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Research Article

Ocimum Gratissimum Influence on Lead-Induced Changes on the Hepatobiochemical Parameters in Wistar Rats

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ABSTRACT

Background: Lead is deleterious to hematological, biochemical and hepatic parameters and researches have focused on antioxidants with therapeutic potentials to resolve and prevent these changes.

Objective: To study the influence of *Ocimum Gratissimum* (OG) extract on lead-induced hepatotoxicity and hemato-biochemical parameters of adult Wistar rats.

Methodology: Thirty-five adult Wistar rats were assigned into seven groups of five rats each. Group A served as Control, Group B received 120 mg/kg of lead while Group C received 375 mg/kg of OG. Groups D and E received 120 mg/kg of lead before 375 mg/kg and 750 mg/kg of OG respectively. Group F received 375 mg/kg of OG extract then 120 mg/kg of lead while Group G received 120 mg/kg of lead and 1,190 mg/kg of ascorbic acid. All administrations lasted for 21 days orally, after which the animals were sacrificed; blood and liver tissues were collected for biochemical and histological analysis.

Results: The result showed increase in the mean body weight in the Control but a decrease in B. The result of biochemical analyses showed significant increase in Liver enzymes namely: ALT, AST, GGT and ALP in Group B compared to the Control and other treatment groups ($p \leq 0.05$). The result of histological analysis of the liver tissues showed degenerative changes with focal necrosis and aggregated inflammatory cells in B and liver treated with the extract showed ameliorative changes as in Groups D, E, F and G.

Conclusion: The extract of OG could be used as a therapeutic agent for intervention in lead poisoning.

Keywords: Hepatobiochemical; Lead; Liver; *Ocimum gratissimum*; Wistar rats; Parameters

INTRODUCTION

Lead is a heavy metal occurring in a several of organic and inorganic compounds that are used in the manufacturing industries such as paints used to guide against rust in iron and steel, explosives, rodenticide, batteries, X-ray apparatus, eye cosmetics, gasoline, electric cables, water pipes, tanks and the manipulation of lead for the above purpose has contaminated the environment [1]. Lead can accumulate in different tissues and organs of the body such as the nervous tissues, kidneys and liver to cause lead poisoning referred to as painter's colic [2-3]. According to Kim, et al. (2015), lead is one of the causes of anemia by blocking the activity of three vital enzymes related to haem synthesis [1-5]. According to Wu (2006), the maximum level of lead in the blood of a normal adult should be about 25 µg/dL or lower and a threshold of 10 µg/dL for children but was reduced to 5µg/dL or less based on the report of Center for Disease Control (2012) [6-7]. The threshold for occupational lead exposure should not be above 30 µg/dL in random blood testing and blood lead level for adults should be reduced to below 10 µg/dL [8-9].

It has been shown that the liver tissues have about 33% of lead repository and that makes it the largest among soft tissues [10-11]. Lead causes damage to various tissues and alters normal biochemical processes. The mechanism by which lead induces injury is to increase the production of reactive oxygen species and oxidative stress that results in damage to the DNA [11-15].

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Natural plant remedies have been used to ameliorate the toxic damages to the tissue owing mainly to their antioxidant characteristics [16]. *Ocimum gratissimum* is a plant with antioxidant activity and a tropical plant found in India and West Africa. Savannah and coastal areas of Nigeria is mostly the home of this plant. In many Countries, *Ocimum gratissimum* has been used extensively in the traditional medicine and as a condiment and culinary in meal preparation [17]. Researchers have focused a lot on dietary antioxidants that may be a potential therapeutic agent and their uses in preventing certain ailments. These investigations have led to discovering plants several bioactive components such as flavonoids, phenolics, limonoids, carotenoids, coumarins, phytosterols, with a lot of medical and health benefits [16]. Our aim in the present study was to determine the influence of *Ocimum gratissimum* leave extract on lead induced changes in hepato-biochemical parameters in adult Wistar rats.

MATERIALS AND METHODS

Plant collection, identification and extraction

Ocimum Gratissimum (OG) leaves were purchased from the market and were identified and authenticated by a Botanist in the Department of Biology of the University. The leaves were washed in running water and rinsed with clean water to remove any debris, dried under shade before it was grounded into powder. 100 g of the fine powder was soaked in 750 ml of distilled water for 1 hour and concentrated using a rotary evaporator. The paste was stored at a temperature of less than 40°C until used for experiments according to the methods of Okechukwu, et al. (2019) [18].

Animal grouping

Ethical approval was obtained from the University, Ethics and Animal Handling Committee. Thirty-five adult Wistar rats of mean weight (90 ± 30) g used in the study were assigned into 7 groups of 5 animals per group. The animals were acclimatized for two weeks in the Animal house. The rats fed with standard feed and water ad libitum during the experimental period were divided into seven groups of five rats each.

Experimental protocol

The extract dosage was arrived at using the LD_{50} of 1250 mg/kg body weight [19]. The stock solution was prepared by dissolving 700 g of the extract in 100 ml of distilled water, 60% (750 mg/kg body weight) and 30% (375 mg/kg body weight) of the LD_{50} were used for the high and low dose respectively. Group 1 was the Control and took only feed and water. Group 2 received 120 mg/kg body weight of lead only, while Group 3 received 375 mg/kg of OG only. Group 4 received 120 mg/kg of lead and 375 mg/kg of OG extract, while Group 5 received 120 mg/kg of lead and 750 mg/kg of OG extract. Group 6 received 375 mg/kg of OG extract for two weeks followed by 120 mg/kg of lead for one week, while the rats in Group 7 received 120 mg/kg of lead and 1190 mg/kg of ascorbic acid (Vitamin C) according to the methods of Okechukwu, et al. (2019) [18].

Animal sacrifice

After administration, animals were sacrificed after a day of fast by cervical dislocation. The blood was collected through cardiac puncture for hematological and biochemical analyses.

Biochemical analysis

Biochemical parameters studied include Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST); Gama Glutamyl Transferase (GGT) and Alkaline Phosphatase (ALP) were assayed spectro photochemically using commercially available kits according to the methods of Sayed, et al. (2015) [20].

Alanine Aminotransferase (ALT) activity

The activity of ALT was determined using the Reitman-Frankel colorimetric method for *in vitro* determination of ALT in serum using a Quimica Clinica Applicada (QCA) test kit [20]. ALT activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydazine which is proportional to its concentration at 505 nm.

Aspartate Aminotransferase (AST) activity

AST activity was determined by the Reitman-Frankel colorimetric method for *in vitro* determination of AST in serum using a Quimica Clinica Applicada (QCA) test kit [21]. AST activity was measured by monitoring the concentration of Oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydazine at 505 nm.

Gama glutamyl transferase

Serum was separated by centrifugation at 3600 rpm for 15 mins for the determination of serum Gama Glutamyl Transferase (GGT) levels using Qumica Clinica Applicada (QCA) commercial test kits according to the manufacturer's instruction.

Alkaline Phosphatase (ALP) activity

Phenolphthalein monosphosphate method for the *in vitro* determination of alkaline phosphatase in serum using Quimica Clinica Applicada (QCA) test kit. Alkaline phosphatase acts upon the AMP-buffered sodium thymolphthalein monophosphate. Addition of the alkaline reagent stops the enzyme activity and simultaneously develops a blue chromagen which can be measured spectrophotometrically at wavelength of 550 nm.

Statistical analysis

The results were expressed as Mean ± Standard Error of Mean (M ± SEM). Data obtained from biochemical studies were analyzed using one-way Analysis of Variance (ANOVA) for comparison between means for treated Groups and Control group for statistical difference using SPSS package version 20. The values of $p \leq 0.05$ were considered as significant.

RESULTS

The result from Table 1 showed a significant increase in the mean weights of the animals and organs in Groups B and E compared with Group A (Control) ($p<0.005$) while there was no significant difference when compared with Groups C and F.

The result showed a significant increase in WBC in Group B compared with Groups A, C, D, E and F ($p<0.005$). There was no significant difference in hemoglobin in Group B compared with Groups A and E while the result showed a significant decrease when compared with Groups C, D, F and G ($p<0.005$) as shown in Table 2.

The result of the differential white blood cell count showed significant decrease in monocytes and neutrophils in Group B compared with Group A ($p<0.005$), and there was no significant difference when compared to Groups (C-G). There was significant increase in lymphocytes and eosinophils in Group B compared with Groups A, C, D, E and F ($p<0.005$) while there was no significant decrease when compared with Group F as shown in Table 3.

Table 1: The mean weight of the organ and the percentage liver index.

Groups	Final weight (MEAN ± SD) (g)	Liver weight (MEAN ± SD) (g)	Percentage liver index (%)	Relative weight
A	114.5 ± 12.99	4.45 ± 0.62	3.9	0.04
B	153.9 ± 42.84*	6.08 ± 0.47*	3.95	0.04
C	151.9 ± 49.99	6.1 ± 1.28	4.01*	0.04
D	137.7 ± 19.34**	5.60 ± 0.69**	4.06*	0.04
E	201.4 ± 20.27*	6.31 ± 0.41*	3.13	0.03**
F	151.2 ± 24.99	6.09 ± 1.06	4.03*	0.04
G	145.0 ± 11.79**	4.82 ± 0.59**	3.32	0.03

*,** Represent significant increase or decrease at p ≤ 0.05 when compared to the Control (Group A). Values are Mean ± SD.

Table 2: Hematological parameters from the experimental groups.

Groups	RBC (MEAN ± SD) (1012/L)	WBC (MEAN ± SD) (109/L)	Hemoglobin (MEAN ± SD)(g/L)	PCV MEAN ± SD) (L/L)
A	6.67 ± 0.58	17.00 ± 0.00	13.0 ± 0.00	42.33 ± 0.58
B	5.60 ± 0.58**	31.0 ± 1.00*	13.33 ± 0.58	40.67 ± 1.53**
C	6.67 ± 0.58	23.33 ± 6.43	12.33 ± 0.58**	42.00 ± 1.00
D	6.63 ± 0.58	26.00 ± 4.00	12.67 ± 0.58**	41.67 ± 2.31
E	6.33 ± 0.58	26.67 ± 3.51	13.33 ± 0.58	42.33 ± 1.16
F	6.67 ± 0.58	25.67 ± 3.21	12.67 ± 0.58**	40.67 ± 2.31
G	6.33 ± 0.58	28.00 ± 1.00	12.67 ± 1.16**	41.33 ± 1.53

*; ** Represent significant increase or decrease at p ≤ 0.05 when compared to Control (Group A). Values are mean ± SD.

The result of liver function test showed a significant increase in ALP, AST, GGT, and ALT in Group B compared with Groups A. C, D, and G ($p<0.005$). The result showed a significant decrease in RBC and PCV in Group B compared with Groups A, C, D, E, F and G ($p<0.005$) as shown in Table 4.

Histological studies

The results from histological observations showed liver from Group A (Control) with normal hepatic structure with hepatocytes, central vein and sinusoids as shown in Figure 1 While the liver tissues of the rats exposed to lead only (Group B), showed severe distortion with focal aggregation of inflammatory cells as shown in Figure 2. Group C liver tissues showed a normal hepatic structure with Central Vein (CV) and Hepatocytes (H) similar to that of the Control as shown in Figure 3. The liver tissues of Group D rats showed mild congestion of the central vein as shown in Figure 4. The liver tissues of animals in Groups (E and F) showed normal histological structure as represented in Figures 5 and Figure 6 respectively while the liver of animals in Group G showed normal histological structure Figure 7 when compared to that of Group 2.

DISCUSSION

The present study showed progressive increase in the body weight of experimental animal models throughout the period. However, no weight loss was observed throughout the week of experiment which could be as a result of ad libitum method of feeding. The result was in agreement with the reports of Rabelo et al., (2003) and Rossi (2008) [19, 22]. The increase in body weight of the animals could be due to the active components found in the extract which are good for the body [23]. We observed a reduced physical activities and increase rate of respiration which might be attributed to reduction in energy generation due to hypoxia caused by met hemoglobin formation which agrees with that of Philip and Gerson (1994); and Yu et al., (2004) [24-25]. Furthermore, the results showed a significant increase in the liver weight of rats treated with *Ocimum gratissimum* when compared to the Control Group which was not in agreement with Rossi (2008) as increase in the liver of animals have been shown to be a sign of hepatotoxicity [1,22,26]. Balance in the activities of serum enzyme markers such as AST, ALT, GGT and ALP is used to mark the functionality of a normal liver [26-27]. If the liver produces very high level of AST and ALT and releases such into the blood stream, it signifies danger called hepatocellular necrosis. Increased level of ALT presents a sign of liver injury because it represents 90% of whole body total enzymes [1,28]. The activities of ALP relate to the liver cells functioning and it has been shown that increase in its activity as a result of increased production in the presence of increased biliary pressure [29-30]. In analyzing the liver enzymes, we noticed a significant increase in ALT, AST, ALP and GGT in Group B when compared to control. The histological examination revealed the toxic effects of lead on the liver such as distortion, focal necrosis and aggregation of inflammatory cells in Figure 2 while sections of liver treated with extract of OG revealed that the extract has ameliorating effects on. Also Group F when compared with Group G showed normal hepatic architecture with central vein, hepatocytes and sinusoids. The apparent decrease in the mean body weight when Group B were compared with Group A might have resulted from the hypoglycemic and the diuretic effect of the extract. There was a significant reduction in the weight of the animals that received only lead compared to the control during the experimental period, and this decrease may be due to the reduction in food intake [24-25]. Generally, weight loss may be the attribute of low food intake, hormonal balance disturbances or direct cytotoxic effect of lead. In analyzing our hematological parameters, we recorded a decrease in PCV, RBC and Hb concentration in the given lead alone which may be as a result of anemia as a consequence of the toxic effect of lead on bone marrow, spleen and liver [19,31]. Imaizumi et al., (1980) and Wu (2006) had reported that this reduction might be due to lead administration [6,32], which was accompanied by a remarkable increase of met hemoglobin level but increase in white blood cell concentration was also recorded [16]. There was also a significant decrease in monocytes and neutrophils and an increase

Table 3: The Differential count of the white blood cells.

Groups	Monocyte ($10^{9/L}$)	Lymphocytes ($10^{9/L}$)	Neutrophils ($10^{9/L}$)	Eosinophils ($10^{9/L}$)
A	2.00 ± 0.00	11.33 ± 0.58	82.67 ± 0.58	2.00 ± 0.00
B	1.00 ± 0.00**	15.67 ± 0.58*	79.00 ± 1.73**	3.00 ± 0.00*
C	1.67 ± 0.58	12.67 ± 1.16	81.67 ± 0.58	2.67 ± 0.58
D	1.67 ± 0.58	13.00 ± 0.00	80.00 ± 1.00	3.00 ± 0.58
E	1.67 ± 0.58	12.67 ± 0.58	80.33 ± 0.58	2.33 ± 0.58
F	1.67 ± 0.58	12.67 ± 0.58	79.00 ± 2.65	2.67 ± 0.58
G	1.67 ± 0.58	12.33 ± 0.58	78. 67 ± 2.31	2.67 ± 0.58

*,** Represent significant increase or decrease at $p \leq 0.05$ when compared to the Control (Group A). Values are mean ± SD

Table 4: The liver function test in the experimental animals.

Groups	ALP (U/L)	ALT (U/L)	AST (U/L)	GGT (U/L)
A	125.5 ± 2.12	63.15 ± 1.34	126.3 ± 5.94	11.65 ± 0.64
B	177.7 ± 4.04*	72.83 ± 2.03*	158.2 ± 5.37*	12.37 ± 0.12*
C	141.0 ± 5.00	67.23 ± 2.10	132.1 ± 1.94	11.27 ± 1.05
D	148.3 ± 3.22	70.00 ± 0.79	138. 1 ± 4.37	11.03 ± 0.64
E	142.0 ± 5.00	66.90 ± 3.21	133.9 ± 1.44	10.60 ± 0.61
F	145.3 ± 4.16	69.87 ± 1.43	138.9 ± 2.92	10.37 ± 0.12
G	141.0 ± 8.54	68.00 ± 2.77	138.0 ± 3.84	10.20 ± 0.95

*represent significant increase or decrease at $p<0.05$ and $p<0.01$ when compared to the Control (Group A). Values are mean ± SD.

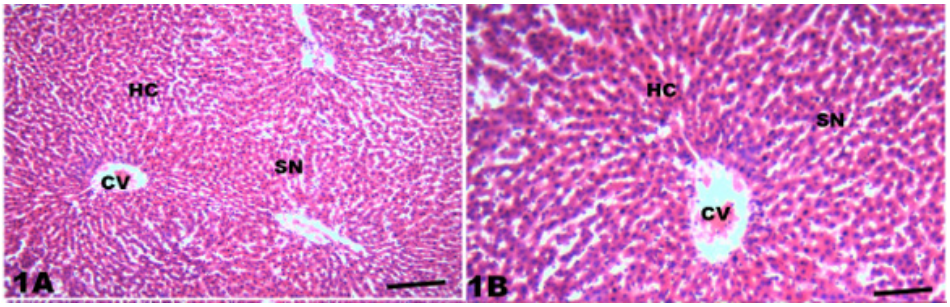


Figure 1: A section of the liver of Group A (Control) animals showing normal hepatic structure with Central Vein (CV), Hepatocytes (HC) and Sinusoids (SN). H and E; Mxg: x100; x400; Scale Bar; 1 µm=5 mm.

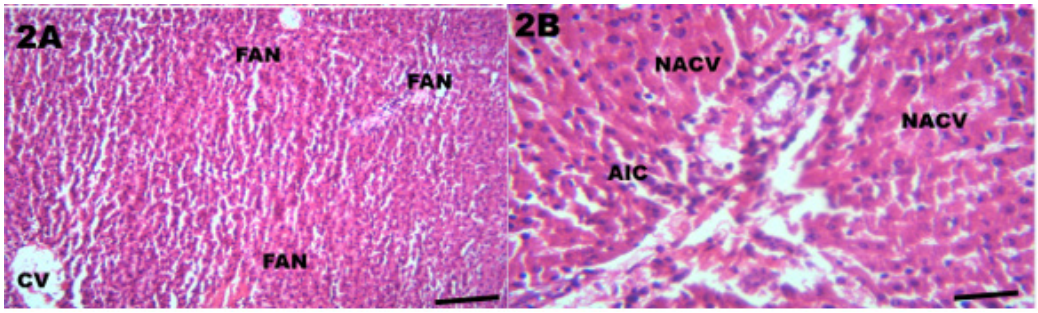
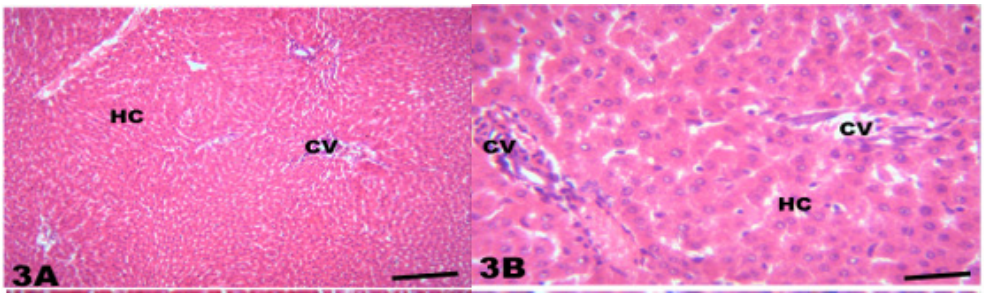
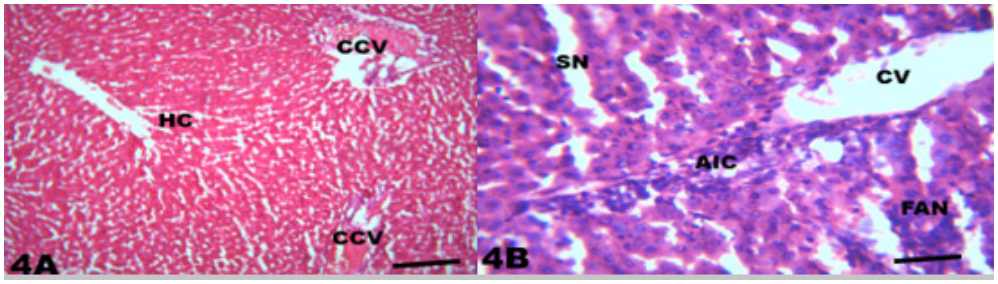


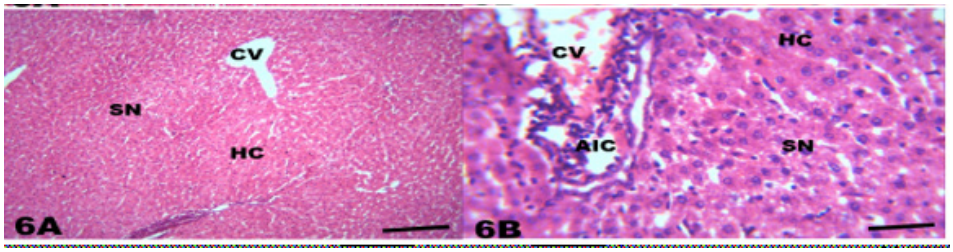
Figure 2: A section of the liver of Group B animals (induced with only lead) showing distortion of hepatic structure, Focal Area of Necrosis (FAN), Aggregate of Inflammatory Cells (AIC) and Necrosis Around Central Vein (NACV). Stain: H and E; Magnification: x100; x400; Scale Bar; 1 µm=5 mm.



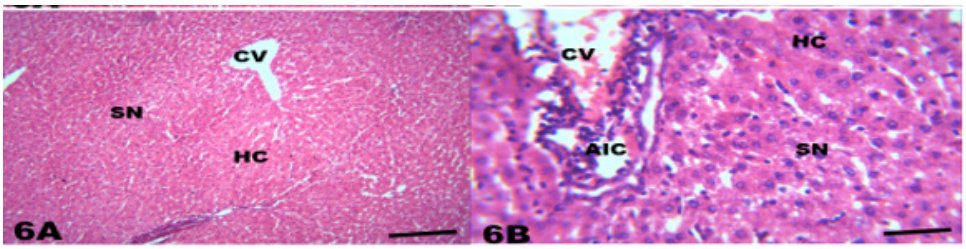
Figures 3: A section of the liver of Group C animals administered with *Ocimum gratissimum* only showing normal hepatic structure with Central Vein (CV) and Hepatocytes (HC). Stain: H and E; Magnification: x100; x400, Scale Bar; 1 µm=5 mm.



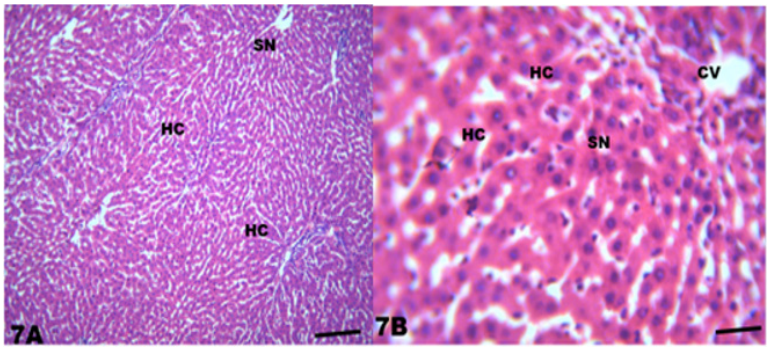
Figures 4: A Section of the liver of Group D animals administered with 375 mg/kg *Ocimum gratissimum* and 120 mg/kg lead acetate, showing mild Congestion of Central Vein (CCV) with normal Hepatocytes (HC) with Aggregate of Inflammatory Cells (AIC), Focal Area of Necrosis (FAN) and enlarged Sinusoids (SN) Stain: H and E; Magnification: x100; x400. Scale Bar; 1 µm=5 mm.



Figures 5: A section of the liver of Group E animals administered with 750 mg/kg of *Ocimum gratissimum* and 120 mg/kg of lead acetate, showing Focal Area of Necrosis (FAN), Central Vein (CV), Enlarged Sinusoids (ESN) and some Area of Necrosis (AN). Stain: H and E; Magnification: x100; x400.



Figures 6: A Section of the liver of Group F animals administered with 375 mg/kg of *Ocimum gratissimum* for two weeks and then 120 mg/kg of lead acetate for one week showing the Central Vein (CV). Normal Hepatocytes (HC), Sinusoid (SN) and Aggregation of Inflammatory Cells (AIC). Stain: H and E; Magnification: x100; x400; Scale Bar; 1 µm=5 mm.



Figures 7: A Section of the liver of Group 7 (fed with vitamin C and 120 mg/kg of lead acetate) showing Sinusoids (S). Stain: H and E; Magnification: x100; Scale Bar; 1 µm=5 mm.

in lymphocyte concentration.

CONCLUSION

The extract showed ameliorative and protective effects on hepatic toxicity in our experiment and as such we strongly recommend its beneficial use in a controlled manner in the management of hepatotoxicity that involves depletion of liver enzymes.

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