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AMELIORATIVE EFFECT OF *Nigella sativa* OIL ON LEAD ACETATE INDUCED HEPATOTOXICITY ON ADULT MALE WISTAR RATS

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ABSTRACT

There is an increasing concern about the rising level of lead acetate in environment mainly due to unsafe mining and extraction of natural resource. Several evidences have indicated the devastating effect of the lead exposure to different parts of the body. The *Nigella sativa* oil is used as natural herbal product for healing of many diseases. Its pharmacological constituents have been shown having anti-oxidant, anti-inflammatory, anti-microbial and anti-parasitic efficacy. This study investigated the ameliorative protective effects of *Nigella sativa* (black seed) oil on lead acetate induced hepatoxicity in adult male wistar rat. A total number of fourty two rats were divided into six groups of seven rats per group. Group I rats (normal control) were administered normal saline equivalent to the volume administered to the highest dose of treated rats. Group II (negative control)) received 120 $mgkg^{-1}$of lead acetate while and group III (positive control) received 120 $mgkg^{-1}$of lead acetate and 160 $mgkg^{-1}$of vitamin C. Groups IV, V and VI were administered with 120 $mgkg^{-1}$ of lead acetate followed by 6 $mlkg^{-1}$, 4 $mlkg^{-1}$, 2 $mlkg^{-1}$of *Nigella sativa* oil respectively via oro-gastric intubation for a period of seven days. The lead acetate caused significant increased (P<0.001) of the liver enzymes (AST, ALT and ALP) in all the negative control groups as compared with the treatment groups, which appeared to be dose dependants. Histopathology observation showed severe liver damage in the lead acetate group. However there is great improvement in the morphology of the liver in the treated groups even at the minimal doses. The changes in the level of biomarkers and improvement in the histomorphological appearance of the liver suggested that, consumption of *Nigella sativa* oil can be used to ameliorate liver damage.

Keywords: *Nigella sativa* oil, hepatotoxicity, lead acetate, liver

INTRODUCTION

Environmental and occupational exposure to lead acetate has been a major concern in most of the developing countries due to its hazardous effect (Wani *et al.,* 2015). Recently, lead exposure has resulted to death of hundred lives in Nigeria (Tirima *et al.,* 2016). There is an increasing concern about the rising level of lead acetate in environment mainly due to unsafe mining and extraction of natural resource (Dooyema *et al.,* 2012; Tirima *et al.,* 2016). The range of the sources of lead exposure is extensive and industrial activities has been identified as one of the major source in many countries (Kar-Purkayastha *et al.,* 2012). Several evidences have indicated the devastating effect of the lead exposure to different parts of the body such as blood (Ibrahim *et al.,* 2012), bone marrow (Haleagrahara *et al.,*

2011), liver and kidney (Offor *et al.,* 2017) among others.

Nigella sativa is a small shrub plant, present in many parts of the world with a large fruit, containing several seeds (Ahmad *et al.,* 2013). Traditionally, different parts of the of *Nigella sativa* has been used to treats many ailments (Ahmad *et al.,* 2013; Yimer *et al.,* 2019). Studies have also reported the antibacterial (Chaieb *et al.,* 2011), antioxidant and anti-inflammatory (Bordoni *et al.,* 2019) and antidiabetic (El Rabey *et al.,* 2017) activities of the *Nigella sativa*. Similarly, its active constituents has been shown to improve learning and memory (Sahak *et al.,* 2016), possess anticancer activity (Agbaria *et al.,* 2015) and also play role as hepato and nephro protective (Hasan *et al.,* 2016) against liver and kidney toxicity.The *Nigella sativa* oil is used as natural herbal product for healing of many diseases (Yimer *et al.,* 2019). Several studies have proved the pharmacological usage of *Nigella sativa* oil as having analgesic, anti-oxidant, anti-inflammatory, anti-microbial and anti-parasitic efficacy (Ahmad *et al.*, 2013; Yimer *et al.,* 2019). Previous studies have demonstrated the ameliorating activities of the *Nigella sativa* oil on other hepatotoxic drugs such as paracetamol (Hasan *et al.,* 2016).

The strategies on ameliorating the toxicity of lead acetate are of clinical benefits and the present study focused on the treatment, based on the fact that *Nigella sativa* oil could be used as an adjuvant therapy in rats with lead acetate ingested substances. It is hypothesized that, treatment with *Nigella sativa* oil will ameliorate the hepatotoxicity caused by lead acetate.

MATERIALS AND METHODS

Ethical Approval and Chemicals Used

The Animal Care and Use Committee (ACUC), Faculty of Health Sciences, Bauchi State University, Gadau approved this study. (Ref. number: BASUG/FHS/ACUC/2019/017). A pure (100% Natural) *Nigella sativa* oil (Hermani International KEPZ, Kerachi-Pakistan) was obtained from a commercial shop (Makkah and Madina shop) in Azare local Government of Bauchi State, Nigeria. The Lead acetate (BDH chemical Ltd Poole England, 29021) was obtained from chemistry department, Bauchi State University, Gadau. The vitamin C (Care Industrial Ltd Debo, Lagos, VC 491) was purchased from a commercial drug store in Azare local government area of Bauchi State, Nigeria.

Animal Husbandry

Adult wister rats (both sexes) weighing 55-88g, postnatal age 6 weeks, were obtained from National Veterinary Research Institute Vom Jos, Plateau State of Nigeria. Animals handling was performed in accordance with ACUC guidelines. The animals were kept for 2 weeks acclimatization period in the animal house facility of Human Anatomy Department, Faculty of Basic Health Science, Bauchi State University, Gadau. The rats were sheltered and caged in plastic cages of dimensions 140 cm x 70 cm x 60 cm under a uniform husbandry condition at room temperature, with 12hr light/dark cycle. Cleaning of the animals cages is done daily and rats were fed with standard laboratory diet (Bendel feeds, Ilorin) and drinking water *ad libitun.*

Experimental Design

Acute Toxicity

The lead acetate acute toxicity was performed following Up and Down procedure as described in guideline for testing of chemicals (Bruce, 1985). A total number of four rats were used and were administered 10 $mlkg^{-1}$, 50 $mlkg^{-1}$, 250 $mlkg^{-1}$ and 1250 $mlkg^{-1}$ body weight of lead acetate respectively at different time as described. The animals were observed for 48-72 hours for neurological, behavioural changes and mortality. Similarly, a total number of five adult, female, non-pregnant rats were randomly selected for the *Nigella sativa* oil acute toxicity following the same method (Bruce, 1985). The rats were administered single oral dose of 10 $mlkg^{-1}$ 20 $mlkg^{-1}$, 40 $mlkg^{-1}$, 80 $mlkg^{-1}$ and 160 $mlkg^{-1}$ body weight of *Nigella sativa* oil orally respectively following the standard protocol for Up and Down procedure (Bruce, 1985). The rats were observed individually at least once during the first 30 minutes after administration and periodically for 72 hours. Toxicological effects were assessed on the basis of mortality.

Experimental Protocol

A total number of fourty two rats were divided into six groups of seven rats per group. Group I rats (normal control) were administered normal saline equivalent to the volume administered to the highest dose of treated rats. Group II (negative control)) were administered with 120 $mgkg^{-1}$of lead acetate while and group III (positive control) received 120 $mgkg^{-1}$of lead acetate and 160 $mgkg^{-1}$of vitamin C. Groups IV, V and VI were administered with 120 $mgkg^{-1}$ of lead acetate followed by 6 $mlkg^{-1}$, 4 $mlkg^{-1}$, 2 $mlkg^{-1}$of *Nigella sativa* oil respectively via oro-gastric intubation for a period of seven days. All the controls and treated groups were studied in parallel with administration done between 0900hr-0100hr daily. After the administration, the rats were allowed free access to feed and clean water. The body weights were recorded daily using an electronic balance (OHAUS AX150/E). Clinical signs such as weakness, poor respond to physical activities and fluctuation in convulsion of the rat was observed throughout the study.

Animal Euthanization and Sample Collection

To eliminate perception of pain, the rats were deeply anaesthetized using chloroform. The rats were disinfected with 70% ethanol and the abdominal skin was incised to expose the abdominal cavity. Blood sample (2ml-5ml) was collected through cardiac puncture from each rat. The blood were dispensed into a specimen container (Lithium heparin) and subjected to further treatment for determination of the liver biomarkers following the standard procedure. The liver tissues were excised and fixed in 10% formalin for histopathological assessment.

Tissue Processing, Staining and Microscopic Examination

Tissue processing was performed according to standard paraffin embedded procedure. The tissues was cut into thin sections and placed in a tissue cassette with label for processing (dehydration, clearing and infiltration) using automatic tissue processor (LUPETEC, PT09 TS). Tissues were embedded using embedding machine (BIOBASE, BK CPII) and the samples were sectioned at 7 µm using rotary microtome (BIOBASE, BK-MT268M). Serial sections of the tissues were collected and mounted onto a microscope slides (Hecate, 7105) and slides were labelled accordingly and dried at 40^{o}C on hot plate. The haematoxylin and eosin (H & E) (TissuePro Technology, H08-500R) stain was performed following the

manufacturer's protocol to assess the general morphology of the liver. Micrographs of the liver tissues were capture using a bright-field light microscope (BIOBASE, BMB-300M) with CCD digital camera attached to the microscope. From each slide, three images were obtained and assessed for any histopathological conditions.

Statistical Analysis

Numerical data obtained from the study were analysed using SSPS (Version 25). The difference in the significance level between the control groups and treated groups was determined using analysis of variance (ANOVA) and the probability values of $P \leq 0.05$ were considered to be statistically significant. The results were expressed as mean ± SEM.

RESULTS

Acute Toxicity

There was signs of drowsiness and loss of appetite for the first six hours in all the rats. The rats survived the highest doses of both lead acetate and *Nigella sativa* oil, with zero mortality rates.

Effect of *Nigella sativa* Oil on the Mean Body Weight

There was change in the body weight of the rats over the period of treatment in both the control and treated groups. However, analysis showed no significant differences significant (P=0.08) in the body weight gained between the control groups and the treated groups (Table 1).

Table 1: Effect of *Nigella sativa* oil on lead acetate induced hepatotoxicity on the mean body weight gained.

Treatments	Initial body weight (g)	Final body weight (g)	Body weight difference (gm)
Normal saline	70.71±5.07	74.05±5.07	3.34±0.17
Normal saline + Lead (120 $mgkg^{-1}$)	64.71±5.59	66.82±5.59	2.11±0.55
Lead + Vitamin C (160 $mgkg^{-1}$)	80.40±4.75	81.23±4.75	0.83±0.10
Lead + *Nigella sativa* oil (6 $mlkg^{-1}$)	74.86±4.41	76.45±3.24	1.569±0.94
Lead + *Nigella sativa* oil(4 $mlkg^{-1}$)	68.86±5.65	69.63±5.51	0.770±0.12
Lead + *Nigella sativa* oil(2 $mlkg^{-1}$)	71.86±4.12	73.07±3.74	1.210±0.64

**Statistically significant, N*=7.

Nigella sativa oil ameliorates the effect of lead acetate induced hepatotoxicity in rats.

The activity of the liver enzymes was used to assess the ameliorative potential of the *Nigella sativa* oil on lead acetate hepatotoxicity in rats. Analysis of the result showed that, aspartate transaminase (AST) level increased significantly in all the treated groups as compared to the normal control group. Analysis within the groups showed that, AST level was significantly greater in both group II (86.67±1.67, n=6) and group III (68.00±3.00, n=5) as compared with group I (42.33±1.45, n=6,P<0.001). Similarly, there was a change in the AST level in all the treated groups compared with the control groups. The analysis (ANOVA) revealed a significant difference (P<0.001) in the AST level between the treated groups [(group IV = 66.00±4.93, n=5), (group V = 74.33±5.24, n=6) and (group VI = 81.67±5.21, n=6)] compared with the negative control group (86.67±1.67, n=6). Also, analysis within the treated groups showed that, AST level in the group IV (66.00±4.93) reduced significantly compared to that of group VI (81.67±5.21, P<0.05) (Table 2).

The alanine transaminase (ALT) level was markedly reduced following treatment of the rats with different doses of the *Nigella sativa* oil. The result showed a higher level of ALT in the negative control group compared with the treated groups which appeared to be statistically significant (P=0.04). Also, analysis within the treated groups showed no significant difference (P<0.08) in the level of ALT [(group IV = 32.33±5.90, n=5), (group V =30.00±5.51, n=6) and (group VI= 31.67±3.20, n=6)] (Table 2).

The effect of the *Nigella sativa* oil on the alkaline phosphatase (ALP) level showed an increase in the activities of the enzyme in the negative control group (68.86±6.33, n=6) as compared with the normal control group (31.86±3.03) and was found to statistically significant (P<0.001). There is significant difference in the ALP level between the negative control group (68.86±6.33, n=6) and the treated groups [(group IV = 33.55±3.66, n=5, P<0.001), (group V = 43.15±3.66, n=6, P<0.05) and (group VI=56.05±4.26, n=6)]. Similarly, analysis within the groups showed the level of ALP in the rats that received lowest dose of *Nigella sativa* oil (group VI =56.05±4.26, n=6) was significantly higher as compared with the group IV rats (33.55±3.66, n=5, P<0.01) (Table 2).

Table 2: Effect of Nigella sativa oil treatment on lead acetate induced hepatotoxicity on rats

Treatments	AST (μL^{-1})	ALT (μL^{-1})	ALP (μL^{-1})
Normal saline	42.33±1.45	36.33±2.19	31.86±3.03
Normal saline + Lead (120 $mgkg^{-1}$)	86.67±1.67	45.67±0.33	68.86±6.33
Lead + Vitamin C (160 $mgkg^{-1}$)	68.00±3.00	37.33±5.90	45.85±2.93
Lead + *Nigella sativa* oil (6 $mlkg^{-1}$)	66.00±4.93	32.33±5.90	33.55±3.66
Lead + *Nigella sativa* oil(4 $mlkg^{-1}$)	74.33±5.24	30.00±5.51	43.15±3.66
Lead + *Nigella sativa* oil(2 $mlkg^{-1}$)	81.67±5.21	31.67±3.20	56.05±4.26

**Statistically significant, N*=7

Nigella sativa oil improved liver histomorphology on lead acetate induced hepatotoxicity

Histomorphological assessment of the liver stained with H and E showed normal architecture of liver, with the central vein, hepatocytes and sinusoids in the normal control group (Figure 1A). Examination of the liver sections administered with 120 $mgkg^{-1}$ body weight of lead acetate showed degenerated histology of hepatocytes and sinusoids, with occlusion of some central vein (Figure 1B). However, there was improvement in the architecture of the liver in group III rats (standard drug) with sinusoids and hepatocytes. Derangement within the tissues was observed more especially along the central vein (Figure 1C). Moreover, there was improvement in the morphology of the liver treated with 6 $mgkg^{-1}$*Nigella sativa* oil. The radiating hepatic cords and sinusoids were restored, with normal central veins (Figure 1D).

It is apparent that, the appearance of the liver treated with 4 $mgkg^{-1}$ body weight *Nigella sativa* oil showed improvement in the histomorplogy as compared with that of the negative control. The morphology of the central vein, the hepatocytes and the sinusoids were improved (Figure 1E). Similarly, the liver section of the group treated with 2 $mgkg^{-1}$ body weight *Nigella sativa* oil also showed improvement in the morphology of the hepatocytes and sinusoids. Although, there is appearance of the merged central veins (Figure 1F). This

also indicates improvement in the appearance of the liver sections even at the lower dose of the *Nigella sativa* oil.

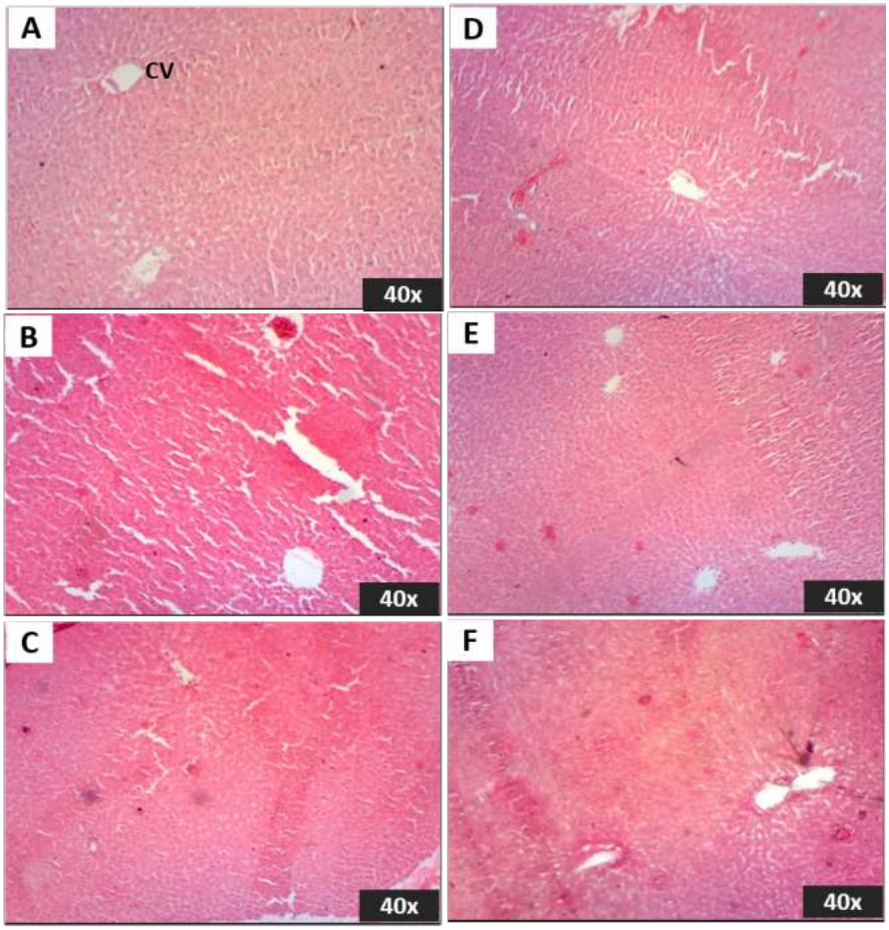

Figure 1: Haematoxylin and Eosin staining of the liver sections.Histomorphological assessment of the liver section from the normal control group(A) showed normal arrangement of central vein (CV), sinusoids and hepatocytes. Liver sections from group II (B) showed distortion of the sinusoids and hepatocytes with congestion of some central veins. Assessment of the liver section of group III showed improvement in the histomorphology of the sinusoids and hepatocytes (C). The liver section from the treated groups [Group IV (D), Group (V) and group VI (F)] showed an improvement in the histomorphology of the liver even at the lower dose of 2mgkg (F).

DISCUSSION

Treatment of rats with *Nigella sativa* oil at different doses was observed to ameliorate the effect of lead acetate induced hepatotoxicity. It is known that, exposure to lead acetate is one of the primary cause of liver damage and its corresponding biomarker alteration. Emerging evidences from plant study have indicated their role in modulations the toxic effects of lead acetate (Arti e*t al.,* 2010) particularly those with antioxidant constituents (Bordoni *et al.,* 2019). Also, findings have demonstrated the safety of *Nigella sativa* oil as several studies has reported the absence of toxicity in mice treated with this oil (Zaoui *et al.,* 2002). Evaluating the activity of the marker

enzymes in tissues have been used in assessment of plant for safety, toxicity risk or medicinal benefits. Similarly, it plays a significant role in disease investigation. The AST and ALT considered in this study are useful marker enzymes of liver cytolysis or damage to plasma membrane (Gowda *et al.,* 2009) and their expression level provides an insight to the severity of the tissue damage or its improvement after treatment.

The AST appeared to increase in all the treated groups as compared with the normal control. Similarly, its level increased significantly in the negative control group indicating injury and impaired function of liver as a result of lead acetate intoxication. Following treatment of the liver with *Nigella sativa* oil, the level of the AST have reduced significantly compared with that of the negative control. It was further observed that, the level of the AST in the rats treated with high dose of the *Nigella sativa* oil, reduced significantly, thus indicating the ameliorating effect of the *Nigella sativa* oil at this dose. A similar finding was observed following treatment of *Nigella sativa* oil on paracetamol induced hepatotoxicity in rats (Offor *et al.*, 2017). An increase in the level of AST is usually accompanied by the elevation in the ALT level. Both the two enzymes are excellent markers of liver damage caused by exposure to toxic substance (McGill, 2016). AST can be located in other organs like kidney, heart, brain and skeletal muscles (Huang *et al.,* 2006), thus is not specific for liver alone. However, the ALT is more specific to liver and widely used for diagnostic purposes.

When the integrity of the hepatocellular membrane is compromised, there is extrusion of this enzyme in to the plasma (Arun *et al.,* 2012), hence the significant increased level of ALT in the negative control. This increase is an indication of hepatocelluar damage caused by the lead acetate (Contreras-Zentella & Hernández-Muñoz, 2016) as it is well known that, an elevated ALT level is a sensitive clinical indicator of hepatocellular injury (Kim *et al.*, 2008). Following the treatment of the rats with *Nigella sativa* oil, the level of ALT have reduced significantly especially in the group treated with highest dose of *Nigella sativa* oil. The current findings is also in line with other studies that reported reduced level of ALT following treatment with *Nigella sativa* oil (Mosbah *et al*., 2017; Hasan *et al.,* 2016). The level of ALT in the normal control group is higher as compared with that of the treatment groups. These observations could perhaps due to the oro-gastric method of administration, which could result to an increase in the oxidative stress . However decrease in the level of ALT enzyme observed in the treatment groups, following administration of *Nigella sativa* oil, was due to the anti-oxidants effect and free radical scavenging role of *Nigella sativa* oil (Bordoni *et al.,* 2019). Previous studies also reported similar findings that, *Nigella sativa* seeds /oil extract increased the activity of ALT and AST enzymes (Arti *et al.*, 2010; Hasan *et al.,* 2016).

The ALP is a marker enzyme for plasma membrane and it is often used to assess its integrity (Sharma *et al.,* 2014). Increases in the level of ALP especially in the negative control group may be an indicator of liver disease or damage, as it is one of the main source of ALP (Sharma *et al.,* 2014). The decrease in the level of ALP observed in the treated group indicates the ameliorating effect of the *Nigella sativa* oil on the damaged liver and this effect is dose dependent. The current findings is in line with other studies that reported an reduced level of ALP following treatment with

Nigella sativa oil (Arti *et al.*, 2010; Hasan *et al.*, 2016).

The histopathological changes observed in liver appeared to be dose dependent. The presence of the pathological lesions especially observed in the negative control group may not be surprising since liver is the main organ for biotransformation. Thus, the organ may have been exposed to the toxic substance. The improvement in the histomorphological appearance of the liver section of the rats treated with different doses of the *Nigella sativa* oil indicates the ameliorating potential of the oil. Our findings are in accordance with previous reports of other related studies (Arti *et al.,* 2010; Hasan *et al.,* 2016)that have suggested the protective role of *Nigella sativa* oil extract, possibly due to the antioxidative effect of flavonoids present in the seeds (Bordoni *et al.,* 2019), that act as strong superoxide radicals and singlet oxygen quenchers.

CONCLUSION

The study showed that, treatment of rats with *Nigella sativa* oil even at minimal dose moderately mitigate lead acetate induced hepatotoxicity and regulate the activities of liver biomarkers. This amelioration perhaps is due the antioxidative constituent of the *Nigella sativa* oil which usually binds with free radicals release by lead acetate, thus impairing the mechanism that causes organ toxicity. In view of the improvement in the histomorphological appearance of the liver tissue and the changes in the level of biomarkers, it is therefore suggested that, consumption of *Nigella sativa* oil by human is safe and can be used to ameliorate liver damage.

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