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 platensis* (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 platensis*, 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 platensis* 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 (Erythrogram, 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 straw 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 -20°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+ (RILEE 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 [18-20], 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 dehydrated 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, Kidlington, UK) and kidney caspase-3 (AB-20074b, Sangon Biotech, China)-positive cells overnight at 4°C by an avidin-biotin-peroxidase (ABC) method as previously mentioned by [24] following deparaffinization of sections.
treatment with 3% H2O2 for 10 min to inactivate endogenous peroxidases and subsequent heating in 10-mM citrate buffer at 121°C 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),

<p>| Table 1: Changes in erythrogram of <em>spirulina platensis</em> (SP) and/or lead acetate (LD)-exposed animals groups comparing with the control (C). |</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>SP</th>
<th>LD</th>
<th>SP+LD</th>
<th>P-value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (×10^6/µl)</td>
<td>7.33±</td>
<td>7.40±</td>
<td>3.90±</td>
<td>5.80±</td>
<td>0</td>
<td>0.96</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.16±</td>
<td>14.16±</td>
<td>7.40±</td>
<td>10.66±</td>
<td>0</td>
<td>1.91</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>44.66±</td>
<td>45.00±</td>
<td>29.00±</td>
<td>36.00±</td>
<td>0</td>
<td>4.64</td>
</tr>
<tr>
<td>MCV(fl)</td>
<td>6.01±</td>
<td>60.79±</td>
<td>74.64±</td>
<td>61.97±</td>
<td>0.003</td>
<td>4.27</td>
</tr>
<tr>
<td>MCHC(%)</td>
<td>31.72±</td>
<td>31.52±</td>
<td>25.17±</td>
<td>29.76±</td>
<td>0.014</td>
<td>2.07</td>
</tr>
<tr>
<td>Retics(%)</td>
<td>2.81±</td>
<td>2.63±</td>
<td>10.71±</td>
<td>6.01±</td>
<td>0</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at P ≤ 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 *spirulina platensis* (SP) and lead acetate (LD) for 30 days on leukogram in rats compared to the control group (C). |
| Parameters                  | C             | SP            | LD            | SP+LD         | P-value | LSD |
| WBCs (×10^3/µl)             | 10.83±        | 11.00±        | 6.00±         | 7.83±         | 0.001   | 1.5 |
| Neutrophils (×10^3/µl)      | 7.16±         | 7.33±         | 2.50±         | 3.90±         | 0       | 1.47|
| Lymphocytes (×10^3/µl)      | 2.66±         | 2.66±         | 2.50±         | 2.93±         | 0.597   | 0.23|
| Eosinophils (×10^3/µl)      | ±0.33±        | ±0.16±        | ±0.01±        | ±0.23±        | NS      | 0.035|
| Monocytes (×10^3/µl)        | ±0.05±        | ±0.05±        | ±0.06±        | ±0.04±        | NS      | 0.036|
| Basophils (×10^3/µl)        | ±0.00±        | ±0.00±        | ±0.00±        | ±0.00±        | NS      | 0   |

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at (P ≤ 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), intraperitoneal injection of rats with lead acetate for four weeks with a concentration of 50 mg/kg b.wt caused a significant (p < 0.05) hypoproteinemia, 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 kidney 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 1A,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).

**DISCUSSION**

Lead (Pb2+) 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. Pb2+ is divalent cation with a propensity to settle 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. Pb2+ is divalent cation with a propensity to settle.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>SP</th>
<th>LD</th>
<th>SP+LD</th>
<th>P-value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>22.33a</td>
<td>24.00c</td>
<td>49.00a</td>
<td>33.66b</td>
<td>0</td>
<td>7.34</td>
</tr>
<tr>
<td>± 1.45</td>
<td>± 2.51</td>
<td>± 2.08</td>
<td>± 2.72</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.94a</td>
<td>0.93b</td>
<td>3.16c</td>
<td>1.70b</td>
<td>0.04</td>
<td>0.67</td>
</tr>
<tr>
<td>± 0.029</td>
<td>± 0.106</td>
<td>± 0.100</td>
<td>± 0.208</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA(nmol/ml)</td>
<td>3.08c</td>
<td>3.00c</td>
<td>13.00c</td>
<td>6.25c</td>
<td>0</td>
<td>2.49</td>
</tr>
<tr>
<td>± 0.36</td>
<td>± 0.14</td>
<td>± 1.01</td>
<td>± 0.38</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH(mmol/L)</td>
<td>27.91a</td>
<td>28.50c</td>
<td>11.91a</td>
<td>18.75b</td>
<td>0</td>
<td>4.86</td>
</tr>
<tr>
<td>± 1.50</td>
<td>± 2.03</td>
<td>± 2.23</td>
<td>± 1.15</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD(U/ml)</td>
<td>11.33c</td>
<td>11.75a</td>
<td>4.25c</td>
<td>8.33c</td>
<td>0</td>
<td>2.1</td>
</tr>
<tr>
<td>± 0.46</td>
<td>± 1.01</td>
<td>± 0.38</td>
<td>± 0.82</td>
<td>**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at (P ≤ 0.05).

**Abbreviations:** MDA: Malondialdehyde; SOD: Superoxide Dismutase; GSHH Reduced Glutathione; C: Control; SP: Spirulina platensis; LD: Lead Acetate; Least Significant Difference
Table 4: Protein-gram of all experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>LD</th>
<th>SP+LD</th>
<th>P-value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proteins (g/dl)</td>
<td>7.16±</td>
<td>7.16±</td>
<td>3.23±</td>
<td>4.83±</td>
<td>0</td>
<td>1.14</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.61±</td>
<td>4.33±</td>
<td>1.90±</td>
<td>2.83±</td>
<td>0.003</td>
<td>0.83</td>
</tr>
<tr>
<td>Total globulins (g/dl)</td>
<td>±0.10±</td>
<td>±0.16±</td>
<td>±0.10±</td>
<td>±0.14±</td>
<td>±0.14±</td>
<td>0.39</td>
</tr>
<tr>
<td>α-1-globulin (g/dl)</td>
<td>0.28±</td>
<td>0.35±</td>
<td>0.33±</td>
<td>0.33±</td>
<td>0.949</td>
<td>0.08</td>
</tr>
<tr>
<td>α-2-globulin (g/dl)</td>
<td>±0.01±</td>
<td>±0.14±</td>
<td>±0.02±</td>
<td>±0.16±</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>β-globulin (g/dl)</td>
<td>0.50±</td>
<td>0.58±</td>
<td>0.51±</td>
<td>0.56±</td>
<td>0.831</td>
<td>0.008</td>
</tr>
<tr>
<td>γ-globulin (g/dl)</td>
<td>±0.05±</td>
<td>±0.08±</td>
<td>±0.06±</td>
<td>±0.08±</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Total IgG (mg/dl)</td>
<td>13.36±</td>
<td>13.00±</td>
<td>4.16±</td>
<td>8.33±</td>
<td>0</td>
<td>2.65</td>
</tr>
<tr>
<td>Total IgM (mg/dl)</td>
<td>±0.53±</td>
<td>±0.90±</td>
<td>±0.19±</td>
<td>±0.35±</td>
<td>0</td>
<td>1.08</td>
</tr>
</tbody>
</table>

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

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...
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 H₂O₂ [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 animal and human studies of [35-38] and [39] as they reported 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 histopathological 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 (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 decreases 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].

**Table 5: Renal caspase-3 and splenic-CD8 scoring.**

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>SP</th>
<th>LD</th>
<th>SP+LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal caspase-3</td>
<td>+ (25)</td>
<td>+ (25)</td>
<td>+++ (50-75)</td>
<td>++ (25-50)</td>
</tr>
<tr>
<td>Splenic-CD8</td>
<td>+++ (50-75)</td>
<td>+++ (50-75)</td>
<td>+ (25)</td>
<td>++ (25-50)</td>
</tr>
</tbody>
</table>
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 Pb2+ provided near complete protection, same as in the negative control group.

REFERENCES


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