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Research Article

Ameliorative Potential of *Spirulina platensis* against Lead Acetate Induced Immuno-Suppression and Kidney Apoptosis in Rats

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Abstract

Background: Environmental toxicants have been found to induce multiple adverse effects on human and animal's body organs including, kidney and immune system. Presently, this experiment was designed to assess the nephro-protective and immune-stimulant effects of *spirulina platensis* microalgae against lead acetate induced attenuated body immune response and oxidative renal damages.

Methods: 40 male albino rats were randomly divided into equal 4 groups. In comparison with control (C) group. Animals were received *spirulina platenesis* (300 mg/kg b. wt, orally) and/or lead acetate (50 mg/kg b.wt, IP) for 4 weeks. Samples were collected at the end of the experimental period. Protective effects of SP were checked by measuring selective hematological, immunological and biochemical tests, besides histopathological and immunohistochemical investigations.

Results: Co-exposed SP/LD rats showed significant ($P < 0.05$) suppression in the levels of LD induced –elevated urea, creatinine, MDA, MCV, retics%, and caspaes-3 over-expression, in contrast, they evoked significant elevation in LD-induced depleting GSH, SOD, IgG, IgM, hypo-proteinemia, hypo-albuminemia, hypo-gamma-globulinemia, RBCs, Hb, PCV, MCHC, WBCs, Lymphocytes, and CD8 down-expression. Additionally, SP restored the renal histological structure near the normal.

Conclusion: SP protects from toxic immunological, hematological, and nephrotoxic impacts of LD through its powerful free radical-scavenging, antioxidant, and immunostimulant activities.

INTRODUCTION

Industrial -delivered products are very potent environmental pollutants that act as dangerous cancer-bearing substances [1]. Lead (Pb2+) is considered the most established and world-wide occupational, industrial and environmental contaminants that affect human and animal are health [2]. Lead exposure occurred from multiple sources such as soil, air, water and industrial pollutants. It has been utilized in drugs, paintings, pipes, and ammunition [3,4]. On a large scale, it found to induce behavioral, biochemical and physiological disturbances in the living organisms. Additionally, it induced hematological alterations and renal failure in laboratory animals [5] by generation of reactive oxygen species (ROS) and depletion of anti-oxidant enzymes activities.

Herbal medication assumes a vital part in pharmaceutical medicine for a long time. Recently, a wide range of people all over the world depends on herbal preparations and extractions to meet their wellbeing demands because their low cost and minimal side effects [6]. The *cyanobacterium spirulina* is a filamentous blue-green alga belonging to the *Oscillatoriaceae* family that is generally found in tropical and subtropical regions of warm alkaline water. *Spirulina* is characterized by high nutritional value as it contains high protein (60–70% by dry weight), plenty of vitamins, amino acids, gamma-linoleic acid, and minerals [7]. The consumption of *spirulina* as a diet supplement has health benefits in preventing or managing hypercholesterolemia, hyperglycerolemia [8], obesity, inflammation [9], cancer [10], and cardiovascular disease [11]. In addition, Spirulina has antidiabetic effect [12], radio protective activity [13] and a potential therapeutic option to protect the testicular tissue from oxidative damage caused by some heavy metals [14]. Despite the above pharmacological and therapeutic properties of SP, there is a diminishing of information about the role of SP against the harms of lead acetate-induced immuno suppression. Therefore, the current study has been undertaken to find out the possible nephron-protective and immune-stimulant potential of the SP against lead-induced immune system and kidney damage in experimental animal model.

MATERIAL AND METHODS

Tested substances and chemicals

SP is a bright, blue-green tablet with a characteristic odour produced by power nutritional, Jin Shun, Guangzhou, Trading Co., USA. It was purchased from Delta Trade Company, Alexandria. Lead acetate (LD) obtained from El-Nasr Pharmaceutical Chemical Company, Egypt. All chemicals, reagents, and stains were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louise, MO, and USA) and El-Gomhoria Company.

Animals and experimental design

Forty male albino rats weighing 150- 200 g were used in the current study. The animals were obtained from the Laboratory Animal's Farm, Faculty of Veterinary Medicine, Zagazig University. The animals were clinically healthy, kept under hygienic condition, housed in metal cages with hard wood shavings as bedding. They were maintained on balanced ration composed of barley, milk, green fodder. Water and feed were given *ad-libitum* throughout the experimental period, and were accommodated to the laboratory conditions for two weeks before being experimented.All animals were treated in accordance with the guidelines of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals and were confirmed by Ethics of Animal Use in Research Committee (EAURC), Cairo University. In a controlled experimental study, rats were divided randomly into four equal groups, each consisting of 10 animals.

The first group (C)

The rats in this group were received distilled water (0.5 ml / animal).

The second group (SP)

The rats in this group were orally administered with *Spirulina platenesis*, once daily for 30 days via gastric tube at a dose of 300 mg/kg body weight (dissolved in distilled water) [13].

The third group (LD)

The rats in this group were treated with lead acetate (50 mg/kg body weight; intraperitoneally) every other day for 4 weeks [15].

THE FOURTH GROUP (SP+LD)

The rats in this group were co-exposed to *Spirulina platenesis* and lead acetate at the same time with the same previously mentioned doses, duration, and routes.

Blood sampling and tissue preparation

At the end of the experiment, before sacrificing of animals, blood samples were collected from the retro-orbital venous plexus of rats into EDTA-tubes for hematological examination (Erythrogam, leukogram, and reticulocytes count) and another blood sample from each rat was taken on clean, dry, sterile and labeled centrifuge tubes to separate clean non-hemolysed strew yellow serum for biochemical analysis (selective kidney function tests, IgG, IgM, protein electrophoresis). Spleen and kidney were rapidly removed and then divided into 2 portions; the 1st portion kept on -20º C until used for measuring the oxidative stress biomarkers meanwhile, the 2nd portion was kept on 10%neutral formalin for HE-histopathological and immune-histochemical examination of CD8 and caspase-3.

Hematological picture

Erythrogram and leukogram were evaluated by using an automated hematology analyzer (Hospitex Hema Screen 18 analyzer, Italy). Reticulocytes (retics) were counted on blood smear stained with brilliant cresyl blue stain and expressed in percentage using the equation of reticulocytes counting per total number of RBCs x 100%.

Biochemical analysis

Selective kidney function tests such as serum urea and creatinine were measured using commercial kits of Diamond-Diagnostic, Egypt on semi-automated Photometer 5010 V5+ (RIELE GmbH & Co, Berlin, Germany) according to the method of Fawcett and Scott, (1960) [16] and Henry, (1974) [17], respectively.

Oxidative stress biomarkers assessment

Superoxide dismutase activity (SOD), reduced glutathione (GSH) activities and malondialdehyde (MDA) concentration were measured by using kits of Biodiagnostics-Egypt , according to methods described by [18-201], respectively.

Protein electrophoresis

Serum IgG and IgM were measured using a specific Rat IgG, IgM ELISA Kit (ab189578, ab157738) of abcam, Co., United Kingdom following the method of [21]. Electrophoretic pattern of serum protein was determined in the rats by means of cellulose acetate electrophoresis to show five protein bands, namely albumin, alpha-1, alpha-2, beta and gamma [22].

Histopathological investigation

Spleen and kidney specimens were quickly collected then immersed rapidly into 10% neutral buffered formalin for 7 days. The formalin-fixed samples were continuously transferred to freshly prepared fixative every day. Following fixation, the specimens were then preserved in 70% ethyl alcohol. The preserved samples were briefly dehyderated in a graded series of ethanol, cleared in 3 changes of xylene, then embedded in paraffin wax. Paraffin blocks were sectioned into 4-5 µm thick sections. The paraffin sections were then subjected to haematoxylin and eosin stain (H&E) according to Suvarna et al. (2013) [23] to be examined by light microscopy for histopathological changes.

Immuno-histochemical examination

Another group of embedded paraffin sections was also prepared for immunodetection of spleen CD8, B-lymphocytes marker, using primary monoclonal antibodies anti-CD8 (MCA1768, Serotec, Kidlingtone, UK) and kidney caspase-3 (AB-20074b, Sangon Biotech, China)-positive cells overnight at 4ºC by an avidin-biotin-peroxidase (ABC) method as previously mentioned by [24] following deparaffinization of sections , treatment with 3% H_2O_2 for 10 min to inactivate endogenous peroxidases and subsequent heating in 10-mM citrate buffer at 121ºC for 30 min to permit antigen retrieval, and then blockage in 5% normal goat serum (Life Technologies) for 20 min. After three extensive washes with PBS, sections were incubated for 20 min at 32 °C with goat anti-rabbit-IgG-biotin-conjugated secondary antibody (1:2,000; Cat. No. sc-2040; Santa Cruz Biotechnology, Inc.). After further incubation with horseradish peroxidase-labeled-streptavidin, Ab binding was visualized using diaminobenzidine, and the sections were counterstained with haematoxylin [25]. According to the diffuseness of the staining, sections were graded as 0= no staining; 1= staining ≤ 25%; 2= staining between 25% and 50%; 3= staining between <50%. According to staining intensity, sections were graded as follows: 0= no staining; 1= weak but detectable staining; 2= distinct; 3= intense staining. Immunohistochemical values were obtained by adding the diffuseness and intensity scores [26].

Statistical analysis

Data were expressed as mean ± SE. Statistical comparisons were performed by Student-*t* test to compare mean values between lead treated groups versus control and lead treated groups versus SP/lead-treated group, using the SPSS 16.0 computer program. A value of $p< 0.05$ was considered as statistically significant [27].

RESULTS

Changes in erythrogram

Regarding the erythrogram results as shown in Table (1) and in comparison with the negative control group (I), LD-intoxicated rats (gp. III) showed a significant *(p< 0.05)* decrease in the values of RBCs, Hb, PCV, and MCHC with a significant *(p< 0.05)* increase in MCV. However, SP/LD-treated group (IV) showed an elevation in the values of RBCs, Hb, PCV, and MCHC with a decrease in MCV compared to the LD-only treated group but not return towards the normal values of the negative control group.

Leukogram results

Concerning to the leukogram data present at Table (2),

Table 1: Changes in erythrogramat *spirulinaplatensis* (SP) and/or lead acetate (LD)-exposedanimals groups comparing with the control (C).

	Groups					
Parameters	**C**	**SP**	**LD**	**SP+LD**	**P -value**	**LSD**
RBCs (×10⁶/μl)	7.33[a]	7.40[a]	3.90[c]	5.80[b]	0	0.96
	± 0.24	± 0.15	±0.20	± 0.11	**	
Hb (g/dl)	14.16[a]	14.16[a]	7.40[c]	10.66[b]	0	1.91
	± 0.44	± 0.20	± 0.70	± 0.17	**	
PCV (%)	44.66[a]	45.00[a]	29.00[c]	36.00[b]	0	4.64
	±1.45	±1.52	±0.57	±2.08	**	
MCV(fl)	60.91[b]	60.79[b]	74.64[a]	61.97[a]	0.003	4.27
	±0.79	±1.30	±2.69	±2.37	**	
MCHC(%)	31.72[a]	31.52[a]	25.17[b]	29.76[a]	0.014	2.07
	±0.31	±0.66	±1.88	±1.18	*	
Retics(%)	2.81[c]	2.63[c]	10.71[a]	6.01[b]	0	2.25
	±0.20	±0.68	±0.93	±0.53	**	

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at $P \leq 0.05$.
Abbreviations: RBCs: Red Blood Cells; Hb: Hemoglobin; PCV: Packed Cell Volume; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; Retics: Reticulocytes; LSD: Least Significant Difference

Table 2: Effect of single or double exposure to spirulinaplatensis (SP) and lead acetate (LD) for 30 days on leukogramin rats compared to the control group (C).

	Groups					
Parameters	**C**	**SP**	**LD**	**SP+LD**	**P -value**	**LSD**
WBCs(×10³/μl)	10.83[a]	11.00[a]	6.00[b]	7.83[b]	0.001	1.5
	± 0.33	± 0.76	± 0.28	± 0.44	**	
Neutrophils(×10³/μl)	7.16[a]	7.33[a]	2.50[b]	3.90[ab]	0	1.47
	± 0.44	± 0.88	± 0.28	± 0.20	**	
Lymphocytes(×10³/μl)	2.66[a]	2.66[a]	2.50[a]	2.93[a]	0.597	0.23
	±0.33	±0.16	±0.01	±0.23	NS	
Eosinophils(×10³/μl)	0.60[a]	0.65[a]	0.68[a]	0.61[a]	0.688	0.035
	±6.05	±1.02	±0.05	±0.03	NS	
Monocytes (×10³/μl)	0.40[a]	0.35[a]	0.31[a]	0.38[a]	0.687	0.036
	±0.05	±0.05	±0.06	±0.04	NS	
Basophils(×10³/μl)	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0	0
	±0.0	±0.0	±0.0	±0.0	NS	

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at ($P \leq 0.05$).
Abbreviations: WBCs: White blood cells; LSD: Least Significant Difference; NS: Non-Significant

animals group (III) orally exposed to lead acetate showed a significant *(p<0.05)* leukopenia, and neutropenia comparing with the control group with non-significant changes in the counts of lymphocytes, monocytes, and eosinophils. Meanwhile, oral administration of *spirulina platensis* for 4 weeks with lead acetate resulted in marked elevation of total leukocytic counts and neutrophils compared to the positive control group (III) but not reach towards the values of control group.

Protein profile alterations

As shown in Table (3), intraperiotoneal injection of rats with lead acetate for four weeks with a concentration of 50 mg/kg b.wt caused a significant ($p< 0.05$) hypoproteinemmia, hypoalbuminemia, hypoglobulinemia with lowering of the serum levels of gamma-globulina, IgG, and IgM when compared with the control rats. *Spirulina platensis* showing a potential protective role against lead acetate induced protein-gram alterations by elevation of serum total proteins, albumin, total globulin, and gamma globulin, IgG, and IgM concentrations near to the control values. Alpha1, 2- and beta-globulin levels showed non-significant changes in rats of all experimental groups.

Oxidant, antioxidant status and kidney function

Statistically, as tabulated in Table (4), lead acetate (LD) administration in rats for 4 weeks able to induce oxidative nephrotoxic impacts which represented by elevated serum creatinine, urea, and MDA with lowering serum SOD, and GSH contents. Lead acetate intoxicated rats orally administered *spirulina platensis* showed partial restoration of the above mentioned parameters near to the control level.

Histopathological and immunohistochemical observations

Microscopical examination of H&E-stained kiney section of negative control rats of group (I) and spirulina-alone-treated rats (gp.II) showed normal renal tubules, glomeruli and bowman's capsule (Figure 1 A,B). Meanwhile, rats of group (III) IP injected with lead acetate showed necrotic glomeruli with presence of hyaline casts in the lumen of some renal tubules (Figure 1C). The combination (SP+LD) group (IV) showed cellular and hyaline casts and peritubular congestion (Figure 1D).

The spleen of the control group (I) and spirulina-alone-treated group (II) showed a normal splenic structure with normal white and red pulp integrity and absence of hemosiderosis (Figure 2 A,B). Lead acetate exposed group (III) showed severe lymphoid depletion and reduction of the lymphoid follicles size (Figure 2C). The co-exposed group (IV) treated with both *spirulina platensis* and lead acetate showed an increase in the size of lymphoid follicles in between control and lymphoid depletion (Figure 2D).

Immunohistochemical scoring of renal caspase-3 and splenic-CD8, as found in table (5) revealed weak caspase-3 (Figure 3 A,B) and strong CD8 (Figure 4A,B) expression for rats at the negative control and spirulina-alone-treated groups, strong caspase-3 (Figure 3C) and weak CD8 (Figure 4C) expression in rats exposed only to lead acetate, and moderate caspase-3 (Figure 3D) and moderate CD8 (Figure 4D) expression in the co-exposed group (SP+LD).

DISCUSSION

Lead (Pb^{2+}) is a widely distributed environmental pollutant, as it found in several industrial and non-industrial sources. The industrial forms are the accumulator battery industry, lead smelters, lead or silver ore mining and lead refining. Non-industrial sources are air-borne lead from leaded gasoline fumes and lead-based paints. Pb^{2+} is divalent cation with a propensity to settle in the proximal tubule of the nephron, leading to nephrotoxicity in human, animals [28], and poultry. Oxidative stress plays a significant role in lead-induced immunosuppression [29]. The current study was initiated to determine the involvement of oxidative stress in reduced immune function and induced nephrotoxicity in lead-intoxicated rats and also to evaluate the potential of *spirulina platensis* as exogenous supplementation in reducing blood and tissue Pb levels via chelation or by bolstering cellular antioxidant defenses.

Table 3: Selective renal function tests and oxidative stress biomarkers at different experimental groups 4 weeks post-treatment.

	Groups					
Parameters	**C**	**SP**	**LD**	**SP+LD**	**P -value**	**LSD**
Urea (mg/dl)	22.33[c]	24.00[c]	49.00[a]	33.66[b]	0	7.34
	± 1.45	± 2.51	± 2.08	± 2.72	**	
Creatinine (mg/dl)	0.94[b]	0.93[b]	3.16[a]	1.70[b]	0.004	0.67
	± 0.029	± 0.106	± 0.100	± 0.208	*	
MDA(nmol/ml)	3.08[c]	3.00[c]	13.00[a]	6.25[b]	0	2.49
	±0.36	±0.14	±1.01	±0.38	**	
GSH(mmol/ l)	27.91[a]	28.50[a]	11.91[c]	18.75[b]	0	4.86
	±1.50	±2.03	±2.23	±1.15	**	
SOD(U/ml)	11.33[a]	11.75[a]	4.25[c]	8.33[b]	0	2.1
	±0.46	±1.01	±0.38	±0.82	**	

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at ($P \leq 0.05$).
Abbreviations: MDA: Malondialdehyde; SOD: Superoxide Dismutase; GSHH Reduced Glutathione; C: Control; SP: Spirulinaplatensis; LD: Lead Acetate; Least Significant Difference

Table 4: Protein-gram of all experimental groups.

	Groups					
Param-eters	**C**	**SP**	**LD**	**SP+LD**	**P -value**	**LSD**
Total proteins (g/dl)	7.16[a]	7.16[a]	3.23[c]	4.83[b]	0	1.14
	± 0.44	± 0.16	± 0.14	± 0.44	**	
Albumin (g/dl)	4.61[a]	4.33[a]	1.90[b]	2.83[b]	0.003	0.83
	± 0.53	± 0.33	± 0.10	± 0.44	**	
Total globulins (g/dl)	2.55[a]	2.23[a]	1.33[c]	2.00[b]	0	0.39
	±0.10	±0.16	±0.16	±0.001	**	
α 1-globulin (g/dl)	0.28[a]	0.35[a]	0.33[a]	0.33[a]	0.949	0.08
	±0.11	±0.07	±0.04	±0.08	NS	
α 2-globulin (g/dl)	0.26[a]	0.36[a]	0.30[a]	0.33[a]	0.813	0.07
	±0.01	±0.14	±0.02	±0.04	NS	
β -globulin (g/dl)	0.50[a]	0.58[a]	0.51[a]	0.56[a]	0.831	0.008
	±0.05	±0.08	±0.06	±0.08	NS	
γ-globulin (g/dl)	1.50[a]	1.53[a]	0.38[c]	0.66[b]	0	0.4
	±0.12	±0.13	±0.04	±0.08	**	
Total IgG (mg/dl)	13.36[a]	13.00[a]	4.16[c]	8.33[b]	0	2.65
	±0.32	±1.15	±0.72	±1.20	**	
Total IgM (mg/dl)	4.26[a]	4.56[a]	1.02[c]	2.30[b]	0.007	1.08
	±0.53	±0.90	±0.19	±0.35	*	

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at P ≤ 0.05.
Abbreviations: LSD: Least significant difference; NS: Non-Significant; C: Control; SP: *Spirulinaplatensis*; LD: Lead Acetate

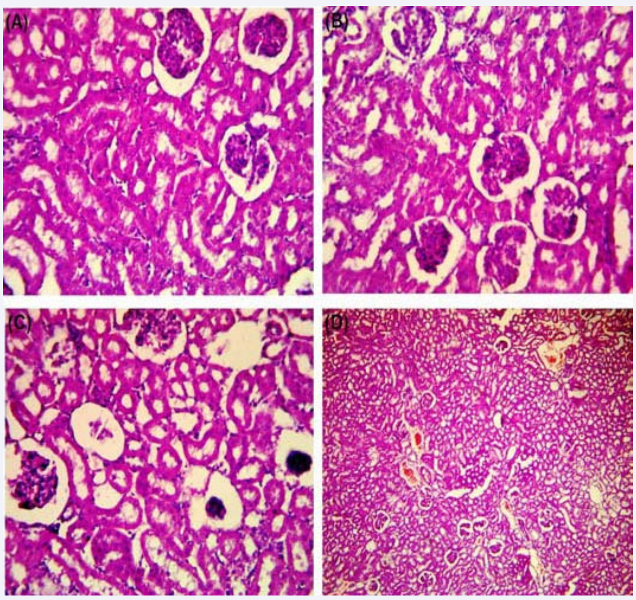

Figure 1 Photomicrograph of H&E-stained kidney section of negative control rats of group (I) and spirulina-alone-treated rats showing normal renal tubules, glomeruli and bowman's capsule (A, B) (400 X). Meanwhile, rats intoxicated with lead acetate showing necrotic glomeruli with presence of hyaline casts in the lumen of some renal tubules(C) (400 X).The combination group showing cellular and hyaline casts and peritubular congestion (D) (100 X).

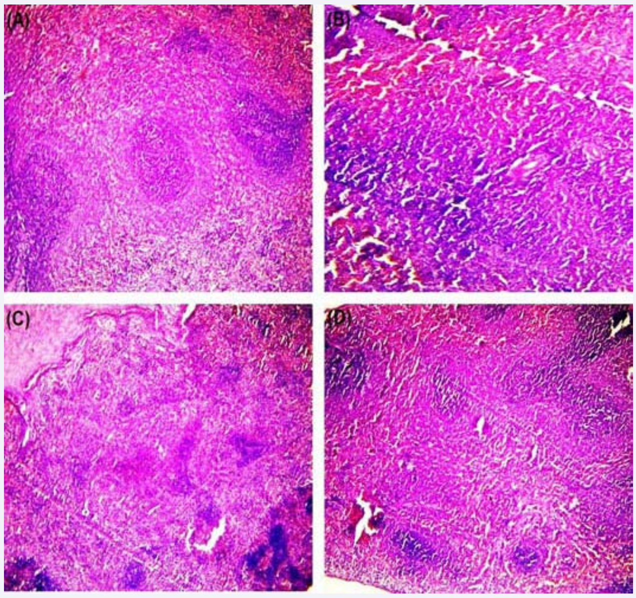

Figure 2 Photomicrograph of H&E-stained spleen section of control group (I) and spirulina-alone-treated group showing normal splenic structure with normal white and red pulp integrity and absence of hemosiderosis (A,100x B,400x). Lead acetate exposed group showing severe lymphoid depletion and reduction of the lymphoid follicles size (C, 400x). The co-exposed group treated with both *spirulina platensis* and lead acetate showing increase in the size of lymphoid follicles (D, 400X).

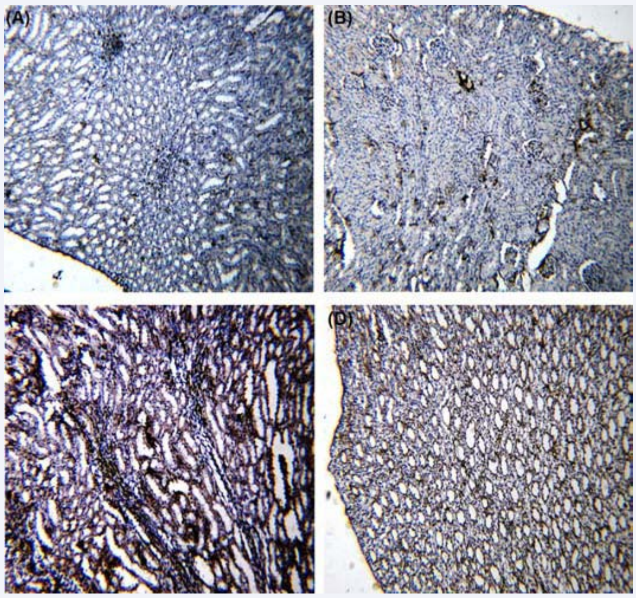

Figure 3 Immunohistochemical –stained kidney section showing weak caspase-3 (A, B) expression in rats of the negative control and spirulina-alone-treated groups, while, strong caspase-3 (C) expression in rats exposed only to lead acetate, and moderate caspase-3 (D) expression in the co-exposed group (SP+LD) (100X).

In the present work, significant reduction in serum IgM, IgG and splenic CD8 levels in lead-intoxicated rats (gp. III) discussed the ability of lead to alter B-and T-lymphocytes function *in vivo* which may contribute to its role in inhibiting

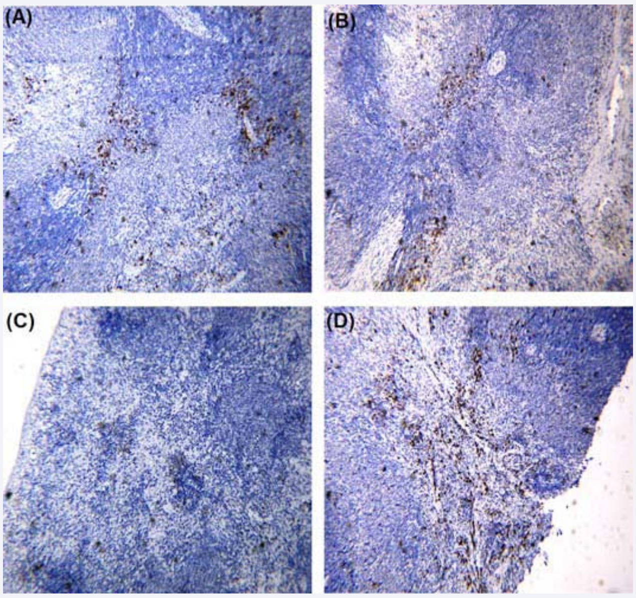

Figure 4 Immunohistochemical –stained spleen section showing strong CD8 (A, B) expression in rats of the negative control and spirulina-alone-treated groups, however weak CD8 (C) expression in rats exposed only to lead acetate, and moderate CD8 (D) expression in the co-exposed group (SP+LD) (100X).

the cellular thiol antioxidant capacity, based on glutathione content measurements [30,31] which confirmed in our results by declining serum GSH content and previously by [32] who reported decreased whole blood GSH content in Sprague–Dawley rats-intoxicated with lead. In addition, lead may cause oxidative stress by inhibiting d-aminolevulinic acid dehydratase (ALAD) leading to the buildup and autooxidation of d-aminolevulinic acid to form H_2O_2 [30,33], this is consistent with our present study and previous reports of increased serum MDA levels following lead exposure (34) Decreases in serum immunoglobulins levels following lead exposure have been previously reported in both animal and human studies of [35-38] and [39] as they reported suppressing in serum immunoglobulin (IgG and IgM) Levels in different animals models and non-immunized lead workers with chronic lead exposure. These results confirmed microscopically by severe lymphoid depletion and reduction of the lymphoid follicles size with few scattered CD8 ($CD8^+$ T-cells are a critical component of the cellular immune response) in lead intoxicated group(II),this agreed with the data previously seen by[40] and [41].

Lead is readily removed via proximal tubules of kidney, where it caused mitochondrial damages resulted in losing of its function, and subsequently failure of the tubular cells absorptive activities, causing renal failure [42]. Furthermore, long-period of Pb2+ exposure by various routes enhance the lipid peroxidation (LPO) in kidney and deplete glutathione (GSH). Also, it has been found to suppress the sulfhydryl-dependent enzymes, interfere with metals responsible for antioxidant enzymes activities, and/or increase the resistance of cells to oxidative radicals by impairing the cells integrity and fatty acid composition of renal cell membranes [43]. The bad effect of lead acetate on kidney, due to its oxidative generation activity, is a result of the ROS production, which induces cell injury and apoptosis [43]. These above mentioned facts about Pb-induced oxidative nephrotoxic impacts discussed our results belongs to the kidney function, oxidant, and antioxidant assay tests which showed significant increases in serum levels of MDA, urea, and creatinine and marked decreases in serum content of GSH and SOD enzyme in addition to, over expression of caspase-3 as apoptotic marker at rats group injected IP with lead acetate at a dose of 50 mg/dl daily for 30 days. Our results were in the same line with those obtained by [44-47].

Lead acetate exposure has reported to reduced natural antioxidant defense system present within the erythrocytes during laboratory investigation [48] and during occupational exposure. Lead is known to increase oxidative stress in different cell culture based study model as reported previously [29]. Since oxidative stress is a major pathway of lead acetate induced hemolysis in erythrocytes, antioxidant treatment must be effective in ameliorative the toxicological effects of lead. In the present work, lead acetate intoxicated group showed marked macrocytic hypochromic anemia with reticulocytosis . The term hemolysis refers to the destruction of the red blood cells (RBC) and reticulocytosis is an important responsive bone marrow and regenerating the anemia index for assessment and management of hemolytic anemia [49]. In line with this observation, several investigators have reported the efficacy of antioxidant treatment in ameliorating lead ($Pb2^+$) toxicity [50]. Furthermore, treatment with a metal chelator or a thiol antioxidant as *spirulina platensis* following lead exposure reduced oxidative stress in PMCs and kidney and normalized serum Ig and tissue CD8 levels, indicating a reversal in lead-induced effects on B, T lymphocytes function so, elevate the humeral and cellular immunity [51]. Additionally, it reduced ROS production [52] and subsequently decreased the cell apoptosis [53] and restoring the blood constituents and kidney function [54-56] towards the normal values and relief the histopatological alterations.

CONCLUSION

Oxidative stress plays a vital role in Pb-induced adverse effects on immune system, kidney and blood cellular constituents. Natural antioxidants such as spirulina have been found to be powerful in ameliorating Pb -induced toxicity in many previous scientific articles against many known environmental toxicants. In the current work, it is obvious that long standing Pb- exposure caused variable degrees of ROS generation, lipid per oxidation

Table 5: Renal caspase-3 and splenic-CD8scoring.

	C	SP	LD	SP+LD
Renal caspase-3	+ (25)	+ (25)	+++ (50-75)	++ (25-50)
Splenic-CD8	+++ (50-75)	+++ (50-75)	+ (25)	++ (25-50)

over-production, antioxidant enzymes activity inhibition resulted in immune-system suppression, hematological disorders besides histological alterations of renal tissue as a result of renal failure. SP co-exposure at the same time with Pb^{2+} provided near complete protection, same as in the negative control group.

REFERENCES

1. Babu BR, Parande AK, Raghu S, Kumar PT. Textile Processing and Effluent Treatment. J Cott Sc. 2007; 3: 143-153.
2. Soliman MM, Baiomy AA, Yassin MH. Molecular and histopathological study on the ameliorative effects of curcumin against lead acetate-induced hepatotoxicity and nephrototoxicity in Wistar rats. Biol Trace Elem Res. 2015; 167: 91-102.
3. Garza A, Vega R, Soto E. Cellular mechanisms of lead neurotoxicity. Med Sci Monit. 2006; 12: 57-65.
4. Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. Interdiscip Toxicol. 2012; 5: 47-58.
5. Abdel Moneim A E. Indigofera oblongifolia prevents lead acetate-induced hepatotoxicity, oxidative stress, fibrosis and apoptosis in rats. Plos One. 2016; 11.
6. Ogbera AO, Dada O, Adeyeye F, Jewo PI. Complementary and alternative medicine use in diabetes mellitus. West Afr J Med. 2010; 29: 158-162.
7. Hosseini SM, Khosravi-Darani K, Mozafari MR. Nutritional and medical applications of spirulina microalgae. Mini Rev Med Chem. 2013; 13: 1231-1237.
8. Deng R, Chow TJ. Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. Cardiovasc Ther. 2010; 28: 33-45.
9. Coskun ZK, Kerem M, Gurbuz N, Omeroglu S and Pasaoglu H. The study of biochemical and histopathological effects of Spirulina in rats with TNBS-induced colitis. Bratislavske Lekarske Listy. 2011; 112: 235-243.
10. Ismail MF, Ali DA, Fernando A, Abdraboh ME, Gaur RL, Ibrahim WM, et al. Chemoprevention of rat liver toxicity and carcinogenesis by Spirulina. Int J Biol Sci. 2009; 5: 377-387.
11. Khan M, Shobha JC, Mohan IK, Naidu MU, Sundaram C, Singh S, et al. Protective effect of Spirulina against doxorubicin-induced cardiotoxicity. Phytother Res. 2005; 19: 1030-1037.
12. Karkos PD, Leong SC, Karkos CD, Sivaji N, Assimakopoulos DA. Spirulina in clinical practice: evidence-based human applications. Evid Based Complement Alternat Med. 2011; 2011: 531053.
13. Wafaa A M, Shimaa A I,Yasmina MA. Spirulina platensis ameliorative effect against GSM 900-MHz cellular phone radiation-induced genotoxicity in male Sprague-Dawley rats. Comp Clin Pathol. 2014; 23: 1719-1726.
14. Samir AE B , Sally A E, Hossam E, Ibrahim M A. Antioxidant potential of Spirulina platensis mitigates oxidative stress and reprotoxicity induced by sodium arsenite in male rats. Oxid Med Cell Longev. 2016: 1-8.
15. Ahmed YF, Eldebaky HA, Karima GM, Nawito M. Effects of lead exposure on DNA damage and apoptosis in reproductive and vital organs in female rabbits. Glob Veter. 2012; 9: 401-408.
16. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. J Clin Pathol. 1960; 13: 156-159.
17. Henry RJ, Canon DC, Winkelman JW. Clinical chemistry principles and techniques. 2nd Edn. Lange Medical Publication. 1974.
18. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometry assay of superoxide dismutase. Indian J Biochem Biophys. 1984; 21: 130-132.
19. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61: 882-888.
20. Esterbauer H, Cheeseman KH, Dianzani MU, Poli G, Slater TF. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2+ in rat liver microsomes. Biochem J. 1982; 208: 129-140.
21. Whicher JT, Price CP, Spencer K. Immunonephelometric and immunoturbidimetric assays for proteins. Crit Rev Clin Lab Sci. 1983; 18: 213-260.
22. Bracht A, Silveira SS, Castro-Ghizoni CV, Sá-Nakanishi AB, Oliveira MR, Bersani-Amado CA, et al. Oxidative changes in the blood and serum albumin differentiate rats with monoarthritis and polyarthritis. Springerplus. 2016; 5: 36.
23. Suvarna S K, Layton C, Bancroft J D. Bancroft's Theory and Practice of Histological Techniques. Elsevier, Churchill Livingstone. 2013.
24. Abd-Elhakim YM, Mohamed AA, Mohamed WA. Hemato-immunologic impact of subchronic exposure to melamine and/or formaldehyde in mice. J Immunotoxicol. 2016; 13: 713-722.
25. Soliman M M , Mohamed A N , Tamer A I. Immunohistochemical and molecular study on the protective effect of curcumin against hepatic toxicity induced by paracetamol in Wistar rats. BMC Complement Altern Med. 2014; 14: 457.
26. Lin M, Guo L M, Liu H, Du J, Yang J, Zhang L J, Zhang B. Nuclear accumulation of glioma-associated oncogene 2 protein and enhanced expression of forkhead-box transcription factor M1 protein in human hepatocellular carcinoma. Histol Histopathol. 2010; 25: 1269-1275.
27. SPSS. Statistical package for social science, computer software. 2008.
28. Assi MA, Hezmee MN, Haron AW, Sabri MY, Rajion MA. The detrimental effects of lead on human and animal health. Vet World. 2016; 9: 660-671.
29. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem. 2001; 1: 529-539.
30. Gürer H, Ozgünes H, Neal R, Spitz DR, Erçal N. Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. Toxicology. 1998; 128: 181-189.
31. Ercal N, Treeratphan P, Hammond TC, Matthews RH, Grannemann NH, Spitz DR. *In vivo* indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with meso-2,3-dimercaptosuccinic acid or N-acetylcysteine. Free Radic Biol Med. 1996; 21: 157-161.
32. El-Sherbini E , Gehad E , Rehab E , Nervana G , Mohamed A ,Steve M H , Gamal I K . Ameliorative effects of l-carnitine on rats raised on a diet supplemented with lead acetate. Saudi J Biolog Sci. 2016.
33. Monteiro HP, Abdalla DSP, Faljoni-Alario A, Bechara EJH. Generation of active oxygen species during coupled autooxidation of oxyhemoglobin and delta-aminolevulinic acid. Biochem Biophys Acta. 1986; 881: 100-106.
34. Farooq H, Jamil Y, Ahmad MR, Khan MAA, Mahmood T, Mahmood Z, et al. Lead pollution measurement along national highway and motorway in Punjab, Pakistan. J Basic appl Sci. 2012; 8: 463-467.
35. Luster MI, Faith RE, Kimmel CA. Depression of humoral immunity in rats following chronic developmental lead exposure. J Environ Pathol Toxicol. 1978; 1: 397-402.
36. Lawrence DA. Heavy metal modulation of lymphocyte activities--II. Lead, an in vitro mediator of B-cell activation. Int J Immunopharmacol.

1981; 3: 153-161.

37. Undeger U1, Başaran N, Canpinar H, Kansu E. Immune alterations in lead-exposed workers. Toxicology. 1996; 109: 167-172.

38. Ewers U, Stiller-Winkler R, Idel H. Serum immunoglobulin, complement C3, and salivary IgA levels in lead workers. Environ Res. 1982; 29: 351-357.

39. Ercal N, Neal R, Treeratphan P, Lutz PM , Hammond TC , Dennery PA, et al. A role for oxidative stress in suppressing serum immunoglobulin levels in lead-exposed Fisher 344 rats. Arch Environ Contam Toxicol. 2000; 39: 251-256.

40. Al-Naimi RA, Abdul-Hadi D, Zahroon OS, Al-Taae E H. Toxicopathological study of lead acetate poisoning in growing rats and the protective effect of cysteine or calcium. Al-Anbar J Vet Sci. 2011; 4: 26-39.

41. Aldahmash BA, El-Nagar DM. Antioxidant effects of captopril against lead acetate-induced hepatic and splenic tissue toxicity in Swiss albino mice. Saudi J Biol Sci. 2016; 23: 667-673.

42. El-Khishin IA, El-fakharany YM, Abdel Hamid OI. Role of garlic extract and silymarin compared to dimercaptosuccinic acid (DMSA) in treatment of lead induced nephropathy in adult male albino rats. Toxicol Rep. 2015; 2: 824-832.

43. Abdel Moneim AE, Dkhil MA, Al-Quraishy S. The protective effect of flaxseed oil on lead acetate-induced renal toxicity in rats. J Hazard Mater. 2011; 194: 250-255.

44. Rong J, Chang W, Lv L, Chen J. Study on the roles of nuclear factor-kappaB, p53 and Bcl-2 gene in lead acetate induced apoptosis in PC12 cells. Wei Sheng Yan Jiu. 2008; 37: 262-268.

45. Agarwal S, Roy S, Ray A, Mazumder S, Bhattacharya S. Arsenic trioxide and lead acetate induce apoptosis in adult rat hepatic stem cells. Cell Biol Toxicol. 2009; 25: 403-413.

46. Yedjou CG, Milner JN, Howard CB, Tchounwou PB. Basic apoptotic mechanisms of lead toxicity in human leukemia (HL-60) cells. Int J Environ Res Public Health. 2010; 7: 2008-2017.

47. Dkhil MA, Al-Khalifa MS, Al-Quraishy S, Zrieq R, Abdel Moneim AE. Indigofera oblongifolia mitigates lead-acetate-induced kidney damage and apoptosis in a rat model. Drug Des Develop Ther. 2016; 10: 1847-1856.

48. Patra RC, Swarup D. Effect of lead on erythrocytic antioxidant defence, lipid peroxide level and thiol groups in calves. Res Vet Sci. 2000; 68: 71-74.

49. Barcellini W. New Insights in the Pathogenesis of Autoimmune Hemolytic Anemia. Transfus Med Hemother. 2015; 42: 287-293.

50. Aykin-Burns N, Franklin EA, Ercal N. Effects of N-acetylcysteine on lead-exposed PC-12 cells. Arch Environ Contam Toxicol. 2005; 49: 119-123.

51. Ragab AR, Mohamed AE, Basem YS, Hany NB. Antioxidant and tissue-protective studies on Ajwa extract: Dates from Al Madinah Al-Monwarah, Saudia Arabia. J Environ Anal Toxicol. 2013; 3: 1.

52. Finamore A, Maura P, Sarra B, Ilaria P. Antioxidant, immunomodulating, and microbial-modulating activities of the sustainable and ecofriendly Spirulina. Oxid Med Cellu Longev. 2017: 1-14.

53. El-Tantawy WH. Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. J Tradit Complement Med. 2015; 6: 327-331.

54. Abdel-Daim MM, Abuzead SM, Halawa SM. Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. PLoS One. 2013; 8: 72991.

55. Gürer H, Ozgünes H, Saygin E, Ercal N. Antioxidant effect of taurine against lead-induced oxidative stress. Arch Environ Contam Toxicol. 2001; 41: 397-402.

56. Wenn-Chyau L, Bruce R, Yee-Ling L, Mun-Yik F, Cindy C, Kanlaya S, et al. Giemsa-stained wet mount based method for reticulocyte quantification: A viable alternative in resource limited or Malaria endemic settings. PLOS ONE. 2013; 8: 3: e60303.

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