (n=6). Superscripted items indicate significant values (* P<0.05, ** P<0.01). ^aabsolute counts of the differential in ($\times 10^9/L$).

liver and then transferred into 10% buffered formalin. The organs were dehydrated in ethanol (70 to 100%), cleared in xylene and embedded in paraffin. Tissue sections were examined under a light microscope after staining with haematoxylin and eosin (H and E) (Culling, 1963; Lillie, 1965).

Statistical analysis

The data obtained from the experiment were presented as mean \pm standard error of mean (SEM) and analyzed using the one-way analysis of variance (one-way ANOVA). The

group means were separated by Duncan Multiple range Tests at 95% confidence interval using GraphPadInstat@ software.

RESULTS

Acute toxicity study

Result showed that no mortality was observed in the treated and untreated groups (Table 1). From the observations, there was slight diarrhoea at the higher dose of 2000 mg/kg.

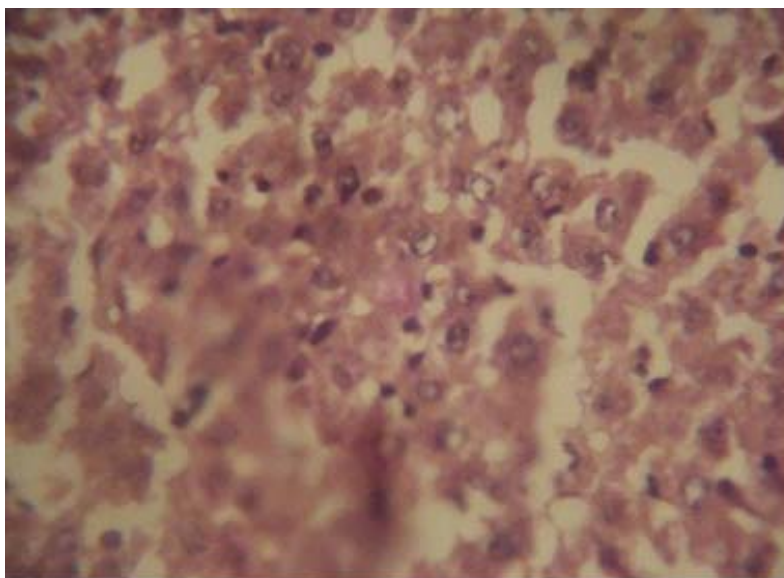
Effect of the aqueous extract of *Waltheria indica* on haematological parameters of the rats

Result in Table 2 showed the effect of graded doses of *W. indica* on haematological parameters of rats. The extract doses of 400 and 800 mg/kg b.w (Groups C and D) caused significantly increase (P < 0.01) in RBC counts. The extract dose of 400, 800 and 1600 mg/kg b.w (groups C, D and E) also showed significant increased (P < 0.01) in white blood cell counts. The lymphocyte

Table 3. Effects of the aqueous extract of *W. indica* on serum biochemical parameters of rats (n=6).

Parameter	A	B (200 mg/kg)	C (400 mg/kg)	D (800 mg/kg)	E (1600 mg/kg)	F (2000 mg/kg)
Total protein (g/dl)	8.30±0.10	8.04±0.17	7.49±0.16	7.66±0.09*	7.72±0.06*	7.52±0.21**
Albumin (g/dl)	5.04±0.09	4.42±0.25	4.42±0.17	4.44±0.16	4.58±0.10	4.08±0.36*
Globulin (g/dl)	3.28±0.05	3.64±0.09	3.52±0.14	3.22±0.11	3.20±0.09	3.30±0.08
AG ratio	1.56±0.09	1.18±0.10*	1.22±0.07	1.40±0.11	1.38±0.07	1.26±0.11
AST (U/L)	40.20±0.37	42.8±0.37**	44.0±0.89**	45.2±1.02**	45.6±0.51**	47.40±0.68**
ALT (U/L)	28.2±0.58	29.6±0.93	30.8±1.11	32.0±0.55**	31.6±0.40*	33.4±0.40**
ALP (U/L)	93.8±2.78	89.4±4.40	80.4±1.33	118.8±3.39**	83.8±6.56	98.2±5.27
BUN (mg/dl)	13.2±0.37	15.6±0.24**	15.4±0.51**	14.6±0.40	15.8±0.38**	16.6±0.25**
Creatinine (mg/dl)	0.54±0.06	0.82±0.07*	0.92±0.08**	1.00±0.06**	0.80±0.03	0.90±0.11**

Results are reported as mean± standard error of mean (SEM) and analyzed using the one-way analysis of variance (one-way ANOVA) and Duncan Multiple range Tests (n=6). Superscripted items indicate significant values (*P< 0.05, **P<0.01).

**Figure 1.** Liver of the control showing no visible lesion. M ×100 H&E

count was also significantly increased ($P < 0.05$) for the groups E and F. In addition, the neutrophils counts for the groups B and C were significantly increased ($P < 0.05$) compared to the control group. There were no significant difference in PCV, Hb count, MCV, MCHC, MCH and platelets counts between the treated groups (group B, C, D, E and F) and the control group (group A).

Effects of the aqueous extract of *Waltheria indica* on serum biochemical parameters of the rats

The result of the effects of the graded doses of *W. indica* on serum biochemical parameters was presented in Table 3. There was significant decrease in total protein for the groups D, E ($P < 0.05$) and F ($P < 0.01$) compared to the control (group A). Furthermore, there was a significant ($P < 0.05$) decrease in albumin for group F

compared to the control. Level in AST showed significantly increase ($P < 0.01$) in the treated groups (B to F) compared to the control. More so, the level of BUN showed significant increase ($P < 0.01$) in the treated groups (B, C and F) compared to the control. Nevertheless, the levels of ALP was significantly increased ($P < 0.01$) for group D. The level of creatinine was significantly increased ($P < 0.01$) for group B, C, D and F compared to the control. However, there were no significant difference in globulin between the control (A) and treated groups (B to F).

Histopathological effects

Histopathological evaluation of rats treated with aqueous leave extract of *W. indica* is presented in Figures 1 to 4. No visible lesion was observed in the liver (Figure 1, showed

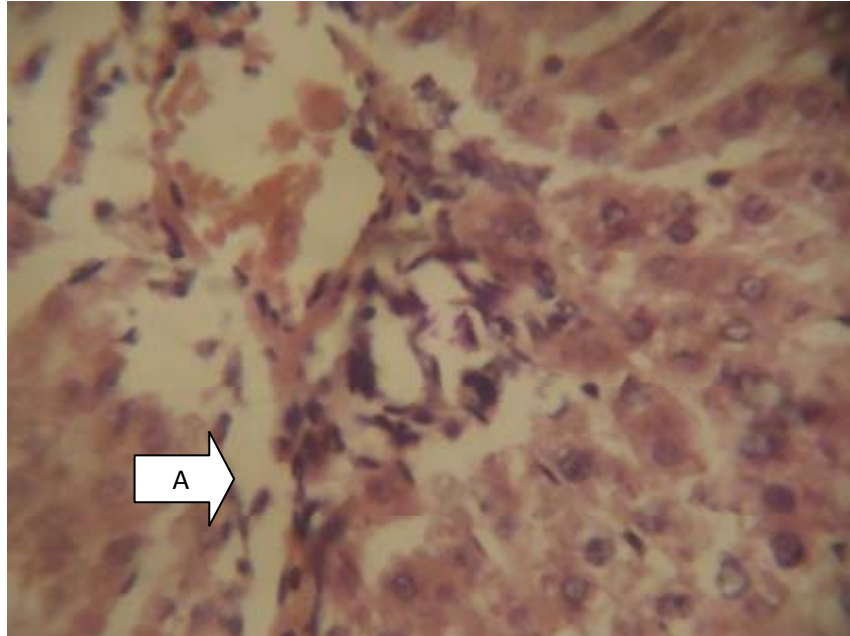


Figure 2. Liver of group B (200 mg/kg) showing periportal cellular infiltration A, by mononuclear cells. M \times 100 H&E.

showed by an arrow) of the control group. Periportal cellular infiltration by mononuclear cells was observed in the liver of group B (Figure 2, showed by an arrow A) and group E (Figure 4, showed by an arrow D). The liver of group C (400 mg/kg b.w) has diffuse hydropic degeneration of hepatocytes (Figure 3, showed by an arrow C).

DISCUSSION

There was no mortality observed except for the slight diarrhoea that was observed at the 2000 mg/kg b.w dose. This shows that *W. indica* has wide safety margin. This finding is supported by the result of the haematological effect of *W. indica* leaves in albino rats. There were no significant changes observed in the values of PCV, haemoglobin, MCH, MCHC and MCV between the treated groups and the control group. The extract caused significant increase in red blood cell counts at 400 and 800 mg/kg doses. This could be due to the presence of iron and proteins in *W. indica* plant as reported by Oladiji et al. (2005) and iron is a major component of red blood cell. This observation is in agreement with the ethnobotanical use of this plant in treatment of anaemia (Gbadamosi et al., 2012).

The oral administration of aqueous extract of *W. indica* leaves also caused significant increase in white blood cell counts. The increase in neutrophils and lymphocytes accounted for the increase in white blood cell counts. This observation of increase in the white blood cells counts by this plant extracts shows that the principal function of

phagocytes which is to defend against microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes will be enhanced (Paul, 1993; Swenson and Reece, 1993; Adedapo et al., 2005). This is attested to by the antibacterial activity of this plant (Olajuyigbe et al., 2011; Mongalo et al., 2012). The increase in white blood cell counts may be due to the presence of cardiac glycosides in this plant extract. It has been demonstrated that plants with high composition of cardiac glycosides has been found to inhibit microbial growth and is capable of protection against microbial infection, thereby increasing the white blood cell counts (Gonzolel and Mather, 1982).

However, we observed from the results that the extract caused significant reduction in total protein at doses 800, 1600 and 2000 mg/kg b.w (groups D, E and F, respectively). This reduction showed that the plant inhibit protein biosynthesis. Serum albumin is a good criterion to assess the function and secretory capacity of the liver (Yakubu et al., 2005). This may indicate hepatic toxicity of this extract at higher doses. Aminotransferases (ALT and AST) are produced in the liver and are good markers of damage to liver cells but not necessarily the severity of the damage (Rej, 1989). They are normally present at low levels in the blood, however, if the liver cells are damaged, it would be expected that some of the enzymes leak into the blood and increase in levels. Increase in serum level of AST and ALT as observed in this study may reflect damage to liver cells. Increase serum ALT is known to occur in liver disease and it has been used as a tool for measuring hepatic necrosis (Bush, 1991).

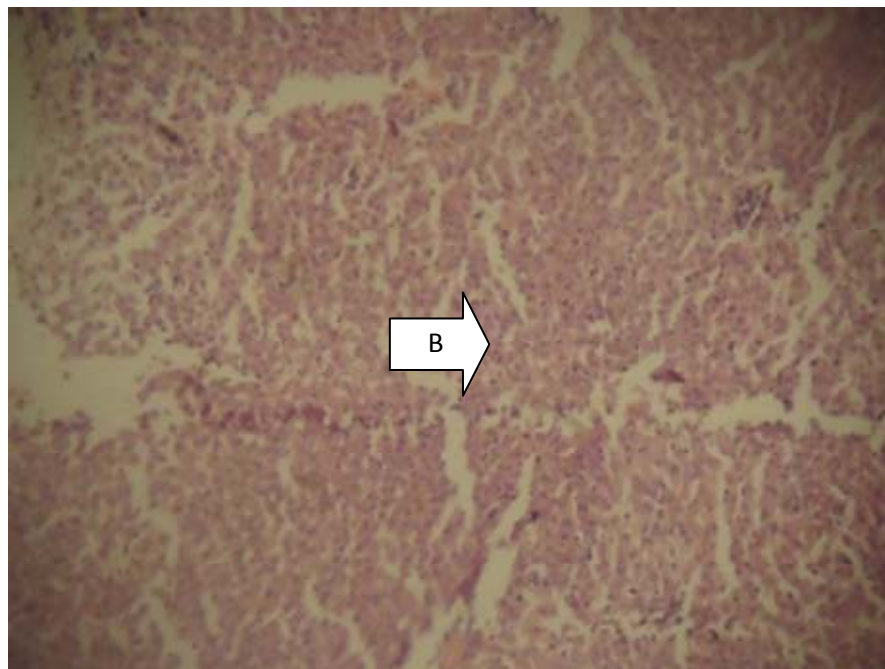


Figure 3. Liver of group C showing diffuse hydropic degeneration, B of hepatocytes. M \times 100 H&E.

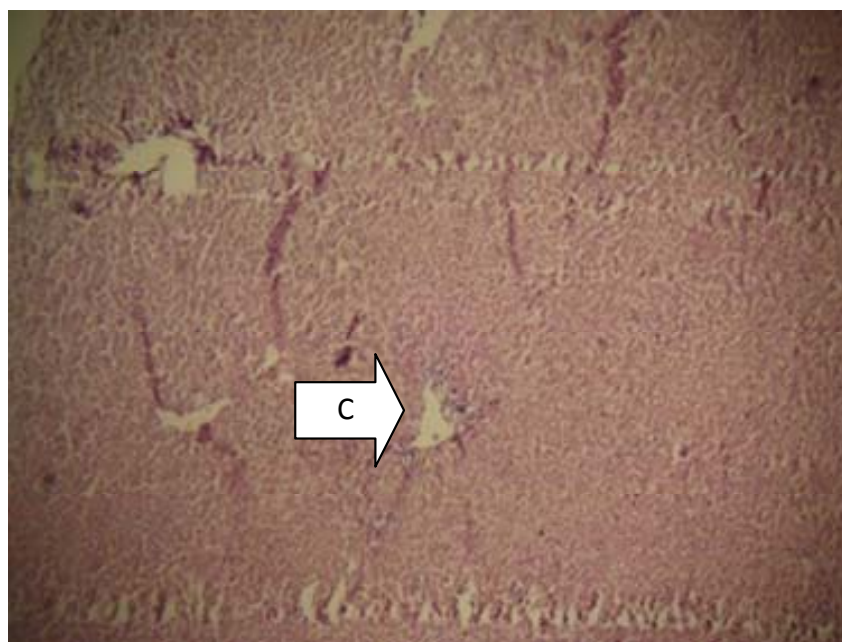


Figure 4. Liver of group E showing periportal cellular infiltration C, by mononuclear cells. M \times 100 H&E.

The result obtained in this study is in agreement with findings of Ajibade et al. (2011) who also reported increase levels of ALT and AST by methanolic seed extract of *Moringa oleifera*. Urea is one of the non-protein

nitrogenous substances that accumulate in the plasma when renal excretion is reduced. The causes of increased blood urea levels include: high protein diet, intestinal haemorrhage, dehydration, severe haemorrhage and

shock among others. Urea level could be decreased due to the following: liver failure, low protein diet, anabolic steroids, diabetes insipidus, etc. (Bush, 1991). The increased blood urea nitrogen level observed in this study may be due to the high protein content of *W. indica* plant. Gbadamosi et al. (2012) reported that *W. indica* contained 11.9% crude protein. This increased urea level may point to renal dysfunction which resulted in reduced urea excretion.

Creatinine is measured primarily to assess kidney function. A rise in blood creatinine is observed only with marked damage to functioning nephrons. The plasma level of creatinine is independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of plasma creatinine is indicative of under-excretion, suggesting kidney damage (Gross et al., 2005). The increase level of creatinine observed in this study shows that the extract may cause renal dysfunction.

The study concludes that excessive use of aqueous extract of *W. indica* leaves can be hepatotoxic; therefore caution should be applied to the use of *W. indica* leaves despite its numerous medicinal values.

Conflict of Interest

The author(s) declared that there is no conflict of interest as regards this paper.

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