Possible role of *Arthrospira platensis* in reversing oxidative stress-mediated liver damage in rats exposed to lead

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Environmental pollutants, particularly metallic elements, mobilized and released into the environment, eventually accumulate in the food chain and thus pose a serious threat to human and animal health. In the present study, the role of *Arthrospira* (Spirulina platensis; SP) as a protector against oxidative stress-mediated liver damage induced by an exposure to lead acetate (LA; as a metallic pollutant) was assessed. To achieve this aim, rats were orally administered with 300 mg/kg bw SP for 15 days, before and concurrently with an intraperitoneal injection of 50 mg/kg bw LA (6 injections throughout 15 days). As a result, co-administration of SP with LA reduced the amount of lead that accumulated in both blood and liver tissue of the exposed rats and minimized the increased levels of lipid peroxidation, protein oxidation, DNA oxidative damage, and liver enzyme endpoints. In addition, because of SP administration, the levels of depleted biomarkers of antioxidant status and total antioxidant capacity in LA-exposed rats improved. Moreover, SP protected the liver tissue against the changes caused by LA exposure and also decreased the reactivity of HSP70 in the cytoplasm of hepatocytes. Collectively, our data suggest that SP has a potential use as a food supplement in the regions highly polluted with heavy metals such as lead.

1. Introduction

*Arthrospira* is a genus of microscopic, multicellular, filamentous blue-green algae (cyanobacteria) and is one of the most primitive forms of photosynthetic prokaryotes. It is also known as a super health food [1], gaining the consideration of medical scientists because of the capacity in securing the body physiological system against oxidative damage as well as nutraceutical and source of potential pharmaceuticals. It acts as a food supplement in human and as many animal species [2], as it’s rich in proteins, lipids, carbohydrate and elements [3]. It is likewise a source of bioactive compounds such as ß-carotene, phycocyanin, and allophycocyanin with anti-inflammatory and antioxidant properties [4]. Spirulina is generally thought to be safe for consumption owing to its long history of usage as food source and its favorable safety profile in human and animal studies. Regardless, rare cases of side-effects in human have been reported, including hepatotoxicity, rhabdomyolysis and development of a mixed immunoblistering disorder were possibly associated with Spirulina consumption [5–7]. It had no detectable unfavorable consequences on reproductive performance, embryo and fetus development and growth at the doses much higher than any expected human consumption [8]. In addition, it did not alter behavior, food and water intake, growth, health status and measurements of clinical chemistry when consumed either in relatively long-term feeding or at high dose [9,10]. The most intensively explored species of are *Arthrospira platensis* (Spirulina platensis), *Arthrospira maxima* (Spirulina maxima) and *Arthrospira fusiformis* (Spirulina fusiformis) [11].

The studies conducted in the past using *Spirulina platensis* (SP) as a supplement have proved that it has an ability to encounter toxicity to organs caused by medications and chemicals, particularly those containing heavy metals [12,13]. SP reversed the deltamethrin-induced toxicity by its potent free radical scavenging and antioxidant activities [14] and modulated the immune response altered by the herbicide atrazine in the common carp [15]. Moreover, an SP polysaccharide activated the hematopoietic system in rat [16]. Additionally, it provided a protective effect against GSM 900-MHz cell phone radiation in...
Moreover, SP could help manage numerous medical conditions including diabetes and its related complications by reducing blood glucose level and diminishing insulin resistance [18].

Lead is a persistent environmental contaminant that can be recognized in the environment and biological systems as it is widely used in industry on account of its malleability, resistance to corrosion, and low melting point [19]. Despite lead being one of the most useful metals, it is in like manner one of the most toxic metals [20]. Both environmental and occupational exposures remain a significant and serious health problem in many developing and industrializing countries [21]. The major sources of lead are industries where lead and lead-based components are used, such as lead acid battery manufacturing, cable and wire products industries, rubber and plastic industries, and soldering activities [22].

Lead can enter the body by inhaling and ingesting contaminated soil and water and consuming processed, preserved, and canned food. After absorption, lead accumulates in various body organs and influences many biological activities at the molecular, cellular, and intracellular levels, which may result in morphological alterations that can remain even after the levels of lead have decreased in the body [23]. In humans, among the soft tissues, the liver is the largest depot (33%) of lead, followed by the cortex and medulla of the kidney [24]. Earlier studies with both animals and humans have revealed that lead toxicity can cause hematological alterations and kidney damage [25], cardiovascular damage [26], immunological response disturbances [27], reproductive disorders [28], and nerve dysfunction [29].

The present study was conducted to examine the role of SP in protecting against hepatic impairment elicited by the exposure to a metallic pollutant, such as lead, in rats through biochemical evaluations and immunohistochemical analysis.

### 2. Material and methods

#### 2.1. Tested compounds and chemicals

Lead acetate (LA; 99.6% purity) was purchased from El-Nasr Pharmaceutical Chemical Co. (Qaliubiya, Egypt). SP is a bright, blue-green powder and it was purchased from EL-Hellowa for Biological Products, Egypt. For experimental use, a working stock solution of tested compounds was prepared by diluting them in distilled water. All other chemicals used in the study were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

#### 2.2. Animals and experimental design

Forty male Sprague-Dawley rats (each weighing 150–200 g),
Laboratory Animals in the National Institutes of Health (NIH) for the Care and Use of Animals procedures were conducted following the general guidelines and investigations and approved by the Ethics of Animal Use in Research Committee (Zagazig University, Egypt).

The animals were randomly divided into four groups of ten each. The control group consisted of rats that were intraperitoneally (IP) injected with distilled water as a vehicle. SP-treated animals orally received SP at a dose of 50 mg/kg bw for 30 days through a gastric tube [12]. LA-treated animals were injected IP with LA at a dose of 50 mg/kg bw (three times a week) for two weeks [30]. The animals in the SP/LA co-treated protective group were orally administered with SP for 15 days before and 15 days concomitant with an IP injection of LA at a dose of 50 mg/kg bw (three times a week) [30]. The animals in all groups for estimating body weight changes.

At the time of sacrifice, blood samples were collected from the median canthus (orbital vessels) of experimental rats without anticoagulant, centrifuged at 3000 rpm for 15 min for separating serum, and stored at −20 °C for biochemical analysis of liver function markers. Another whole blood sample was collected in heparin-containing tubes for evaluating the amount of lead. Liver tissue specimens were obtained, dissected, rinsed with sterile physiological saline (0.9% NaCl), weighed, and then divided into four parts. The first part was purchased from the farm of laboratory animals (Faculty of Veterinary Medicine, Zagazig University, Egypt), were used in the current study. The animals were housed in stainless steel cages with a photoperiod of a 12-h light/12-h dark cycle and relative humidity of 50% at 22 – 28 °C. The animals had free access to water and feed throughout the experimental period, and they were accommodated to the laboratory conditions for two weeks before being used in the experiment. The experimental procedures were conducted following the general guidelines of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals in scientific investigations and approved by the Ethics of Animal Use in Research Committee (Zagazig University, Egypt).

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2.3. Blood and hepatic tissue collection

At the time of sacrifice, blood samples were collected from the median canthus (orbital vessels) of experimental rats without anticoagulant, centrifuged at 3000 rpm for 15 min for separating serum, and stored at −20 °C for biochemical analysis of liver function markers. Another whole blood sample was collected in heparin-containing tubes for evaluating the amount of lead. Liver tissue specimens were obtained, dissected, rinsed with sterile physiological saline (0.9% NaCl), weighed, and then divided into four parts. The first part was homogenized and the homogenates were centrifuged at 3000 rpm at 4°C for 15 min for obtaining the supernatants, which were used for determining antioxidant and oxidative stress indices. The second part was used for determining the level of lead. The third part was used to prepare the cells for DNA damage investigation (comet assay), by setting in 1 mL of cold Hank’s balanced salt solution containing 20 mM ethylenediaminetetraacetic acid and 10% dimethyl sulfoxide and was minced into fine pieces to acquire cell suspensions. The fourth part was fixed in 10% neutral buffered formalin for histopathological and immunohistochemical analysis.

2.4. Assessment of lead level in blood and hepatic tissue

For lead level assays, whole blood (0.2 mL) and tissue samples (100 mg) were digested with 4 mL of nitric/perchloric acid mixture for 24 h at room temperature. Then, the samples were heated at 80 °C for 2 h in a water bath. They were then cooled to room temperature, filtered, and diluted with deionized water before analysis [31]. The amount of lead was quantified using Buck scientific model 210VG flame atomic absorption spectrophotometry; the suitable wavelength for the lead was 220.35 nm.

2.5. Evaluation of antioxidant biomarkers and total antioxidant capacity in hepatic tissue

The activities of superoxide dismutase (SOD) and catalase (CAT) and the level of reduced glutathione (GSH) were estimated according to methods described previously [32–34]. Total antioxidant capacity (TAC) was determined using kit reagents from Biodiagnostic Co., Egypt (Cat. No. TA 25 13) following the method of Koracevic et al. [35].

2.6. Evaluation of oxidative stress biomarkers (lipid peroxidation, protein oxidation, and DNA damage) in hepatic tissue

Lipid peroxides (malondialdehyde; MDA) were measured by a colorimetric assay as described previously by Ohkawa et al. [36]. Protein carbonyl (PC), as a marker of protein oxidative damage, was estimated using a colorimetric assay kit (Cayman’s Chemical Company; Ann Arbor, MI, USA) according to Levine et al. [37]. The comet assay was performed according to Singh et al. [38]. A total of 50 randomly selected cells per slide were investigated. Imaging was performed using a fluorescence microscope (Zeiss Axiovert L410 Inc., Jena, Germany) equipped with a CCD camera (Olympus Inc., Tokyo, Japan). The comets were analyzed by a visual scoring method and computer image analysis using the Comet Assay Project Software.

2.7. Serum biochemical analysis

Commercial kits for the quantitative determination of total protein, albumin, total and direct bilirubin, and the levels of liver enzymes, serum glutamic-oxaloacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) were used (kits supplied by Diamond Diagnostics, Egypt) according to Reitman and Frankel [39]. Alkaline phosphatase (ALP) level was measured using kits from Spectrum Diagnostics, Egypt according to Bel and Goldberg [40]. Lactate dehydrogenase (LDH) level was measured according to Burtis et al. [41]. For this, a diagnostic kit was procured from Spinreact Co., Santa Coloma, Spain. The quantitative detection of these indices was done following manufacturers’ instructions.

Serum total protein and albumin measurements were conducted by biuret method and bromocresol purple method, respectively [42, 43]. Serum total and direct bilirubin were measured according to Kaplan et al. [44].
2.8. Histopathological study

2.8.1. Light microscopy

Fixed liver tissue specimens were processed for histopathological investigation using a light microscope (Olympus BX51 microscope, Olympus Inc., Tokyo, Japan) according to Bancroft and Gamble [45]. This process involved sectioning the tissues, staining with hematoxylin/eosin (H&E) dye, and viewing under the light microscope.

2.8.2. Immunohistochemical analysis

Liver tissue sections were deparaffinized and treated with 3% hydrogen peroxide for 10 min to inactivate peroxidases. Then, the sections were heated in 10 mM citrate buffer at 121 °C for 30 min for antigen retrieval, blocked in 5% normal serum for 20 min, and incubated with a rabbit polyclonal anti-heat shock protein 70 (HSP70) antibody (1:100) in phosphatebuffered saline (PBS) overnight at 4 °C. After three extensive washes with PBS, the sections were incubated with a goat anti-rabbit IgG biotinconjugated secondary antibody (1:2000) for 20 min at 32 °C. After further incubation with horseradish peroxidase-labeled streptavidin, antibody binding was visualized using 3,3′-diaminobenzidine, and the sections were counterstained with hematoxylin.

2.9. Data analysis

Data were expressed as mean ± SE. Statistical comparisons were performed by one way-ANOVA using the SPSS 16.0 computer program.
Table 2
Liver tissue injury biomarkers in the serum of rats in response to LA exposure (50 mg/kg b.w, IP) and/or SP administration (300 mg/kg b.w via gastric tube).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Spirulina</th>
<th>Lead acetate</th>
<th>Spirulina/Lead acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT (U/l)</td>
<td>28.00 ± 1.53²</td>
<td>27.66 ± 1.76³</td>
<td>113.00 ± 9.29⁴</td>
<td>86.00 ± 6.08⁵</td>
</tr>
<tr>
<td>SGOT (U/l)</td>
<td>78.80 ± 3.32³</td>
<td>84.00 ± 6.11¹</td>
<td>136.66 ± 15.05¹</td>
<td>84.31 ± 7.37¹</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>126.00 ± 5.56²</td>
<td>114.33 ± 6.33¹</td>
<td>182.17 ± 8.55¹</td>
<td>150.67 ± 11.05¹</td>
</tr>
<tr>
<td>LDH (IU/l)</td>
<td>837.67 ± 27.28³</td>
<td>849.00 ± 30.51⁵</td>
<td>2784.7 ± 41.49⁶</td>
<td>1625.7 ± 625.2⁷</td>
</tr>
<tr>
<td>Total protein (gm/dl)</td>
<td>6.03 ± 0.17²</td>
<td>5.85 ± 0.19²</td>
<td>3.74 ± 0.47⁵</td>
<td>5.39 ± 0.47⁵</td>
</tr>
<tr>
<td>Globulin (G)</td>
<td>2.11 ± 0.16</td>
<td>2.36 ± 0.32</td>
<td>1.44 ± 0.37</td>
<td>2.25 ± 0.40</td>
</tr>
<tr>
<td>Albumin (A)</td>
<td>3.88 ± 0.22²</td>
<td>3.60 ± 0.20³</td>
<td>2.28 ± 0.25⁵</td>
<td>3.14 ± 0.08³</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.88 ± 0.26</td>
<td>1.58 ± 0.24</td>
<td>1.82 ± 0.53</td>
<td>1.49 ± 0.28</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.41 ± 0.11²</td>
<td>1.34 ± 0.05⁶</td>
<td>2.49 ± 0.42³</td>
<td>1.80 ± 0.19⁹</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>0.72 ± 0.06</td>
<td>0.68 ± 0.03</td>
<td>1.37 ± 0.32</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>Indirect bilirubin</td>
<td>0.71 ± 0.12</td>
<td>0.66 ± 0.07</td>
<td>1.12 ± 0.09</td>
<td>0.90 ± 0.18</td>
</tr>
</tbody>
</table>

Values are mean ± E for six samples/group. Means within the same row (in each parameter) carrying different superscripts (a,b,c) are significantly different at p < 0.05.

Table 3
Pearson's correlation coefficient (r) between liver Lead level, antioxidant biomarkers and oxidative stress indices in rats in response to LA exposure (50 mg/kg b.w, IP) and/or SP administration (300 mg/kg b.w via gastric tube).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SOD</th>
<th>GSH</th>
<th>CAT</th>
<th>TAC</th>
<th>Comet%</th>
<th>PC</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver lead level</td>
<td>1</td>
<td>−0.875**</td>
<td>−0.880**</td>
<td>−0.834**</td>
<td>−0.892**</td>
<td>0.927**</td>
<td>0.726**</td>
</tr>
<tr>
<td>SOD</td>
<td>1</td>
<td>0.902**</td>
<td>0.761**</td>
<td>0.916**</td>
<td>−0.889**</td>
<td>−0.797**</td>
<td>−0.898**</td>
</tr>
<tr>
<td>GSH</td>
<td>1</td>
<td>0.793**</td>
<td>0.989**</td>
<td>−0.954**</td>
<td>−0.885**</td>
<td>−0.947**</td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>1</td>
<td>0.812**</td>
<td>0.806**</td>
<td>−0.716**</td>
<td>−0.875**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>1</td>
<td>−0.976**</td>
<td>−0.886**</td>
<td>−0.962**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comet %</td>
<td>1</td>
<td>0.845**</td>
<td>0.973**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>1</td>
<td>0.788**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level.

Tukey’s multiple comparison post-hoc test was performed to compare mean values between treated groups and corresponding control. A value of p < 0.05 was considered as statistically significant. Proc COR was used to estimate Pearson’s correlation coefficient to find out the correlation between hepatic tissue Lead level versus antioxidant and oxidative stress biomarkers level. Differences were considered significant at p < 0.01 and p < 0.05 for two-tailed tests.

3. Results

3.1. General observation, body weight changes, and liver/body weight ratio of rats in response to LA exposure and/or SP administration

During the experiments, no deaths were recorded in all groups. However, anorexia and low activity were recorded mainly in the LA-exposed group. At necropsy, white spots were observed in the liver of rats exposed to LA, which were more noticeable in the LA-exposed rats than in the SP/LA co-treated rats. The LA-exposed rats showed a significant decrease of body weight changes relative to the control rats. SP administration exhibited an improvement through lowering the decline in body weight gain in the SP/LA co-treated rats. In addition, a significant increase in the liver/body weight ratio was observed in the LA-exposed group (140.2%), which was significantly modulated by SP supplementation in the co-treated group (121.2%) compared with the control group (Table 1).

3.2. Lead level in blood and hepatic tissue of rats in response to LA exposure and/or SP administration

The levels of lead in blood and hepatic tissue samples from control and treated rats are presented in Table 1. The highest levels of lead were recorded in blood and hepatic tissue samples of the LA-exposed rats (67.5% and 4516.6%, respectively). In the SP/LA co-treated rats, SP supplementation diminished the elevated level of lead in blood (187.5%), but the level still showed a significant increase in hepatic tissue (2666.6%) when compared with the control and SP-administered groups, which recorded the lowest levels of lead.

3.3. Antioxidant biomarkers and TAC in hepatic tissue of rats in response to LA exposure and/or SP administration

Enzymatic activities of SOD and CAT, as well as GSH level in liver tissue homogenates, significantly decreased in the LA-exposed rats (58%, 20%, and 58%, respectively) compared with the control rats. The co-treatment of SP with LA significantly improved both SOD activity and GSH level in co-treated rats but not as much as that in the control rats. While, CAT activity in SP/LA co-treated rats was not significantly different from that in the control rats. A significant depletion of TAC was evident in the LA-exposed rats by 35.4% relative to the control rats. Although SP administration significantly improved TAC in the SP/LA co-treated rats (68%), it did not help to attain TAC value in the control rats (Fig. 1).

3.4. Levels of MDA, PC, and DNA damage in hepatic tissue of rats in response to LA exposure and/or SP administration

Both MDA and PC levels were significantly elevated in hepatic tissue of the LA-exposed rats by 377.6% and 216.7%, respectively, compared with the control rats. The level of MDA was significantly decreased to 256% in response to administration of SP in the SP/LA co-treated rats, but it did not attain the value in the control rats. The formation of PC in the SP/LA co-treated rats was not significantly different from that in control rats, indicating that SP administration prevented carbonyl formation in the SP/LA co-treated group (Fig. 2).

The results of DNA damage are shown in Fig. 3, which show a significant elevation in the comet percentage, tail length, tail DNA percentage, and tail moment by 281.3%, 129.2%, 146.4%, and 195%, respectively, in hepatic tissue of the LA-exposed rats compared with the control group.
Fig. 4. Light microscopy of liver tissue from rat of (A, B): control group, SP-administered group showing normal histological structure (Bar 100 μm). (C): LA-exposed group, showing many apoptotic hepatocytes, severe hydropic degeneration, individual coagulative necrosis and focal replacement of necrotic hepatic cells by mononuclear cells (Bar = 100 μm). (D): LA-exposed group showing portal congestion, proliferation of the bile duct with newly formed bile ductules and slight fibrosis (Bar = 100 μm). (E): SP/LA co treated group, showing mild hepatic lesions including slight perivascular mononuclear cell aggregation, few apoptotic hepatocytes (Bar = 40 μm). (F): SP/LA co treated group, showing areas of telangiectasia (Bar = 100 μm).

Fig. 5. Photomicrograph of liver tissue (Anti HSP-70 Immunohistochemistry) showing, (A, B): negative reactivity in the cytoplasm of the hepatocytes in both control and SP-administered group. (C): LA-exposed group, many hepatocytes showed positive reactivity in their cytoplasm that either observed in groups of hepatocytes or in scattered cells. (D): SP/LA co treated group, only a few, scattered hepatocytes showed positive reactivity (Bar = 100 μm).
control rats. The SP/LA co-treated rats exhibited a reduction in the degree of DNA damage, represented by a significant reduction in comet %, while showed a non-significant decrease in both tail length and tail % on the other hand, tail moment did not elicit a modulation in response to SP co-administration.

3.5. Biochemical indices for hepatic tissue injury and serum protein profile in response to LA exposure and/or SP administration

As shown in Table 2, the activities of hepatic enzymes, SGPT, SGOT, ALP, and LDH, were significantly increased (P < 0.05) in LA-exposed rats (403.5%, 173.3%, 119.6%, and 332.4%, respectively) compared with those recorded in control rats. By contrast, SP administration significantly reversed the elevation of SGOT and ALP in SP/LA co-treated rats compared with the LA-exposed rats, while SGPT and LDH showed a non-significant modulation as the levels of these enzymes did not attain the control values. Moreover, the total bilirubin level showed highly significant increases in the LA-exposed rats (174%), which was significantly modulated in response to the administration of SP in the SP/LA co-treated rats, compared with the control rats. Neither LA nor SP treatment had a significant effect on the direct and indirect bilirubin levels.

Significant decreases in the total protein and albumin levels (62% and 58.8%, respectively) in the serum of LA-exposed rats were observed, but nonsignificant decreases in the globulin fraction were observed when compared with control rats. However, no differences were observed in albumin/globulin value among all animals. SP co-treatment with LA had a significant modulating effect on the levels of serum proteins and fractions.

3.6. Correlation coefficient of hepatic lead level versus antioxidant biomarkers and oxidative stress indices

A significant negative correlation was found between the levels of lead in the liver and those of antioxidants (P < 0.01), but a significant positive correlation was found between the levels of lead in the liver and oxidative stress indices (P < 0.01) (Table 3).

3.7. Histopathological and immunohistochemical findings

Histopathological observations of the H&E-stained paraffin liver sections revealed a normal histological structure in both control and SP-administered rats (Fig. 4A, B). In LA-exposed rats, the extensive liver injury was observed. Hepatic damage was characterized by the presence of many apoptotic hepatocytes. Moreover, most hepatic cells showed severe hydropic degeneration, individual coagulative necrosis, and focal replacement of necrotic hepatic cells by mononuclear cells (Fig. 4C). Additionally, the portal area showed portal congestion, the proliferation of the bile duct with newly formed bile ductules, and slight fibrosis (Fig. 4D). However, the SP/LA co-treated rats showed mild hepatic lesions including slight perivascular mononuclear cell aggregation and few apoptotic hepatocytes (Fig. 4E). In addition, the areas of telangiectasia (which is the dilatation of sinusoids and filled with blood because of the death of hepatocytes) were frequently observed (Fig. 4F).

Immunohistochemical observations of HSP70 in liver sections showed negative reactivity in the cytoplasm of hepatocytes in both control and SP-administered group rats (Fig. 5A, B). However, in the LA-exposed group, many hepatocytes showed positive reactivity in their cytoplasm, which was observed in groups of hepatocytes or in scattered cells (Fig. 5C). In the SP/LA co-treated rats, only a few scattered hepatocytes showed positive reactivity (Fig. 5D).

4. Discussion

The naturally occurring sources of antioxidants, such as extra virgin olive oil, ascorbic acid, and rosemary have been used and have given promising outcomes with regard to their effects on metal-induced toxicity in vitro studies and in animal models [25,46-48]. The present study deals with the efficacy of SP on modulating hepatic impairments as consequences of subacute exposure to a metallic pollutant such as LA.

The main outcome of this study is that an IP injection of LA resulted in a significant oxidative stress-mediated damage and hepatic injury by altering the biochemical and histopathological indices under investigation. The supplementation of SP provided a limited protective efficacy through the modulation of these impairments.

In the study, LA exposure to animals elicited a significant reduction in body weight changes. This might be a possible direct impact of LA on the gastrointestinal tract, resulting in the malabsorption of nutrients or a decreased food intake as LA affects food satiety signals by producing premature termination of food intake during a meal [49]. However, other studies have demonstrated that exposure to lead has no effect on body weight [50,51] and Faulk et al. [52] showed that exposure to lead increased body weight. SP significantly minimized weight loss, which might be because of providing the body with essential nutrients present in SP, such as high-quality proteins, vitamins, and amino acids [53]. These nutrients had a beneficial role in restoring body weight and health. The liver/body weight ratio was significantly higher in LA-exposed rats than in control rats which may be attributed to the accumulation of lead in liver tissue as recorded in our findings, increasing the liver weight. Such effects of LA appeared to be reversed on the administration of SP as evidenced by a reduction in this ratio. The mechanism by which this protection takes place is the subject of further research, however, the significant decrease in the deposition of lead by the liver tissue as a result of SP coadministration observed in this study may explain this reduction in the ratio.

It has been long known that dietary calcium insufficiency improves lead absorption [54] and that the vulnerable site for localization of lead is the calcified matrix of the bones. The major concern, nonetheless, has been with the disposition of lead in other more toxicologically critical tissues, e.g. liver, lung, heart, kidney, brain and the haemopoietic system. Following exposure, the concentration of lead in the soft tissues falls rapidly, generally because of transfer to the skeleton. An approximate steady state with respect to inter-compartmental distribution is accomplished in about 14 days and the profile of distribution is independent of dose over a wide range [55].

In this study, elevated lead levels were found in the whole blood and liver tissue of LA-exposed rats, which diminished on SP supplementation indicating that SP could reduce the lead levels in both blood and liver tissue in rats. The liver is an active metabolic organ involved in the storage, biotransformation and detoxification of toxic substances, is of interest in heavy metal poisoning [56]. Through its role in the detoxification of toxicants from the body, this explains why large amounts of heavy metals released from other tissues during the detoxification process are accumulated in the organ involved in detoxification.

Kaushal et al. [57] recorded that lead levels in the blood, liver, and kidney peaked within 24 h of an IP injection of lead, which was not excreted completely and nearly 15% of the administered dose remained in the body of rat. Furthermore, the reduction of lead level on SP administration mainly involved the chelating ability of SP with heavy metals such as cadmium, iron, and fluoride [10,58,59]. This ability is because SP has a rapid lead adsorption rate and high lead adsorption capacity [60] and it enhances the elimination of heavy metals from the body [13].

Lead toxicity is believed to be multifactorial but it widely mediated through oxidative stress through 5-aminolevulinic acid (ALA) accumulation followed by the auto-oxidation of ALA with the formation of a superoxide anion and hydrogen peroxide [61]. Lead toxicity also involves the impairment of mitochondrial functions by increasing the intracellular levels of calcium, which can also result in free radical generation [62]. In addition, lead toxicity involves the reduction of the...
activities of the electron transport chain components, altering mitochondrial energy metabolism resulting in free radical generation [63]. It is well known that the disruption of the prooxidant/antioxidant balance is the core mechanism by which lead damages various tissues [64–66].

Such damage was clearly shown in our results by the promotion of the formation of lipid peroxide (MDA) and protein oxidation (PC) products, which was accompanied by exhaustion of antioxidant enzymes, GSH, and TAC level in hepatic tissue of rats exposed to lead. Many enzymes in antioxidant defense systems may secure against the imbalance between prooxidants and antioxidants induced by lead exposure, and most of these enzymes become inactive because of a direct binding of lead to the sulfhydryl group [67], interrupting their function and inhibiting their activities (SOD, CAT, and glutathione) [68]. TAC includes enzymes such as SOD, CAT, and glutathione peroxidase and macromolecules such as ferritin, ceruloplasmin, and albumin. Hence, TAC may give more applicable biological information compared with that acquired by measuring its individual components, because TAC considers the cumulative impact of all antioxidants. Furthermore, we observed that SP administration exerted a reversal effect in LA-induced alterations in antioxidant enzymes, these findings were consistent with other previous studies [69,70]. Such effect was achieved by improving the level of liver tissue antioxidant capacity mechanisms because of antioxidant supply; thus, reducing the oxidative damage represented by the reduction of MDA and PC formation.

The extreme generation of ROS not only causes alterations in subcellular structures, such as proteins and lipids of polynsaturated fatty acids of the cell membrane but also induces DNA damage. Here, lead promotes DNA damage, the percentage of which is expressed by DNA adduct formation and disturbs Ca2+ homeostasis because of impairment of the endoplasmic reticulum membrane [71]. Lead has been reported to be clastogenic, aneugenic, and mutagenic [72]. It induces chromosomal aberrations, DNA damage, and micronuclei and nuclear alterations [73]. In the present study, treatment with SP significantly reduced DNA damage caused by LA. Bhat and Madyastha [74] revealed that the polysaccharides of SP improve both the repair activity of damaged DNA excision and unscheduled DNA synthesis. Similarly, phycocyanin and phycocyanobilin in SP have potent anti-cyclooxygenase-2, antioxidant activity to scavenge peroxidedirtrate and they can lessen OONO-induced oxidative damage to DNA [75].

By an exposure to environmental toxicants, the formation of free radicals increases extremely, compromising the body’s capacity to counteract them. In such situations, supplementation with antioxidants can give the protection to the intracellular and cellular machinery. SP ameliorated the effects of lead on the liver by its potential antioxidant efficacy, and facilitated the displacement of lead, resulting in reduced lead accumulation in the body. The radical-scavenging ability of SP was evident from a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and β-carotene linoleic acid assay [13], where the strong antioxidant ability of SP was shown. The ability of SP to counteract oxidative stress could be linked to the collection of antioxidants, including phycocyanins, β-carotene, vitamins E and C, and chlorophyll. Additionally, SP contains riboflavin, α-lipoic acid, xanthophyll phytytogens, magnesium, selenium, and manganese, which present antioxidant potency [59].

The release of cellular enzymes such as GOT, GPT, ALP, and LDH from the liver into the circulation mainly reflects the severity of hepatic injury because there is damage to the cell membrane of the hepatic cells [76]. GPT enzyme is one of the indices of the degree of cell membrane damage, whereas GOT is one of the indicators of mitochondrial damage because mitochondria contain 80% of GOT [77]. The induced hepatic damage is mainly related to lipid peroxidation, which rapidly breaks down the structure and affects the function of membranes such as those of the endoplasmic reticulum, mitochondria, and lysosomes, leading to plasma membrane damage [78]. The disturbances in hepatic function biomarkers induced by lead may be associated with lead accumulation in the liver or triggered by oxidative stress causing cellular damages [79].

In the present study, the pronounced increases in serum liver enzymes were recorded consequently to LA exposure, which were significantly reduced by the treatment with SP. This decline might be due to the stabilization of the hepatocyte membrane through the prevention of lipid peroxidation, amelioration of activities of the antioxidant enzymes, inhibition of inflammation, and radical scavenging activity of phycocyanin and phenolic compounds in SP [80]. Hence, SP prevents the discharge of cellular contents into the blood stream. Furthermore, the recorded increase in bilirubin level because of LA exposure is demonstrated in cases of hepatocellular injury, and the decline in bilirubin level on treatment with hepatoprotective agents reflect the repair and recovery of hepatic cells [81].

The biochemical data together with histopathological observation (an altered normal tissue architecture of the liver) clearly demonstrated that LA injured the liver, but the condition of the liver was restored with co-treatment with AP, proposing its ability to rescue the liver from lead-induced damage. These histopathological findings suggest that lead induces cell death, which was confirmed by the increase of LDH and MDA levels in LA-exposed animals demonstrating membrane damage.

In a response to cellular stress, the expression of HSPs elevates dramatically. It is well known as a pervasive adaptation mechanism of organisms that enable them to survive and adapt to various environmental stressors. The immunohistochemical data obtained for HSP70, whose expression was increased in hepatic tissue in response to the stress generated by LA exposure as shown by positive staining for HSP70 in the hepatocyte cytoplasm, reflecting the activation of this intracellular buffer system, which responds to oxidative stress when antioxidant enzyme exhaustion occurs [82].

In conclusion, SP has shown a protective role against LA-induced hepatic damage probably by scavenging free radicals and chelating lead; thereby, reducing the lead burden in rats. Thus, it is worthwhile using SP as a supplement in geographical areas with high lead exposure for minimizing the risk of hepatic alterations. Furthermore, SP can be studied further for elucidating the molecular mechanism by which SP exhibits this protective effect against sub-acute toxicity of lead.

References


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