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HISTOLOGICAL CHANGES AND IMMUNOSUPPRESSION INDUCE BY LEAD IN FEMALE RATS

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Abstract

The study aimed to investigate the effect of whole sonicated *Enterococcus faecalis* (WSEF Ages) and cysteine on the lead acetate, also 75 white rats from female sexes at average age 8-10 weeks were getted and divided randomly into 5 groups equally and treated as following: 1st group : immunized with 0.5ml of whole sonicated Ags (WSEF) (protein concentration18mg/ml) subcutaneous two doses, two weeks interval, 2nd group: administrated with cysteine 200mg/kg diet for 90 days and immunized as 1st group. 3rd group: immunized as 1st group and at the same time oral administrated with lead acetate 75mg/kg for 90 days. 4th group: rats, which immunized as 1st group and treated as 2nd and 3rd. 5th group which seted as control group, at day 28-30 post-immunization, delayed hypersensitivity and indirect passive haemagglutination tests were done. The results showed Immunological examination revealed that the lead acetate induced depressed in both arms of immune response are cell mediated immunity and humeral immunity as comparing with immunized non-treatment animals, the results showed increase significant ($p<0.05$) in immunized group with cysteine treatment. The pathological examination revealed that the lead acetate induced, liver showed lung of rat treated thickening alveolar septa and infiltration mononuclear cells, in addition, section of intestine showed show villi sloughing and increase number of goblet cells and eosinophil cells. In conclusion, cysteine can decreased the damage of lung cells and intestine from oxidative effect induced by lead, and that related to their antioxidant effects.

Key words : Lead, cysteine, *Enterococcus faecalis*, histopathology, antioxidant.

Introduction

Lead is common contaminants and toxic heavy metals, dangerous even in small quantities. Humans and animals may be exposed to lead via contaminated food or water and fuel additives (Gidlow, 2004). Heavy metal is mainly absorbed by the gastrointestinal tract. Higher gastrointestinal absorption and poorly established potential for detoxifying and eliminating damaging substances, Megaoesophagus may rarely be present in cats and may be secondary to neurological processes (Jayaprakash *et al*., 2019). Mechanism of haematopoietic pollution The impact of lead on haematopoiesis is connected with suppression of important enzymes in the heme formation pathway. Immunotoxic characteristics of metals have also been identified and some studies have confirmed that there is a connection between metal exposures and IgEs. Positive associations respectively mercury (Hg), lead (Pb) and serum IgEs exposures have already been reported in human studies (Kim *et al*., 2016). Cysteine is important for protein synthesis, detoxification, and diverse metabolic functions. Found in beta-keratin, the main protein in nails, skin, and hair (Vallee *et al*., 2017), Cysteine is important in collagen production, as well as skin elasticity and texture. Also required in the manufacture of amino acid taurine (Breilkreutz *et al.,* 2000), Cysteine is a component of the antioxidant glutathione and plays a role in the metabolism of essential biochemicals such as coenzyme A, heparin, and biotin (Zahroon, 2009).

Materials and Methods

Animals and treatments

75 adult Wistar albino female rats 8 weeks old and weighing 250 ± 10 gms, they were obtained from (Animal house colony of veterinary college in Baghdad University). Females rats were housed in temperature controlled rooms (24°±3ºC) with constant humidity (40 - 70%) and 12h/12h light/ dark cycle prior to use in experimental protocols. Rats were left for 1 week before

experimentation to adapt the laboratory conditions. Experimental Animals were treated as following :

1. 1st group was immunized with sonicated *Enterococcus faecalis* (WSEF Ages), two doses, with two weeks intervals.
2. 2nd group was immunized as in 1st group and at the same time it was given daily cysteine, via oral route 200mg\kg, b.w for 90 days.
3. 3rd group was immunized as in 1st group and administrated with the Lead acetate is produced by (Estrin fine chemicals LTD, Italy), supplied in granular form in a dose of 75 mg / kg b. w. (Batra *et al*., 2004), and dissolved in distilled water.
4. 4th group was immunized as in 1st group and administrated with lead acetate as in 3rd group and treated with cysteine as in 2nd group
5. 5th group was administrated daily with 0.3 ml of sterile normal saline orally for 90 days and it was served as control group.

Group Cysteine group treated with 200 mg/kg/rat of cysteine. All treatments were for 90 days. The Lead acetate is produced by (Estrin fine chemicals LTD, Italy), supplied in granular form in a dose of 75 mg/kg b. w. (Batra *et al*., 2004) and dissolved in distilled water. The cysteine was purchased from the local market. Lead acetate at a dose of 75 mg/Kg.BW. has been given as a solution which prepared by dissolving of 3 grams of lead acetate in sterile distilled water then completed to 400 ml. This dose of 75 mg/kg.BW of lead acetate has been used by previous studies to show the toxic effects of lead poisoning (zahroon, 2009). L-cysteine was used in a dose of 200 mg/Kg. BW. (Appel *et al*., 2006) and prepared by dissolving of 600 mg of pure L-cysteine in a sterile distilled water and completed to 30 ml. The prepared drug was given orally by using stomach tube at a dose level of 1 ml/100g.BW. Animal sacrifice and collection of samples: Blood samples were collected at the end of the experiment via cardiac puncture from each anaesthetized rat (Ketamin hydrochloride with Xylazin) after fasting 8-12 hours, using disposable syringes. Samples were centrifuged at 3500 rpm for 15 minutes, then the clear serum was collected in sterilized disposable plastic tubes and stored in a freezer set at -20ºC for subsequent measurement of ALT and AST in serum is done by using of AST and ALT kits (Reitman, 1957) depending on the following reactions. Biochemical Analysis of Plasma Samples. Total bilirubin (TB) determinations in plasma were total bilirubin, in the presence of a suitable solubilizing agent is coupled with a diazonium ion in a strongly acidic medium. acid Bilirubin + diazonium ion azobilirubin The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically (Kirk, 2008). Total Protein Extracts Total protein in serum can be measured by a variety of methods, including chemical methods, turbidimetry and nephelometry, The most widely used is a method based on the biuret reaction (Yýlmaz *et al*., 2004).

Histopathological study

The intestine and lung of different groups were removed and fixed in 10% formal saline. Paraffin sections of 5 nm thick, were routinely stained with haematoxylin and eosin (H&E) (Luna, 1968) and assessed in a light microscope (CYAN, China).

Statistical analysis

Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses (SAS, 2001).

Results

Foot pad - Delayed type hypersensitivity (DTH)

Results at 24hrs. post-inoculation reveals that mean values of foot pad thickness against WSEFAg when in the 2nd group (2.22±0.30) were twice as high than those in the 1st group (1.82±0.31), 3rd group (1.41±0.25) and 4th group (1.7±0.15). All of these values decreased at 48hrs. The major qualities of the 2nd group at this period (48hr. sub-inoculation) (2.12±0.48) also were larger than for the 1st group (1.46±0.90), the 3rd group (1.05±1.30) and the 4th group (1.30±0.52) shown in the table 1.

Passive Haemagglutination Test

Humoral reaction was detected in vaccinated individuals (4 rats in each group) with WSSTAgs (without millipore filtering). Table 2 did show that mean titers of antibodies significantly increased ($p \leq 0.05$) in the vaccinated groups 1st (480 ± 198.12) and 2nd (768 ± 147.8) and 3rd (105.5 ±1.5) simultaneously. There was a labeled decline in titration of antibodies and lastly both the 4th group (282 ± 11.31) was slightly greater than the whole 3rd group and lower than the 1st and 2nd group. While the 5th group (as control group) actually showed no titer antibody rate (0 ±0).

Histopathological

The histopathological study of both immunized activated lymphoid follicls and control group rats' have a normal histology of lung and small intestine. lead acetate

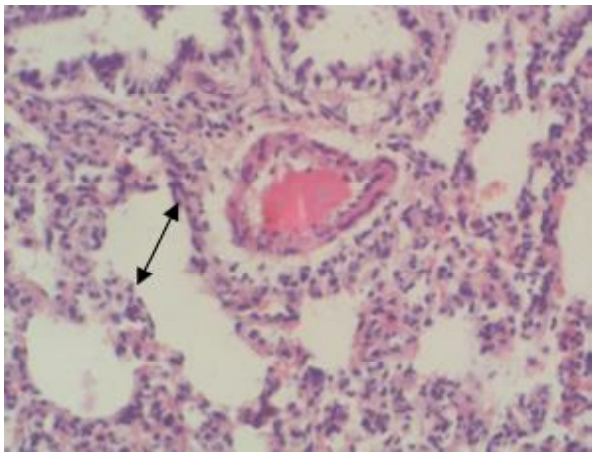

Fig. 1 : Lesions section of lung of rat treated with 75 mg/Kg.BW/day of lead acetate for 90 days show thickening alveolar septa and infiltration mononuclear cells (⟷) and congestions (H & E X 400).

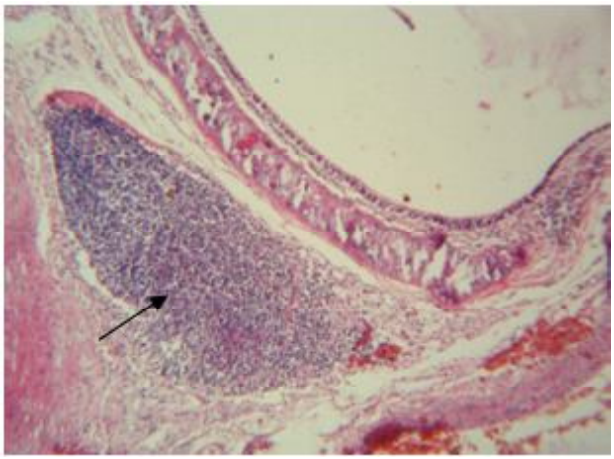

Fig. 2 : Histopathological section of lung of rat treated with 75 mg/Kg.BW/day of lead acetate and 200 mg/Kg.BW/day of cysteine for 90 days show thickening of lymphoid tissues (⟶) (H & E X 400).

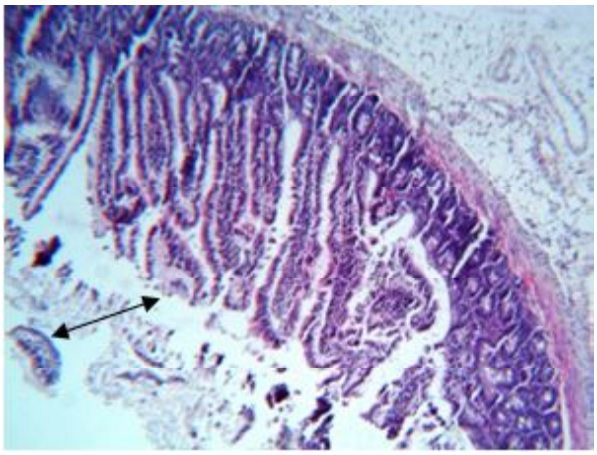

Fig. 3 : Main lesions of intestine of rat treated with 75 mg/Kg.BW/day of lead acetate for 90 days show villi sloughing and epithelial cells damage and congestions (⟷) (H & E X 200).

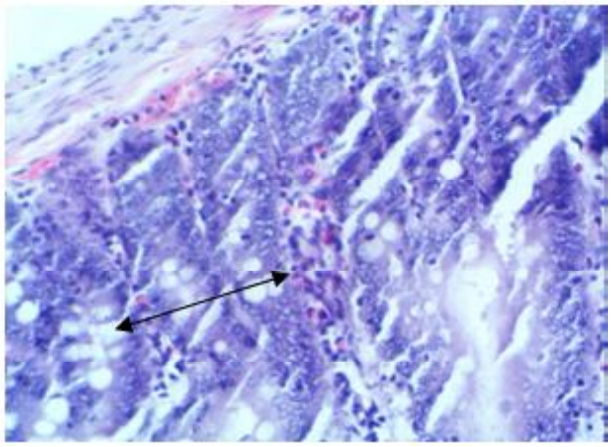

Fig. 4 : Main lesions of intestine of rat treated with 75 mg/Kg.BW/day of lead acetate for 90 days show villi sloughing and increase number of goblet cells and eosinophil cells (⟷) (H & E X 200).

Table 1 : Mean scores of the foot pad density of the animal after 28 days of vaccination.

Group	Foot pad thickness (mean ± SE)	
	24 hr.	48 hr.
G1	B 1.82±0.31	B 1.46±0.90
G2	A 2.22±0.30	A 2.12±0.48
G3	B 1.41±0.25	C 1.05±1.30
G4	Bc 1.7±0.15	B 1.30±0.52
G5	E 0 ± 0	D 0 ± 0

Different capital letters means significant ($p<0.05$) results between groups.

Table 2 : Mean antibody titer values and principles in immunized and control-negative groups 30 days only after immunization.

Group	Treated	Mean ± SE
1st group	Immunization only	B 490.0± 13.24
2nd group	Immunization + cystein	A 789.0±43.31
3rd group	Immunization + lead	D 108.0± 4.42
4th group	Immunization + cystein + lead	C 326.6± 15.74
5th group	Negative control	0 ± 0

The distinctly different vertical letters written refer to the important variations at ($p \leq 0.05$).

feeding group rat lung showed congested blood vessels with infiltration inflammatory cells (fig. 1), while the main lesions in immunized group was lung thickening of lymphoid tissues (fig. 2). Small intestine and its disruption reduces nutrients utilization and feed efficiency, regarding Pb-induced oxidative stress (fig. 3), in addition increase number of eosinophil's and goblets cells (fig. 4). Using cysteine as a protective agent with lead acetate in lung

and intestine appear pathological changes recorded in the viscera of the animals were less in severity compared with the toxic groups.

Discussion

scientific study has shown that the whole ultrasonic *Enterococcus faecalis* antigens (WSEFAgs) overstimulated cell-mediated innate immune response in the 1st group (immunocompromised group), since DTH is a single arm of cell-mediated immune response mediated by CD4 + and CD8 + T cell cytokines (Theilacker *et al*., 2006). Outcome revealed that WSEFAgs sparked T helper1 (Th1) cells that naturally produce interferon gamma (IFNΔ), which is a powerful enhancer and activation of macrophages as well as other immune cells at the location of antigen immunization, this proof was shared with Masao *et al*. (2019), who observed that DTH reaction was used as a cell-mediated immune response marker and seems to be dependent on both T helper1 (Th1)-driven stimuli as well as cell recruitment and local chemotaxis.

Accumulation of immune cells as a outcome of lymphokines derived from the effector stage of the Th1 subset, which activates macrophages to generate monokines that cause and promote vasodilatation, the foot pad thickness that happens after inoculation of WSEF Ags may be caused by swelling of the blood vessels and highly vascularized exudates and Chemoattraction of immune cells to the foot of immunocompromised rats and these occurrences lead in significant swelling of inoculated locations during 24-48hrs. Post antigen awareness. Those same findings were endorsed by Dahliatul (2019), who confirmed that the growth of extremely enhanced macrophages depends on lymphokines synthesized by the Th1 subset and were linked with the onset of DTH. On the other side, the current research have shown that WSEFAgs contain all the antigens of *Enterococcus faecalis* and the sonication method has made, it much easier for the antigen presenting cells (APCs) to phagocyte and to destroy Ags and to show the significant histocompatibility class II (MHC II) on their surface. APCs and natural killer cells are therefore activated, which initiate the production of proinflammatory cytokines, including interleukin-12 (IL-12) and IFNΔ, which are essential in the innate immune response to influence intracellular pathogen disease. (Kretschmer *et al*., 2017).

In just the immunized 2nd group immunizing WSSTAg and injecting alpha lipoic acid (200mg\kg) orally once daily, the current research reported a significant increase in the foot pad density of more than 1st immunized group. immunomodulator, it can recruit both arms of immunity (CMI and HI) through direct and indirect effect (Sevgi *et al*., 2013).

In cell-mediated immunity, cysteine has a significant impact on both the maintenance of lymphocytes and increases the amount of interferon, which is a powerful enhancer and is accountable for recruiting macrophages and other immune cells to move to the Ags inoculation place Often, the impact of cysteine is defined by its capacity to improve T career cellular activity and could increase the antibody response because it is a disulfide molecule. Similarly, Parodi *et al*. (2007), who tried to prey for disulfide substances that could be administered as an immunostimulant to the skin and discovered that cysteine could increase the antibody reaction in it in vivo and in vitro mice.

Pranav (2011) also show that sulfur-containing molecules have an efficient immunostimulant that is great for medicinal use Penny (2015) imply that cysteine have been shown to strengthen the antibody reaction in immunodeficient animals. In particular, it has significant indirect immunological effects. The capacity to biosynthesize other intracellular antioxidants such as glutathione, vitamin C and vitamin E is due to cysteine **(**Minich and Brown, 2019). This proof was consistent with Tremellen (2019), which stated that cysteine could boost immunity via its role in the manufacture of other antioxidants such as plasma vitamin C amounts, maximum glutathione concentrations and complete blood thiol concentrations.

All the above antioxidants, especially glutathione, play a key role in cell-mediated immunity by increasing levels of cytokines such as IFNΔ, 1L-10, 1L-6, which induce the immune response of Th1 and increase the levels of T-assistant lymphocyte. As well Ghezzi *et al*. (2019); stated that glutathione provides an major role in cell-mediated immunity Increased production of cytokines such as IFNΔ and 1L-10. Also, the 2nd group did show a marked increase in titer antibodies (768±147.8), that may be due to immunization with WSEFgs, but also receiving alpha lipoic acid as an immune amplifier. The 3rd cluster immunizing with WSEFAg and receiving lead (orally) experienced a significant decline in foot pad density and antibody titer concentrations less than any other immunized group this results agreed with Mohamed *et al*. (2019). The indication for these outcomes is that the immunized animals of this group got lead acetate, that also acts as immunosuppressive agents that prevent the skin test and the antibody titer quality of this group.

The processes through which lead inhibits the

immune response are by causing apoptosis of immune cells such as T cells, leading in a significant weakening of the immune system. This proof was consistent with Kelainy *et al*. (2019), who proposed which apoptosis could be an significant mechanism for lead-induced immune suppression Many mechanisms suggest the suppressive function of lead in immunity, which may decrease the amount of secreted cytokines by T cells that play an significant part in cell-mediated reaction and humoral reaction, such as TNF-alpha, IFNΔ, 1L-2, 1L-10, 1L-5 and 1L-4, proof agreed with Emilia *et al*. (2018), which proved that lead is exposed to people may cause immune suppression through Significant decreases in T cell proliferation owing to decreased levels of naturally produced T-cell cytokines (TNF-alpha, IFNΔ, 1L-2, 1L-10, 1L-5 and 1L-4). As well as Liao *et al.* (2015) have shown that both dermal and systemic immune dysfunction has been shown to be influenced by the heavy metals, *viz*., As, Cd, Pb and Hg are most toxic to all human beings, animals, fishes and environment. The excess levels of heavy metals cause severe toxicity. Though some heavy metals are essential for animals, plants and several other organisms, all heavy metals exhibit their toxic effects via metabolic interference and mutagenesis. The Pb and Hg cause severe toxicity in Both cutaneous and systemic immune dysfunctions have been shown, likely due to decreased expression of CD4 + cells, to be significant factors for the identification of antigens on the surface of virus-infected cells, the information did agree with Jarad (2012), who discovered that Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead induced toxic effects interfered with T-cell activation, influencing the pathway of activation of the T-cell receptor. Lead acetate can suppress humoral immunity through inhibition of antibody-forming cell order to respond and cytotoxic result against T-cell precursors. This science was did agree with Dayong *et al*. (2019). It has been shown that B6C3F1 rodents given to a single dose of lead (75mg\kg) decreased the reaction of IgM and IgG antibody-forming cells, inhibited T-cell proliferation and macrophage functioning. Also, Emilia *et al*. (2018) reported immunological effects of lead compounds on mouse spleen cells *in vitro*.

The 4th group that was immunocompromised with WSEFAg and injected orally with cysteine also continued to receive lead (75mg\kg B.W orally) with a slightly better elevation than the 3rd immunized group in both foot pad and foot pad test results. Antibodies titer, but that the values of this group were less than 1st group and 2nd group. In addition to the immunization of mammals in this group with WSEFAg, the possible explanation for these results was that cysteine was expected to receive. Immunosuppressive adversely effect of lead, given the increase in both the foot pad and titer antibodies by the immune modulator influence of cysteine on the cell-mediated order to respond And the humoral response. A certain evidence was congruent with Stefanie *et al*. (2019), which had shown that cysteine plays an immune amplifier effect. It also did agree with Panel *et al.* (2019), which exposure to lead may cause systemic immunodepression in so many animal and human studies.

Results have shown that daily supplementation of cysteine at the same dose of 200mg\kg B.W plays an significant role in immunomodulation, especially for immunized pets, and in protecting the body. Actually effect of cysteine to improve the immunotoxicity of lead . The 5th group (control group) showed no obvious differences (0±0) in both the foot pad test and the antibody titer concentration.

All tissue pieces examined show interstitial thickening pneumonia, which is the main pathological change that occurs in the end of the experiment. Pneumonia in lead poisoning may be contributing to secondary infection due to aspiration of gastric material in addition to impairment of the body's defence system similar (Bassim and Al-Wan, 2012) reported that lead concentration toxicopathological in internal organs in mice.

While the lead and cysteine group lesions may cysteine role of the SH group in the L-cysteine matrix gives it the ability to act as a chelating agent this agreed with the Bjørklund *et al*. (2017) found insertion of metallothionein (MT) synthesis is an affective and protective response to toxic lead exposure. Also, cystein contains certain compounds such as sulfhydryl group and the hydroxyl groups in the side chains of other polar amino acids that play an important role in normalizing the oxygen utilization in the cells (Cassano and Trombino, 2019).

Hyperplasia of goblet cells by increasing the number of mucin are the significant histopathological changes in the lead-treated group and may be due to the inhalation hazard and corrosive effect of lead acetate; at extremely high doses, it could cause nausea, vomiting and diarrhea as accepted (AIC, 2007) to which the lead acetate is an irritant.

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