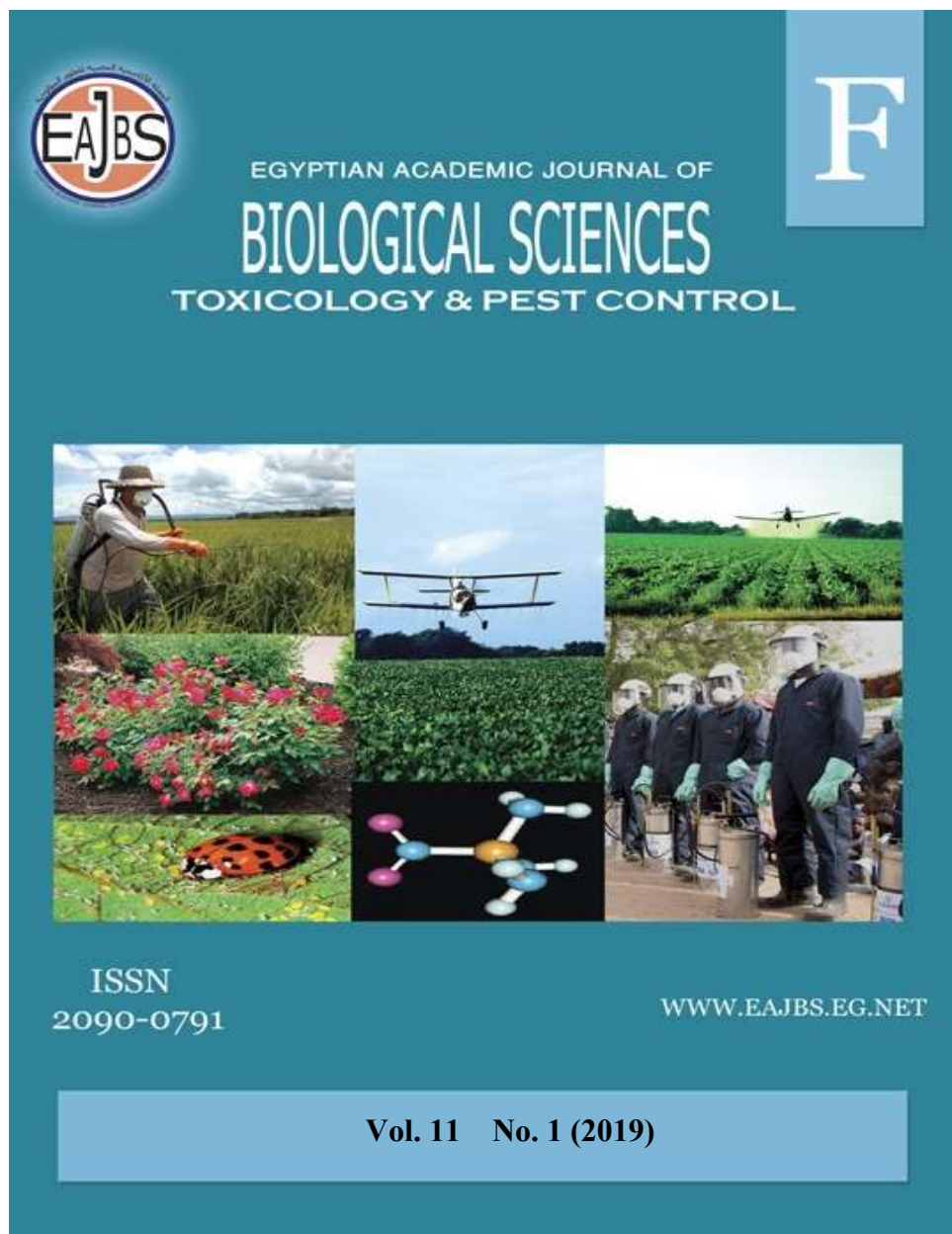


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Alleviation of Lead-Induced Immunotoxicity by *Moringa oleifera* in Albino Rats

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ABSTRACT

The existing study was designed to evaluate the ameliorative influences of *Moringa oleifera* on several immune parameters in male rats subsequent to lead administration. Twenty eight adult male rats were randomly assigned equally into 4 groups; control group was given distilled water. Lead treated rats were administered a dose of 44 mg of lead acetate/kg BW. Moringa group was treated with 50 mg/ kg BW of *Moringa oleifera* leaf extract. Lead and moringa treated group was given a dose of 44 mg/kg of lead acetate and 50 mg/ kg of moringa extract. Treatments were given orally by gavage tube for one month. By the termination of the experimental period, rats were immolated; spleen and thymus weights were recorded in addition to the collection of blood and tissue samples. Tumor necrosis factor alpha (TNF- α), interferon gamma (IF- γ), interleukin-2 (IL-2) and total antioxidant capacity (TAC) were assayed in serum. Complete blood picture was evaluated for rats. Body weight gain was reduced significantly ($P<0.05$) in lead and moringa treated rats in comparison to control. A significant ($P<0.05$) increase in spleen weights was observed in lead and moringa co-administered group compared to moringa treated group. Lead administration produced higher ($P<0.001$) levels of TNF- α , IL-2 and IF- γ compared to that in other groups. In contrary, the level of TAC was significantly ($P<0.001$) reduced in lead treated rats. A significant ($P<0.01$) reduction in RBCs and low level of hemoglobin ($P=0.07$) were observed in lead-treated rats. Spleen of rats receiving lead showed widespread hyperplasia of lymphoid follicles in white pulp and hemosiderin pigment in red pulp. Thymus of rats receiving lead showed marked proliferation in the cortical region. Spleen and thymus of rats receiving lead showed a higher ($P<0.01$) immune reactivity for NF- κ B and CD8⁺ parallel to that in other groups. In conclusion, the administration of *Moringa oleifera* extract ameliorates the immunotoxicity induced by lead exposure in rats.

INTRODUCTION

Exposure to environmental compounds is a causal factor for various health problems that have a worldwide provocation. Increased domestication and the resultant industrialization may lead to continuous human exposure to several toxic environmental contaminants (Omotoso et al. 2015). Lead is rated one of the major circumferential pollutants (Karrari et al. 2012), and the prolonged exposure to low doses of lead subsequently gave rise to biochemical, hematopoietic and immune systems dysfunctions in mammals (Lawrence and McCabe 1995; Skerfving and Bergdahl 2007). The immunotoxicity of lead is an essential issue of extensive research. Lead may modify inflammatory reactions (either early or late), varying the number of circulating B and T lymphocytes and may stimulate the cytokines release (Skoczyńska et al. 2002). The elevated count of CD8⁺ lymphocytes, expressing cytotoxic response, and the reduced count of B lymphocytes were detected in policemen of road services exposed to lead (Skoczyńska et al. 2002).

Inflammation is a serious point of organism perturbation resulting from heavy metals exposure. Nuclear factor kappa B (NF- κ B) is an inducible transcription factor in lymphocytes and considered as a key transcription factor in terms of inflammatory responses as well as it regulate cellular stress in different cell types and innate immunity responses (Vallabhapurapu and Karin 2009). It is renowned to control the expression of inducible nitric oxide synthase (iNOS) and tumor necrosis factor alpha (TNF- α) genes (Cogswell et al. 1994). TNF- α is a macrophage proinflammatory cytokine (Raabe et al. 1998) that activates NF- κ B (Mandrekar and Szabo 2009). *In vitro*, lead provoked the liberation of IL-2, and TNF- α from splenocytes and adherent peritoneal cells (Krocova et al. 2000).

Moringa oleifera possesses a variety of potential uses as the leaves of this trees has been reported to regulate thyroid hormones (Tahiliani and Kar 2000) and act as antioxidant (Nikkon et al. 2003); via reduction of lipid peroxidation and inhibition of free radicals (Sreelatha and Padma 2009). *Moringa oleifera* enhanced positively several blood indices, leucocytes (WBCs) counts, hemoglobin (Hb) and platelets (Chinwe and Insitua 2010). *Moringa oleifera* has been recited to have been utilized in traditional medicine to cure splenic enlargement and necrotic conditions (Muselin et al. 2010).

Few studies have handled the effect of *Moringa oleifera* in alleviating the deleterious influence of lead poisoning on spleen and thymus. Therefore, the existing study was proceeded to assess the toxic action of lead on the spleen, thymus and various immunological parameters with a trial to diminish this toxicity using *Moringa oleifera* extract in rat model. Moreover, the influence of lead exposure on the expression of NF- κ B and CD8⁺ in spleen and thymus of rats were investigated by immunohistochemistry.

MATERIALS AND METHODS

Rats:

Total of twenty eight adult male Wister rats, weighing from 130-150 g, were left over in plastic cages (4 rats / cage) at Laboratory Animal House, Faculty of Veterinary Medicine, Suez Canal University, Egypt. They were kept under natural day light rhythm with a temperature of 26°C ($\pm$ 1°C) and have free access to diet and *ad libitum* water supply. The experimental animals were handled and cared according to ethical guidelines described by Faculty of Veterinary Medicine, Suez

Canal University (Approval number #2018065).

***Moringa oleifera* Aqueous Extract Preparation:**

Moringa oleifera aqueous extract was prepared by mixing 10 g of dried powdered leaves of *Moringa oleifera* with 100 mL of distilled water for 24 h and then stored at 4 °C. Afterward, the mixture was filtered two times by using a 2- μ m pore filter paper. The stock solution of this aqueous extract (100 mg/mL) was kept at 4 °C for up to 5 days, or prepared as fresh solution for each set of experiment (Tuorkey 2016).

Experiment Design:

After 10 days of acclimatization, rats were split into four equal groups; control group (n=7), they were given distilled water by gavage tube daily for one month. Lead treated rats (n=7) were administrated a dose of 44 mg/kg BW of lead acetate (# No. 6080-56-4, Ava Chemicals Private Limited Co., India) 5% solution by gavage tube for one month. Moringa group (n=7), they were received daily an oral dose of 50 mg/ kg BW of *Moringa oleifera* leaf extract by gavage tube for one month. Lead and moringa treated group (n=7), they were given a dose of 44 mg/kg of lead acetate 5% solution and 50 mg/ kg of moringa leaf extract by gavage tube for one month.

Body Weight Gain:

Experimental rats were weighed weekly during the experimental period. The final body weight was subtracted from the initial one to obtain the weight gain.

Sampling:

After 30 days of treatments, overnight fasted rats were euthanized and blood samples were collected in sterile plain tubes for serum collection and in EDTA coated tubes for hematology. The collected sera were stored at -20°C. Spleen and thymus of each experimental rat were excised, weighed, washed with cold phosphate buffer saline, dried with filter paper. Relative weights of both spleen and thymus were obtained in relation to rats' body weights. Moreover, tissues of spleen and thymus were immersed in 10% neutral formalin buffered saline for histopathological examination and immunohistochemistry.

Tumor Necrosis Factor Alpha (TNF- α), Gamma Interferon (IF- γ) and Interleukin 2 (IL-2):

Serum TNF- α (IBL Co., Japan), IF- γ (R&D systems, China) and IL-2 (IBL Co., USA) were assessed using rat enzyme-linked immunosorbent assay (ELISA) kits. The procedures were done as stated by the manufacturers.

Total Antioxidant Capacity (TAC):

Sera were subjected to TAC estimation according to the manufacturer protocol (Labor Diagnostika Nord GmbH & Co. KG Co., Germany).

Hematology:

Whole blood collected in EDTA tubes and submitted to red blood cell count, hemoglobin estimation, packed cell volume (PCV) evaluation in addition to blood indices calculations. Also total and differential leukocyte counts were performed. All procedures were performed according to Pierson et al. (2000).

Histopathology:

Formalin fixed spleen and thymus were put in paraffin wax, and, several 5- μ m sections were sliced then stained with hematoxylin and eosin (H&E) stain according to Drury and Wallington (1980).

Immunohistochemistry:

Paraffin embedded spleen and thymus were sliced into 4 μ m sections on positive charged slides. Sections were subjected for xylene deparaffinization after that they were rehydrated with descending ethanol concentrations series, followed by

water. For NF- κ B, the slides were incubated with primary monoclonal anti-NF- κ B p65 (F-6) antibody (Cat #: sc-8008, sc-8008) at a rate of 1:100, for overnight at 4°C according to Meng *et al.* (2005). For CD8⁺, the slides were incubated with monoclonal Anti-CD8⁺ antibody clone OX-8, (Cat #: CBL1507, Chemicon, Chandlers Ford, UK) at a rate 1:200 at room temperature for one hour (Randall and Pearse 2008). After incubation of NF- κ B and CD8⁺ spleen and thymus slides, they were washed 3 times with PBS. Biotinylated polyvalent secondary antibody (Cat #: 32230, Thermo Scientific Co., UK) co-incubated for 30 min with tissue sections then slides were subjected to wash three times using wash buffer. Visualization of the immunoreaction was performed by adding Metal Enhanced DAB Substrate Working Solution to the tissue for 10 min. The slides were rinsed twice with wash buffer then counterstained by hematoxylin stain.

Image Analysis:

The IHC stained area percentages were performed via Image J program for NF- κ B and CD8⁺ (Abdelrazek *et al.* 2018) after subtracting light background. Briefly, seven fields of spleen and thymus were randomly chosen. The stained immunohistochemistry (IHC) areas and the percentages of IHC stained regions were calculated (Elgawish *et al.* 2014).

Statistical Analysis:

Values were set as mean $\pm$ standard error of the mean. The differences among groups were analyzed by ANOVA followed by Tukey's test for inter-group comparisons using GraphPad Prism (Version 5.01, GraphPad Software, San Diego, USA). A $P < 0.05$ indicates a significant difference between groups.

RESULTS

Body Weight Gain, Spleen and Thymus Weights:

The weight gain was reduced significantly ($P < 0.05$) in lead and moringa co-administrated rats compared to that of control. While there was no significant differences in thymus weights among rats in different groups, a significant ($P < 0.05$) increase in relative and absolute spleen weights were noted in rats treated with lead plus moringa extract compared with *Moringa oleifera* treated group (Table 1).

Table 1. Body weight gain (g), absolute (g) and relative (%) spleen and thymus weights in rats administrated different treatments

	Control	Lead	Moringa	Lead and Moringa
Body weight gain	69 $\pm$ 5.5 ^a	52.1 $\pm$ 3.5 ^{ab}	61.7 $\pm$ 5.9 ^{ac}	47.1 $\pm$ 5.2 ^{bc}
Spleen weight (absolute)	1.1 $\pm$ 0.05 ^a	1.0 $\pm$ 0.06 ^{ab}	0.8 $\pm$ 0.09 ^b	1.2 $\pm$ 0.1 ^a
Spleen weight (relative)	0.5 $\pm$ 0.02 ^{ab}	0.4 $\pm$ 0.04 ^a	0.4 $\pm$ 0.04 ^a	0.6 $\pm$ 0.08 ^b
Thymus weight (absolute)	0.3 $\pm$ 0.04	0.3 $\pm$ 0.03	0.3 $\pm$ 0.02	0.3 $\pm$ 0.02
Thymus weight (relative)	0.1 $\pm$ 0.01	0.1 $\pm$ 0.02	0.1 $\pm$ 0.01	0.2 $\pm$ 0.01

Significant differences are expressed by the different subscripts within the same row

TNF- α , IL-2, IF- γ and TAC:

Lead administration induced remarkably higher ($P < 0.001$) levels of TNF- α , IL-2 and IF- γ in comparison to the control and other treated groups. In contrary, the level of TAC was significantly ($P < 0.001$) reduced in lead treated rats relevant to other groups (Table 2).

Table 2. Effect of lead and *Moringa oleifera* on TNF- α , IL-2, IF- γ and TAC levels in the serum of rats

	Control	Lead	Moringa	Lead and Moringa
TNF- α (pg/mL)	5.2 $\pm$ 0.04 ^a	8.1 $\pm$ 0.01 ^b	5.1 $\pm$ 0.01 ^a	6.3 $\pm$ 0.01 ^c
IL-2 (pg/mL)	1.3 $\pm$ 0.01 ^a	1.9 $\pm$ 0.01 ^b	1.3 $\pm$ 0.01 ^a	1.5 $\pm$ 0.01 ^c
IF- γ (pg/mL)	667.5 $\pm$ 4.9 ^a	826.2 $\pm$ 3.8 ^b	666.9 $\pm$ 3.4 ^a	700.1 $\pm$ 8.1 ^c
TAC (U/mL)	1.8 $\pm$ 0.03 ^a	1.2 $\pm$ 0.02 ^b	1.8 $\pm$ 0.03 ^a	1.5 $\pm$ 0.01 ^c

Significant differences are expressed by the different subscripts within the same row

Hematology:

Non-significant changes were detected in haematological parameters and blood indices among control and treated rats. However, a significant ($P<0.01$) reduction in RBCs count as well as low level of hemoglobin ($P=0.07$) were observed in lead-treated rats compared to other groups (Table 3).

Table 3. Effect of lead and *Moringa oleifera* on different blood indices in rats

	Control	Lead	Moringa	Lead and Moringa
Hb (g/dl)	13.4 $\pm$ 0.9	11.3 $\pm$ 0.6	14.2 $\pm$ 0.4	12.7 $\pm$ 0.6
RBCs ($10^6/\mu\text{L}$)	4.7 $\pm$ 0.1 ^a	3.9 $\pm$ 0.1 ^b	4.8 $\pm$ 0.1 ^a	4.4 $\pm$ 0.1 ^a
PCV (%)	40.0 $\pm$ 3.1	36.8 $\pm$ 1.1	42.2 $\pm$ 2.9	37.2 $\pm$ 1.6
MCV (fl)	88.53 $\pm$ 4.1	80.67 $\pm$ 1.4	91.1 $\pm$ 5.9	83.87 $\pm$ 2.6
MCH (pg)	29.77 $\pm$ 1.1	29.8 $\pm$ 1.3	30.67 $\pm$ 1.0	28.6 $\pm$ 1.0
MCHC (%)	33.7 $\pm$ 0.3	31.3 $\pm$ 0.8	33.9 $\pm$ 1.8	34.2 $\pm$ 1.3
TLC ($10^3/\mu\text{L}$)	20.8 $\pm$ 1.5	21.2 $\pm$ 3.0	19.8 $\pm$ 2.5	15.0 $\pm$ 2.7
Neutrophil ($10^3/\mu\text{L}$)	13.4 $\pm$ 0.9	14.7 $\pm$ 3.4	14.4 $\pm$ 2.7	13.9 $\pm$ 2.8
Lymphocyte ($10^3/\mu\text{L}$)	6.0 $\pm$ 1.1	5.4 $\pm$ 1.0	4.4 $\pm$ 0.4	4.3 $\pm$ 0.8
Monocyte ($10^3/\mu\text{L}$)	0.9 $\pm$ 0.3	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.04
Eosinophil ($10^3/\mu\text{L}$)	0.6 $\pm$ 0.1	0.3 $\pm$ 0.04	0.4 $\pm$ 0.04	0.4 $\pm$ 0.03

Significant differences are expressed by the different subscripts within the same row

Histopathology:

Spleen of control and moringa-treated rats showed normal architecture. The parenchyma consisted of white pulp (lymphatic nodules and periarterial sheaths), red pulp (splenic sinus and splenic cord) and marginal zone (Fig. 1a, 1f). However, spleen of lead-treated rats showed widespread hyperplasia of the lymphoid follicles in white pulp (Fig. 1b) and focal area of lymphocytosis (Fig. 1d). There was ill-defined spleen architecture, due to diffusion of white pulp into red pulp and presence of tangible body macrophage (Fig. 1c) and hemosiderin pigment in red pulp. Moderate congestion of red pulp with dilatation and congestion of some blood vessels with hemolysed RBCs was observed (Fig. 1 d, e). Improvement was detected in lead and moringa-treated rats with reduction of the hyperplastic lymphoid follicles in spite of some diffusions of red pulp into white pulp were still detected (Fig. 1g).

Thymus of control and moringa-treated rats had normal architecture. Parenchyma has outer, more darkly staining and highly cellular cortical region and inner, lighter staining and less cellular medullary region (Fig. 2a, 2e). However, thymus of lead-treated rats showed marked proliferation in cortical region and complete disappearance of medulla in some lobules (Fig. 2b). Mild hemorrhage in cortical region (Fig. 2c) and tangible body macrophages in cortex giving starry sky appearance were detected (Fig. 2d). Improvements were detected in rats given lead with moringa (Fig. 2f).

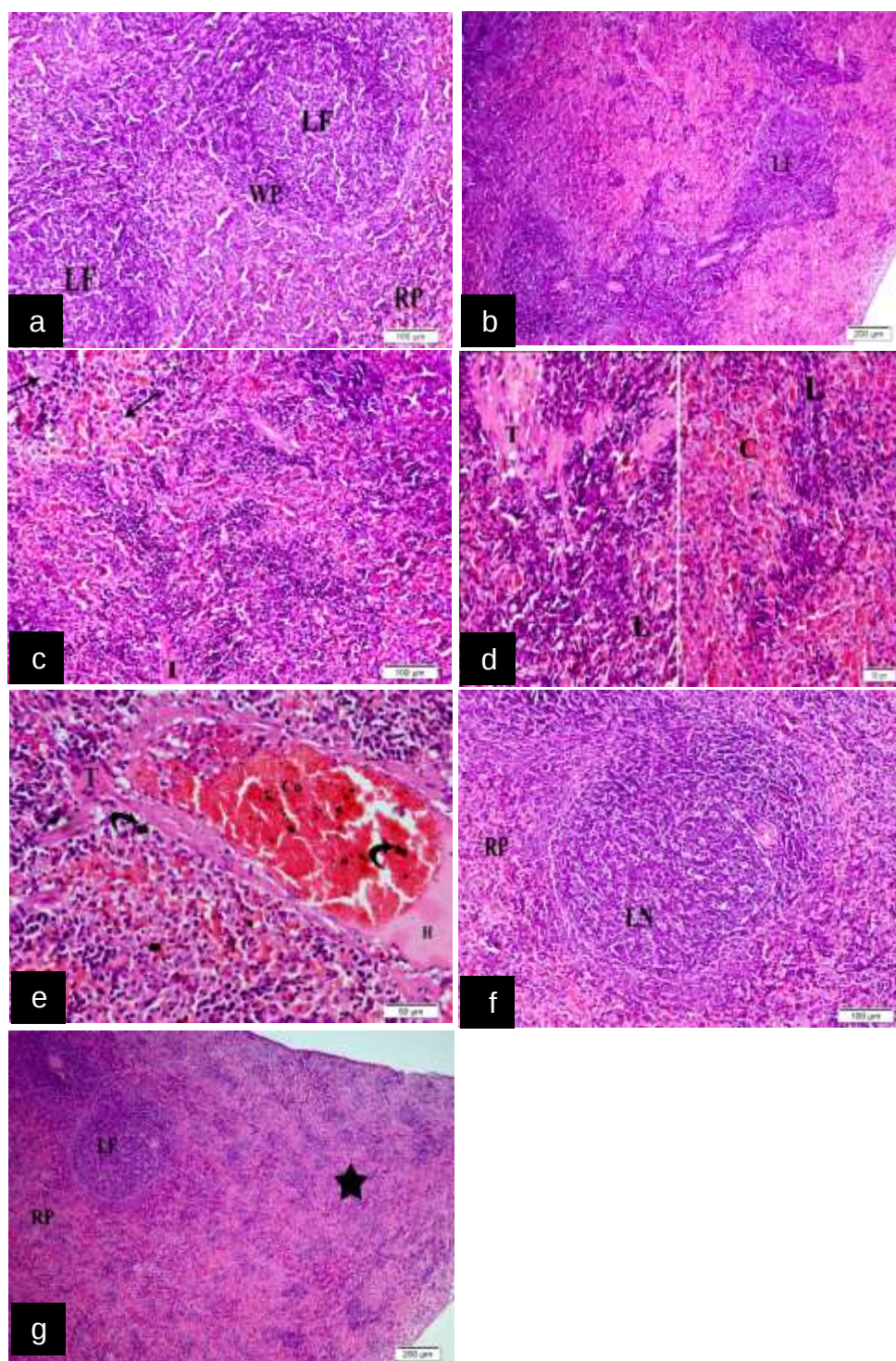


Fig. 1. Photomicrographs of spleen stained with H&E. Control rats (a): showed normal structure of the spleen represented by white pulp (WP) that has lymphatic follicle (LF), and red pulp (RP). Lead-treated rats showed (b): hyperplasia of the lymphatic nodules (LF); (c): ill-defined spleen architecture trabeculae (T) and tangible body macrophages (arrow); (d): lymphocytosis (L), congestion of the red pulp (C) and trabeculae (T) and (e): congestion and dilatation of the blood vessel (Co), hemolyzed RBCs (H), hemosiderin pigment (curved arrow) and trabeculae (T). Moringa-treated rats (f) showed lymphatic follicle (LF) and red pulp (RP). Lead and moringa-treated rats (g): showed ill-defined spleen architecture (star), lymphatic follicle (LF), and red pulp (RP).

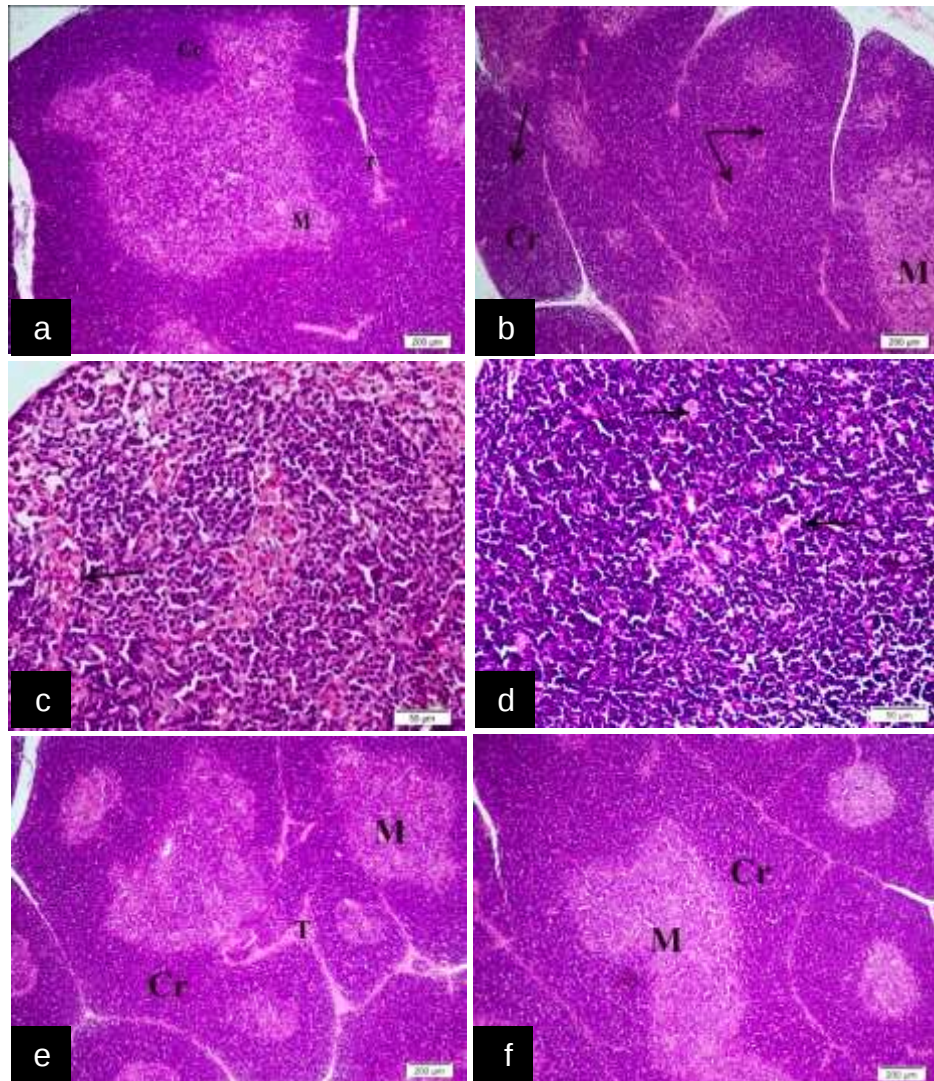


Fig. 2. Thymus stained with H&E. Control group (a): showed normal structure of thymus represented by cortex (Cr) medulla (M) and trabeculae (T). Lead-treated rats showed (b): cortex (Cr) and disappearance of the medulla in some lobules (arrow); (c): mild hemorrhage (arrow); (d): Tangible body macrophage imparted starry sky appearance (arrow). Moringa-treated rats (e): showed cortex (Cr) medulla (M) and trabeculae (T). Lead and moringa-treated rats (f): showed cortex (Cr) and medulla (M).

Immunohistochemistry of NF- κ B and CD8⁺ in Spleen and Thymus:

NF- κ B and CD8⁺ in the spleen and thymus revealed a higher ($P < 0.01$) immune reactivity in rats receiving lead compared with control and treated groups. On the other hand, the immune reactivity of NF- κ B and CD8⁺ were declined significantly ($P < 0.01$) in rats given moringa alone or in combination with lead (Figs. 3 - 5).

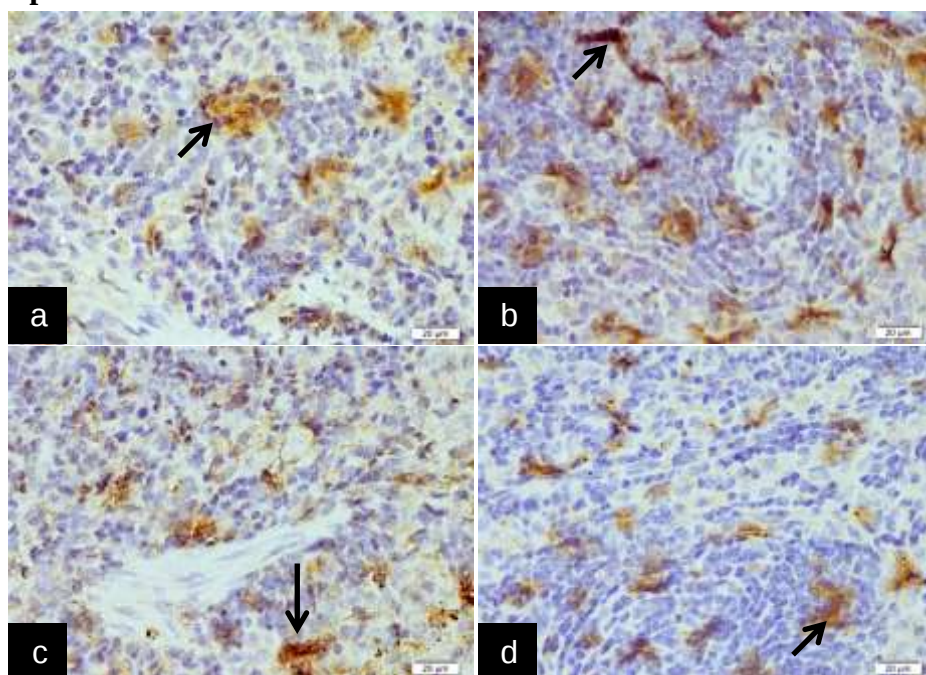
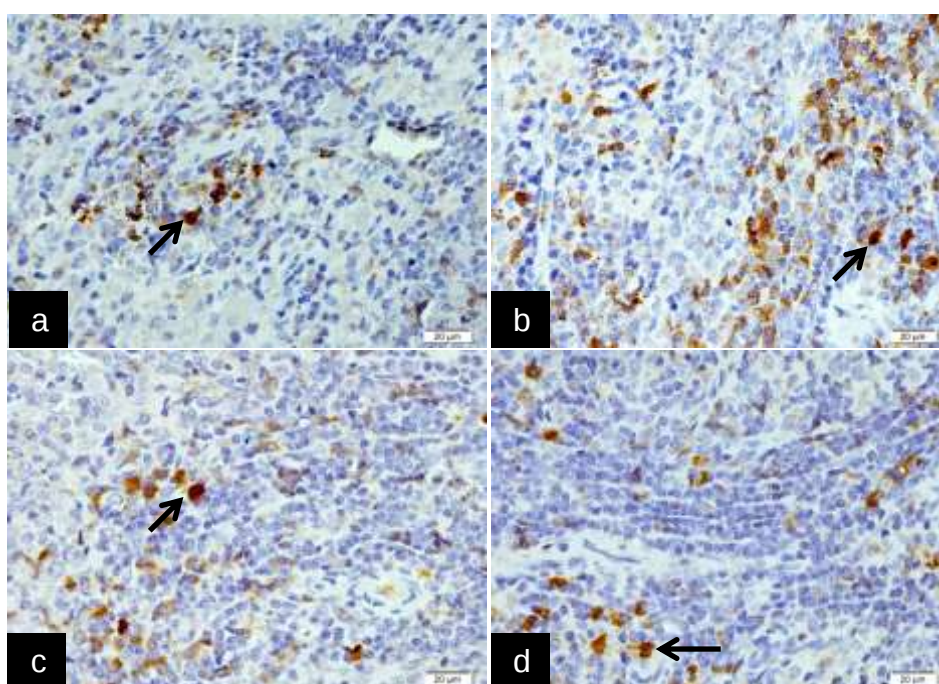
Spleen NF- κ B**Spleen CD8⁺**

Fig. 3. Photomicrograph of spleen represented NF- κ B and CD8⁺ expression in control rats (a); lead- treated group (b); *Moringa oleifera*- treated rats (c) and lead and *Moringa oleifera*- treated rats (d).

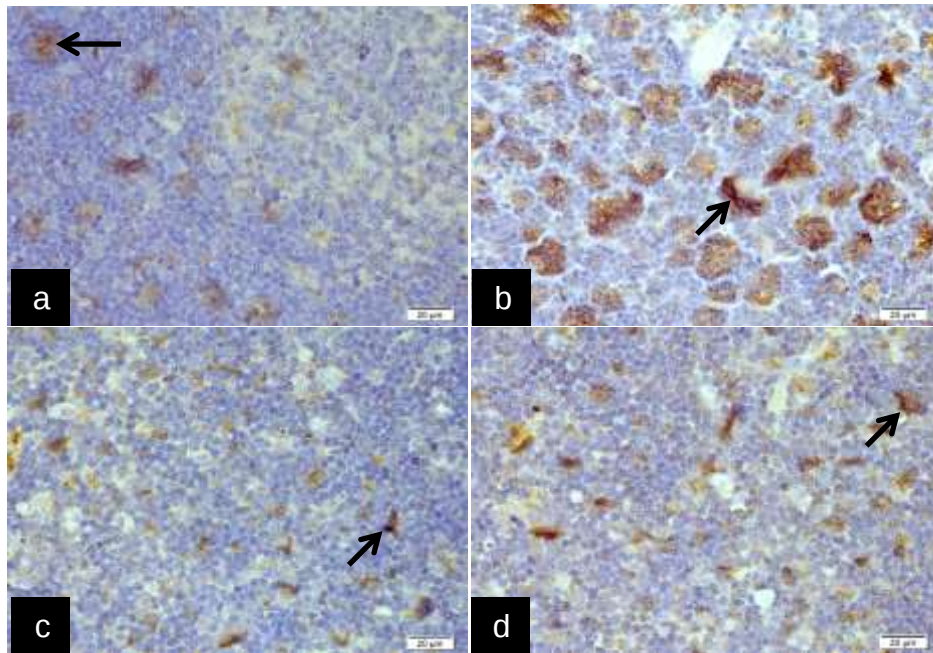
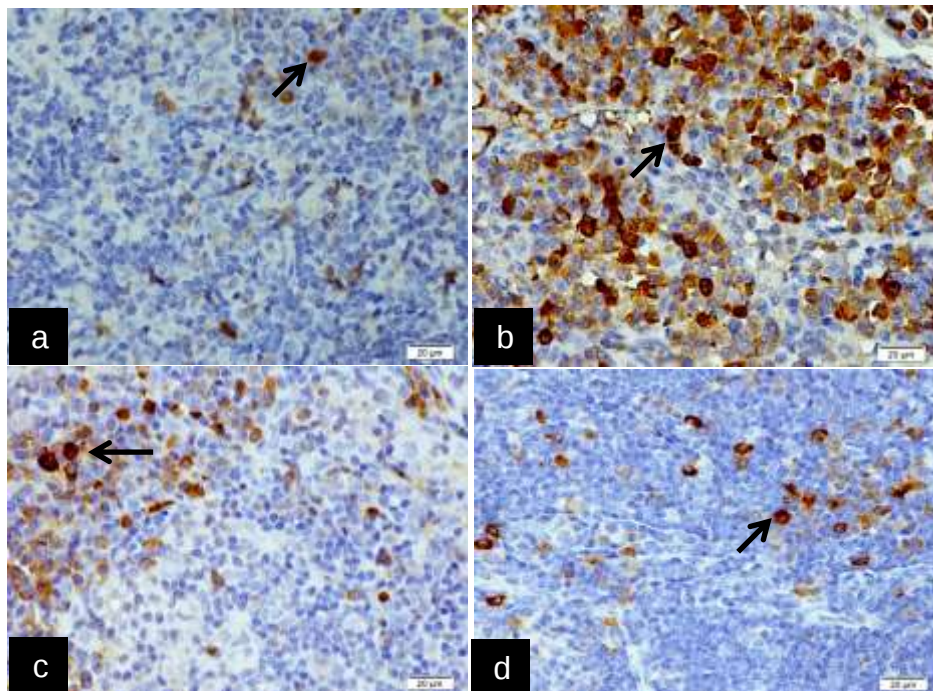
Thymus NF- κ B**Thymus CD8⁺**

Fig. 4. Photomicrograph of thymus represented NF- κ B and CD8⁺ expression in control rats (a); Lead- treated rats (b); *Moringa oleifera*- treated rats (c) and lead and *Moringa oleifera*- treated rats (d).

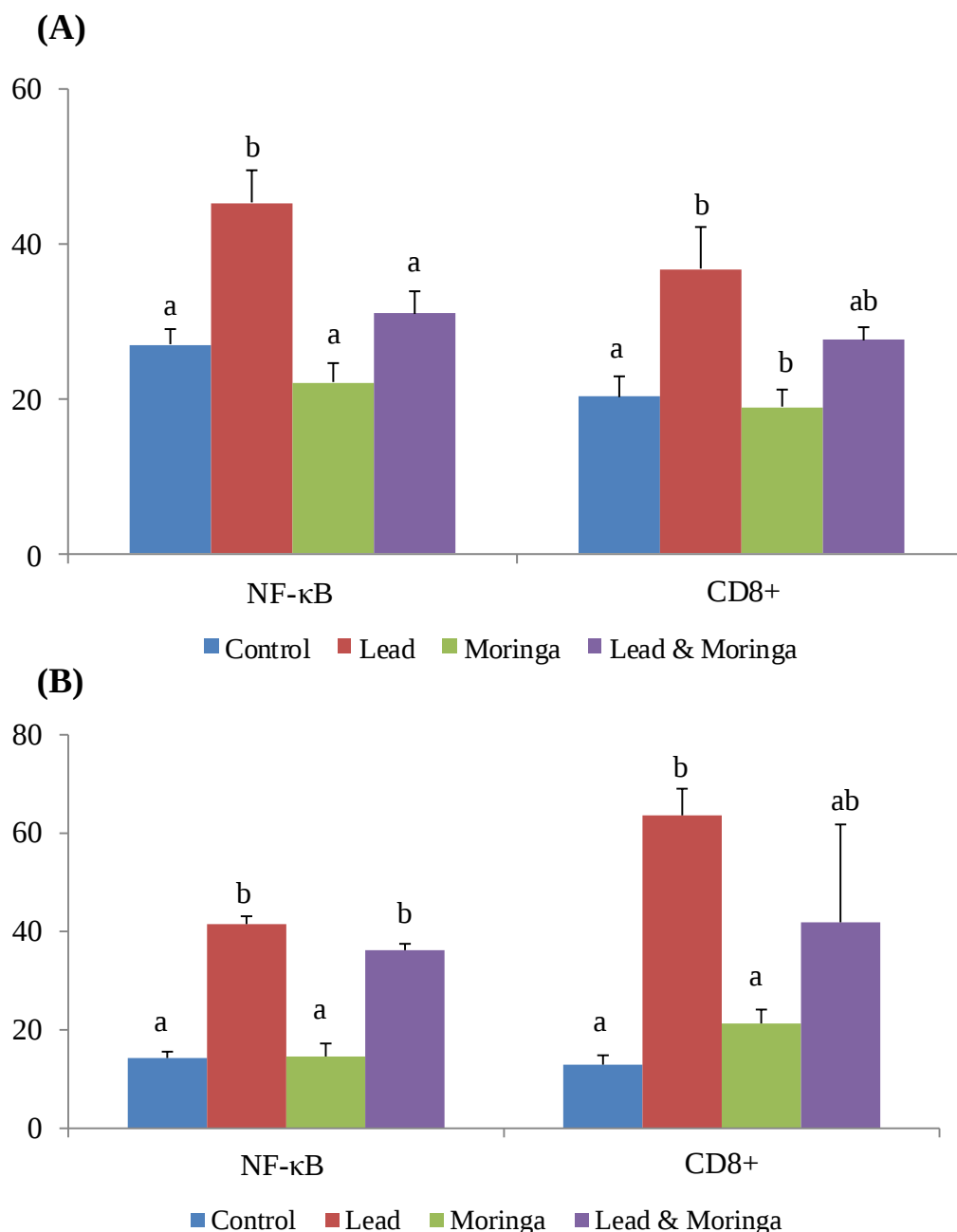


Fig. 5. Expression levels of NF- κ B and CD8⁺ in spleen **(A)** and thymus **(B)** of control, lead and moringa-treated rats. Positive proportions of NF- κ B and CD8⁺ were significantly ($P < 0.01$) higher in spleen and thymus of lead-administered rats in comparison with that in other rats groups. Values represented are means $\pm$ SE. Means having the same letters are not significantly different from each other, $P < 0.05$.

DISCUSSION

In the existing work, the body weight gain was reduced significantly ($P < 0.05$) in rats treated with lead and *Moringa oleifera* in comparison to that of control rats. However there was no significant alterations in thymus weights among rats in different groups, a significant ($P < 0.05$) increase in relative and absolute spleen weights were observed in rats treated with lead and *Moringa oleifera* compared with that in *Moringa oleifera*-administered rats. Lead brought about significant decline in

the body weight gain (Ibrahim et al. 2012) as the growth rate in rats was declined when they fed on lead (Seddik et al. 2010). This decline in the body weight gain might be attributed to imbalance in the metabolism due to lead administration by means of impairing zinc utilization in zinc-dependent enzymes which are essential for various metabolic processes (Ibrahim et al. 2012). Additionally, *Moringa oleifera* might reduce the weight gain through down-regulation of leptin gene expression (Metwally et al. 2017).

In the current study, lead administration motivated remarkably higher ($P < 0.001$) TNF- α , IFN- γ and IL-2 levels, however, the level of TAC was significantly ($P < 0.001$) reduced in lead-administered group. Cytokines are biomarkers of heavy metal-induced immune toxicity. A former study has stated that lead upregulated the TNF- α levels in a mouse microglial cell line (Kumawat et al. 2014). Lead has been articulated to rise significantly TNF- α , both *in vitro* by macrophages (Flohe et al. 2002) and rat spleen cells (Krocova et al. 2000) and *in vivo* in rats (Chen et al. 1999; Liu et al. 2000) and cows (Kaminska et al. 1998). Sun et al. (2016) found that levels of NF- κ B, and TNF- α mRNA were significantly higher in chicken received lead than that of control, suggesting that excess lead could give rise to inflammation of chicken peripheral blood lymphocytes. Miller et al. (1998) found that offspring born to dams treated with 250 ppm of lead had an increase in TNF- α and nitric oxide production; however, IFN- γ levels were decreased in offspring born to dam received 500 ppm of lead treatment. Excess lead increased IL-6 and caused inflammation in kidney of rats (Liu et al. 2012). Lead poisoning during the rat's late gestation and elevated IL-12 level resulted in offspring splenic cells immune toxicity (Bunn et al. 2001). Away from our findings, lead decreased IFN- γ and suppressed immunity in rat T cells (Fang et al. 2012). Additionally, Jiao et al. (2017) found that lead diminished IFN- γ and IL-2 mRNA levels in bursa Fabricius of chicken. Lead was in charge of a significant shift in the morphology and a weakened cell function as well as TNF- α release which enhanced the inflammatory process in mice testicular macrophages (Barbhuiya et al. 2013). The differences observed in the immune response to lead treatment might be relevant to the reason that lead may possibly favors development of T- helper subset and/or function, causing imbalance and changing the immune response of type 1 and type 2 cells (Miller et al. 1998). It was clear that lead acetate in the current study induced oxidative damage that depleted TAC and promoted TNF- α and IL-2 over production. Oxidative stress can cause deleterious effects that damage lipids, proteins and DNA which directly associated to inflammation and over production of tyrosine kinase mediated cytokines as IL-2 (Sánchez et al. 2015). Also promotion of NF- κ B is obvious in case of oxidative stress that resulted in upregulation of TNF- α (Karin and Delhase 2000). Lead has a negative impact on the immune system and the most recent evidence indicated that lead can enhance inflammatory response (Metryka et al. 2018). Current experiment suggested that moringa extract could alleviate the lead-produced oxidative stress thus reducing IL-2 and TNF- α compared with that in lead group and this might be due to the antioxidant activity and total phenolic contents of moringa (Sreelatha and Padma 2009).

In the present work, a significant reduction in RBCs count and low level of hemoglobin were observed in lead-treated rats, despite the absence of any statistical variations in other blood pictures in control and other treated groups. These results indicated that lead administration could lead to anaemia in treated rats. These findings were similar to that of Ibrahim et al. (2012), who found that hemoglobin level was reduced by lead ingestion, although the WBCS count wasn't significantly altered in relation to the control. In a recent study by Gani et al. (2017), they reported

a remarkable low level to total erythrocytes counts and hemoglobin percentage in rats after exposure to low doses of lead (6 mg/ ml) in drinking water for about two months. In the present work, moringa improved the hemoglobin level in treated rats. Other studies explained the beneficial effect of the administration of the *Moringa oleifera* at different doses on the production of white blood cells and they attributed this effect to the possible stimulation of *Moringa oleifera* to the immune defense system (Kashinath 1990), as well as the presence of flavonoids and saponins in the extract of *Moringa oleifera* which might have profound effects on the immune and inflammatory cells functions (Evans 2006).

Current study declared that spleen of rats receiving lead showed widespread hyperplasia of lymphoid follicles in white pulp and hemosiderin pigment in red pulp. Thymus of rats receiving lead showed marked proliferation in the cortical region. These results were in accordance with the serum increase in IL-2 in this group where it exerts a substantial role in T-cell proliferation in an autocrine manner (Smith 1988). Spleen and thymus of rats receiving lead showed a higher ($P<0.01$) immune reactivity for NF- κ B and CD8⁺ than in other treated groups. Teijón *et al.* (2003) found that oral intervention of lead caused histological alterations in spleen, such as edema as well as increasing the number of lymphocytes, moreover, intraperitoneal lead administration resulted in more evident histopathological modifications beside increase in lymphocytes number, and also promoted an increase in CD8⁺ cells. A marked preservation in the splenic pulps were restored or preserved to a large extent after moringa administration (Owolabi *et al.* 2014). A significantly higher percentage of CD8⁺ cells and lower percentage of CD4⁺ cells were found in preschool children exposed to environmental lead (Li *et al.* 2005). The elevations in expression values of thymic and splenic NF- κ B have a direct contribution in the observed lymphoid proliferation in such organs as NF- κ B is considered a vital signal necessary for lympho-proliferation (Gerondakis and Siebenlist 2010). Increased levels of IF- γ , in lead treated rats was in harmony with the elevated expression of splenic and thymic CD8⁺ cells. IF- γ is considered a powerful pro-inflammatory modulator to cytotoxic CD8⁺ cells action. These results suggested the pro-inflammatory effect of lead that was ameliorated via moringa extract administration.

CONCLUSION

Lead exposure augmented the cytokines levels in serum, activated the CD8⁺ and NF- κ B expression in the tissue of spleen and thymus as well as depressed the TAC level. *Moringa oleifera* supplementation had a mitigative influence on the activation of the NF- κ B and CD8⁺ pathways produced by lead exposure. Finally, these results suggested that *Moringa oleifera* could diminish the activation of the NF- κ B and CD8⁺ pathways and reduce the level of inflammatory markers in the serum of rats under lead exposure.

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ARABIC SUMMARY

تخفيف التسمم المناعي الناجم عن الرصاص بواسطة المورينجا اوليفرا في الجرذان البيضاء

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تم تصميم الدراسة الحالية لتقييم التأثيرات المحسنة للمورينجا اوليفرا على عدة معايير مناعية في ذكور الجرذان البالغة المسممة بالرصاص. ثمانية وعشرون ذكرا من الجرذان البالغين ، تم تقسيمهم عشوائيا إلى أربع مجموعات متساوية: المجموعة الضابطة أعطيت ماء مقطر، المجموعة المعالجة بالرصاص و تم اعطائها خلات الرصاص بجرعة 44 ملجم/كجم و مجموعة المورينجا و تم معالجتها بـ 50 ملجم/كجم من مستخلص اوراق المورينجا و اخيرا مجموعة الرصاص والمورنجا معا و التى تم معالجتها بـ 44 ملجم / كجم من خلات الرصاص و 50 ملجم / كجم من مستخلص أوراق المورينجا. تم إعطاء جميع العلاجات عن طريق الفم بواسطة الأنبوب المعوي وذلك لمدة شهر واحد. بحلول نهاية التجربة ، تم قتل الجرذان و تم تسجيل وزن كلا من الطحال والغدة التيموسية بالإضافة إلى تجميع عينات الدم والأنسجة. تم قياس كل من معامل نخر الورم ألفا (TNF-α) و الإنترفيرون جاما (IF-γ) و انترلوكين- 2 (IL-2) و السعة التأكسدية الاجمالية (TAC) في مصل الدم. تم تقييم صورة الدم كاملة للجرذان. اسفرت النتائج عن نقص معدل زيادة وزن الجسم بشكل معنوى في الجرذان المعالجة بالمورينجا والرصاص مقارنة بالمجموعة الضابطة. لوحظت زيادة معنوية في أوزان الطحال في المجموعة التى تم معالجتها بكل من الرصاص والمورنجا معا مقارنة بالمجموعة المعالجة بالمورينجا فقط. أسفر العلاج بخلات الرصاص عن زيادة معنوية معامل نخر الورم ألفا (TNF-α) و الإنترفيرون جاما (IF-γ) و انترلوكين- 2 (IL-2) مقارنة مع المجموعات الأخرى. في المقابل ، انخفض مستوى السعة التأكسدية الاجمالية (TAC) معنويا في الجرذان المعالجة بالرصاص. لوحظ انخفاض طفيف في مستوى خضاب الدم (الهيموجلوبين) في مجموعة الرصاص. أظهرت النتائج تضخم واسع الانتشار في الحويصلات الليمفاوية باللب الأبيض داخل نسيج الطحال بالإضافة الى وجود صبغيات الهيموسيدرين في اللب الاحمر. كما نجم عن التعرض للرصاص زيادة في حجم قشرة الغدة التيموسية. أسفر التعرض للرصاص عن زيادة معنوية في مساحة التفاعل المناعي لكل من NF-κB و CD8⁺ في القطاعات النسجوية- كيميائية لكل من هذين القياسين عنها في المجموعات الأخرى. في الختام: يخفض مستخلص نبات المورينجا اوليفرا السمية المناعية الناجمة عن التعرض للرصاص في الجرذان.