Protective Role of Curcumin Against Oxidative Stress, Immunosuppressive and Cytotoxic Effects of Lead Exposure

Mahmoud El-Sherbiny, Azza Araffa, Mona Mantawy and Hany M. Hassan

Abstract: Lead occurs naturally in the environment. However, most lead concentrations that are found in the environment are a result of human activities. The objective of the present investigation was to investigate the effects of lead exposure on the immunocompetence, DNA damage and antioxidant enzymes in albino rats. Moreover, the study elucidates the possible corrective role of curcumin in lead exposed rats. Thirty Adult male rats were divided into 6 equal separate groups. 1st group kept as control, 2nd group received diet supplemented with curcumin (0.2%), 3rd group treated with 8 mg lead acetate, while 4th group take 16 mg lead acetate. 5th group & 6th groups treated with 8 mg and 16 mg of lead acetate simultaneous with 0.2 % curcumin, respectively. To evaluate the effect of lead and curcumin on immunity, lymphocyte transformation assay (blastogenesis), IL-6, TNF-α and IL-2 levels were measure. Moreover, Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (G-Px) and Malondialdehyde (MDA) were assayed to assess their oxidative stress effect. Comet assay was performing to determine the degree of DNA damage that induces by lead acetate and curcumin. The obtained results revealed that lead treatment reduce SOD, CAT, G-Px and lymphocyte transformation index. Moreover, IL-6, TNF-α and IL-2 and DNA damage increased due to lead exposure. On the opposite side, curcumin has antioxidant, anti-inflammatory effect and reduce DNA damage in lead exposed rats. It was concluded that curcumin has protective role against cytotoxic, oxidative and immunosuppressive profile that perform due to lead acetate exposure.

Key words: DNA damage • Lymphocytes • Cytokines and antioxidant

INTRODUCTION

Lead (Pb^{2+}) is a heavy metal that can be toxic when introduced into the human and animal bodies by ingestion or inhalation in sufficient quantities. It causes various destructive effects [1]. In human, increased levels of lead causes many serious diseases and dysfunction of organs [2]. The toxicity of lead remains a matter of public health concern [3] due to its perserviveness in the environment and the awareness about its toxic effects [4] at exposure levels lower than what was previously considered harmful [5]. Immunotoxicological evaluation of lead exposed animals has demonstrated alterations in the humoral [6] and cell mediated immunity [7]. The effects of lead on interferon have shown that lead does not inhibit the action of interferon both in vivo [8] and in vitro [9]. Other studies have documented the direct immunotoxicity caused by short-term exposure to heavy metals [10]; still others observed genotoxic [11], apoptosis [12], or even chronic effects resulting from long-term exposure [13]. The turmeric (Curcuma longa) plant, a perennial herb belonging to the ginger family, is cultivated extensively in south and southeast tropical Asia. The rhizome of this plant is also referred to as the “root” and is the most useful part of the plant for culinary and medicinal purposes. Curcumin is an antioxidant, antiinflammatory active principal ingredient of the curry spice turmeric. For the past two decades, the compound has attracted interest as a cancer preventive agent. The compound is generally regarded as safe in a phase I clinical trial of cancer patients and marketed as a dietary supplement [14, 15].

The main purpose of this study was to investigate the effects of lead exposure on the immunocompetence, DNA damage and antioxidant enzymes of albino rats. Moreover, the study cast the light on the possible corrective role of curcumin in lead exposed rats.
MATERIALS AND METHODS

Experimental Animals: Thirty adult male rats, each with average weight 220-250 g, were divided into 6 equal separate groups. 1st group kept as control. 2nd group receive diet supplemented with 2000 ppm curcumin (0.2%). The preparation of curcumin used here was obtained from LKT Laboratories, Inc.(St. Paul, MN,USA). Diets were supplied in the cage in a standard feeding bowl. Water was provided ad libitum. 3rd group treated with 8 mg lead acetate, while 4th group take 16 mg lead acetate [16]. lead acetate [Pb(C₂H₃O₂)₂, 3H₂O; Fisher Scientific Co.]; using a 16 gauge gavage needle to administer the contaminant in a volume of 1.0 ml deionized water. 5th group & 6th group were treated with 8 and 16 mg of lead acetate simultaneously with 0.2 % curcumin, respectively.

Blood Samples: Heparinized blood samples and serum were collected from each rat, 30 days post exposure.

Lymphocyte Transformation Assay (Blastogenesis): Estimation of lymphocyte transformation index was carried out to evaluate the cellular immune response, using concanavaline -A(Con-A) cell mitogen. Isolated Lymphocytes were setting into a 96 well tissue culture plate. The 1st row of wells was left free as blank (culture medium only), while each of the other wells received 100µl of treated lymphocyte culture suspension (10⁶ lymphocytes/ml) with 50µl of Con-A . The culture plate was incubated for 48 hours at 37 °C in humid incubator (5 % CO₂). After 48 hours, MTT dye [3-(4,5-dimethylthiazol-2)-2,5-diphenyl tetrazolium bromide] was added in a ratio of 1:10 of the total sample then incubated at 37°C for 4 hours. After incubation 50µl of lysing buffer (2.5ml sulfuric acid and 25g sodium dodecyl sulfate /250 ml Distilled water) were added and incubated overnight. Optical density was recorded at 470 nm using ELISA reader ( Dynalink-USA) to estimate lymphocyte transformation index (LT). The difference of optical density readings between the treated and control samples were subjected to statistical analysis [17, 18].

Comet Assay: The technique was applied on lymphocytes that isolated from heparinized blood samples. The alkaline comet assay was applied to determine the percentage of damaged DNA concentration in the comet tail by measuring total intensity of ethidium bromide fluorescence in cells[19].

Measurement of IL-6, TNF-α and IL-2 Levels by Enzyme Linked Immunosorbent Assay (ELISA): Serum IL-6, TNF-α and IL-2 levels were measured by using a polyclonal ELISA kits (RapidBio Lab. Calabasas, California, USA) following the manufacturer’s instructions. Briefly, the anti-IL-6 capture polyclonal antibody (pAb) was absorbed on a polystyrene 96-well plate and the IL-6 present in the sample was bound to the antibody coated wells. The biotinylated anti-IL-6 detecting pAb was added to bind the IL-6 captured by the first antibody. After washing, avidin-peroxidase (Sigma, USA) was added to the wells to detect the biotinylated detecting antibody and finally 2,2'-azino-bis (3-thylibenzthiazoline-6-sulfonic acid) (ABTS; Sigma, USA) substrate was added and a colored product was formed in proportion to the amount of IL-6 present in the sample, which was measured at optical density 405 nm (OD405) with an ELISA microplate reader (model 450, Bio-Rad, Chicago, Illinois, USA). A standard curve was generated and the IL-6 concentration (in pg/ml) of the samples was calculated. The measurement of TNF-α and IL-2 are similar to that of IL-6. All determinations were performed by full-time technical personnel.

Serum Biochemical Investigations: Superoxide dismutase (SOD) activity was determined by the epinephrine method [20]. Catalase (CAT) activity was measured by the previous method [21]. The activity of glutathione peroxidase (G-Px) was assayed by following the oxidation of NADPH at 340 nm with t-butyl-hydroperoxide [22]. Malondialdehyde (MDA) were assayed colorimetrically, where as MDA was determined by using 1 ml of trichloroacetic acid (10%) and 1 ml of thiobarbituric acid (0.67%). Consequently, they were then heated in a boiling water bath for 30 min. Thiobarbituric acid reactive substances (TBARS) were determined by the absorbance at 535 nm and expressed as malondialdehyde formed [23].

Statistical Analysis: The data were expressed as the mean ± S.E. and were analyzed by means of one-way analysis of variance (ANOVA). Statistical evaluation of data was done following Student’s t-test. A difference was considered significant at p<0.05.

RESULTS

Table 1 reveals that curcumin induced an significant (p<0.05) elevation of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (G-Px) and significantly (p<0.05) reduce the level of malondialdehyde in compare to control group.
Table 1: Effect of lead acetate and curcumin treatment on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (G-Px) and Malondialdehyde (MDA) in serum.

<table>
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<tr>
<td>G-Px (U/ml)</td>
<td>102.38±3.88</td>
<td>116.11±5.88*</td>
<td>93.26±6.56</td>
<td>84.52±3.18*</td>
<td>98.41±1.25</td>
<td>100.69±2.25</td>
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<tr>
<td>SOD (U/ml)</td>
<td>89.27±3.29</td>
<td>99.47±3.38*</td>
<td>82.52±9.21</td>
<td>73.05±2.95*</td>
<td>87.55±1.55</td>
<td>90.51±3.94</td>
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<tr>
<td>CAT (U/ml)</td>
<td>64.22±3.05</td>
<td>78.59±2.05*</td>
<td>60.07±2.15</td>
<td>52.05±1.88*</td>
<td>60.21±1.77</td>
<td>58.71±4.58</td>
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<tr>
<td>Malondialdehyde (mmol/ml)</td>
<td>8.52±0.29</td>
<td>7.21±0.17*</td>
<td>9.28±0.46*</td>
<td>11.25±0.35*</td>
<td>9.02±0.35</td>
<td>9.22±0.29*</td>
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</table>

1st group : control, 2nd group: curcumin (0.2%) treated group, 3rd group treated with 8 mg lead acetate, 4th group take 16 mg lead, 5th group & 6th group treated with 8 mg and 16 mg of lead acetate respectively simultaneous with 0.2 % curcumin. *: significant at p=0.05 in compare with control group.

Table 2: Effect of lead acetate and curcumin treatment on of IL-6, TNF-α and IL-2 levels in serum

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<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>133.74±2.28</td>
<td>125.03±1.18*</td>
<td>135.41±4.51</td>
<td>160.62±4.51*</td>
<td>130.17±5.89</td>
<td>140.51±3.89</td>
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<tr>
<td>TNF-α (pg/ml)</td>
<td>56.29±2.07</td>
<td>50.39±0.39*</td>
<td>59.05±4.33</td>
<td>77.88±4.89*</td>
<td>58.54±1.57</td>
<td>60.28±3.28</td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td>157.52±1.11</td>
<td>146.28±3.66*</td>
<td>161.05±8.28</td>
<td>182.33±5.37*</td>
<td>156.51±4.08</td>
<td>161.45±2.31</td>
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</tbody>
</table>

1st group : control, 2nd group: curcumin (0.2%) treated group, 3rd group treated with 8 mg lead acetate, 4th group take 16 mg lead, 5th group & 6th group treated with 8 mg and 16 mg of lead acetate respectively simultaneous with 0.2 % curcumin. *: significant at p=0.05 in compare with control group.

Table 3: Effect of lead acetate and curcumin treatment on Lymphocyte Transformation ( LT index), COMET tail length and COMET tail DNA %.

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<tbody>
<tr>
<td>LT index</td>
<td>1.75±0.06</td>
<td>2.91±0.21*</td>
<td>1.41±0.16*</td>
<td>1.08±0.11*</td>
<td>1.58±0.31*</td>
<td>1.59±0.05*</td>
</tr>
<tr>
<td>COMET tail length (µ/ml)</td>
<td>1.92±0.33</td>
<td>1.88±0.14</td>
<td>4.67±1.59*</td>
<td>12.95±2.58*</td>
<td>3.25±0.05*</td>
<td>7.88±1.46*</td>
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<tr>
<td>COMET tail DNA %</td>
<td>1.81±0.25</td>
<td>1.51±0.15</td>
<td>4.05±1.27*</td>
<td>8.28±2.31*</td>
<td>2.81±0.29*</td>
<td>6.81±1.30*</td>
</tr>
</tbody>
</table>

1st group : control, 2nd group: curcumin (0.2%) treated group, 3rd group treated with 8 mg lead acetate, 4th group take 16 mg lead, 5th group & 6th group treated with 8 mg and 16 mg of lead acetate respectively simultaneous with 0.2 % curcumin. *: significant at p=0.05 in compare with control group.

Treatment with lead acetate (8 mg) did not record any significant alteration of antioxidant enzymes levels in compare to control group. Meanwhile, rats treated with 16 mg lead acetate elucidate significant (p<0.05) reduction of antioxidant enzymes and significantly (p<0.05) enhance malondialdehyde in compare to control group. Concomitant treatment of lead treated groups with curcumin provoke levels antioxidative enzymes and reduce malondialdehyde level in comparison with lead non curcumin treated groups.

Moreover, Curcumin produced an significant (p<0.05) decrease of IL-2, IL-6 and TNF- α in compassion with control group. Treatment with lead acetate (8 mg) did not record any significant alteration of proinflamatory cytokines in compare to control group. Meanwhile, rats treated with 16 mg lead acetate elucidate significant(p<0.05) increase of proinflamatory cytokines.

Simultaneous treatment of lead acetate exposed group with curcumin produce remarkable reduction of proinflamatory cytokines in compare with lead non curcumin treated groups (Table 2).

Lymphocyte transformation index of curcumin treated group was significantly(p<0.05) higher than control group. Meanwhile, all remaining group record significant (p<0.05) reduction of Lymphocyte transformation index in compare control group. Curcumin treatment induces non-significant change in comet tail length or comet DNA %. On the opposite side, lead treatment induce significant (p<0.05) elevations of
comet tail length or comet DNA%. Curcumin and lead simultaneous treatment produce significant ($p<0.05$) low (Table 3 & Photo 1).

**DISCUSSION**

Lead is a poisonous metal that can damage nervous connections (especially in young children) and cause blood and brain disorder. Lead poisoning typically results from ingestion of food or water contaminated with lead; but may also occur after accidental ingestion of contaminated soil, dust, or lead based paint. Long-term exposure to lead or its salts (especially soluble salts or the strong oxidant PbO$_2$) can cause nephropathy and colic-like abdominal pains. The effects of lead are the same whether it enters the body through breathing or swallowing. Lead can affect almost every organ and system in the body [24].

The obtained results display that lead treatment induced reduction of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (G-Px) and significant elevation of Malondialdehyde (MDA) in serum samples of albino rats. The recorded data are on the same line of that previously obtained [25]. They reported that lead enhances lipid peroxidation and nitric oxide production in both serum and testes with concomitant reduction in GSH and antioxidant enzymes as CAT, SOD, glutathione reductase (GR), glutathione-S-transferase (GST) and GPx. Moreover, lead acetate induced DNA fragmentation in testes of rats. Moreover, the exposure to lead at the concentration of 500 mg/dm$^3$ for 6 weeks caused a decrease in the activities of SOD, GSH-PX and GR as well as an increase in MDA concentrations in the serum [26]. Lead is well known to produce oxidative damage by enhancing lipid peroxidation [27]. Lipid peroxidation inactivates cell constituents by oxidation or causes oxidative stress by undergoing radical chain reaction, ultimately leading to loss of membrane integrity [28].

The present investigation disclosed that lead elucidate significant elevations of pro inflammatory cytokines (IL-6, TNF-$\alpha$ and IL-2) especially with 16 mg lead acetate administration. These results agree with the pervious findings recorded that lead (Pb) may affect humoral and cellular immunity, acting on lymphocytes as well as on granulocytes and monocytes in lead workers, were found to have significantly higher plasma TNF-$\alpha$, IL-2 and IL-6 levels compared to non-exposed workers [29].

The current study showed that lead produce significant reduction of lymphocyte transformation indices and augmentation of DNA damage of isolated lymphocytes as appear throughout comet assay. These data agree with the findings recorded that lead treatment for a duration of proved to be most effective in suppressing the lymphocyte proliferation immune response [30, 31]. This may be attributed to a direct effect of lead on the DNA structure, oxidative mechanisms [32] or indirectly due to another mechanism involving the activation of caspases in the process of cell death [4]. In fact, participation of lead compounds to damage DNA directly is not yet reported. However, evidences indicate that lead ions can apparently take part in a Fenton reaction to generate damaging oxygen radicals and can cause DNA strand breaks [33]. Moreover, lead ions are believed to decrease the fidelity of DNA synthesis. Also, some indirect mechanisms leading to inhibition of DNA polymerase B, by lead induced reactive oxygen species (ROS), possibly indicate the failure of DNA repair mechanisms [34, 35].

Curcumin, a yellow pigment obtained from turmeric (*Curcumin longa*), is a dietary polyphenol that has been reported to possess anti-inflammatory and antioxidant properties [36].

The recorded data display that curcumin enhance SOD, CAT and G-Px and significantly reduce MDA in serum samples of albino rats. Also, curcumin reduce pro-inflammatory cytokines (IL-6, TNF-$\alpha$ and IL-2). Moreover, curcumin enhance lymphocyte transformation indices and reduce DNA damage of isolated lymphocytes especially in lead exposed rats. These data were supported with the findings reported that curcumin exhibited protective action against the As III induced depletion of antioxidants like CAT, SOD, GPx, GR, GST and GSH in mice liver tissue. Thus the present work provided a direct evidence for the involvement of curcumin in reducing As III induced oxidative stress in Swiss albino mice by virtue of its antioxidant potential and trapping of free radicals. Moreover, Curcumin increases proliferation of splenic lymphocytes and CD4+T cells [37 &38]. On the similar ground, curcumin's benefits on tumorigenesis are thought to be mediated by its antiinflammatory activity; however, these effects have not been well characterized in a mouse model of colon cancer [39]. Briefly, curcumin is efficacious for chronic nonbacterial prostatitis in rats and the action mechanism may be associated with its decreasing effect on the proinflammatory cytokines IL-8 and TNF-$\alpha$ in the blood and tissues [40]. Curcumin has protective effect
on DNA of pulmonary cells [41]. There was direct evidence for an involvement of curcumin in reducing arsenic and lead induced oxidative stress in Swiss albino mice by virtue of its antioxidant potential and trapping of free radicals [42,43].

The current investigation concluded that curcumin has protective role against cytotoxic, oxidative and immunosuppressive profile that perform due to lead acetate exposure.

REFERENCES


