The modulating effect of melatonin against the genotoxicity of lead acetate

Dalia Demerdash Abd El-Monem

Zoology Dept., Girls College for Arts, Science and Education, Ain Shams University, Egypt

Received 21 February 2012; accepted 8 March 2012
Available online 6 September 2012

Abstract Lead represents a significant ecological and public health concern due to its toxicity and its ability to accumulate in living organisms. The present investigation was designated to assess the modulating effect of melatonin (MLT) against lead acetate (LA) genotoxicity. Three cytogenetic end points were considered: the frequencies of micronucleated polychromatic erythrocyte cells (MnPCEs) in the bone marrow, the chromosomal aberration in the primary spermatocytes and the frequency of sperm abnormalities. Male mice were used in this experiment; animals were divided into 8 groups of 6 animals each. First group received an oral gavage of solvent (4% ethanol) and served as control and the other groups received an oral gavage of MLT (10 mg/kg) and/or 10, 50, 100 mg/kg body weight of lead acetate for 11 days. Mice were scarified 24 h after the last treatment. Examination and analysis of MnPCEs and meiotic metaphases showed no mutagenic effect of melatonin. Meanwhile LA induced a significant ($P < 0.01$) increase in the three end points used in this investigation. Results showed that melatonin caused a significant reduction in MnPCEs and chromosomal aberrations in meiotic cells. PCE/NCE ratios in bone marrow also increased in relation to melatonin and LA treatments. Moreover, melatonin decreased the % of sperm abnormality by 28.3%. The data obtained in this study suggest that melatonin administration confers protection against damage inflicted by LA, and support the contention that melatonin protection is achieved by its ability as a scavenger for free radicals generated by LA.

Introduction Melatonin (MLT) is an endogenous molecule produced in the human and vertebrate pineal gland, the retina and possibly in some other organs. It is known as the “hormone of darkness,” and is secreted in darkness in both diurnal and nocturnal animals (Challet, 2007). Melatonin is a radical scavenger and functions as an antioxidant; it is proven to be a more potent antioxidant than vitamin E and vitamin A (Korkmaz et al., 2009). Thus, for its antioxidant potential, MLT has been proposed to use for the protection against molecular damage by oxygen and nitrogen-based toxic reactants (Reiter et al., 2004). Synthetic melatonin supplements have been used for a variety of medical conditions, most notably for disorders related to sleep (Rajaratnam et al., 2009). In addition, MLT displayed a marked role in the protection of testicular toxicity induced by radiation and different mutagenic and clastogenic agents (Anjum et al., 2011; Ji et al., 2012).
In an in vivo and in vitro micronucleus test, MLT significantly reduced micronuclei formation in both peripheral blood and bone marrow cells of lipopolysaccharide treated rats (Sewerynek et al., 1996). MLT inhibited the paraquat-induced increase in MnPCEs in mice bone marrow cells (Melchiorri et al., 1998) and in both peripheral blood and bone marrow cells of mice (Ortiz et al., 2000). MLT also caused a significant reduction in MnPCEs in bone marrow cells of albino mice.

Table 1  Frequency of micronucleated polychromatic erythrocytes (MnPCEs) and PCE/NCE ratio in mouse bone marrow post treatment with lead acetate and/or melatonin.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total MnPCEs/12000PCEs</th>
<th>Mean MnPCEs/2000PCEs</th>
<th>± SD</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17</td>
<td>2.83</td>
<td>1.7</td>
<td>0.926</td>
</tr>
<tr>
<td>Melatonin</td>
<td>16</td>
<td>2.67</td>
<td>1.6*</td>
<td>1.03</td>
</tr>
<tr>
<td>Lead acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg bw</td>
<td>39</td>
<td>6.5</td>
<td>1.87b</td>
<td>0.735</td>
</tr>
<tr>
<td>50 mg/kg bw</td>
<td>135</td>
<td>22.5</td>
<td>1.76c</td>
<td>0.663</td>
</tr>
<tr>
<td>100 mg/kg bw</td>
<td>129</td>
<td>21.5</td>
<td>1.87d</td>
<td>0.703</td>
</tr>
<tr>
<td>Melatonin + Lead acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg bw</td>
<td>31</td>
<td>5.16</td>
<td>1.69*</td>
<td>0.835</td>
</tr>
<tr>
<td>50 mg/kg bw</td>
<td>110</td>
<td>18.3</td>
<td>2.65g</td>
<td>0.738</td>
</tr>
<tr>
<td>100 mg/kg bw</td>
<td>109</td>
<td>18.17</td>
<td>1.94g</td>
<td>0.760</td>
</tr>
</tbody>
</table>

*a P > 0.05 compared with control.
*b P < 0.05 compared with control.
*c P < 0.01 compared with control.
*d P < 0.0001 compared with control.
*e P > 0.05 compared with 10 mg/kg LA.
*f P < 0.01 compared with 50 mg/kg LA.
*g P < 0.01 compared with 100 mg/kg LA.
** P > 0.05 compared with LA.

Table 2  Percentage of the different types of chromosomal aberrations in primary spermatocytes of male mice after treatment with lead acetate and/or melatonin.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Examined metaphases</th>
<th>Time intervals</th>
<th>Aberrant metaphases</th>
<th>Different types of chromosomal aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Mean % S.D.</td>
<td>AU X Y U AU + X YU CIII + I CIV CVI F Pol</td>
</tr>
<tr>
<td>Control</td>
<td>600</td>
<td>24 h after 11 days of treatment</td>
<td>26 4.33 1.21 6 4 2 – 8 – 6</td>
<td></td>
</tr>
<tr>
<td>Melatonin 10 mg/kg 600</td>
<td></td>
<td></td>
<td>28 4.67 1.37a 7 5 3 2 7 – – 4</td>
<td></td>
</tr>
<tr>
<td>Lead acetate</td>
<td>10 mg/kg bw 600</td>
<td></td>
<td>58 9.67 2.17b 16 10 5 – 17 2 – 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/kg bw 600</td>
<td></td>
<td>145 24.17 2.63c 34 17 3 11 39 13 9 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 mg/kg bw 600</td>
<td></td>
<td>156 26 2.37d 39 12 15 7 45 12 7 19</td>
<td></td>
</tr>
<tr>
<td>Melatonin + Lead acetate</td>
<td>10 mg/kg bw 600</td>
<td></td>
<td>34 5.67 1.64e 9 6 – – 10 – – 9</td>
<td></td>
</tr>
<tr>
<td>50 mg/kg bw 600</td>
<td></td>
<td></td>
<td>116 19.33 2.5f 20 11 – 3 37 16 3 26</td>
<td></td>
</tr>
<tr>
<td>100 mg/kg bw 600</td>
<td></td>
<td></td>
<td>136 22.67 2.4g 30 7 12 10 40 18 5 14</td>
<td></td>
</tr>
</tbody>
</table>

*AU = autosomal univalent.
*X – Y U = X – Y univalent.
*CIII + I = chain of three plus one.
*CVI = chain of four.
*CVI = chain of six.
*F = fragment.
*Pol = Polyploidy.
*a P > 0.05 compared with control.
*b P < 0.001 compared with control.
*c P < 0.0001 compared with control.
*d P < 0.01 compared with 10 mg/kg bw LA.
*e P < 0.01 compared with 50 mg/kg bw LA.
*f P < 0.05 compared with 100 mg/kg bw LA.

In an in vivo and in vitro micronucleus test, MLT significantly reduced micronuclei formation in both peripheral blood and bone marrow cells of lipopolysaccharide treated rats (Sewerynek et al., 1996). MLT inhibited the paraquat-induced increase in MnPCEs in mice bone marrow cells (Melchiorri et al., 1998) and in both peripheral blood and bone marrow cells of mice (Ortiz et al., 2000). MLT also caused a significant reduction in MnPCEs in bone marrow cells of albino mice.
The modulating effect of melatonin against the genotoxicity of lead acetate

Figure 1  The frequency of Mn-PCEs of mice treated with lead acetate and/or melatonin.

Figure 2  Mean percentage of the different types of chromosomal aberrations in primary spermatocytes of male mice after treatment with lead acetate and/or melatonin.

Figure 3  The percentage of sperm abnormalities induced after lead acetate and/or melatonin treatment.
(Badr et al., 1999); in peripheral blood of rats (Ortega-Gutiérrez et al., 2009); in peripheral blood and bone marrow cells of CD2-F1 male mice (Vijayalaxmi et al., 1999) and rats (Assayed and Ab-del-aty, 2009) exposed to radiation. In addition, MLT decreased the frequency of CA and SCEs in human lymphocytes (Awara et al., 1998) and in spermatogonial and primary spermatocyte chromosomes of mice exposed to radiation (Badr et al., 1999).

Lead represents a significant ecological and public health concern due to its toxicity and its ability to accumulate in living organisms. Earlier studies have demonstrated that lead can pass through the blood–testis barrier, accumulate in the testis and/or epididymis and affect the germinal cells at different levels of differentiation (spermatogonia, primary spermatocytes, spermatids or spermatozoa) (Apostoli et al., 1999). Several studies assessed the genotoxic effect of lead acetate (LA) by means of chromosomal aberrations and micronucleus test. Regarding the induction of chromosomal aberrations, LA induced significant increase of aberrant cells and numerical aberrations in bone marrow cells of Wistar rats (Lorenz et al., 1996; Nehéz et al., 2000). Additionally, Aboul-Ela (2002) detected a significant increase of structural chromosomal aberrations in bone marrow cells and primary spermatocytes of albino mice treated with LA. In addition, LA proved to be a potent micronuclei inducer in vivo and in vitro test systems: LA induced micronuclei in kidney cells of Sprague–Dawley albino rats (Robbiano et al., 1999); in human melanoma cell (Poma et al., 2003); in Chinese hamster V79 cells (Thier et al., 2003; Bonacker et al., 2005); in Wistar rats’ leukocytes, reticulocytes and erythrocytes (Kašuba et al., 2004; Çelik et al., 2005); in rats’ erythrocytes (Piao et al., 2007; Alghazal et al., 2008); in peripheral blood erythrocytes, gill and fin epithelial cells of Carassius auratus auratus (Çavas, 2008) and in bone marrow cells of Algerian mice (Tapisso et al., 2009). Moreover, positive results indicate the induction of SCE with LA was obtained by Poma et al. (2003) in human melanoma cells and in bone marrow cells of Algerian mice (Tapisso et al., 2009).

The objective of this study is to assess the protective effect of melatonin to modulate the frequency of lead acetate-induced genotoxicity in male mice.

![Micronuclei induced after lead acetate and/or melatonin treatment.](image-url)
Materials and methods

Animals and treatments

The present study was carried out using 48 adult male mice 9–12 weeks’ age and 25–30 g in weight which were purchased from the animal house of The National Research Center, Cairo, Egypt. Animals were kept in groups of six in different cages, and acclimatized for 7 days before dosing. Standard laboratory chow and fresh tap water were provided ad libitum. Mice were divided into 8 groups of 6 animals each. Animals in the first group were given the solvent (4% ethanol) via oral gavage and served as control. Mice in the other groups received oral gavage of 10 mg/kg bw melatonin (Vijayalaxmi et al., 1999) and or 10, 50 and 100 mg/kg bw lead acetate for 11 days. Mice were sacrificed 24 h after the last treatment.

Chemicals

Lead acetate, also known as lead diacetate, plumbous acetate, sugar of lead, salt of Saturn, and Goulard’s powder, is a watersoluble white crystalline chemical compound with a sweetish taste and was purchased from Sigma–Aldrich, Melatonin (5-Methoxy-N-acetyltryptamine), and fetal calf serum were purchased from Sigma–Aldrich. Melatonin solution was made by dissolving 1 mg in 4% ethanol.

Micronucleus test

Both femurs were dissected and bone marrow was flushed from the femoral cavity with fetal calf serum. The cells were dispersed by gentle pipetting and collected by centrifugation at 1000 rpm for 10 min the pellet was resuspended in a small

![Figure 5](image_url) Abnormal meiotic metaphases in 1ry spermatocytes of male albino mice treated with lead acetate.
volume of fetal calf serum and used for smear preparation. After air-drying, the smears were stained by Giemsa (Schmid, 1976). From each animal, 2000 PCEs were examined for micronucleated polychromatic erythrocytes (Mn-PCEs) under 1000 magnification using light microscope. In addition, the number of PCEs among 500 total erythrocytes (PCE + NCE) per animal was recorded to evaluate bone marrow toxicity.

Meiotic preparation and scoring criteria

Mice were sacrificed 12 days from the beginning of treatment, where sample MI cells were at preleptotene at the beginning of the treatment as recommended by Ciranni and Adler (1991). Chromosomal preparations were made according to the air-drying method of Evans et al. (1964). Mice were injected (i.p.) with colchicines (0.1%) 2 h before killing by cervical dislocation. The testes were transferred to 2.5 ml of a 2.2% citrate solution in Petri dishes and the tunica removed. The contents of the tubules were gently teased out with curved forceps. The cell suspension produced was aspirated well and centrifuged at 1000 rpm for 10 min the supernatant was discarded, and the pellet was resuspended in 2 ml of hypotonic solution (1% sodium citrate) at 37 °C. After 12 min, the suspension was centrifuged for 10 min at 1000 rpm. Then the supernatant was removed. The cells were fixed 3 times with cold fixative solution (3:1 of methanol and glacial acetic acid). Slides were stained with Giemsa in phosphate buffer (pH 6.8) for 8 min. Hundred primary spermatocytes/mouse at diakinesis-metaphase I were scored. Abnormalities recorded included univalents (x–y univalent, autosomal univalent), reciprocal translocations, polyploidy and fragments.

Sperm shape abnormality assay

Evaluation of sperm-shape abnormality was made according to the technique described by Wyrobek and Bruce (1978). After the animal was sacrificed cauda epididymis was dissected out and placed in a Petri-plate containing 0.5 ml of saline solution (0.9% NaCl) at room temperature. The epididymis was cut into small portions to allow the sperms to swim out. After that, the smears were prepared using 2–3 drops of the solution, air dried overnight, fixed with absolute methanol for 15 min and stained with hematoxylin and eosin. One thousand sperms per animal were examined to determine the morphological abnormalities under oil immersion. Sperm shape abnormalities were classified as sperm head morphology which was categorized as normal, quasi-normal, amorphous, triangular, collapsed, deformed orientation, acute curvature, coiled head, banana shape, and without hook and tail abnormality. Data are shown in terms of % of abnormal sperms.

Statistical analysis

Data from control and treated animals for all tests were analyzed statistically to assess the significant differences using student’s t-test.

Results and discussion

Results showed that melatonin had no significant effect on the induction of micronuclei in bone marrow cells and chromosomal aberrations in primary spermatocytes which showed nearly the same values of control as shown in Tables 1 and 2. This indicates that melatonin is neither clastogenic nor mutagenic and can be used as a protective agent without any side effects, this observation agrees with Vijayalaxmi et al. (1995), Musatov et al. (1997) and Badr et al. (1999).

Meanwhile, lead acetate induced significant (P < 0.01) increases in bone marrow micronuclei when compared with control group (Table 1 and Fig. 1). The most frequent types of micronuclei observed were dot like and round shape (Fig. 4). The middle dose of LA (50 mg/kg bw) induced the highest frequency of MnPCEs. In what concerns the polychromatic to normochromatic ratios, lead acetate (50 and 100 mg/kg)

### Table 3 Percentages of abnormal sperms in mice post-treatment with lead acetate and/or melatonin.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time intervals</th>
<th>No of examined sperms</th>
<th>Types of sperm head abnormalities</th>
<th>Tail abnormalities</th>
<th>Total %</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24 h after the last treatment</td>
<td>6000</td>
<td>q bh am t bs do ac v-h col c-h wh</td>
<td>Total %</td>
<td>137</td>
<td>2.28</td>
</tr>
<tr>
<td>Melatonin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg bw Lead acetate</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10 mg/kg bw</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg/kg bw</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>100 mg/kg bw</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melatonin + Lead acetate</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg bw</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>50 mg/kg bw</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg bw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

q = quasi-normal, bh = big head, am = amorphous, t = triangular, bs = banana shape, do = deformed orientation, ac = acute curvature, col = collapsed head, ch = coiled head, wh = without hook, SD = standard deviation.

a P > 0.05 compared with control.

b P < 0.001 compared with control.

c P < 0.0001 compared with control.

d P < 0.05 compared with 10 mg/kg bw LA.

e P < 0.01 compared with 50 mg/kg bw LA.

f P < 0.001 compared with 100 mg/kg bw LA.
significantly \((P < 0.01)\) reduced these ratios when compared with control, which indicates the toxicity of lead acetate on bone marrow cells and coincides with Jagetia and Aruna (1998).

Additionally, the mean percent of primary spermatocyte chromosomal aberrations in control samples was 4.33. This value increased after lead acetate treatment into 9.67, 24.17 and 26 (Table 2 and Fig. 2). These highly significant \((P < 0.01)\) increases indicate the clastogenic effect of lead acetate. Concerning the types of aberrations, both autosomal and \(x-y\) univalent were observed (Fig. 5). Reciprocal translocations were also detected as trivalent \((\text{CIII} + \text{I})\), quadrivalent \((\text{chain IV})\) and hexavalent \((\text{chain VI})\). Additionally, in few diakinesis-metaphases I, cells with fragments were also detected as listed in Table 2. The above results are in accordance with other studies using the micronucleus test (Aboul-Ela, 2002; Tapisso et al., 2009) as well as chromosomal aberration assay in primary spermatocytes (Aboul-Ela, 2002).

Moreover, a significant increase in the \% of abnormality in sperm head morphology was found in animals treated with lead acetate as compared to control group (Table 3 and Fig. 3). This result revealed that lead acetate caused clastogenic effect which coincides with Aboul-Ela (2002). In addition, results showed that abnormalities such as acute curvature, triangular and sperms with big head were the most frequently observed types after lead exposures (Fig. 6). On the contrary, the two deformed orientation and vacuolated head were the least common morphological abnormalities (Table 3).

Several in vitro and in vivo studies have shown that different LA metabolic pathways result in ROS generation and

![Figure 6](image_url) Different types of head or tail abnormalities after lead acetate and/or melatonin treatment. (a) Quasi-normal, (b) acute curvature, (c) big h, (d) amorphous, (e) hookless, (f) banana shape, (g) collapsed, (h) triangular, (i) deformed orientation, (j) vacuolated, (k) coiled tail and (l) forked tail.
alteration of antioxidant defense systems in animals (Hsu et al., 1997; Aykin-Burns et al., 2005; Xu et al., 2008). Haleagrahara et al. (2011) reported that exposure to lead acetate caused a marked increase in lipid peroxidation and a reduction in free radical scavenging enzymes in bone marrow. In the present investigation, the genotoxic effect of lead acetate in the three cytogenetic end points used could be due to the induction of lipid peroxidation and reactive oxygen species (ROS) which has been considered as one of the direct mechanisms underlying lead-mediated DNA damage (Acharya et al., 2003). The generation of highly reactive oxygen species (ROS), such as superoxide radicals (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals (OH) and lipid peroxides (LPO), in the aftermath of heavy metal ions are known to damage various cellular components including proteins, membrane lipids and nucleic acids (Halliwell and Gutteridge, 1989).

Additionally, Monterio et al. (1995) suggested that lead-induced oxidative stress by disrupting the delicate prooxidant/antioxidant balance exists within mammalian cells. Moreover, Aitken and Roman (2008) stated that both spermatogenesis and Leydig cell steroidogenesis are vulnerable to oxidative stress. In the present investigation, control samples showed 2.28% of abnormal sperms which presumed to be a result of naturally occurring errors in the differentiation process, or the consequence of an abnormal chromosome complement. As well, significant increases in the percents of sperm head abnormalities were detected after lead acetate treatment. These increases might be due to the effect of lead acetate on the spermatogonial cells, Leydig cells and lastly sertoli cells where increases might be due to the effect of lead acetate on the spermatocytes from 9.67, 24.17 and 26 to 5.67, 19.33 and 22.67 (Tables 5,16, 18.3 and 18.17 and decreased CA% in primary spermatocytes from 6.5, 22.5 and 21.5 following LA treatment into 593.

In conclusion, this study showed that lead acetate has a potent clastogenic effect and melatonin has the ability to evacuate that clastogenic effect as recorded by the percentage of the abnormalities which retained to control percentages.

References


