The current study was conducted to investigate the protective effect of spermine, a natural polyamine against toxicity of lead and/or gamma irradiation in male rats. Eight groups of rats were used in this study (control, irradiated group (6 GY), lead (40 mg/kg bw), spermine (10 mg/kg bw), lead plus irradiation, irradiation plus spermine, lead plus spermine, irradiation plus lead co-treated with spermine) for consecutive 14 days. Blood samples were used for complete blood count (CBC) and glucose-6-phosphate-dehydrogenase (G6PD) levels. Moreover, malondialdehyde (MDA), glutathione (GSH), metallothionein (MT) levels and catalase (CAT) activity were investigated in liver, kidney and brain. G6PD activity significantly decreased post exposure to lead and/or gamma irradiation. Hepatic, renal and brain MDA, GSH, MT and CAT were significantly increased in lead intoxicated group, while GSH, MT and CAT activity were significantly decreased in gamma-irradiated group. Spermine administration alleviated changes in CBC, G6PD, MDA, MT and CAT to normal control levels, but with significant increase in G6PD activity and platelets count. In conclusion, spermine acts as an antioxidant and plays a prophylactic role against intoxication with lead and/or gamma irradiation exposure.
INTRODUCTION

Heavy metal pollution is a global public health challenge due to its stable and persistent environmental contamination. Lead is considered to be one of the most common ubiquitous and industrial pollutants and at low concentration it exerts extensive damages to the tissues (Sajitha et al., 2016).

Lead was reported to cause lipid peroxidation (LPO) in the liver and deplete glutathione (GSH). Consistently, Pb has been found to derail the antioxidant defense system by interfering with the metals that are essential for antioxidant enzyme activities, supporting the role of oxidative stress in lead toxicity (Moneim, 2016). Pathogenesis of lead poisoning is mainly attributed to lead-induced oxidative stress. Chronic lead exposure is known to disrupt the pro oxidant/antioxidant balance existing within the mammalian cells. Lead was reported to induce oxidative stress by generating reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides (El-Tantawy, 2015).

Living organisms are continually exposed to ionizing radiation in nature as well as from medical procedures which contribute most whole-body background radiation. Ionizing radiation (IR) is a form of radiation with sufficient energy to remove electrons from their atomic or molecular orbital shells in the tissues they penetrate (Mohamed et al., 2015). Radiation is known to produce various reactive oxygen species (ROS) in biological systems such as superoxide, hydrogen peroxide and hydroxyl radical reaction. Detrimental effect of ionizing radiation occurs mainly due to free radicals generated through the decomposition of cellular water. However, organisms have protective systems against free radical reaction including endogenous enzymatic and nonenzymatic antioxidative systems (Abdel-Shafi et al., 2016).

The direct and indirect damaging effects of ionizing radiation lead to peroxidation of macro-molecules, particularly those existing in lipid-rich membrane structures, lipoproteins and chromatin lipids. Phospholipids in membranes are highly vulnerable to free radical attacks. Once the process of lipid peroxidation is started, it proceeds as a free radical-mediated chain reaction involving initiation, propagation, and termination (Gago-Dominguez et al., 2005). During lipid peroxidation, several end products are formed such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), pentane and ethane, 2, 3 transconjugated dienes, isoprostanes and cholesteroloxides (Catalá, 2009; Tuma, 2002). These aldehydes are highly reactive and bind with DNA and proteins and form adducts which hinder proteins functions and can interrupt nuclear events. Products of lipid peroxidation are measured as thiobarbituric acid reactive substances (TBARS) in biological materials using thiobarbituric acid reaction (Møller and Loft, 2010).

As referenced data suggest, the generation of reactive oxygen species such as superoxide anions, hydrogen peroxides, and hydroxyl radicals or products of lipid peroxidation (lipid hydroperoxides, lipid aldehydes) has been implicated in lead and/or gamma irradiation toxicity. Therefore, compounds with antioxidant properties probably may have a beneficial effect on therapy or/and prophylactic in lead and/or gamma irradiation intoxication.

The natural polyamine, spermine, is present in virtually all living cells and a wide variety of polyamine are formed in nature according to species. Mammals produce only spermine, spermidine, and their precursor, the diamine putrescine (Pegg, 2014).

Spermine, a polyamine, plays an important role in many cellular processes including the regulation of transcription and translation, control of the activity of ion channels, modulation of kinase activities, effects on the cell cycle, protection from oxidative damage, the maintenance of membrane structure/function, and contributing to nucleic acid structure and stability against both thermal as well as alkaline denaturation and radiation-induced injury and mutagenesis (Pegg, 2009; Park et al., 2010; Newotarski et al., 2013). Moreover, spermine acts as
Prophylactic Role of Spermine in Rats Intoxicated With Lead and/or Gamma

a biologically important antioxidant in vitro (Shoji et al., 2005; Rider et al., 2007; Toro-Funes et al., 2013), and an anti-inflammatory agent (Løvaas and Carlin, 1991). Spermine administration can enhance the jejunum antioxidant properties of suckling rats (Cao et al., 2015) and serum antioxidant capacity of suckling piglets (Fang et al., 2016), and alleviate serum oxidative stress in weaned rats (Liu et al., 2014). Therefore, spermine has potential functions against oxidative stress.

In this study, the protective effect of spermine on liver, kidney and brain of rats exposed to whole-body gamma irradiation (6Gy) as acute dose and/or lead acetate was examined.

MATERIAL AND METHODS

Animals

Male albino rats (n=80, weighing 120 – 150 g), were obtained from El-Nile Company for Drugs, Cairo, Egypt. The animals were kept for 15 days for laboratory acclimatization, were fed commercial pellets, and were provided with tap water. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Center conformed to the “guide for the care and use of laboratory animals” (NIH publication, No.85-23, 1996).

Irradiation process

Animals were γ-irradiated at an acute 6 Gy (0.708 rad/sec) using the biological irradiator Gamma cell-40 (Cesium-137 source). Whole-body γ-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo.

CHEMICALS

Lead acetate trihydrate, Ellman’s reagent [5,5-dithio-bis (2-nitrobenzoic acid); DTNB], trichloroacetic acid (TCA), disodium ethylenediaminetetraacetate (Na₂EDTA), n-butanol, malondialdehyde bis (diethyl acetal) [1,1,3,3-tetraethoxypropane; TEP], thiobarbituric acid (TBA), silver nitrate (AgNO₃), glycine, sodium hydroxide (NaOH), potassium chloride (KCl) and hydrochloric acid (HCl) were purchased from (Sigma Chemical Co.; St. Louis, MO, USA). All chemicals and solvents were of the highest grade commercially available.

Experimental design

Male albino rats were divided into eight groups (10 rats of each) and were classified as follows:

Group I: Rats received distal water.

Group II: Rats were exposed to whole body γ-irradiation, as a single dose (6 Gy).

Group III: Rats received orally 40 mg lead (Pb) / kg bw orally for 14 consecutive days.

Group IV: Rats received 10 mg spermine (Spm) / kg bw intraperitoneally for 14 consecutive days.

Group V: Rats received Pb as in group III for 14 consecutive days; and then exposed to γ-irradiation irradiation (6 Gy) at day 14 of treatment.

Group VI: Rats received Spm as in group IV for 14 consecutive days, and then exposed to whole-body γ-irradiation (6 Gy) at day 14.

Group VII: Rats received orally 40 mg Pb / kg bw and intraperitoneally 10 mg Spm / kg bw for 14 consecutive days.

Group VIII: Rats received Pb and Spm for consecutively for 14 days, then exposed to γ-radiation (6 Gy) at day 14.

All rats were sacrificed 24 hours post irradiation.

Blood sampling

At the end of 14 days, animals were sacrificed after 12 hrs fasting. Whole blood was collected by cardiac puncture after light anesthesia. Blood from each rat was collected in an EDTA tube. EDTA blood samples collected were used for glucose-6-phosphate dehydrogenase(G6PD) activity determination and complete blood count (CBC), where the hematological parameters including RBC’s, WBC’s, and platelets counts, Hb concentration were evaluated using blood counter (Techo, American).
**Tissue sampling**

Tissues (liver, kidney and brain) were prepared for biochemical analysis according to the method of Becciolini et al., (1972). After dissection, liver, kidney and brain were washed with ice cold isotonic 0.9% NaCl and blotted between 2 filter papers and weighed. For each tissue, a 10% (W/V) homogenate (in ice-cold 0.9% NaCl) was prepared using a Tri-R STIR-R model K41 homogenizer. The homogenates were centrifuged at 4,000 rpm for 10 min at 4°C; using cooling centrifuge (Universal 16 R, Germany) and the supernatants were used for lipid peroxidation, reduced glutathione (GSH), and metallothionein (MT) levels estimation and catalase activity determination.

**Biochemical analyses**

The catalase (CAT) activity was evaluated using catalase kits (Biodiagnostic, Egypt) according to the methods of Aebi, (1984) and Fossati, et al., 1980. Lipid peroxidation was estimated according to Yoshioka et al., (1979). Reduced glutathione was measured based on the method of Ellman, (1959) and metallothionein levels were determined by Ag-saturation hemolysate method according to Schuehammer and Cherian, (1986)and Bienengräber et al., (1995).

**Statistical analysis**

Statistical analysis was performed by Graphpad prism 5 software package. Data (n=10) were expressed as mean values ± SEM. p values less than 0.05 were considered significant. All data were subjected to one-way ANOVA test to determine differences among groups.

**RESULTS**

As shown in Table (1&2), compared to normal control, γ-irradiation exposure or treatment with lead resulted in a significant decrease in levels of Hb, counts of PLT, RBCs, and WBCs as well as G6PD activity. Spermine per se resulted in a significant increase in PLT count (28.06 %) and G6PD activity (17.67 %) compared to control. Spermine administration prior to irradiation ameliorated all hematological parameters to normal control level with the exception of Hb levels. Lead and spermine treated group significantly increased Hb, counts of PLT, RBCs and WBCs by 18.05 %, 35.46 %, 21.9 % and 160 %, respectively compared to lead group. Rats exposed to lead and γ-irradiation co-treated with spermine exhibited amelioration in MCV, PLT, RBCs and WBCs and a significant decrease in Hb level and G6PD activity by 22.14 % and 25 %, respectively compared to normal control.

As shown in Table (3), γ-irradiation exposure resulted in a significant decrease in liver GSH, MT levels and catalase activity by 37.81 %, 46.15 %, and 17.7 %, respectively. On the other hand, treatment with lead significantly increased GSH, MT levels and catalase activity in liver by 58.92 %, 167.9 % and 17.2 %, respectively compared with normal control value. Exposure to γ-irradiation or treatment with lead significantly increased MDA levels by 138.01 % and 57.12 %, respectively. Compared to control group, spermine treatment per se resulted in a significant increase in metallothionein by 39.1 % compared to control. Spermine administration with γ-irradiation significantly increased GSH, MT levels and catalase activity in liver in comparison with irradiated group which were increased by 64.4 %, 179.07 % and 15.41%, respectively and significantly decrease MDA level by 52.89 %. Treatment with lead and spermine significantly decreased levels of MDA, GSH, and MT as well as CAT activity in liver tissues by 17.6 %, 26.04 %, 22.9 % and 11.05 %, respectively compared to lead Treatment, whereas a significant increase by 29.43 %, 17.06 %, 129.45 % and 4.25 %, respectively was observed in comparison with control group. Compared with lead and γ-irradiation group, rats exposed to lead, γ-irradiation and treated with spermine exhibited a significant decrease in MDA, GSH, and MT and catalase activity in liver tissues by 15.25 %, 25.66 %, 21.55 % and 11.83 %, respectively.

As shown in Table (4), compared to control group, γ-irradiation exposure significantly decreased kidney levels of GSH, MT and catalase activities by
Prophylactic Role of Spermine in Rats Intoxicated With Lead and/or Gamma

45.45%, 33.1% and 13.97%, respectively while exposure to lead *per se* resulted in a significant increase by 44.53%, 261.6% and 12.9%, respectively. Exposure to γ-irradiation or treatment with lead significantly increased MDA levels by 178.02% and 108.33%, respectively. Spermine treatment recorded a significant increase in MT by 28.85% and non-significant changes in levels of MDA, and GSH and catalase activity. Administration of spermine with γ-irradiation significantly increased kidney levels of GSH, and MT and catalase activity by 96.02%, 99.77% and 15.16%, respectively, while it significantly decreased MDA value by 57.19% in comparison with γ-irradiated group. Combined treatment with lead and spermine significantly decreased kidney levels of MDA, GSH and MT by 37.25%, 25% and 47.9% in comparison to lead treatment. However, lead and spermine treated group resulted in a significant increase in MDA and MT levels by 30.72% and 136.11%, respectively in comparison with control group. Rats exposed to lead, γ-irradiation and treated with spermine exhibited amelioration in kidney levels of MDA, GSH, and MT levels and catalase activity with a significant increase in MDA and GSH by 21.9% and 12.82%, respectively.

**Table (1)** Effect of spermine (10 mg/kg bw) on complete blood count (CBC) in rats intoxicated with lead and/or γ -irradiation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>PLT (*10^9/l)</th>
<th>RBCs (*10^12/l)</th>
<th>WBCs (*10^9/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.98 ± 0.146</td>
<td>49.68 ± 1.073</td>
<td>412.3 ± 11.05</td>
<td>6.994 ± 0.108</td>
<td>6.415 ± 0.285</td>
</tr>
<tr>
<td>Rad.</td>
<td>10.20 ± 0.363a</td>
<td>45.08 ± 1.6a</td>
<td>364.4 ± 1.92a</td>
<td>5.920 ± 0.09a</td>
<td>2.205 ± 0.135a</td>
</tr>
<tr>
<td>Pb</td>
<td>10.36 ± 0.562a</td>
<td>47.16 ± 0.785</td>
<td>335.0 ± 5.00a</td>
<td>5.325 ± 0.319b</td>
<td>3.275 ± 0.175b</td>
</tr>
<tr>
<td>Spm</td>
<td>14.12 ± 0.497</td>
<td>49.42 ± 0.731</td>
<td>508.0 ± 3.215a</td>
<td>7.296 ± 0.243</td>
<td>6.764 ± 0.553</td>
</tr>
<tr>
<td>Pb+Rad.</td>
<td>9.450 ± 0.152a</td>
<td>47.27 ± 0.35</td>
<td>350.2 ± 5.229a</td>
<td>4.913 ± 0.198a</td>
<td>1.585 ± 0.055ab</td>
</tr>
<tr>
<td>Spm +Rad.</td>
<td>11.08 ± 0.536a</td>
<td>55.25 ± 0.355e</td>
<td>399.3 ± 6.884</td>
<td>6.320 ± 0.241</td>
<td>5.9 ± 0.03c</td>
</tr>
<tr>
<td>Spm+Pb</td>
<td>12.23 ± 0.218ab</td>
<td>47.02 ± 0.406</td>
<td>453.8 ± 19.27b</td>
<td>6.492 ± 0.087b</td>
<td>8.515 ± 0.445ab</td>
</tr>
<tr>
<td>Spm+Pb+Rad.</td>
<td>10.90 ± 0.28a</td>
<td>51.33 ± 1.163c</td>
<td>395.0 ± 8.66b</td>
<td>6.246 ± 0.367b</td>
<td>6.57 ± 0.055bc</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SEM, n = 10. a, b and c indicate significant changes from control, lead and γ-ray, respectively at p ≤ 0.05 using ANOVA followed by Tukey-Kramer as a post ANOVA test. Underlined percentage indicated to percentage change from γ-irradiated group. Percentage in round brackets indicated to percentage change from Pb group. Abbreviation Spm: spermine, Pb: lead, γ-ray: gamma-irradiation.
Table (2) Effect of spermine (10 mg/kg bw) on glucose-6-P dehydrogenase (G6PD) activity in rats exposed to lead and/or γ-irradiation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>G6PD (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.74 ± 1.08</td>
</tr>
<tr>
<td>Rad.</td>
<td>38.10 ± 1.103a</td>
</tr>
<tr>
<td>Pb</td>
<td>31.68 ± 1.023a</td>
</tr>
<tr>
<td>Spm</td>
<td>63.24 ± 1.056a</td>
</tr>
<tr>
<td>Pb+Rad.</td>
<td>25.95 ± 0.8657ab</td>
</tr>
<tr>
<td>Spm+Rad.</td>
<td>50.47 ± 1.089c</td>
</tr>
<tr>
<td>Spm+Pb</td>
<td>44.10 ± 1.79ab</td>
</tr>
<tr>
<td>Spm+Pb+Rad.</td>
<td>40.30 ± 1.794ab</td>
</tr>
</tbody>
</table>

Legends are as table (1)

Table (3) Effect of spermine (10 mg/kg bw) on liver levels of MDA, GSH, MT and Catalase activity in rats intoxicated with lead and/or γ-irradiation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (µmole/g tissue)</th>
<th>GSH (mg/g tissue)</th>
<th>Metallothionin (mg/l)</th>
<th>Catalase (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.07 ± 1.801</td>
<td>39.8 ± 2.766</td>
<td>140.9 ± 5.177</td>
<td>143.5 ± 1.421</td>
</tr>
<tr>
<td>Rad.</td>
<td>59.67 ± 0.778a</td>
<td>24.75 ± 0.634a</td>
<td>75.87 ± 7.556a</td>
<td>118.1 ± 1.564a</td>
</tr>
<tr>
<td>Pb</td>
<td>39.39 ± 1.719a</td>
<td>63.00 ± 1.237a</td>
<td>236.6 ± 10.42a</td>
<td>168.2 ± 0.8609a</td>
</tr>
<tr>
<td>Spm</td>
<td>25.25 ± 1.403</td>
<td>38.61 ± 1.349</td>
<td>196 ± 10.76a</td>
<td>146.1 ± 1.568</td>
</tr>
<tr>
<td>Pb+Rad.</td>
<td>52.12 ± 0.679abc</td>
<td>50.23 ± 1.538bc</td>
<td>102.5 ± 7.528b</td>
<td>158.1 ± 1.294abc</td>
</tr>
</tbody>
</table>

Legends are as table (1)
### Table 4

Effect of spermine (10 mg/kg bw) on kidney levels of MDA, GSH, MT and Catalase activity in rats intoxicated with lead and/or γ-irradiation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (µmole/g tissue)</th>
<th>GSH (mg/g tissue)</th>
<th>Metallothionin (mg/l)</th>
<th>Catalase (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>28.67 ± 1.011</td>
<td>45.63 ± 2.005</td>
<td>202.4 ± 17.85</td>
<td>140.3 ± 2.273</td>
</tr>
<tr>
<td><strong>Rad.</strong></td>
<td>79.71 ± 0.487a</td>
<td>24.89 ± 1.625a</td>
<td>135.4 ± 13.43a</td>
<td>120.7 ± 2.051a</td>
</tr>
<tr>
<td><strong>Pb</strong></td>
<td>59.73 ± 2.164a</td>
<td>65.95 ± 1.732a</td>
<td>529.5 ± 12.30a</td>
<td>158.4 ± 2.734a</td>
</tr>
<tr>
<td><strong>Spm</strong></td>
<td>30.63 ± 1.166</td>
<td>42.46 ± 1.526</td>
<td>260.8 ± 4.934a</td>
<td>144.9 ± 1.593</td>
</tr>
<tr>
<td><strong>Pb+Rad.</strong></td>
<td>69.23 ± 0.571abc</td>
<td>54.77 ± 0.4466abc</td>
<td>420.4 ± 8.101abc</td>
<td>158.3 ± 2.27abc</td>
</tr>
<tr>
<td><strong>Spm + Rad.</strong></td>
<td>34.12 ± 0.898abc</td>
<td>48.79 ± 0.674</td>
<td>270.5 ± 9.094abc</td>
<td>139 ± 5.62abc</td>
</tr>
<tr>
<td><strong>Spm + Pb</strong></td>
<td>37.48 ± 1.336abc</td>
<td>49.46 ± 1.369abc</td>
<td>275.5 ± 9.801abc</td>
<td>149.4 ± 2.36abc</td>
</tr>
<tr>
<td><strong>Spm + Pb + Rad.</strong></td>
<td>34.95 ± 0.87abc</td>
<td>51.48 ± 1.052abc</td>
<td>198.5 ± 10.5bc</td>
<td>151.7 ± 2.152abc</td>
</tr>
</tbody>
</table>

Legends are as Table (1)
As shown in Table (5), compared to control group, γ-irradiation exposure significantly decreased brain levels of GSH and catalase activity by 20.23 % and 16.42 %, respectively. However, treatment with lead significantly increased levels of GSH, MT and catalase activity by 50.4 %, 218.69 % and 29.06 %, respectively. Moreover, exposure to γ-irradiation or lead significantly increased MDA level by 136.58 % and 78.1 %, respectively. Compared to control value, administration of spermine alone recorded a significant increase in MDA, GSH and MT levels by 28.89 %, 15.54 %, and 130 %, respectively. In comparison with irradiated group, spermine administration prior to irradiation significantly increased GSH, and MT levels and catalase activity in brain of rats by 18.32 %, 37.33 % and 9.92 %, respectively, while it significantly decreased MDA value by 53.6 %. Compared to lead treatment, combined treatment with lead and spermine significantly decreased MDA and MT levels, while it resulted in a significant increase in GSH level and catalase activity by 13.73 % and 11.65 %, respectively in comparison with control group. Exposure to γ-irradiation after treatment with lead and spermine ameliorated MDA, GSH, MT levels and catalase activity in brain tissues with a significant increase in GSH level and catalase activity by 13.65 % and 14.12 %, respectively.

Table (5) Effect of spermine (10 mg/kg bw) on brain levels of MDA, GSH, MT and Catalase activity in rats intoxicated with lead and/or γ-irradiation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (µmole/g tissue)</th>
<th>GSH (mg/g tissue)</th>
<th>Metallothionin (mg/l)</th>
<th>Catalase (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.71 ± 0.549</td>
<td>24.76 ± 0.62</td>
<td>258.9 ± 10.12</td>
<td>121.8 ± 3.186</td>
</tr>
<tr>
<td>Rad.</td>
<td>58.46 ± 0.778&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.75 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>251.5 ± 6.865</td>
<td>101.8 ± 3.112&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pb</td>
<td>44.01 ± 1.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.24 ± 0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>566.2 ± 13.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>157.2 ± 3.884&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spm</td>
<td>31.85 ± 1.941&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.61 ± 0.989&lt;sup&gt;a&lt;/sup&gt;</td>
<td>336.6 ± 8.491&lt;sup&gt;a&lt;/sup&gt;</td>
<td>137 ± 5.057</td>
</tr>
<tr>
<td>Pb+Rad.</td>
<td>51.06 ± 1.337&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>31.49 ± 2.43&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>483.3 ± 14.27&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>140.4 ± 2.02&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spm +Rad.</td>
<td>27.12 ± 0.417&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.37 ± 1.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>345.4 ± 18.08&lt;sup&gt;ec&lt;/sup&gt;</td>
<td>111.9 ±2.544</td>
</tr>
<tr>
<td>Spm+Pb</td>
<td>30.06 ± 0.7385&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.16 ± 2.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>276 ± 5.067&lt;sup&gt;b&lt;/sup&gt;</td>
<td>136 ± 2.431&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spm+Pb+Rad.</td>
<td>28.77 ± 1.067&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>28.14 ± 1.024&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>288.8 ± 16.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>139 ± 2.557&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legends are as table (1)
Discussion

Exposure to lead leads to various deleterious effects on the hematopoietic system, mainly via increased oxidative stress. Alternation of hematological parameters may also be due to the effect of lead on the activity of δ-aminolevulinic acid dehydratase (ALAD; EC 4.2.1.24) a crucial enzyme of heme production which resulted in decline in heme formation and a shortened life expectancy of erythrocytes by increasing RBCs fragility and destruction (Klassen, 2013). Failure of normal functioning of ALAD to convert two molecules of δ-aminolevulinic acid (δ-ALA) into porphobilinogen (PBG) resulted in increased accumulation of δ-ALA and decreased formation of PBG, thus inducing formation of reactive oxygen species (ROS) and lipid peroxidation which facilitate the depletion of antioxidants in lad exposed RBCs (Saxena et al., 2005; Flora et al., 2007). δ-ALAD is the enzyme that is most sensitive to this depression (Trombini et al., 2015). Moreover, lead inhibits ferrochelatase (EC 4.99.1.1), the mitochondrial enzyme that catalyzes the insertion of iron into protoporphyrin to form heme. Inhibition of ferrochelatase results in replacement of iron by zinc in porphyrin ring and formation of zinc protoporphyrin (ZPP), the concentration of which is used as gauge to monitor levels of lead exposure (Jangid et al., 2012). These findings are in accordance with Helmy et al., (2000) who observed significant decrease in in hematological parameters following exposure of rats to lead acetate.

The results of the present study revealed a significant decline in number of WBCs (leukopenia) may be due to the direct toxic effect of lead on leukocytes synthesis in lymphoid organs. Decrease in total leukocytes count is directly associated with either their decreased production from germinall center of lymphoid organs or increased lysis due to the accumulation of lead in the body (Abdou and Hassan, 2014).

Gamma-irradiation revealed a significant depression in RBCs, WBCs, MCV and hemoglobin content which could be attributed to the destruction of the erythrocyte precursors in bone marrow (Ashry and Hussein, 2007). Furthermore, the increased RBCs lysis by γ-irradiation could to be due to a decreased production of erythropoietin (EPO); a hormone produced by the kidney and is essential for RBCs production or erythropoiesis (Hassan et al., 2015). RBCs are considered to be a main target for free radicals owing to the existence of both extraordinary membrane contents of polyunsaturated fatty acids (PUFA) and oxygen transport related with redox active hemoglobin molecules, which are effective promoters of activated oxygen species (Ebrahimzadeh et al., 2009).

The cellular elements of the blood are predominantly sensitive to oxidative stress because their plasma membranes contain a high content of PUFA. Therefore, the decrease in white blood cells differential count recorded in the irradiated rats may be the result of radiation-induced lipid peroxidation and destruction of their cell membranes (Chew and Park, 2004).

In spermine group, there was a significant increase in platelets count which could be due to enhancement of the differentiation process by spermine per se. This was reinforced by the previous study of Heby, (1989) and Seiler, (2000) that clearly showed that treatment of cancer patients with Diflouro methyl ornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase (ODC), revealed inhibition of differentiation of hematopoietic cells to end stage platelets and erythropoietic cells. Moreover, long term inhibition of ODC by DFMO resulted in a decrease in platelets counts. Hence, administration of exogenous spermine enhanced the differentiation of hematopoietic cells and causes an increase in platelets count.

The evidence of spermine protection to RBCs might be due to red blood cells lack DNA and were totally without RNA so no renewal of damaged protein, likewise the vast majority of circulating spermine were essentially carried by RBCs by polyamine transporter which concentrate spermine (Chabanon et al., 2000; Cooper et al., 1976) which reflect fortification of spermine to antioxidant system of the RBCs (Farriol et al., 2003). In addition, RBCs may accumulate spermine to quench glycating agents as...
RBCs live 120 days with no renewal of their protein and also were exposed to sacral oxidative stress (Gugliucci and Menini, 2003).

Glucose-6-phosphate dehydrogenase (EC 1.1.1.4) is an important rate limiting enzyme of pentose phosphate pathway (PPP), which catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconolactone and finally to ribose-5-phosphate. It results in biosynthesis of reducing equivalent in the form of NADPH to meet cellular needs for reductive biosynthesis of fatty acids and maintenance of the cellular redox balance (Maurya et al., 2016).

A significant decline in G6PD activity by lead may be due to lead ions acts as a potent inhibitor to glucose-6-phosphate dehydrogenase (G6PD) activity in rats which suggests a harmful interaction between lead levels and enzymes for the pentose phosphate pathway. Inhibition of the pentose phosphate pathway may then cause the lead-treated RBC to be more liable to oxidative damage (Demirdag et al., 2015). These results coincide with Cocco, (1998) who indicated that formation of a complex between lead and sulfahydryl (SH) groups of G6PD which plays a vital role in maintaining the enzyme tertiary structure, is suggested as an acceptable and plausible mechanism for G6PD activity inhibition. However; contradicting results were also reported by Gürer et al., (1998) who showed that the increase in G6PD activity in RBCs of lead exposed rats could be implicated as the defense of the animal against the increase of the oxygen active forms in lead toxicity.

Gamma-irradiation exposure led to a significant decrease in G6PD activity. These changes may result from alternation of the redox state of the RBCs by γ – irradiation that subsequently decrease formation of NADPH, which in turn inactivates G6PD (Pari and Venkateswaran, 2003). Decreased activity of G6PD resulted in increased sensitivity of red blood cells to ionizing radiation, so reduced survival time (Agarwal et al., 2007).

Spermine administration to lead and/or irradiation ameliorated G6PD activity may be due to radical scavenging properties, metal chelation properties of spermine. In addition, spermine has the capacity to prevent superoxide generation by stimulated neutrophils (løvaas and Carlin, 1996). Furthermore, the more reactive hydroxyl radical generated by Fenton reaction was efficiently scavenged by spermine (Drolet et al., 1986). Such data were in agreement with another study that noticeably showed that polyamines protected against radiation damage (Hillebrand et al., 1990).

In the current study, spermine enhanced activation of glucose 6-phosphate dehydrogenase, the NADPH-generating enzymes in RBCs which revealed that spermine acted as an antioxidant by stimulating the regeneration of reduced glutathione, the principal reactive oxygen scavenger. Spermine dependent activation of G6PD presented a new perception on the antioxidant properties of this compound in addition to the eminent properties of scavenging reactive oxygen species.

In the current work, a significant increase of oxidative biomarkers was associated with alternation in the antioxidant status of the liver, kidney and brain. The significant increase in reduced glutathione levels and the activity of catalase in lead intoxicated group might be attributed to increased expression of these enzymes as a self-defense mechanism against oxidative stress (Guo et al., 2003). Enhanced synthesis of glutathione and catalase in liver, kidney and brain tissues may be due to induction by lead to abolish free radicals as a compensatory mechanism and an attempt to cope up with oxidative stress. Induction of GSH by lead could be related to the proposed role of GSH in the active excretion of lead by binding to thiol group of GSH and then being excreted (Senapati et al., 2001). In a similar study, Wang et al. (2013) demonstrated that exposure to lead induced expression of nuclear factor 2-related factor 2 (Nrf2) in testis. Induction of Nrf2 may maintain transcriptional activation of various antioxidant genes to balance redox hemeostasis, thereby promoting survival of liver, kidney and brain in lead exposed rats. In another similar study of Moneim (2016) indicated that rats exposed to lead acetate upregulated expression of heme oxygenase 1 (HO-1) which may be an
adaptation that protect hepatocyte from lead-induced oxidative stress, suggesting that the hepatocytes have the ability to restrain oxidative stress by HO-1 induction. The results of our study coincide with Tandon et al. (2002) who suggested that catalase activity increased in lead-exposed animals. On the other hand, a significant decrease recorded in reduced glutathione levels and catalase activity in the group exposed to γ-irradiation could be attributed to the uncontrolled production of ROS and buildup of \( \text{H}_2\text{O}_2 \) whereby oxidative injury to enzymes can cause a retardation of their activity (Kregel and Zhang, 2007).

Lipid peroxidation is considered to be a molecular mechanism of oxidation of cellular polyunsaturated fatty acids based macromolecules. Overproduction of ROS augments lipid peroxidation and subsequently increases the lipid peroxidation products such as malondialdehyde (MDA) and other TBARS levels which lead to degradation of cellular macromolecules (Das et al., 2010).

Increased levels of MDA in liver, kidney and brain on lead administration may be a crucial factor in oxidative deterioration of membrane polyunsaturated fatty acids and may finally be responsible for disturbances in membrane integrity (Moneim, 2016). The results obtained are in agreement with those of El-Tantawy (2015) who reported an increase in MDA levels of lead-intoxicated rats. The ability of lead to enhance tissue lipid peroxidation may be attributed to the indirect effect of lead on reactive oxygen species quenching enzymes and not to a direct effect of lead on lipid peroxidation as lead does not participate in the oxidation-reduction cycle of lead-intoxicated rats. The ability to enhance tissue lipid peroxidation may be attributed to the indirect effect of lead on reactive oxygen species quenching enzymes and not to a direct effect of lead on lipid peroxidation as lead does not participate in the oxidation-reduction cycle (El-Nekeety et al., 2009). Oteiza and Bechara (1993) indicated that lead induced lipid peroxidation may result from increased level of aminolevulinic acid (ALA), which accumulates after lead exposure. Moreover, these changes may result from the interplay of lead with sulfhydryl group or the interaction with essential metal cofactors resulted in disturbances in membrane integrity and function.

The present study revealed that γ-irradiation produced significant oxidative damage accompanied by a decrease in the activity of the antioxidant enzyme catalase and in the level of the non-enzymatic antioxidant GSH. This damage was indicated by a significant increase of malondialdehyde (MDA) level (a marker of lipid peroxidation) in liver, kidney and brain tissues as compared with the corresponding control group. The elevated level of MDA in γ-irradiated rats might be due to the interaction of free radicals with polyunsaturated fatty acids in the phospholipids of cellular membranes (Prasad et al., 2005). These results not coincide with Sridharan and Shyamaladevi (2002) who reported that low and high doses of radiation increased catalase activity and endogenous GSH level in the liver in order to protect from lethal damage of ionizing radiation.

Administration of Spm with Pb or γ-irradiation has significantly ameliorated oxidative stress in liver, kidney and brain tissues. This could be ascribed to anti-lipid peroxidation capacity of spermine and improvement of the antioxidant recovery systems functions to different extents via improving enzymatic antioxidant activities in rats (Cao et al., 2015). The protective effect of Spm on lipid peroxidation may be due to the role of Spm binding to membranes that can suppress lipid peroxidation through electrostatic interaction with phospholipid polar head, the free radical scavenging activity of polyamine to one or more of the following oxygen species: superoxide ion (\( \text{O}_2^- \)), hydroxyl radical (\( \text{OH}^- \)), alkoxy radical (\( \text{RO}^- \)), peroxy radical (\( \text{ROO}^- \)), singlet oxygen (\( \text{O}_2^* \)) (Sava et al., 2006; Khan et al., 1992). These results coincide with Kitada et al. (1979) who observed that polyamines inhibited NADPH- and ascorbic acid-dependent lipid peroxidation in rat liver microsomes. It was suggested that the effect was related to close interaction between open chain structure of spermine and anionic site on membrane (phospholipids). Adsorption of spermine on negatively charged biomolecules resulted in increased localization of spermine at sites of oxidation attack. Kitada et al. (1979) noted that spermine also prevented the loss of the catalyzing activity of cytochrome P450 caused by lipid peroxidation.

Metallothionein (MT) is a cysteine-rich, low
molecular weight protein, has been known to be involved in many physiological and pathophysiological processes, such as intracellular storage, transport and metabolism of heavy metals in order to regulate essential trace metal homeostasis and protect against heavy metal toxicity (Fujiwara and Satoh, 2013).

Induction of MT by lead in liver, kidney and brain has been proposed as an important adaptive mechanism in response to environmental stimuli. Induction of MT acts as a free radical scavenger protecting against oxidative damage and protects against heavy metal toxicity (Klaassen and Liu, 1998). Our results are consistent with the previous study of Liu et al. (2005), which suggested that lead is a weak inducer of MT. The results of Ghoniem et al. (2012) revealed that lead mildly upregulated mRNA expression in kidneys of Pb treated rats which returns to the oxidative stress caused by ROS generated by lead toxicity. Lead may also induce expression of metal regulatory transcription factor 1 (MTF-1) which acts as a ubiquitous expressed zinc finger protein essential for basal and heavy metal induced expression of metallothionein. MTF-1 binds to MRE (metal responsive element of metallothionein) in the MT promoter, thus stimulating its transcription and inducing the expression of metallothion and glutamate cysteine ligase (GCL, EC 6.3.2.2), the rate limiting enzyme in the synthesis of glutathione. It catalyzes the ligation of a reaction of L-glutamate and L-cysteine to form the dipeptide gamma-glutamyl-cysteine (γ-GC) that forms glutathione upon coupling with glycine. This may explain increase in GSH levels by lead (pamiter, 1994; Kojima et al., 2005).

Another possible reason behind depletion of MT in γ-irradiated rats was that γ-irradiation increased reactive oxygen species/reactive nitrogen species (ROS/RNS). MT has cysteinyl thiolate groups that confer oxidoreductive properties (Rutt-key-Nedecky et al., 2013), responsible for MT encountered ROS/RNS. This results in Zn release and formation of MT-disulfide bonds which is subjected to degradation due to depletion of GSH, a modulator of Zn release and transfer from MT, by γ-irradiation. Hence, resynthesis of the apo form of MT, thionein is prevented (Kang, 2006). These data were not in agreement with those reported by Nada et al. (2013) who stated that the induction of metallothioneins by γ-irradiation appeared to be due to an increased synthesis in order to protect tissues against various forms of oxidative stress. Induction of MTs biosynthesis is involved in a protective mechanism against radiation injuries (Azab et al., 2004).

Conclusions:

Study has revealed that spermine intraperitoneal administration (10 mg/kg bw) with lead and/or gamma irradiation, tackled the oxidative stress parameters, signifying the importance of spermine as antioxidant in liver, kidney and brain tissue. On the other hand, administration of spermine per se may adversely affect the brain by induction of MDA, GSH, MT levels and catalase activity which could imply brain excitotoxicity, but spermine effect is more profound by administration with lead and/or gamma irradiation dramatically restored MDA, GSH, MT contents and catalase activity of the brain, a major sign for reversion of brain toxicity. The antioxidant action of Spm may be directly by detoxifying ROS (hydroperoxide, hydroxyl radical and by-product of lipid peroxidation) and indirectly by enhancing G6PD activity in RBCs and MT synthesis in liver, kidney and brain which can provide some beneficial effect against lead and/or gamma irradiation.

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الدور الوقائي للإسبيرمين في الجرذان المتعرضين للتسمم بالرصاص و/أو المشعّين الجامى

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اجريت الدراسة الحاليّة لدراسة الدور الوقائي للإسبيرمين ضد سمية التعرض للرصاص و/أو المشعّين الجامى في ذكور الجرذان. استخدمت ثمان مجموعات من الجرذان في هذه الدراسة وشملت المجموعة الضابطة، المجموعة المعالجة بالرصاص (6 جرّاء)، المجموعة المعالجة للإشعاع (10 مجم/كم/كم من وزن الجسم)، مجموعة الإسبيرمين (10 مجم/كم/كم من وزن الجسم)، مجموعة الإسبيرمين والرصاص، مجموعة الرصاص والإسبيرمين، مجموعة المعرضة للإشعاع والرصاص، ومجموعة المعرضة للإشعاع والإسبيرمين، وذلك لمدة 4 يوما متتالية. تم قياس الصورة الكاملة للدم ونشاط إنزيم الجلوكوز فوسفات ديهيدروجينز في الدم. بالإضافة إلى ذلك، تم قياس مستوى المالون ثنائي الألدهيد، الجلوتاثيون، الميتالوثيونين ونشاط إنزيم الكتاليز داخل نسيج الكبد، الكلى، والمخ. أظهرت النتائج انخفاض نشاط إنزيم الجلوكوز فوسفات ديهيدروجينز بعد التعرض للرصاص و/أو المشعّين الجامى، بينما حدث انخفاض في مستويات الجلوتاثيون، الميتالوثيونين ونشاط إنزيم الكتاليز في المجموعات المعرضة للرصاص، بينما حدث انخفاض في مستويات الجلوتاثيون، الميتالوثيونين ونشاط إنزيم الكتاليز في المجموعات المعرضة للأشعة. 

وثقت الدراسة أن الإسبيرمين يعمل كمضاد اكسدة ويؤدي دوراً وكفاءة ضد الأضرار الناتجة عن التعرض للإشعاع الجامى و/أو الرصاص.