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REVERSAL OF HEMATO-BIOCHEMICAL ALTERATIONS INDUCED BY LEAD ACETATE TREATMENTS WITH *EURYCOMA LONGIFOLIA* AND *NIGELLA SATIVA* IN RATS

Mohammed Abdulrazzaq Assi[1,2]*

[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

*Corresponding author E-mail: razaq_assi@yahoo.com

Abstract

The current study is planned to evaluate the impact of *Nigella sativa* (NS) and *Eurycoma longifolia* (EL) have both been shown to promote hematopoiesis in laboratory animals. However, the combination of the two in preventing lead acetate (LA) induced hemato-toxicity is yet to be reported. In this study, 30 male Sprague Dawley rats were divided into 5 groups of 6 rats each as following; group one (negative control), group two (20 mg/kg lead acetate; positive control), group three (NS 300 mg/kg + LA 20 mg/kg), group four (EL 500 mg/kg + LA 20 mg/kg) and finally group five (EL 500 mg/kg + LA 20 mg/kg + NS 200 mg/kg). All these were administered for a period of a month on a daily basis. Then the samples of the serum and blood were collected from the rats after being euthanized for complete blood count and biochemistry analysis. The red blood cell count, hemoglobin and packed cell volume were all lower ($p<0.05$) in the positive control (PC) group. Total white blood cell count, band and segmented neutrophil counts, lymphocyte, monocyte and eosinophil counts were all greater ($p<0.05$) in the PC group in comparing with the negative control and treatment groups. The serum concentrations of aspartate transaminase, alanine transaminase, alkaline phosphatase, cholesterol, creatinine, and urea were all greater ($p<0.05$) in the PC group in comparing with the other groups. To sum up, the preventive impact of *Eurycoma longifolia* and *Nigella sativa* administration was shown in this study against the modifications in the alterations of hemato-biochemical that are resulted from LA.

***Keywords*:** Lead acetate, *Nigella sativa* (NS), *Eurycoma longifolia,* hematology, biochemistry.

Introduction

Years ago, human beings used medicinal plants for treating diseases. Medicinal herbs can be defined as the plants that are used to treat or prevent diseases by changing the pathological and physiological events. Lately, the trend to use medicinal herbs has been increased comparing with the use of chemical drugs (Kooti *et al.,* 2016).

Nigella sativa (NS) also known as (black seed) it is one of the most common popular medicinal herbs in many parts of the worldwide. It was used as folk medicine in the world to treat a variety of diseases (Assi *et al.,* 2016). The plant is national to North Africa, Southern Europe, and Southwest Asia. It is also cultured in many Mediterranean and Middle Eastern countries such as Iran, Pakistan, India, Saudi Arabia, Syria and Turkey (Mazaheri *et al*., 2019). *N. sativa* belongs to the botanical family Ranunculaceae (Salem, 2005). *Nigella sativa* has been several active components isolated from its seed as well as its oil contains thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine, and alpha-hederin. The medicinal properties of *N. sativa* and its oil components had been investigated by studies conducted *in vitro and in vivo* on human and laboratory animals that have a broad range uses in pharmacology field (Aljabre *et al.,* 2015). From other known medicinal plants are *Eurycoma longifolia* (EL), it is classified under Simaroubaceae plants family that is broadly recognized as "Tongkat Ali" in Malaysia. The roots of this plant are well known as a popular traditional medication for fever after birth, boil, ulcer wound and also acts as energy booster among men (Balan *et al.,* 2018). There are a lot of medical uses of this plant reported by researchers from which its extract increases the luteinizing hormone production activity (Pratomo, 2017).

Eurycoma longifolia is also well known for treating diseases and attractive health, mainly sexual health cases between men. Owing to the tremendous health benefits of *Eurycoma longifolia* its demand highly increased and its preparations now broadly exist in the health-food market in the form of raw crude powder (George *et al.,* 2018). The ability of *Nigella saliva* seeds and *Eurycoma longifolia* against lead acetate toxicity have wide range in medical and pharmaceutical fields (Ali *et al.,* 2017). The existence of toxic compounds in food products become has worldwide problems, where the most important sources for the toxic compound for men human is food from plant cereals (Winiarska, 2018).

Lead is considering as from the most harmful and cumulative environmental pollutants that have an influence on all biological systems through exposure to air, water, and food sources (Patra *et al.,* 2011). The free reactive radical's created by the heavy metals, including lead, lead to damaging the cell structures, together with DNA and cell membrane (Kosnett, 2006). The enzymes that aid in the vitamin D synthesis and contain the integrity of the cell membrane also affected by lead interferes.

The fragility of red blood cell will be increased due to disrupts of the cell membrane caused by lead and this will lead to anemia (White *et al.,* 2007). Many changes in the biochemical, physiological and morphological functionality have been associated with the toxicity of heavy metals including Lead for example; hematological disorders and dysfunction of the liver (Alwaleedi, 2016). The digestive

tract, respiratory tract, and skin are the main routes to enter the Lead into the human body, where part of it is absorbed into the blood and part of it is bind with red blood cells, and the residual stay in plasma to be spread to other tissues (Stowe *et al.,* 1973). *Nigella saliva* had positive effects against the toxicity of LA as reported (Assi *et al.,* 2016).

Materials and Methods

Preparation of *Nigella sativa*, Lead acetate and *Eurycoma longifolia* solutions

With the purpose of getting the powder (*Nigella sativa*) the black seeds that is soluble in the water, the seeds should be collected, cleaned. To obtain a powder of (*Nigella sativa*) that can dissolve in water, the seeds were taken, cleaned with water, and grounded with an electric grinder (Model HGB2WTS3, U.S.A., National Blender 8011S) for ten minutes. After that, suspension of the powder of *Nigella sativa* is applied with a 30 g/L concentration and is prepared for the present study. Lead acetate body weight with a 30 G/L concentration (Oxford Lab. Co., India) is thawed in distilled water and applied to the rats, while *Eurycoma longifolia* with a 500 mg/kg concentration is thawed in distilled water and orally administered to the rats, all that was done by using a gavage tube.

Ethical Statement

Permission from The Institutional Animal Care and Use Committee (IACUC) has been gained for this study to use the animal experimental protocols with a reference number: UPM/IACUC/AUP-R047/2015, depending on the criteria of how to use laboratory animals carefully.

Animal grouping and Treatment

A number of 30 Sprague Dawley rats were separated into five groups. For keeping hygiene, the bedding was weekly changed. For acclimatization purposes, the animals were kept for 15 days before the study commenced. Group one (NC) was set as the negative control and has been given distilled water. Group two was set as the positive control, and Lead acetate 20 mg/kg/ per day was orally given for a period of 30 days' control (Dorostghoal *et al.,* 2011). 300 mg/kg of the black seeds and 20 mg/kg of LA were orally given to group 3 (T1) on a daily basis for a period of one month (Saheb *et al.,* 2016). 500 mg/kg of *Eurycoma longifolia* and 20 mg/kg of LA were orally administered to group 4 (T2) on daily basis for a period of 30 days (Zanoli *et al.,* 2009). 20 mg/kg of LA, 500 mg/kg of *Eurycoma longifolia* and 300 mg/kg of the black seeds were all orally given to group 5 (T3) for one month on daily basis.

Measurement of Body Weight

The body weight of each animal was recorded every week throughout the experimental period.

Measurement of Testicular Weight: Body Weight Ratio

At the end of 30 days of the experimental period for the experiment, the animals were weighed by using digital weighing balance and anesthetized using an intramuscular (IM) injection of ketamine at a dose of 75-100 mg/kg BW and xylazine at 10 mg/kg BW (Paul, 2011). The testicles were removed and weighed. The testicle weight: body weight ratio was determined.

Testicular weight to body weight ratio was calculated as in the following equation = Testicular weight (g)/body weight (g) ratio = wt. of testes (g)/wt. of animal (g) × 100.

Sample Collection for Hematology and Biochemistry

Blood samples were obtained through cardiac puncture from 6 animals per group on day 30 (Weisbroth *et al.,* 2013), after anesthesia with ketamine at 75– 100mg /kg body weight and xylazine 10 mg /kg body weight (Paul, 2011). Blood was collected in EDTA tubes, while plasma was obtained by centrifugation of whole blood at 2,500 rpm for 10 minutes (Hettich, Germany) as earlier described (Assi *et al.,* 2017).

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) on Leishman's stained slides. Packed cell volume (PCV) was determined by micro hematocrit method (Ghai, 2012).

Evaluation of Biochemical Parameters

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

Statistical analysis

The collected data was analyzed by using (ANOVA) one-way analysis of variance accompanied by the test of Tukey multiple comparison pos hoc. The spermiogram was the source of these data, and they were examined by using Graph Pad Prism 6.0 and briefed as mean ± S.E.

Results and Discussion

There were lower significant in the body weights of rats in the PC and there were significantly higher in the treatments and control groups throughout the experimental period (Figure 1). The body weight is very influenced by the harmful lead acetate effects and this significantly increases with the rise in its dose. However, at day 30 the testicular body weight ratio (TBWR) was lower in the PC and higher (p<0.05) in the treatments and control group. Although the food amounts that are given to animals have not exchanged these finding in accordance with previous studies results (Seddik *et al.,* 2010). The decrease of body weight in rat's cause by lead induced toxicity was being observed (Teijón *et al.,* 2006).

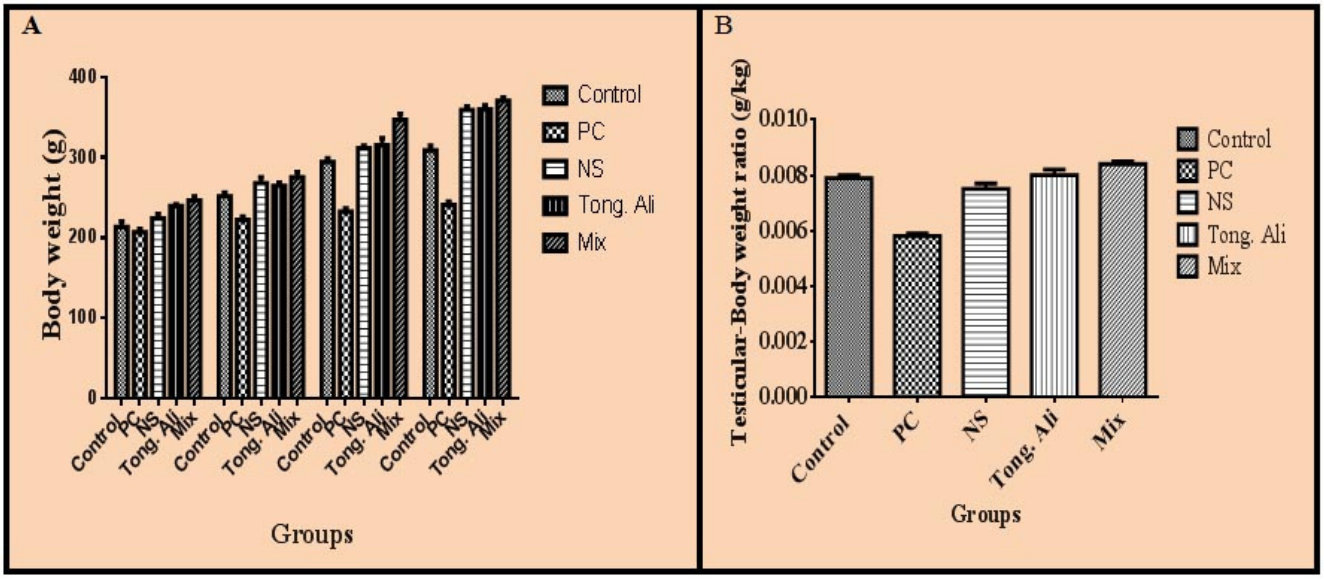

Fig. 1: Body weight of rats throughout the study period (A) and testicular body weight ratio (B) of rats administered Lead acetate, *Nigella sativa* and *Eurycoma longifolia* treatments.

The results of this study indicate that there was a significant increase (P< 0.05) in the enzyme activity of AST and ALT in positive control as illustrated in figure (2- a, b, c and d) respectively. To investigate the liver function and the effect of lead acetate on these enzymes (AST and ALT) were assessed and their activities used as indicators. The high activity of each enzyme paralleled with the rise in the doses of lead acetate, where the stimulation of these enzymes by increasing the dose may be lead to damage of the cell membrane or become high permeability of liver cells. These outcomes correspond with previous studies that reported an increase in AST and ALT levels after management with lead caused by acute hepatitis, jaundice, and liver cirrhosis (Patil *et al.,* 2007). The results showed high increase in the concentration of GGT enzyme in the positive group by increasing the dose of lead acetate in comparison of treated groups which appeared the activity of *Nigella saliva* and *Eurycoma longifolia* against lead acetate toxicity.

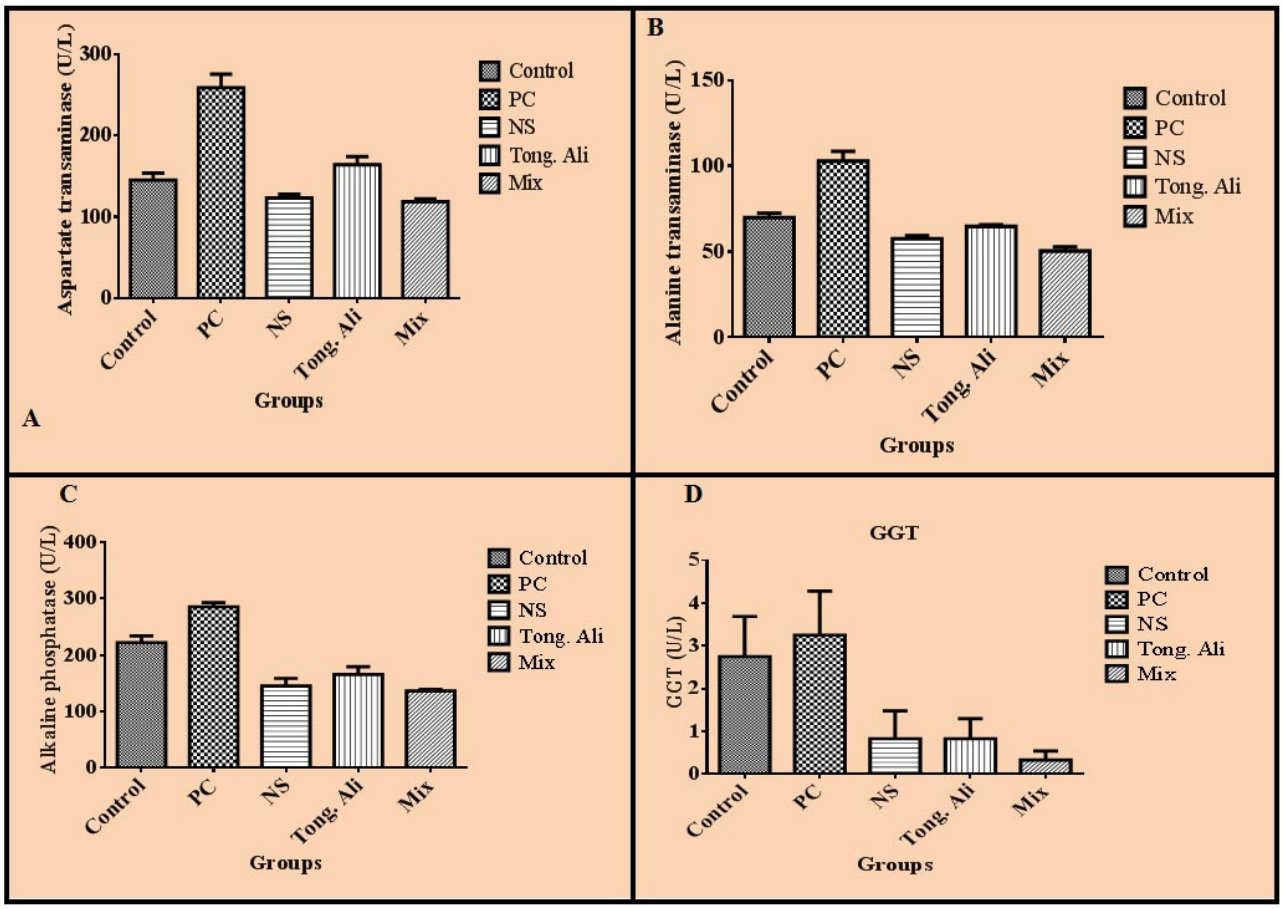

Fig. 2: Graph of serum biochemical parameters showing differences in (A) AST, (B) ALT, (C) ALP and (D) GGT in rats after applying *Eurycoma longifolia,* Lead acetate and *N. sativa*.

The present results indicated to the serum lipids (triglyceride and cholesterol) were significantly increased (P< 0.05) in the positive control (lead acetate) this in agreement with previous studies also conducted on lab animals (Abdou and Hassan. 2014). On the other hand, there is appeared decreasing in groups treated with (Tong. Ali and NS), as shown in figure (3-A and B). The total bilirubin concentration was varied between treated groups and positive control, where it was reverse to other parameters where it found to be increased in (Tong. Ali and NS) groups in comparison to the positive control (lead acetate) as shown in figure (3-C).

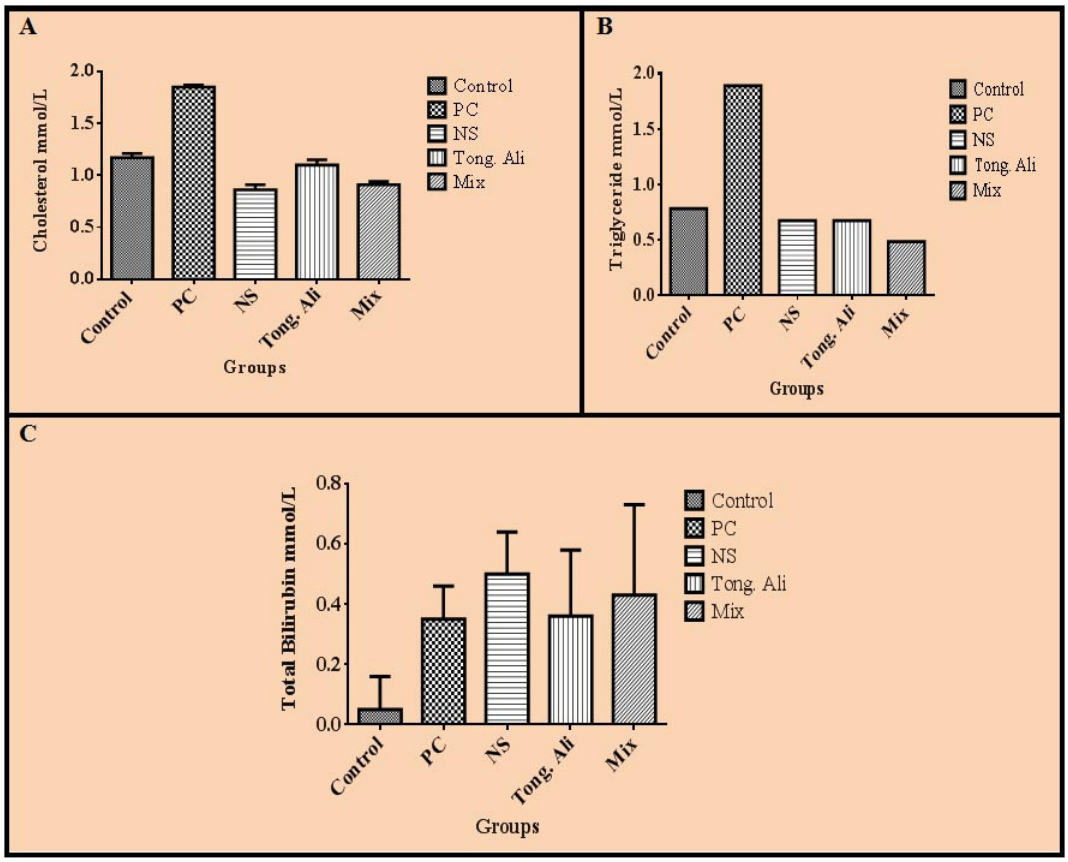

Fig. 3 : Graph of serum biochemical parameters showing differences in (A) Cholesterol, (B) Triglyceride and (C) total bilirubin in rats after applying Lead acetate, *Eurycoma longifolia and* N. *sativa*.

The kidney functions are also assessed by estimating the creatinine and urea concentration for the purpose of confirming the working status of the rats' kidney in case of lead toxicity compared with the healthy rats and what ambit the influence of Tong. Ali and NS on lead toxicity. The serum levels of urea and creatinine were all significantly elevated in the PC and lower in the control and treatment groups (Figure 4). These results similar to other results used lead acetate as toxic material which affected kidney functions (Alwaleedi. 2016 and Abdou and Hassan. 2014).

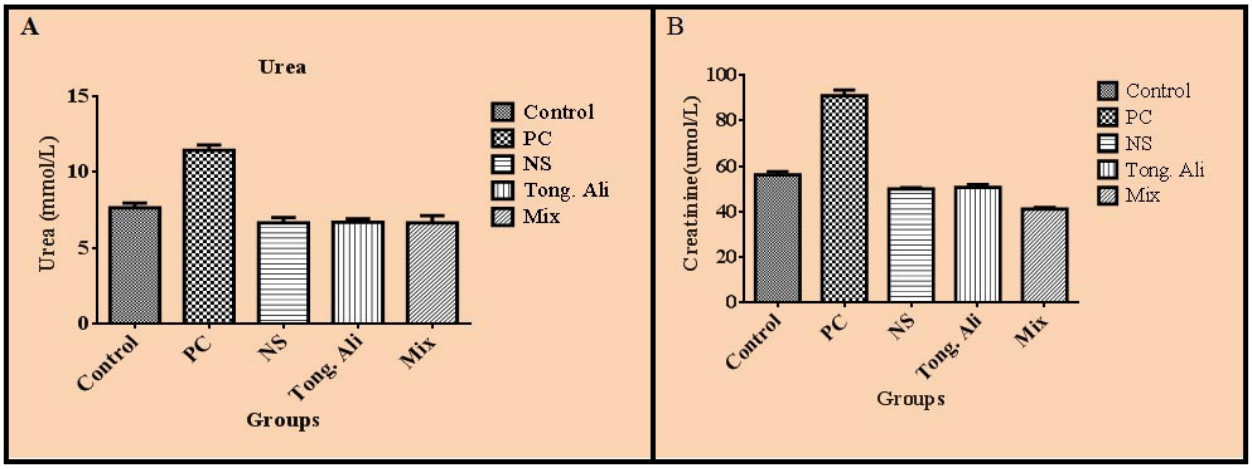

Fig. 4: Graph of serum biochemical parameters showing differences in (A) Urea and (B) Creatinine in rats after applying Lead acetate, *Eurycoma longifolia and* N. *sativa*.

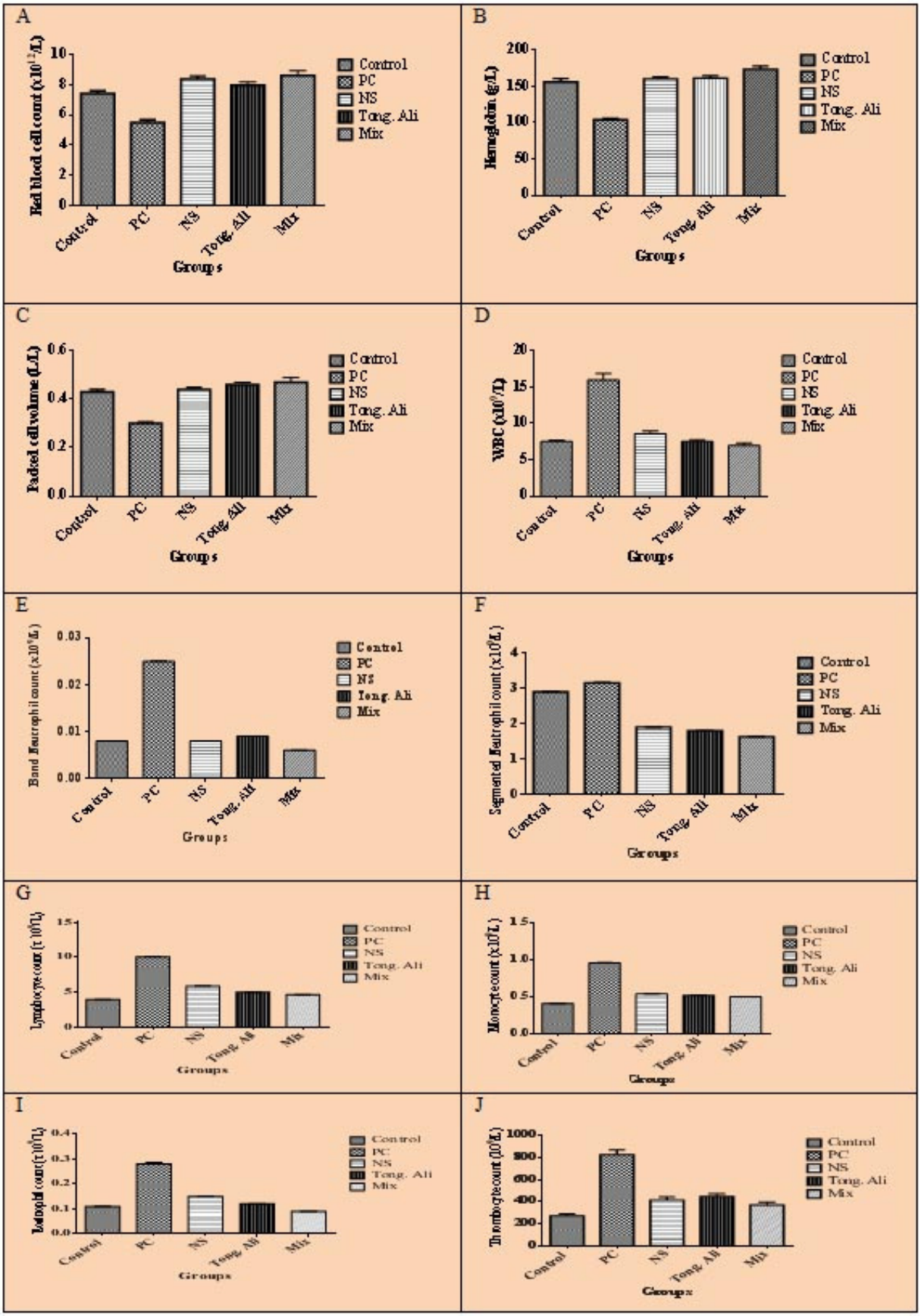

Fig. 5: Graph of hematological parameters showing differences in (A) RBC, (B) hemoglobin, (C) PCV, (D) WBC, (E) band neutrophil, (F) segmented neutrophil, (G) lymphocyte, (H) monocyte, (I) Eosinophil and (J) Thrombocyte in rats after applying Lead acetate, *Eurycoma longifolia and* N. *sativa*.

The red blood cell count was lower in the PC and comparable between the control group and the other groups. The concentration of hemoglobin in the PC was also lower, and higher (p<0.05) in both NS, TA, Mix and control. The PCV was lower in the PC and higher (p< 0.05) in all other groups.

The total WBC count was higher in the PC, and in the treatment and control groups was lower. However, band neutrophil and lymphocyte counts were also higher in the PC but much lower in the treatments group once compared to the control group. Segmented neutrophil was higher in PC comparing with treatments group, and monocyte counts in the PC were much greater comparing with other groups. On the other hand, the count of Eosinophil in the PC was greater (p<0.05), while lower in the control and treatment groups. The count of thrombocyte in the PC was greater, while in other treatment groups, it was lower (Figure 5). On the other hand, with increasing the dose of lead acetate will lead to a significant increase in WBCs and their types at positive control. These changes in hematological parameters may be due to the toxicity of lead on the metabolism of cell or interaction of lead with several reactions for example; enzymatic activities inhibition like dehydratases of levulinic amino acid, that act as key roles at heme synthesis and calcium as being a secondary mediator (Klaassen and Amdur. 2013), and other enzymes of erythrocyte, such as, GA3PD and G6PD (Calderón *et al.,* 1993). The permanent exposure of the animals to lead may harmfully affect the heme synthesis in the body due to the inhibition of enzymes in cell cytoplasm and mitochondria, also one of lead acetate effects is the conversion of copro-porphyrin III into proto-porphyrin IX resulting in limitation of erythrocyte life span and a reduction in the Hb production (Alwaleedi. 2016). The important full in hematological indices referred to the lead acetate binding to RBCs membrane and increases the fragility and finally cell destruction (Rous and Jelínek. 2000). The toxicity of lead also affected significantly on leukocytes count especially lymphocytes and monocytes, this may be due to the direct effect of lead on lymphoid organs especially on vital production sites in these organs, were reported that the lead induces the inflammation and thereby increase in WBCs count. The Platelet count also significantly increases in the positive control (lead acetate) as compared with other treated groups as shown in figure (5- J).

Conclusion

In this study, the lead acetates effects which is one of more hazardous heavy metals was studied by using the biochemical and hematological tests where these tests showed the high toxicity risk of lead on the other hand the study concluded the positive effect of *Nigella sativa* and *Eurycoma longifolia*.

Author Contribution

The author has a significant contribution in this article. Mohammed Abdulrazzaq Assi is the author and he has a contribution in both the conception and the design. The manuscript was drafted and well edited by him, also he reviewed and has a contribution to the intellectual content.

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Competing Interest

A declaration by the author was made to show that there is no competing interest in this piece of work.

References

Abdou, H.M. and Hassan, M.A. (2014). Protective role of omega-3 polyunsaturated fatty acid against lead acetate-induced toxicity in liver and kidney of female rats. BioMed research international.

Ali, H.; Korshom, M.A.; Mandour, A.A.; El-Bessoumy, A.A. and El-Sayed, Y.S. (2017). Hepatoprotective efficacy of Nigella sativa seeds dietary supplementation against lead acetate-induced oxidative damage in rabbit–purification and characterization of glutathione peroxidase. Biomedicine & Pharmacotherapy, 89: 711-718.

Aljabre, S.H.; Alakloby, O.M. and Randhawa, M.A. (2015). Dermatological effects of Nigella sativa. Journal of Dermatology & Dermatologic Surgery, 19(2): 92-98.

Alwaleedi, S.A. (2016). Hematobiochemical changes induced by lead intoxication in male and female albinomice. National Journal of Physiology, Pharmacy and Pharmacology, 6(1): 46.

Assi, M.A.; Hezmee, M.N.; Abba, Y.; Sabri, M.Y.; Haron, A.W.; Baiee, F.H. and Rajion, M.A. (2017). Effect of Nigella sativa pre-treatment on sub-chronic lead acetate induced hematological and biochemical alterations. Journal of Computational and Theoretical Nanoscience, 14(6): 2752-2758.

Assi, M.A.; Hezmee, M.N.M.; Abba, Y.; Yusof, M.S.M.; Haron, A.W.; Rajion, M.A. and Al-Zuhairy, M.A. (2016). Prophylactic effect of Nigella sativa against lead acetate induced changes in spermiogram, reproductive hormones and gonadal histology of rats. Veterinary world, 9(11): 1305.

Assi, M.A.; Noor, M.H.M.; Bachek, N.F. Ahmad, H.; Haron, A.W.; Yusoff, M.S.M. and Rajion, M.A. (2016). The various effects of Nigella sativa on multiple body systems in human and animals. Pertanika Journal of Scholarly Research Reviews, 2(3).

Balan, D.; Chan, K.L.; Murugan, D.; AbuBakar, S. and Wong, P.F. (2018). Antiadipogenic effects of a standardized quassinoids-enriched fraction and eurycomanone from Eurycoma longifolia. Phytotherapy research, 32(7): 1332-1345.

Calderón, V.S.; Hernandez-Luna, C.; Maldonado, M. and Saénz, D. (1993). Mechanisms of the toxic effect of lead. I. Free lead in erythrocyte. Journal of exposure analysis and environmental epidemiology, 3, pp.153-164.

Dorostghoal, M.; Dezfoolian, A. and Sorooshnia, F. (2011). Effects of maternal lead acetate exposure during lactation on postnatal development of testis in offspring wistar rats. Iranian Journal of Basic Medical Sciences, 14(2): 122-131.

George, A.; Kawasaki, Y. and Abas, A. (2018). U.S. Patent Application No. 15/843,593.

Ghai, C.L. (2012). A textbook of practical physiology. JP Medical Ltd.

Klaassen, C.D. and Amdur, M.O. eds. (2013). Casarett and Doull's toxicology: the basic science of poisons (Vol. 1236). New York: McGraw-Hill.

Kooti, W.; Hasanzadeh-Noohi, Z.; Sharafi-Ahvazi, N.; Asadi-Samani, M. and Ashtary-Larky, D. (2016). Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa). Chinese journal of natural medicines, 14(10): 732-745.

Kosnett, M.J. (2006). Lead In Olson, K.R. Poisoning and Drug Overdose (5th ed.) McGraw Hill Professional.

Mazaheri, Y.; Torbati, M.; Azadmard-Damirchi, S. and Savage, G.P. (2019). A comprehensive review of the physicochemical, quality and nutritional properties of Nigella sativa oil. Food Reviews International, 35(4): 342-362.

Patil, A.J.; Bhagwat, V.R.; Patil, J.A.; Dongre, N.N.; Ambekar, J.G. and Das, K.K. (2007). Occupational lead exposure in battery manufacturing workers, silver jewelry workers, and spray painters in western Maharashtra (India): effect on liver and kidney function. Journal of basic and clinical physiology and pharmacology, 18(2): 87-100.

Patra, R.C.; Rautray, A.K. and Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. Veterinary medicine international.

Paul-Murphy, J. (2011). Analgesia, An Issue of Veterinary Clinics: Exotic Animal Practice-E-Book (Vol. 14, No. 1). Elsevier Health Sciences.

Pratomo, H. (2017). *Eurycoma longifolia* extract increases intracellular production activity of luteinizing hormone (LH) in pituitary. In AIP Conference Proceedings (Vol. 1862, No. 1, p. 030112). AIP Publishing.

Rous, P. and Jelínek, P. (2000). The effect of heavy metals boundary contaminated soil on haematological and selected biochemical parameters in blood plasma of rabbits. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 48(3): 93-99.

Saheb, S.H.; Desai, S.D.; Das, K.K. and Haseena, S. (2016). Antioxidant effect of Nigella sativa seed powder and thymoquinone in normal and sterptozotocine induced diabetic albino rats. Int. J. Intg Med Sci, 3: 242-247.

Salem, M.L. (2005). Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. International immunopharmacology, 5(13-14): 1749-1770.

Seddik, L.; Bah, T.M.; Aoues, A.; Brnderdour, M. and Silmani, M. (2010). Dried leaf extract protects against lead-induced neurotoxicity in Wistar rats. Eur J Sci Res, 42(1): 139-151.

Stowe, H.D.; Goyer, R.A.; Krigman, M.M.; Wilson, M. and Cates, M. (1973). Experimental oral lead toxicity in young dogs. Arch. Pathol.; (United States), 95.

Teijón, C.; Olmo, R.; Blanco, D.; Romero, A. and Teijón, J.M. (2006). Low doses of lead. Biological trace element research, 111(1-3): 151-165.

Weisbroth, S.H.; Flatt, R.E. and Kraus, A.L. (2013). The biology of the laboratory rabbit. Academic press.

White, L.D.; Cory-Slechta, D.A.; Gilbert, M.E.; Tiffany-Castiglioni, E.; Zawia, N.H.; Virgolini, M.; Rossi-George, A.; Lasley, S.M.; Qian, Y.C. and Basha, M.R. (2007). New and evolving concepts in the neurotoxicology of lead. Toxicology and applied pharmacology, 225(1): 1-27.

Winiarska-Mieczan, A. (2018). Protective effect of tea against lead and cadmium-induced oxidative stress—a review. BioMetals, 31(6): 909-926.

Zanoli, P.; Zavatti, M.; Montanari, C. and Baraldi, M. (2009). Influence of Eurycoma longifolia on the copulatory activity of sexually sluggish and impotent male rats. Journal of ethnopharmacology, 126(2): 308-313.