# The Protective Effect of Melatonin against Lead Acetate toxicity

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**Abstract:** The present study was designated to evaluate the protective effect of melatonin (MLT) against lead acetate (LA) toxicity through comet assay and histological studies. Male mice were used in this experiment; animals were divided into 6 groups of 4 animals each. First group received orally solvent (4% ethanol) and served as control and the other groups received orally MLT (10mg/kg b.wt) and/or 50, 100 mg/kg body weight of lead acetate for 21 days. Mice were scarified 24 hrs after the last treatment. The results indicated that MLT alone did not induce any significant changes in the DNA tail moment values of liver cells as compared with control. Also MLT showed normal histological picture of liver and kidney. In contrast, LA treated mice showed significant increases in the tail moment values of the liver cells. In addition, LA treated mice exhibited degenerated hepatocytes and portal inflammatory cell infiltrations. Also, the kidneys showed degenerated glomeruli, severe congestion and haemorrhages. Meanwhile, Co-administration of MLT with LA weakened the severity of DNA lesion in liver and the pathological changes in kidney and liver of LA treated mice. These results pointed out the protective effect of MLT against the toxicity of lead acetate.
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**Key words:** lead acetate, melatonin, comet assay, histological changes, liver, kidney, mice.

## 1. Introduction

Lead acetate (LA) is used in dyeing and printing cotton, in varnishes, chrome pigments, the manufacture of pesticides, antifouling paints, analytical reagents, hair dyes and as an astringent and water repellent (**Johonson, 1998**). Several *in vitro* and *in vivo* studies have shown that different LA metabolic pathways result in reactive oxygen species generation and alteration of antioxidant defense systems (**Hsu *et al*., 1997; Aykin-Burns *et al*., 2005 and 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 addition, lead and its conjugated compounds are known genotoxic agents affecting the integrity of chromosomes. Several studies indicated the genotoxic effect of LA, by means of chromosomal aberrations (**Nehéz *et al*., 2000**), micronucleus test (**Çavaş, 2008**) and sister chromatide exchange (**Tapisso *et al*., 2009**). Also, LA induced DNA damage in lung and liver of CD-1 rats (**Valverde *et al*., 2002**); in lymphocytes of humans (**Woźniak and Blasiak 2003**) and mice (**XU *et al*., 2008**); in rat germinal cells (**Nava-Hernández *et al*., 2009**). Additionally, **Woźniak and Blasiak** (2003) suggested that LA may induce single-strand breaks and double-strand breaks in DNA as well as DNA-protein cross-links

Moreover, lead is listed as a human carcinogen on the basis of rodent tests (**ARC, 1994**).

Liver plays a major role in lead's metabolism, which makes it in special risk due to the oxidative action yielding from lead metabolism. **Sivaprasad *et al.* (2004)** revealed that, lead-induced lipid peroxidation of cellular membranes, which plays a crucial role in the mechanisms of its hepatotoxic action. Additionally, lead is known to also affect the kidney, which is another important target (**Garçon *et al*., 2007**). Lead produces oxidative damage in the kidney as evidenced by enhancing lipid peroxidation (**Farrag *et al*., 2007; El-Nekeety *et al.*, 2009**).

On the other hand, melatonin which is the endogenous molecule produced in the human and vertebrate pineal gland in darkness (**Challet, 2007**), possesses immunomodulative, anti-inflammatory and antioxidant properties. MLT was reported to reduce the amount of membrane lipid peroxidation products and DNA damage (**Reiter *et al*., 2003, Konturek *et al*., 2007**). Moreover, MLT has been proven to be 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**).

This study aimed to assess the protective effect of melatonin against the genotoxicity of lead acetate using DNA comet assay in liver and determination of histological changes in both liver and kidney.

## 2. Material and Methods

### A- Animals and treatments

The present study was carried out using 24 adult male mice 9-12 months' 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 4 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 6 groups of 4 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 10mg/kg b.w melatonin (**Vijayalaxmi *et al*., 1999**) and or 50 and 100mg/kg b.w lead acetate for 21 days. Mice were sacrificed 24hrs after the last treatment. After sacrificing, Animals were rapidly dissected and the liver was cleaned and small pieces were left in –4º C for comet assay. Other samples (liver and kidney) were immediately fixed in 10% formalin solution for histological studies.

**B- Chemicals**

Lead acetate and Melatonin (5-Methoxy-N-acetyltryptamine) were purchased from Sigma-Aldrich. Melatonin solution was made by dissolving 1 mg in 4% ethanol. Normal melting agarose, low melting agarose, triton X-100, sodium N-lauryl sarcosine, ethylenediamine tetraacetic acid, trishydroxymethyl aminomethane, ethidium bromide, Giemsa, and other common chemicals were purchased from Sigma-Aldrich.

**D- Comet assay**

The alkaline single-cell gel electrophoresis was performed by the method of **Sasaki *et al.* (1997)**. The liver was minced and suspended in 4 mL of chilled homogenizing solution (0.075 M NaCl and 0.024 M $Na_2EDTA$, pH 7.5), then homogenized gently at 500 to 800 rpm on ice. To obtain nuclei, the homogenate was centrifuged at 700g for 10 min, and the pellet was gently resuspended in 4.0 ml of chilled homogenizing buffer. After that, 75 µl of a mixture containing equal volumes of sample (nuclei preparation) and low melting agarose (2% in phosphate buffer saline) was quickly layered on 1% normal melting point agarose (NMA) (prepared in distilled water) precoated and overnight dried slides and covered with a coverslip. Then the slides were placed on a chilled plate to allow complete polymerization of agarose. Finally 75 µl of 0.5% NMA in PBS was quickly layered in the same manner after removing the coverslip and allowed to solidify on chilled plate. Slides were then immersed in the lysis buffer (containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sarcosine, pH 10.0 adjusted with 10 N NaOH and with 5% DMSO and 1% Triton X-100 was added just before use) for 1 hr at $48^0$C in the dark. After lysis, the slides were transferred on a horizontal electrophoresis platform and immersed in electrophoresis buffer (300 mM sodium hydroxide and 1 mM EDTA, pH > 13.0) for 20 min for unwinding of DNA. Electrophoresis was performed for 15 min at constant voltage (1 V/cm and 300 mA). After electrophoresis, the slides were washed thrice with neutralizing buffer (0.4 M Tris-HCl, pH 7.4) for 5 min each. Slides were dehydrated in absolute methanol for 10 min and left at room temperature to dry. The whole procedure was performed in dim light or dark to minimize artefactual DNA damage. Just before visualization, each slide was stained with 50µl of ethidium bromide (20µl/ml), rinsed with water, and covered with a coverslip. The slides were examined at 200x magnification using Olympus fluorescent microscope. All slides were coded and examined blindly. A total of 100 randomly selected cells from two replicate slides (50 cells per slide) were examined per sample. The analysis of comet cells was done using TriTek Comet Score Freeware v1.5.

The tail moment (a measure of tail length X a measure of DNA in the tail) was taken as the measurement of DNA damage **(Olive *et al*., 1990).**

**Histological studies:**

Liver and kidney samples from mice were carefully dissected and fixed in 10% buffered formalin, then dehydrated through graded series of alcohol and embedded in paraffin. Sections (5µm) were cut and stained with Haematoxylin and Eosin (**Drury and Wallington, 1980**). The stained sections were examined and photographed under a light microscope.

**F- Statistical analysis**

Results of the different treatment groups were compared to data obtained from untreated mice using Students'*t*-test (**Fowler *et al.*,1998**). Significance was indicated by *P* values <0.05**.**

**3-Results and Discussion**

The SCGE assay is a rapid and sensitive procedure for quantitating DNA lesions in mammalian cells (**Fairbairn *et al.*, 1995).** In the present investigation, no significant induction of comets having well separated heads and tails were observed in melatonin treated group (Table1).In contrary, the alkaline comet assay revealed significant dose dependent increases in the tail moment values of LA treated groups as compared to controls (Table 1 ; Figure 1). This result is compatible with the findings of **Woźniak and Blasiak, 2003; Arif *et al*., 2008** and **Xu *et al*., 2008**. Additionally, **Valverde *et al*., 2002** declared that lead acetate inhalation induces systemic DNA damage in liver cells which make it a target organ of this metal. However, MLT Co-treatment significantly reduced the tail moment values from (9.04±1.3) after LA treatment (50mg/kg) to (2.66±3.12). These results are in agreement with **Sliwinski *et al*. (2007**).

Histological changes in animal liver tissue provide a rapid method to detect effects of irritants, not only in liver but in various tissues and organs **(Bernet *et al.*, 1999)**. In the present investigation, examination of H&E liver sections of both control and MLT groups

revealed that the parenchyma was formed of classic hepatic lobules having the central veins in their middle from the central veins branching and anastomosing cords of hepatocytes radiate. The hepatocytes appeared polyhydral in shape, with mildly vacuolated cytoplasm and rounded vesicular nuclei, some hepatocytes appeared binucleated, blood sinusoid were situated between cords of hepatocytes and lined by flattened endothelial cells and VonKupffer cells. Additionally, examination of kidney sections revealed normal cellular architecture with distinct kidney cell. However, examined liver sections of mice treated with LA after three weeks showed vacuolar, hydropic and fatty changes (Fig.2) as well as congestion and mononuclear inflammatory (Fig. 3). Some sections showed cells necrosis changes (Fig.4). Finally, the portal areas showed congestion (Fig. 5), mild fibrosis and degeneration (Fig.6). Similar results were reported by **Shalan *et al.* (2005); Badiei *et al.* (2006) and Khan *et al.* (2008).** Moreover, LA induced marked glomerular, tubular and interstitiail alterations in the kidneys treated groups, such as: hypercellularity, proliferation and glomerular basement membrane thickening (Fig. 7), congestion associated with the inter-tubular blood capillaries dilation (Fig. 8), necrotic proximal tubules (Fig. 9), increased periglomerular space (Fig. 10), severe congestion and haemorrhages, degeneration (Fig.11), these observation are in agreement with **Siddiqui *et al.* (2002).**

Oxidative stress can arise when the production of ROS exceeds the antioxidant capacity of the cell, resulting in damage to membrane lipids, cellular DNA, and cells proteins (**Ames *et al.*, 1993; Conklin, 2000**). In the present investigation, the DNA damage induced after LA administration could be attributed to the excessive generation of highly ROS (**Hsu *et al.*, 1997; Aykin-Burns *et al.*, 2005** and **Xu *et al.*, 2008**) and lipid peroxidation due to the metabolic pathways of LA (**Haleagrahara *et al.*, 2011)** which has been considered as one of the direct mechanisms underlying lead-mediated DNA damage **(Acharya *et al.*, 2003)**. Additionally, **Monterio *et al.*, 1995** suggested that lead-induced oxidative stress by disrupting the delicate prooxidant/antioxidant balance that exists within mammalian cells.

Moreover, alteration of the morphological structure of liver and kidney tissues of LA treated groups may be due to the oxidative damage occurred in the cellular membranes by the accumulation of LA oxidant metabolites, and by direct or indirect inhibition of antioxidant enzymes, reducing the total antioxidant protection of the cell, which affecting membrane structure and function and altering the physiological processes of these organs and tissues (**Rendón-Ramirez *et al.*, 2007**). These damages which are reflected in cellular structural, morphological and molecular changes which appeared in this investigation are in accordance with the observation of **Rendón-Ramirez *et al.*, 2007**; **Suradkar *et al.*, 2010; Ponce-Canchihuamán *et al.*, 2011**.

On the other hand, the powerful antioxidant capacity of melatonin which appeared in the present investigation in the form of reduction in DNA damage and histological recovery of liver and kidney may be attributed to its potential to eliminate free radicals by the donation of electrons (**Hardeland, 2005)** For example, melatonin may neutralize hydroxyl radicals released after LA metabolization by forming 3-hydroxymelatonin, which is excreted in the urine (**Tan *et al.*, 1998).** MLT may also interact with oxygen and nitrogen-based toxic reactants (**Reiter *et al.*, 2004**). Additionally, Metabolites of melatonin, including the major hepatic metabolite 6-hydroxymelatonin, as well as N-acetyl-N-formyl-5-methoxykynuramine and N-acetyl- 5-methoxykynuramine have been shown to detoxify radicals themselves (**Tan *et al.*, 2007**). In addition to these direct interactions with ROS, melatonin may induce upregulation of the activity of antioxidants and antioxidant enzymes, it appears to increase the activity and/or expression of hepatic antioxidant enzymes, such as superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (Gpx) and glutathione reductase (GSR), in the environment of oxidative stress **(El-Missiry, 2000; Meki and Hussein, 2001; Rodriguez *et al.*, 2004**; **Tomás-Zapico and Coto-Montes, 2005**), which involved in repair of lesions in cellular DNA (**Vijayalaxmi *et al.*, 2004**). MLT also has the ability to attenuate hepatic LPO **(Jung *et al.*, 2009).** Moreover, **Sliwinski *et al.*, 2007** reported the protective effect of MLT against oxidative DNA damage by chemical inactivation of a DNA-damaging agent as well as by stimulating DNA repair. In conclusion, melatonin remarkably reduced DNA damage induced by LA as well as, recovery of liver and kidney tissues and this ameliorative action of MLT could be attributed to its powerful antioxidant activity.

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**Table 1:** Means of tail moment in liver cells of mice post-treatment with lead acetate and/or melatonin.

| Treatment / Tail moment | control | melatonin | Lead acetate | | Melatonin + Lead acetate | |
|---|---|---|---|---|---|---|
| | 4% ethanol | 10 mg/kg | 50mg/kg bw | 100 mg/kg bw | 10 mg/kg MLT 50mg/kg bw LA | 10 mg/kg MLT 50mg/kg bw LA |
| mean | 1.11 | 1.37 | 9.04 | 10.4 | 2.66 | 8.97 |
| ± S.D. | 0.54 | 1.03 | 1.30 | 4.255 | 3.12 | 6.62 |
| t-test (*P*-values) | - | 0.67 | 0.0001 | 0.0049 | 0.009 | 0.73 |

**MLT = melatonin LA = lead acetate**

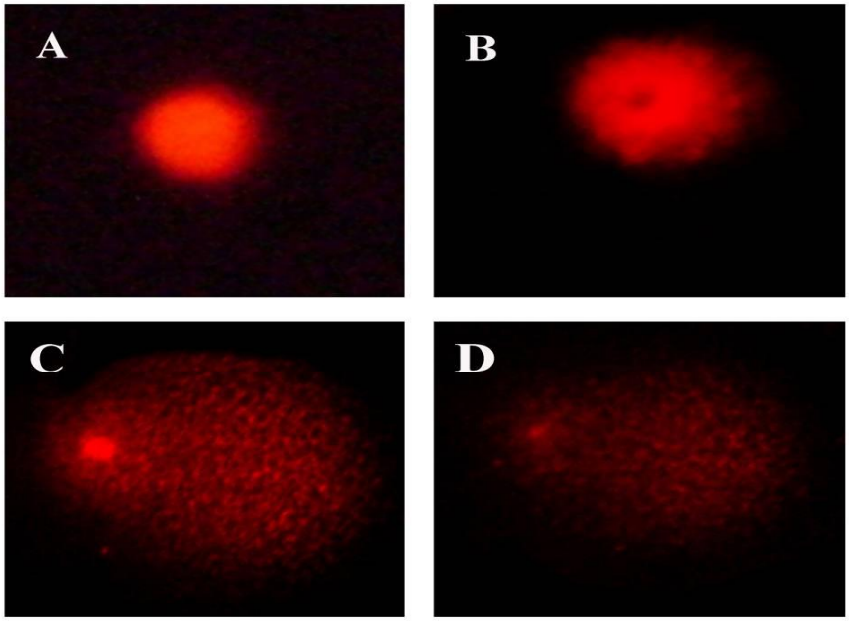


**Fig. 1: Various degrees of DNA damage induced after lead acetate and/or melatonin treatment from (A) undamaged cell; (B) slightly damaged cell to (C&D) highly damaged cells.**

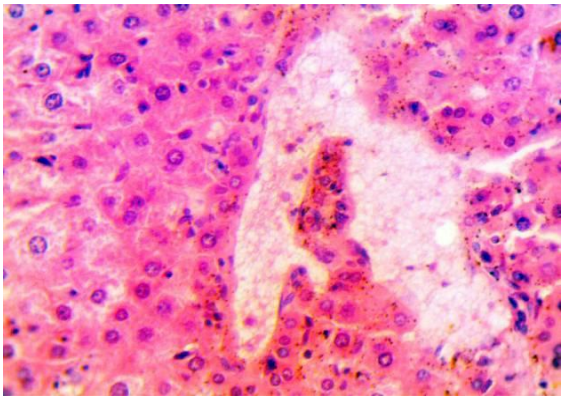

Fig. 2: examined liver sections of mice treated with LA after three weeks showed vacuolar, hydropic and fatty changes (HX&E)x400

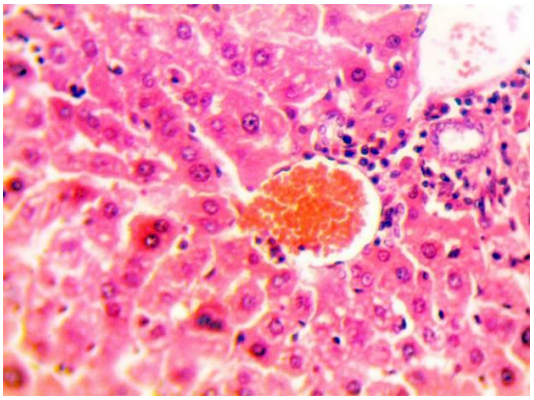

Fig.3: liver section showing congestion and mononuclear inflammatory. (HX&E)x400

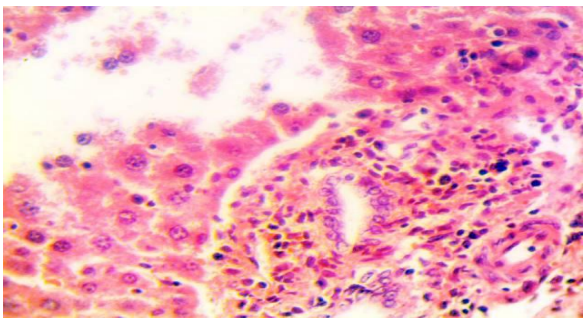

Fig. 4: liver section showing necrosis changes (HX&E)x400

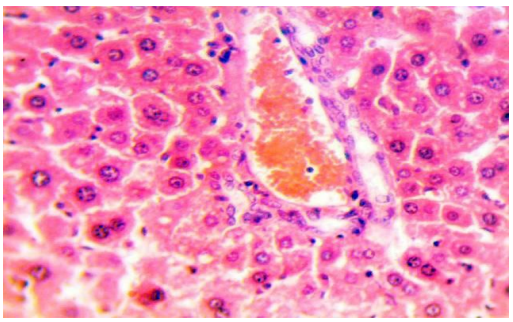

Fig. 5: liver section showing in congestion the portal area (HX&E)x400

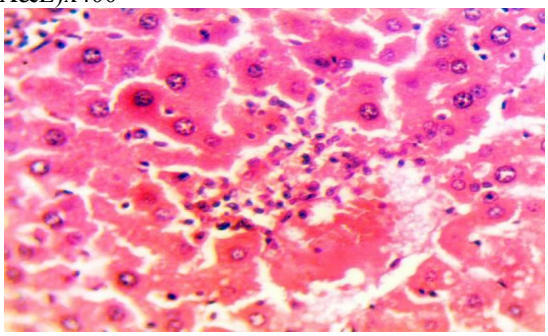

Fig.6: liver section showing mild fibrosis and degeneration (HX&E)x400

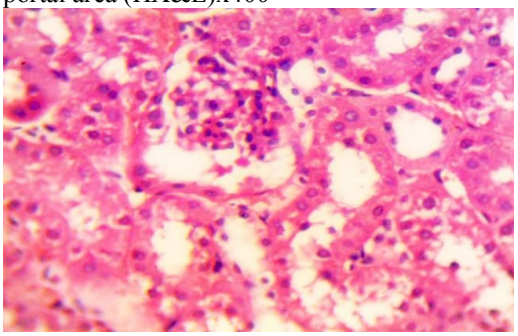

Fig. 7: kidney section showing hypercellularity, proliferation and glomerular basement membrane thickening (HX&E)x400

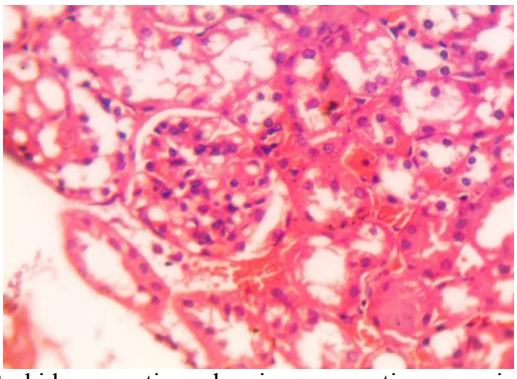

Fig. 8: kidney section showing congestion associated with the inter-tubular blood capillaries dilation (HX&E)x400

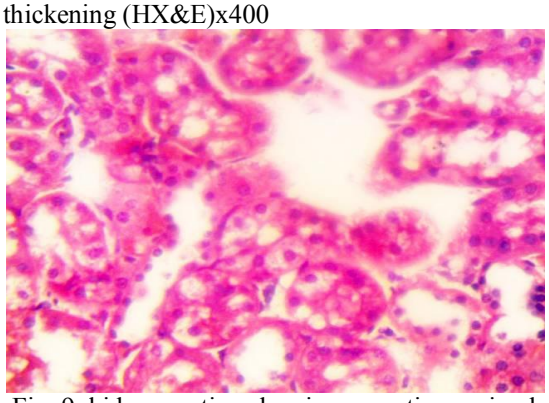

Fig. 9: kidney section showing necrotic proximal tubules (HX&E)x400

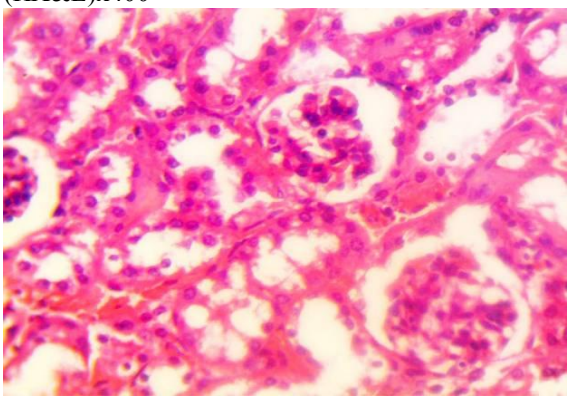

Fig. 10: kidney section showing increased periglomerular space (HX&E)x400

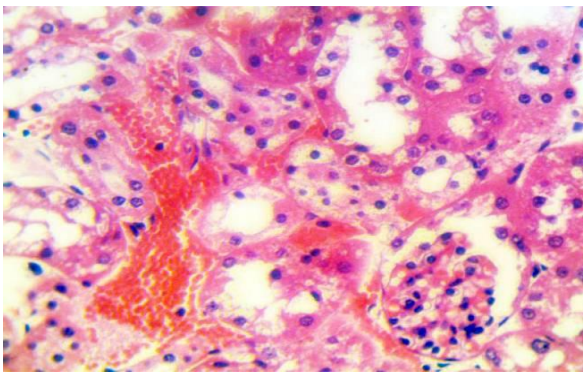

Fig. 11: kidney section showing severe congestion and haemorrhages, degeneration (HX&E)x400

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