Effect of Nigella sativa Pre-Treatment on Sub-Chronic Lead Acetate Induced Hematological and Biochemical Alterations

Article *in* Journal of Computational and Theoretical Nanoscience · June 2017

DOI: 10.1166/jctn.2017.6565

7 authors, including:

Yusuf Abba
University of Maiduguri
125 PUBLICATIONS **90** CITATIONS
SEE PROFILE

Falah Hasan Baiee
University Of Kufa
9 PUBLICATIONS **4** CITATIONS
SEE PROFILE

Journal of
Computational and Theoretical Nanoscience
Vol. 14 (6), 2752–2758, 2017

Effect of Nigella sativa Pre-Treatment on Sub-Chronic Lead Acetate Induced Hematological and Biochemical Alterations

M. A. Assi[1 2], M. N. M. Hezmee[2 *], Y. Abba[3], M. Y. Sabri[3], A. W. Haron[4], F. H. Baiee[4 5], and M. A. Rajion[2]

[1]Department of Community Health, College of Health and Medical Techniques, Al_Furat Al_Awsat Technical University, Iraq
[2]Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[3]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[4]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[5]Department of Clinical Studies, College of Veterinary Medicine, University of Kufa, Iraq

Lead acetate (LA) toxicity can occur either by ingestion or inhalation from contaminated surfaces or from the environment. Nigella sativa is a natural product with immense pharmacological proper-ties. In this study, the effects of N. sativa pre-treatment on lead acetate induced hematological and biochemical changes were evaluated. A total of 20 male Sprague Dawley rats were divided into 4 groups with 5 rats each. Group 1 (NC) was the negative control, group 2 was the lead acetate control (PC) and was administered 10 mg/kg/per day of lead acetate (LA) per OS for 30 days, group 3 (T1) was administered 200 mg/kg/daily of Nigella sativa orally for a month and Group 4 (T2) was pre-treated with 200 mg/kg/daily of Nigella sativa orally for one month, followed by administration of 10 mg/kg/daily of lead acetate (LA) orally for another month. At the end of the experiment, whole blood and serum were collected to evaluate the complete blood profile and serum biochemistry. The haemogram showed lower ($p < 0$ 05) level of hemoglobin, packed cell volume and prothrombin in the PC group, while total white blood cell count, band neutrophils, segmented neutrophils, lymphocytes and monocytes counts were higher ($p < 0.05$) in the PC group than the treatment groups. However, eosinophil count was higher in T2, while no changes were observed in RBC and MCV values. Both alanine and aspartate aminotransferase enzymes were higher in the PC as compared to other groups. Similarly, the levels of alkaline phosphatase, cholesterol, urea and creatinine were all higher ($p < 0.05$) in the PC group and comparable ($p > 0.05$) in the control, T1 and T2 groups. The level of SOD and GSH were lower ($p < 0.05$) in the PC and T2 groups. In summary, this study showed the prophylactic potential of N. sativa extract in modulating both hematological, biochemical and anti-oxidant enzymes alterations induced by sub-chronic lead acetate administration in rats.

Keywords: Lead Acetate, Black Caraway Seed, Hematology, Biochemistry, Anti-Oxidant Enzymes.

RESEARCH ARTICLE

1. INTRODUCTION

Lead (Pb) is a common contaminant of the environment and water bodies found in paints, leaded gasoline, dust and soil in gold mining areas, as well as a result of improper industrial waste disposal. Following exposure to Pb, it binds to the blood, soft tissues and bones, thus remaining in these tissues for an extended time. Lead is particularly deadly to young children, resulting in non-regenerative anemia, neurological signs and development of nutritional Deficiencies[20]. Adults are mostly exposed at work and show clinical signs of microcytic anemia and nervous symptoms.[20] Experimental toxicity studies in laboratory animals have shown various disruptions and alterations in the animal's physiological function. In one study on acute toxicity of lead acetate (LA) in rats, administration of 10 mg or 100 mg of lead acetate caused elevation of alanine aminotransferase enzyme (ALT) after 12 and 24 hours, respectively.[7] In a related study, administration of graded doses of LA to rats for 30 days showed reduction in total protein, albumin, globulin, total

* Author to whom correspondence should be addressed.
Mohd Noor Mohd Hezmee: hezmee@upm.edu.my

bilirubin, red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb) and packed cell volume (PCV) while increasing the levels of ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine.[8] Other researchers have found similar finding following chronic administration of LA to rats for periods lasting from 14 to 24 weeks.[3 4 14] *Nigella sativa* (NS) is a popular medicinal plant whose seeds and leaves have been used to alleviate ailments for centuries. Its seeds and oil have been shown to have low toxicity and its constituents have shown good anti-inflammatory, analgesic, anti-carcinogenic, antidiabetic, antiulcer, antimicrobial and anti-parasitic activities.[2 11] In a related study, the immunomodulatory effects of *N. sativa* was attributed to amelioration of oxidative enzymes such as glutathione and superoxide dismutase, while its anti-inflammatory proper-ties were attributed to inhibition of COX and 5-LO path-ways of arachidonic acid metabolism.[22] Other animal studies have shown the protective effects of *N. sativa* against isoproterenol, tramadol, carbon tetrachloride, cisplastin and sodium valproate toxicities, through regulation of oxidative enzyme levels, hepatic function enzymes, renal function enzymes and blood lipid profiles.[6 9 13 16 18] Previously, *Nigella sativa* has been shown to ameliorate the biochemical imbalances and tissue damage associated with LA toxicity in rats.[10] However, the study lasted for 6 weeks only and does not reflect the true picture associated with chronic LA toxicity.

RESEARCH ARTICLE

2. MATERIALS AND METHODS

2.1. Preparation of *Nigella sativa* and Lead Acetate Solutions

Black seeds (*Nigella sativa*) were obtained, cleaned and grounded using an electric grinder (National Blender 8011S, Model HGB2WTS3, U.S.A.) for 10 min to get a water soluble powder. A suspension of 10 g/L of the pow-der was prepared for this experiment. Lead acetate (Oxford Lab. Co., India) was dissolved in distilled water at a con-centration of 10 mg/kg body weight, and administered to the rats via a gavage tube.

2.2. Ethical Statement

The animal experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee (IACUC) with reference number: UPM/IACUC/AUP-R047/2015, in accordance with the standard guidelines on usage and care of laboratory animals.

2.3. Animal Grouping and Treatment

Twenty adult male rats were randomly divided into 4 groups of 5 animals each. Hygienic condition was main-tained by changing the bedding weekly. The animals were kept for 15 days for acclimatization before commencement of the experiment.

The first group served as the negative control (NC) and received distilled water only, orally. The second group; positive control (PC) was given 10 mg/kg of LA, orally for 30 days.[5 19] The third group (T1) was given 200 mg/kg BW of *Nigella sativa* water suspension, orally for 30 days,[15 25] while the fourth group (T2) were pre-treated with NS 200 mg/kg BW, orally for 30 days,[18] followed by LA 10 mg/kg BW daily for another 30 days.[5 19]

2.4. Sample Collection for Hematology, Biochemistry and Histopathology

Blood samples were obtained through cardiac puncture from 5 animals per group on day 30 and 60, respectively,[24] after anesthesia with ketamine at 75–100 mg/kg body weight and xylazine 10 mg/kg body weight.[22] Blood was collected in EDTA tubes, while plasma was obtained by centrifugation of whole blood at 2,500 rpm for 10 minutes (Hettich, Germany). Tissue samples of the liver and kidneys were collected and kept inside 10% neutral buffered formalin for fixation.

2.5. Evaluation of Hematological Parameters

Whole blood was used for estimation of hemoglobin (Hb) content (Sahli's hemoglobin meter), total WBC, RBC, and platelet count using the Automatic Cell Counter (CELL-DYN 3700, U.S.A.) at the Hematology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Evaluation of differential leucocyte count (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were performed on Leishman's stained slides. Packed cell volume (PCV) was determined by micro hematocrit method.[12] The mean corpuscular volume (MCV), mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration was calculated using the formula by.[23]

$$\text{MCV fl} = \frac{\text{Packed cell volume \%} \times 10}{\text{Red cell count per liter}}$$

$$\text{MCH pg} = \frac{\text{Hemoglobin in gm/dl} \times 10}{\text{Red cell count per liter}}$$

$$\text{MCHC g/dl} = \frac{\text{Hemoglobin concentration gm/dl} \times 100}{\text{Volume of red cells \%}}$$

2.6. Evaluation of Biochemical Parameters

Whole blood, which was collected in a non-heparinized tube and left undisturbed at room temperature for few min-utes to clot and was centrifuged at 10000 rpm for 10 min. At the end of the centrifugation, two layers of serum and clotted cells were harvested. The upper layer (serum) was then separated by automatic pipette. The separated serum was used for evaluation of enzymes specific for liver func-tion (alanine transaminase, aspartate transaminase, alkaline phosphatase and gamma glutamyl transferase), kidney function (urea and creatinine), and cholesterol using an automated chemistry analyzer (Siemens, USA).

3. HISTOPATHOLOGY

3.1. Processing of Tissue Samples for Histopathology Tissue samples of the liver and kidney were collected in 10% neutral buffered formalin and fixed for 48 hours. Formalin fixed tissues sections were processed through serial dehydration in ethanol, embedded in paraffin wax, sectioned at 5–6 m and stained with hematoxylin and eosin for histopathological examination of each animal through light microscopy at 200× and 400 × magnifications. Photomicrographs of microscopic focal fields were taken using Miotic® microscope.

3.2. Evaluation of Superoxide Dismutase and Total Glutathione Concentrations

Superoxide dismutase catalyzes the dismutation of superoxide into H_2O_2 and molecular oxygen. This enzyme was determined using Superoxide dismutase (SOD) determination kit (Sigma, Singapore). The reaction mix was set up in a 96 well plate and incubated for 20 minutes at 37 C. Absorbance was measured using a plate reader (Tecan, Austria) at 450 nm.

Total glutathione activity was measured using the glutathione assay kit (Sigma, Singapore). Glutathione standards ranging from 50 to 3.125 M were prepared by serially diluting glutathione standard solution in 5% 5-sulfosalicyclic Acid (SSA). The reaction mix was incubated at room temperature for 5 minutes before the addition of NADPH solution to each well. Absorbance was measured using a plate reader (Tecan, Austria) at 412 nm.

3.3. Statistical Analysis

Data obtained from the hematology, biochemistry and enzyme evaluations were summarized as mean ± S.E and analyzed with Graph Pad Prism 6.0 using one-way analysis of variance (ANOVA) with Tukey multiple comparison *pos hoc* test.

4. RESULTS

4.1. Haematology Findings

The red blood cell count was lower in the PC and comparable between the control and other groups. Hemoglobin concentration was also lower in the PC and higher

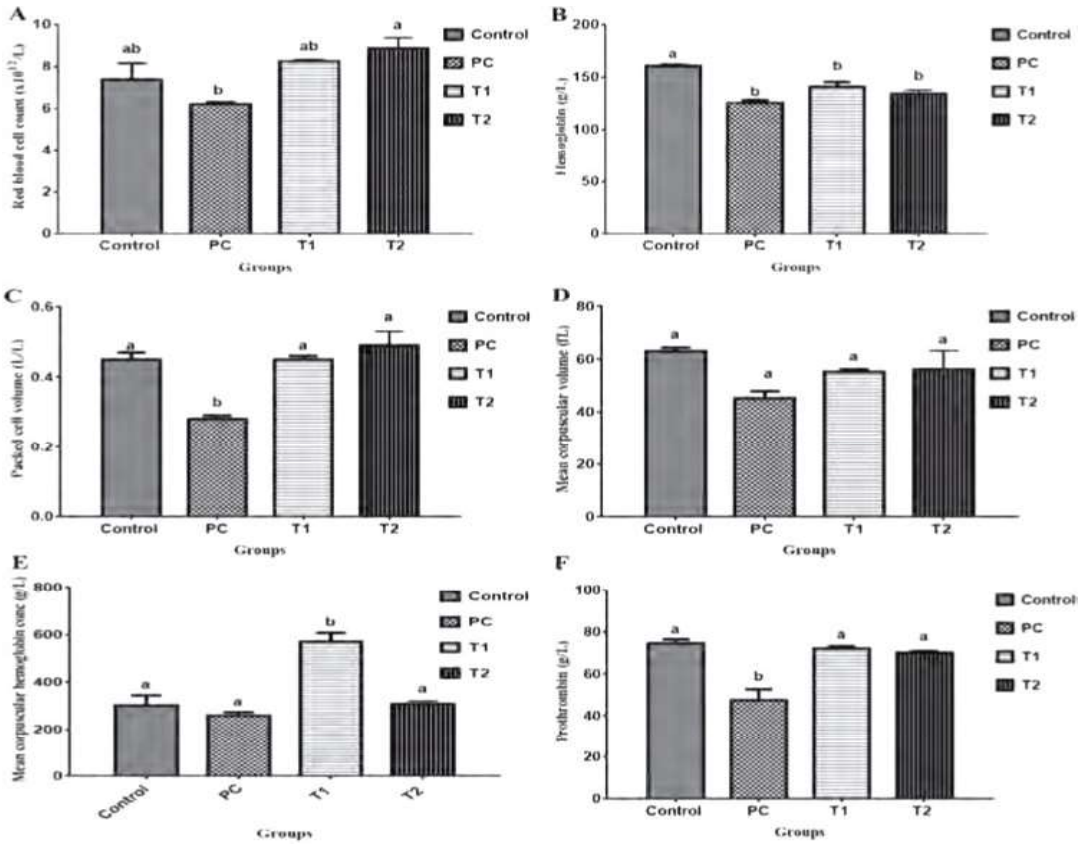

Fig. 1: Hematological parameters showing differences in (A) RBC, (B) hemoglobin, (C) PCV (D) MCV (E) MCHC and (F) prothrombin in rats following pretreatment with *N. sativa* and lead acetate exposure. $^{a\ b\ c}$Bars with different superscript indicate statistical significance at $p < 0.05$.

RESEARCH ARTICLE

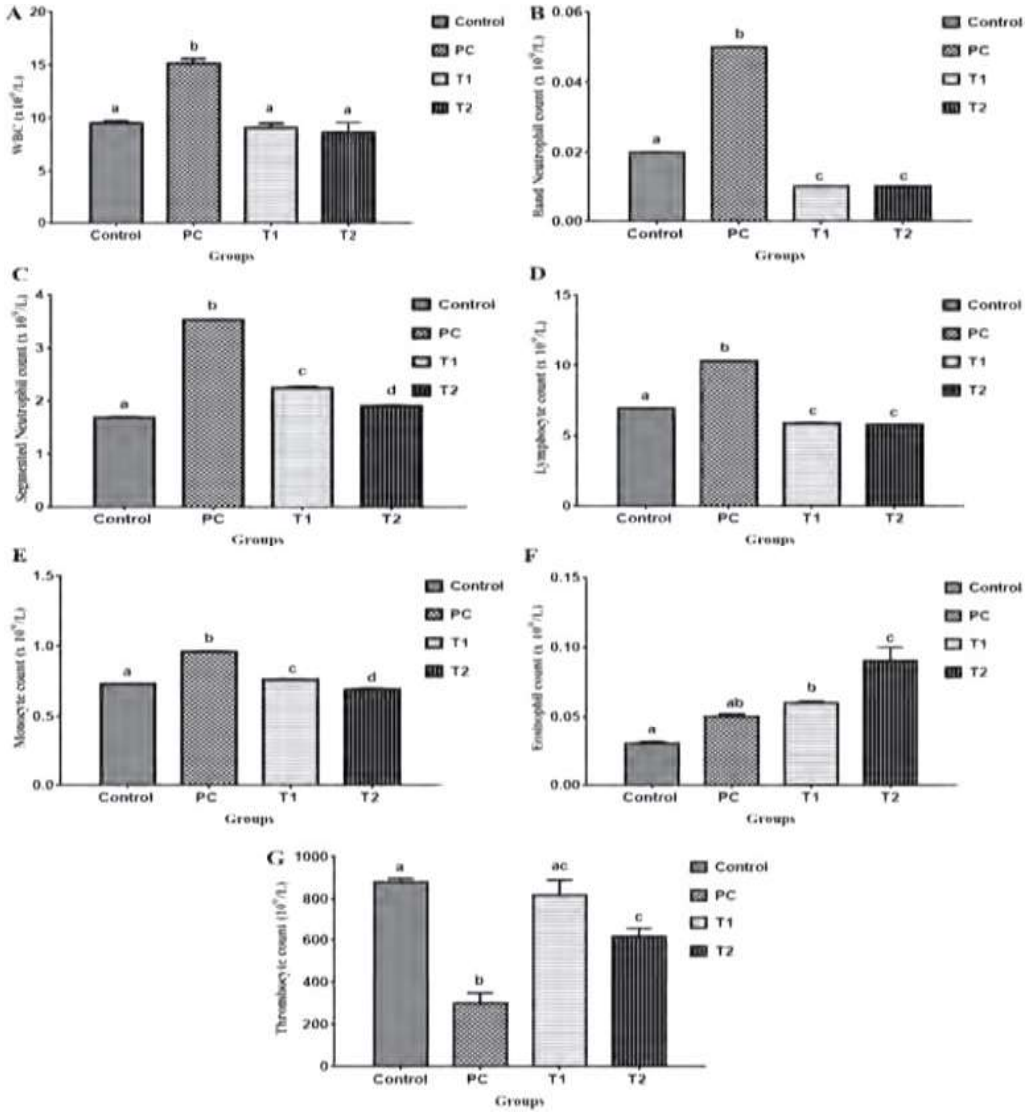

Fig. 2: Hematological parameters showing differences in (A) WBC, (B) band neutrophil, (C) segmented neutrophil (D) lymphocyte (E) monocyte (F) eosinophil and (G) thrombocytes in rats following pretreatment with *N. sativa* and lead acetate exposure. $^{a\ b\ c\ d}$Bars with different superscript indicate statistical significance at $p < 0.05$.

($p < 0.05$) in both T1 and T2, while the control had a value higher than all groups. The PCV was lower in the PC and higher ($p < 0.05$) in all other groups. The MCV value was unchanged ($p > 0.05$) among all groups, while MCHC value was lower in all groups but significantly higher in the T1 group. Prothrombin concentration was lower in the PC and comparable but higher in the other groups (Fig. 1).

The total WBC count was higher in the PC and lower in the control and treatment groups. However, band neutrophil and lymphocyte counts were also higher in the PC but much lower in the T1 and T2 once com-pared to the control group. Segmented neutrophil and monocyte counts were also higher in the PC group than other groups. Eosinophil count was on the other hand was higher ($p < 0.05$) in the T2 and lower in the control, PC and T1 groups. Thrombocyte count was lower in the PC and higher in other treatment groups (Fig. 2).

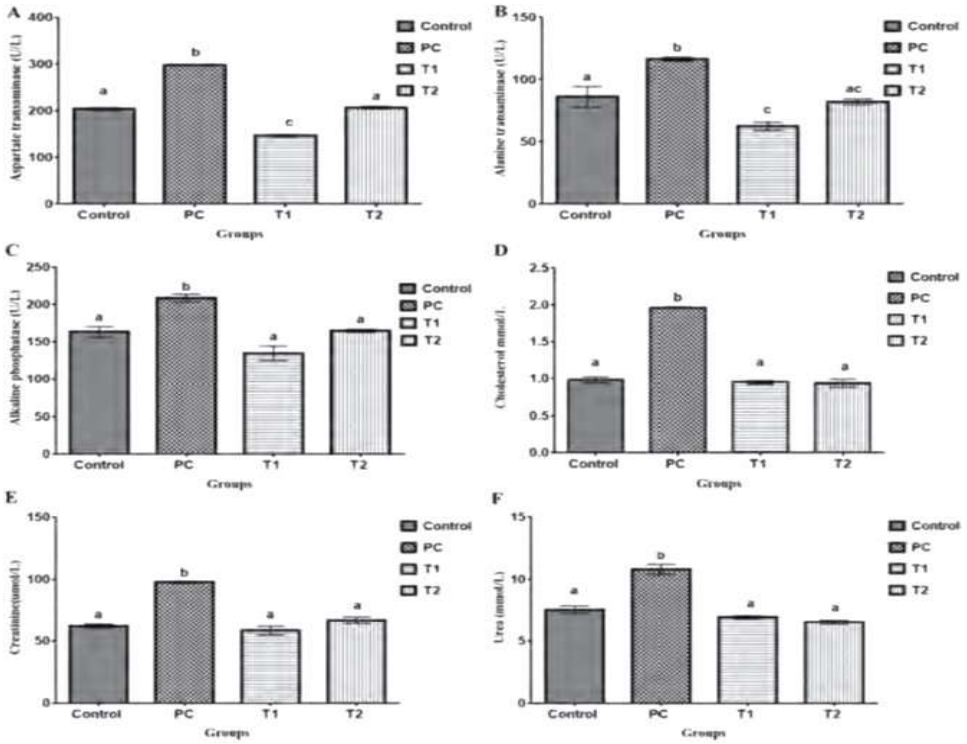

Fig. 3: Serum biochemical parameters showing differences in (A) AST (B) ALT, (C) ALP (D) cholesterol (E) creatinine and (F) urea in rats following pretreatment with *N. sativa* and lead acetate exposure. $^{a\ b\ c}$ Bars with different superscript indicate statistical significance at $p < 0\ 05$.

4.2. Serum Biochemical Findings

The level of liver enzymes AST and ALT were both higher ($p < 0.05$) in the PC group and lower in the control and treatment groups. However, the T1 group had a significantly lower value than the control and T2 groups. The serum levels of ALP, cholesterol, creatinine and urea were all significantly elevated in the PC and lower in the control and treatment groups (Fig. 3).

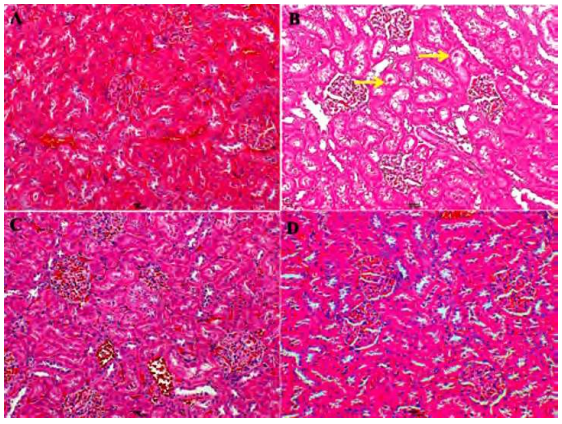

Fig. 4: Photomicrograph of the kidneys of rats following pretreatment with *N. sativa* and exposure to lead acetate (A) NC (B) PC showing evidence of tubular degeneration typified by vacuolation and hyaline deposition within the tubules (yellow arrows) (C) T1 (D) T2 groups, without any obvious lesions, H&E ×200.

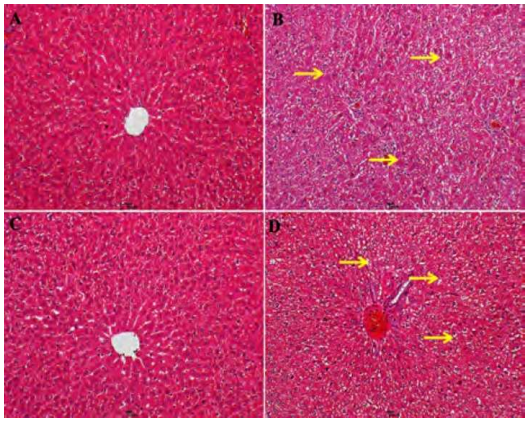

Fig. 5: Photomicrograph of the liver of rats following pretreatment with *N. sativa* and exposure to lead acetate (A) NC (B) PC group with evidence of hepatocyte degeneration and necrosis (yellow arrows) (C) T1 (D) T2 group, showing vacuolar degeneration of hepatocytes (yellow arrows), H&E ×200.

RESEARCH ARTICLE

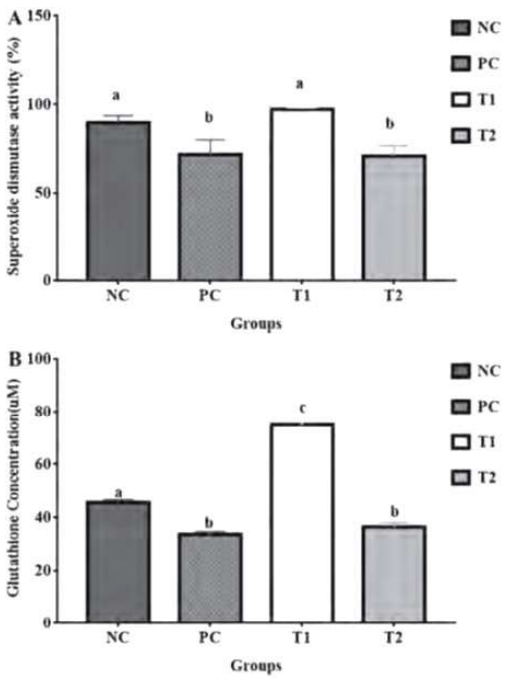

Fig. 6: Concentration of antioxidant enzymes (A) superoxide dismutase (B) glutathione, in rats pretreated with *N. sativa* and exposure to lead acetate. [a b c] Bars with different superscript indicate statistical significance at $p < 0\ 05$.

4.3. Histopathological Findings in the Liver and Kidneys

There were no obvious pathological lesions observed in the kidneys in all groups (Fig. 4). However, the liver of the PC had evidence of hepatocyte degeneration and necrosis, while liver from the T2 group had evidence of vacuolar degeneration (Fig. 5).

4.4. Antioxidant Enzyme Concentrations

The activity of SOD was lower in the PC and T2 groups, while the control and T1 groups had a higher SOD activity, which was comparable. The concentration of GSH was also significantly lower ($p < 0\ 05$) in the PC and T2 groups, while the T1 group had a higher concentration that was significantly different from the control (Fig. 6).

5. DISCUSSION

The ameliorative effects of *N. sativa* against several toxic compounds have been reported in literatures.[6 9 13 16 18] In this study, LA induced decreases in RBCs and PCV counts in the PC group and these effects were reversed by *N. sativa* administration in treated animals. This shows that *N. sativa* can prevent anemia induced by LA. Similarly, we observed a decrease in lymphocyte count and increased neutrophil counts in the PC and T1 groups, both of which were reversed by *N. sativa* administration in the treatment groups. This results supported the earlier toxicity studies showing the negative effects of LA on the hemogram of laboratory animals,[8 14] adding to the therapeutic effects of *N. sativa* in improving the hemogram that have been previously documented.[2 11]

Previous studies have reported the hepato-protective effects of *N. sativa* against several toxic compounds such as tramadol, malathion and carbon tetrachloride.[9 16 17] These effects were attributed to the role of *N. sativa* in restoring deficits in oxidative enzyme levels such as GSH and SOD in the cell.[22] In a related study, rats exposed to LA for 6 weeks were observed to have elevated levels of AST, which was ameliorated by administration of *N. sativa*.[10] However, the study was only able to show the sub-chronic effects of *N. sativa* treatment on LA induced toxicity, while our study evaluated the effect of NS pre-treatment on sub-chronic LA induced toxicity.

The administration of low dose LA has been reported to induce oxidative stress in animals; such resultant effects were attributed to oxidant organ damage and dysfunction associated with LA toxicity in man and animals.[1] The protective effects of *N. sativa* oil and seed extract against oxidant damage have been reported; *N. sativa* oil restored increased malondialdehyde and decreased GSH levels in rats exposed to tramadol toxicity, while 10% extract of *N. sativa* was able to restore an imbalances in CAT, GSH, SOD, MDA, glutathione peroxidase and glutathione reductase in mice exposed to carbon tetrachloride poisoning.[9] These reports concur with the results of this study and might support the fact that *N. sativa* is a potent antioxidant compound.

In this study, the kidney function was affected by LA toxicity, since elevated levels of creatinine and urea were observed in the PC group. Other studies using LA at graded doses also observed a similar or more severe increase in kidney enzymes such as creatinine and urea.[8–10] Here, pre-treatment of rats with 200 mg/kg of *N. sativa* prevented the increase in creatinine in blood on day 30 of LA toxicity. Similarly, histolopathological changes such as degeneration and necrosis was moderate in the liver and kidney of the PC group and mild in the NS pre-treatment group. This also suggests amelioration of the toxic effects of LA by NS pre-treatment.

6. CONCLUSION

To conclude, this study showed that sub-chronic LA administration to rats induces hematological, biochemical, antioxidant enzyme and histopathological changes in rats. The pre-treatment of rats with *N. sativa* at 200 mg/kg was able to prevent these alterations.

RESEARCH ARTICLE

Conflict of Interest

The authors have no conflict of interest.

Acknowledgment: We acknowledged the contribution of the Ministry of Higher Education (MOHE), Malaysia through its Fundamental Research Grant scheme (5524282) for the financial support of this research.

References

1. M. Ahamed and M. K. J. Siddiqui, *Clinical Nutrition Journal* 26, 400 (2007).
2. B. Ali and G. Blunden, *Phytotherapy Research* 17, 299 (2003).
3. L. Allouche, M. Hamadouche, and A. Touabti, *Experimental and Toxicologic Pathology* 61, 503 (2009).
4. L. Allouche, M. Hamadouche, A. Touabti, and S. Khennouf, *Adv. Biol. Res.* 5, 339 (2011).
5. J. K. Arrak, N. J. Al-Khafaji, and A. A. Hassan, *Diyala Journal of Medicine* 3, 90 (2012).
6. E. A. Awadalla, *Biomedicine and Preventive Nutrition* 2, 265 (2012).
7. M. Bharali, *Journal of Global Biosciences* 2, 121 (2013).
8. M. Dalia, *J. Am. Sci.* 6, 541 (2010).
9. A. Elkhateeb, I. E. Khishin, O. Megahed, and F. Mazen, *Toxicology Reports* 2, 512 (2015).
10. A. R. Farrag, K. A. Mahdy, R. G. Abdel, and M. M. Osfor, *Pakistan Journal of Biological Sciences: PJBS* 10, 2809 (2007).
11. H. Gali-Muhtasib, N. El-Najjar, and R. Schneider-Stock, *Advances in Phytomedicine* 2, 133 (2006).
12. C. L. Ghai, A Textbook of Practical Physiology, 5th edn., Jaypee Brothers, New Delhi (1999), pp. 47–69.
13. M. Hala, *Journal of American Science* 7 (2011).
14. N. M. Ibrahim, E. A. Eweis, H. S. El-Beltagi, and Y. E. Abdel-Mobdy, *Asian Pacific Journal of Tropical Biomedicine* 2, 41 (2012).
15. M. H. Jahromy, M. Jalili, A. J. Mohajer, F. K. Poor, and S. M. Dara, *World Journal of Neuroscience* (2014).
16. N. Krishnan and S. Muthukrishnan, *Journal of Acute Medicine* 2, 107 (2012).
17. O. Meyer, L. Blom, and D. Sondergaard, *Laboratory Animals* 16, 271 (1982).
18. M. Murugesan, M. Ragunath, T. Prabu, S. Nadanasabapathi, M. Sakthivel, and V. Manju, *Int. J. Pharmacol. Clin. Sci.* 1, 45 (2012).
19. H. N. Mustafa and A. M. Hussein, *Folia Morphologica* (2015).
20. N. C. Papanikolaou, E. G. Hatzidaki, S. Belivanis, G. N. Tzanakakis, and A. M. Tsatsakis, *Medical Science Monitor* 11, RA329 (2005).
21. F. Paul, Laboratory Animal Anesthesia, Academic Press is an Imprint of Elsevier, Newcastle University (2009), p. 208.
22. M. L. Salem, *International Immunopharmacology* 5, 1749 (2005).
23. B. S. Shirlyn, Clinical Laboratory Hematology, 1st Education, University of Texas Science Center at San Antonio, U.S.A. (2004).
24. S. H. Weisbroth, R. E. Flatt, and A. L. Kraus (eds.), The Biology of the Laboratory Rabbit, Academic Press (2013).
25. O. Yoruk, F. Gur, H. Uyanik, M. Yasar, V. Mutlu, E. Altas, and S. Taysi, *Macedonian Journal of Medical Sciences* 3, 132 (2010).

Received: 5 October 2016. Accepted: 25 October 2016.

RESEARCH ARTICLE