Therapeutic Effects of Melatonin in Lead-Induced Toxicity in Rats

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Abstract

Exposure to lead results in significant accumulation in most of vital organs, and free radical damage has been proposed as a cause of lead-induced tissue damage, where oxidative stress is a likely molecular mechanism. This study was designed to evaluate therapeutic effects of melatonin in lead-induced organ toxicity in rats. The therapeutic effects of melatonin on lead induced toxicity in rats were evaluated using 36 rats, which were allocated into 3 groups and treated as follows: Group I, includes 12 rats injected subcutaneously with 0.2 ml physiological saline for 30 days, followed by treatment with a daily dose of 20mg/kg melatonin, administrated I.P for the successive 30 days; groups II and III, each includes 12 rats , injected with lead acetate 100 mg/kg/day s.c for 30 days, followed by treatment with intraperotoneal injection of physiological saline (0.2 ml) or melatonin 20mg/kg/day for the next 30 days. At the end of treatment period, the rats were sacrificed by an overdose (100mg/kg) of thiopental (twenty-four hour after the last injection). Craniotomy and laparotomy were performed to obtain the brains, livers and kidneys for the assessment of tissue damage. The changes in total body weight, weight of major organs (brain, liver and kidney), oxidative stress parameters, hemoglobin content, liver and renal functions, and histological appearance of the studied organs were evaluated and compared with that of negative and positive controls. Treatment with melatonin reverses the damage induced by lead in many organs and tissues through the reduction of MDA levels in RBCs, brain, liver and kidney; increases GSH levels in all studied organs; in addition to the improvement in the indices of the functions of the organs studied. These findings demonstrated that melatonin is capable of reversing damage of rat tissues caused by successive doses of lead acetate, and animals had restored their organ functions due to treatment with melatonin.

***Key words*: Melatonin, Lead poisoning, Oxidative stress**

الخلاصة

ان التعرض للرصاص يمكن ان يتسبب بتركزه في معظم الاعضاء الحيوية كما ان الاضرار الناجمة عن الجذور الحرة قد تكون هي المسببة للاضرار بهذه الاعضاء ,حيث ان الاجهاد التأكسدي هو الاكثر احتمالية ليكون الميكانيكية المسؤولة .تم تصميم هذه الدراسة لتقييم الفعالية العلاجية للميلاتونين في الفئران المصابة بالتسمم بالرصاص .ان التأثيرات العلاجية للميلاتونين على الفئران المصابة بتسمم الرصاص ليتم تقييمها باستخدام 36 فأرا ,قسمت الى ثلاث مجاميع تلقت المعالجات التالية :المجموعة الاولى تتضمن 12 فأرا .حقنت تحت الجلد ب 2, .مل من المحلول الملحي لمدة ثلاثين يوما ,وبعدها بجرعة 20 ملغم/كغم من الميلاتونين اعطيت في البريتون لثلاثين يوما .و المجموعتين الثانية والثالثة والتي ضمت 12 فأرا لكل منهما تم حقنها بخلات الرصاص بجرعة 100 ملغم /كغم /يوم تحت الجلد لثلاثين يوما اتبعت بجرع من المحلول الملحي)0،2 مل(او الميلاتونين 20 ملغم /كغم /يوم عن طريق حقن البريتون للثلاثين يوما التالية .في نهاية فترة المعالجة تم قتل الحيوانات باستخدام جرعة عالية من ثايوبنتال بعد مرور 24 ساعة من اخر معالجة .تم اجراء التشريح لاستحصال الادمغة و الاكباد والكلى لفحص تضررها .ان التغييرات في معدل اوزان اجسام واعضاء الحيوانات)الادمغة و الاكباد والكلى (و مؤشرات الاجهاد التأكسدي ومحتوى الخضاب فحوصات وضائفكل من الكبد والكلى والتغيرات النسيجية في الاعضاء المدروسة تم تقييمها بعد مقارنتها ب كل من مجاميع المقارنة السالبة والموجبة .ان المعالجة بالميلاتونين يمكن ان يعاكس الضرر الناتج بالرصاص في العديد من الاعضاء والانسجة من خلال تقليل اكسدة الدهون) مستوى المالوندالديهايد(في كل من خلايا الدم الحمر والدماغ والكبد والكلى ، اضافة الى زيادة مستوى الكلوتاثايون المدروسة مع تحسن ملحوظ في مؤشرات وظائف الاعضاء المدروسة .ان هذه النتائج توضح ان الميلاتونين له القابلية على عكس الاضرار الناجمة في انسجة الفئران من التعرض المتعاقب لجرع من خلات الرصاص ، وان الحيوانات قد استعادت وظائف الاعضاء فيها نتيجة المعالجة بالميلاتونين .

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Received : 23/9/2008
Accepted : 22/11/2008

Introduction

Lead Poisoning is one of the oldest occupational hazards in the world. Despite its recognized hazards, lead continues to have a wide spread commercial applications, including the production of storage batteries, pipes and metal alloys such as brass, solders, paints, glass and ceramics [(1)]. Once lead enters the body it binds sulfhydryl (SH) moiety of proteins with consequent impairment of their functions; by disrupting protein structure, it interferes with many enzyme systems in the body, thereby affecting the functions of most organs [(2)]. Lead also interferes with regulatory mechanisms that control the metabolism of many essential cations, particularly calcium, iron, zinc, sodium and potassium; it also alters the integrity of the cellular and mitochondrial membranes, thereby, increasing cellular fragility and facilitate degenerative processes [(3)]. Clinical manifestations of lead toxicity include symptoms referable to the central and peripheral nervous systems, hematopoietic, renal and gastrointestinal systems [(4)]. Lead poisoning is a potential factor in brain damage, mental impairment with severe behavioral problems, as well as anemia, kidney insufficiency, neuromuscular weakness and coma [(5)]. At the molecular level, it disturbs heme biosynthesis leading to accumulation of a variety of heme precursors including δ-aminolevulinic acid (ALA) [(6)]. Lead has effects on the hormonal regulation of calcium absorption, and lead toxicity is exacerbated in the presence of low dietary calcium [(7)]. It also displaces calcium in the mineral bone matrix, which may affect bone quality [(7)]. The effects on heme synthesis are the best studied toxic effects of lead; it inhibits the key enzymes, δ-ALAD and ferrochelatase (heme synthetase) [(8)]. As a result heme synthesis is retarded, and because heme moiety is important for the functions of cytochrome systems and cellular respiration, so lead shows an impact on the entire organism; it inhibits Na^+-K^+-ATPase pump attached to erythrocytes membrane leading to their lyses [(9)]. Many compounds with antioxidant properties have been evaluated for their protective effects against lead-induced toxicity in animal and human models [(10)]; moreover, melatonin has been used successfully to protect the nervous system against lead toxicity in rats [(11)]. The present study was designed to evaluate the therapeutic effects of melatonin in rats intoxicated with successive doses of lead.

Materials and Methods

Thirty six male rats (*Rattus norvegicus*) are used in the present study, weighing 200-250 g, housed in the animal house of the College of Pharmacy, University of Baghdad. The animals were maintained at controlled temperature (25 ± 2ºC) from November 2006 to April 2007, allowed free access to water, and fed standard rat chow *add libitum*. The therapeutic effects of melatonin on lead-induced toxicity in rats were evaluated using 36 rats, which were allocated into 3 groups and treated as follows: Group I, includes 12 rats injected subcutaneously with 0.2 ml physiological saline for 30 days, followed by treatment with a daily dose of 20mg/kg melatonin, administrated I.P for the successive 30 days; group II, includes 12 rats, injected with lead acetate 100 mg/kg/day s.c for 30 days, followed by treatment with intraperotoneal injection of physiological saline (0.2 ml) for the next 30 days; group III, includes 12 rats injected with 100mg/kg lead acetate s.c daily for 30 days, followed by treatment with intraperotoneal injection of melatonin 20mg/kg/day for the latter 30 days. At the end of treatment period, the rats were sacrificed by an overdose (100mg/kg) of thiopental (twenty-four hour after the last injection). Craniotomy and laparotomy were performed to obtain the brains, livers and kidneys for the assessment of tissue damage. After animals were sacrificed, blood samples were obtained by heart puncture and immediately placed into two tubes; an EDTA tube to get whole blood for the estimation of lead by atomic absorption in the Poisoning Consultation Center [(PCC), Medical City/ Baghdad], Hb, PCV, MDA and GSH in RBCs. The second fraction was transferred into plane tube to obtain the serum for analysis of other parameters (ALT, AST, ALP, Urea, and creatinine). In the plane tube, blood allowed to clot and serum was separated after centrifugation for (15-20) minutes at 3000 rpm and the resulted serum was kept frozen at (-18ºC) unless immediately analyzed was. Brains, livers, and kidneys were excised from each animal immediately, placed in chilled saline phosphate buffer solution, blotted with filter paper and accurately weighed. A 10% (W/V) tissue homogenate was prepared in phosphate buffer at 4ºC, using metal head tissue homogenizer which was adjusted at set 3 for one minute. All samples were kept frozen at (-18 ° C) unless analyzed immediately. Specimens from the brain, liver and kidneys were prepared for histopathological examination according to the method of Bauer [(12)], using paraffin sections technique.The significance of differences between mean values was calculated using unpaired Student's ***t***-test and analysis of

variance (ANOVA). *P* values less than 0.05 were considered significant for all data presented in the results.

Results

Administration of 100mg/kg lead acetate s.c for one month and treatment with saline for another month resulted in significant reduction in body weight after two months (25%). Therapeutic treatment with 20 mg/kg melatonin I.P for one month after intoxicated of rats with lead acetate resulted also in significant reduction in total body weight (6%), this level seem to be less than that reported when lead acetate was administered with saline (Table 1) . Malondialdehyde (MDA) levels in the RBCs, brain, liver and kidney tissues were significantly elevated after exposure of animals to 100mg/kg lead acetate (479%, 109%, 178% and 101% respectively, $p<0.05$) compared with 20 mg/kg melatonin treated animals. Therapeutic treatment with 20 mg/kg melatonin resulted in significant decrease in MDA levels in studied tissues (55%, 33%, 54% and 23% respectively, $p<0.05$) compared with animals challenged with 100 mg/kg lead acetate and saline only (Table 2).

Table 1. Effects of therapeutic use of 20 mg/kg melatonin on the total body weight and the weights of brain, liver and kidney in rats previously intoxicated with 100 mg/kg lead acetate.

Treatment groups	Weight (g)		Organ /body weight		
	Pre-treatment	Post-treatment	Brain/body	Liver/body	Kidney/body
Saline +Melatonin (20mg/kg) (n=12)	353.3 ± 1.88	385.8 ± 7.0 ^{a*}	0.004 ± 0.0002 ^{a}	0.027 ± 0.0005^{a}	0.003 ± 0.0001 ^{a}
Lead acetate (100mg/kg) + Saline (n=7)	349.8 ± 1.84^{a}	263.5 ± 9.55 ^{b*}	0.0057 ± 0.0002^{b}	0.05 ± 0.001^{b}	0.005 ± 0.0002^{b}
Lead acetate (100mg/kg) + Melatonin(20mgk) (n=10)	351.0 ± 3.14^{a}	330.0 ± 7.15 ^{c*}	0.004 ± 0.0001^{c}	0.033 ± 0.001^{c}	0.003 ± 0.0002^{c}

Data are expressed as mean ± SEM; n= number of animals;*Significantly different compared to pre-treatement value($P>0.05$) values with non-identical superscripts (a, b, c) within the same variable considered significantly different ($P<0.05$).

Table 2. Effects of therapeutic use of 10 or 20 mg/kg melatonin on the malondialdehyde (MDA) in erythrocytes, brain, liver and kidney in rats previously intoxicated with 100 mg/kg lead acetate.

Treatment groups	Malondialdehyde (MDA)			
	RBC (nmol/g Hb)	Brain (nmol/g tissue)	Liver (nmol/g tissue)	Kidney (nmol/g tissue)
Saline +Melatonin (20mg/kg) (n=12)	5.4 ± 0.12^{a}	48.9 ± 1.62^{a}	52.7 ± 1.31^{a}	24.4 ± 1.21^{a}
Lead acetate (100mg/kg) + Saline (n=7)	31.2 ± 2.48^{b}	101.9 ± 4.71^{b}	144.8 ± 5.56^{b}	49.1 ± 2.17^{b}
Lead acetate (100mg/kg) + Melatonin (20mgkg) (n=10)	13.9 ± 0.83^{c}	68.2 ± 1.89^{c}	66.5 ± 2.13^{c}	37.8 ± 1.87^{c}

Data are expressed as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same variable considered significantly different ($P<0.05$).

Table 3. Effects of therapeutic use of 20 mg/kg melatonin on the glutathione (GSH) levels in erythrocytes, brain, liver and kidney in rats previously intoxicated with 100 mg/kg lead acetate.

Treatment groups	Glutathione (GSH)			
	RBC (µmol/g Hb)	Brain (µmol/g tissue)	Liver (µmol/g tissue)	Kidney (µmol/g tissue)
Saline +Melatonin (20mg/kg) (n=12)	13.9 ± 0.13^{a}	11.8 ± 0.12^{a}	8.9 ± 0.13^{a}	7.8 ± 0.23^{a}
Lead acetate (100mg/kg) + Saline (n=7)	3.2 ± 0.19^{b}	4.4 ± 0.18^{b}	3.3 ± 0.12^{b}	4.1 ± 0.12^{b}
Lead acetate (100mg/kg) + Melatonin (20mgkg) (n=10)	6.1 ± 0.14^{c}	5.9 ± 0.11^{c}	7.0 ± 0.09^{c}	5.8 ± 0.10^{c}

Data are expressed as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same variable considered significantly different ($P<0.05$).

Daily treatment of rats with 100mg/kg lead acetate significantly reduces GSH levels in RBCs, brain, liver and kidney (77%, 63%, 64%, and 48% respectively, $p<0.05$) compared with 20 mg/kg melatonin treated animals. Meanwhile therapeutic treatment with 20 mg/kg melatonin, administered one month after lead acetate results in significant elevation of GSH in the studied tissues (88%, 34%, 115% and 41% respectively, $p<0.05$) compared with lead acetate and saline treated animals (table 3). Administration of 100 mg/kg lead acetate to the rats result in significant decrease in Hb levels and PCV %(12% and 9% respectively, $p<0.05$), when compared with melatonin 20 mg/kg treated group (table 4). Exposure of animals to s.c injections of lead acetate (100 mg/kg) for one month and saline for another month produces significant elevation in the serum levels of hepatic enzymes activity (AST, ALT, ALP)(162%, 232%, and 102% respectively, $p<0.05$) compared with 20 mg/kg melatonin treated animals. Therapeutic administration of melatonin in a dose of 20 mg/kg (39%, 53% and 42%) significantly reduces enzymes activities both with respect to lead acetate and saline treated animal group and between each other (table 5). However, therapeutic treatment of animals with melatonin, one month after lead acetate challenge, significantly reduces serum levels of urea and creatinine in which the reduction were (28% and 25% respectively, $p<0.05$), the reduction in serum level of their parameters was significantly different when compared with lead acetate and saline treated animals and between each others(table 6). Lead acetate, when administered subcutaneously, in a consecutive 100 mg/kg doses for one month and saline for another month produces significant elevation in blood lead levels (513%), and lead levels in brain, liver and kidney of these animals were also significantly elevated (3810%, 4736% and 2849% respectively, $p<0.05$) compared with 20 mg/kg only melatonin treated animals. Melatonin reduces lead levels significanty in all studied compartments (blood 28%, brain 46%, liver 40% and kidney 42%) compared with lead acetate and saline treated animals (table 7).

Table 4. Effects of therapeutic use of 20 mg/kg melatonin on the hematological parameters of rats previously intoxicated with 100 mg/kg lead acetate for one month.

Treatment Groups	Hb (mg/dl)	PCV %
Normal saline + Melatonin (20 mg/kg) (n=12)	14.6 ± 0.20^{a}	44.3 ± 0.81^{a}
Lead acetate (100 mg/kg) + Saline (n=7)	12.2 ± 0.29^{b}	37.3 ± 0.87^{b}
Lead acetate (100 mg/kg) + Melatonin (20 mg/kg) (n=10)	13.6 ± 0.15^{c}	40.7 ± 0.72^{c}

Data are expressed as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same variable considered significantly different ($P<0.05$).

Table 5. Effects of therapeutic use of 10 or 20 mg/kg melatonin on the liver enzymes (AST, ALT, and ALP) of rats previously intoxicated with 100 mg/kg lead acetate for one month.

Treatment groups	Liver enzymes level (U/L)		
	AST	ALT	ALP
Normal saline + Melatonin (20mg/kg) (n=12)	55.0 ± 1.53 [a]	36.0 ± 1.30 [a]	95.6 ± 2.27 [a]
Lead acetate (100mg/kg) + Saline (n=7)	144.2 ± 3.87 [b]	119.8 ± 3.23 [b]	192.9 ± 3.44 [b]
Lead acetate (100mg/kg) + Melatonin (20mgkg) (n=10)	87.7 ± 2.71 [c]	56.8 ± 2.14 [c]	112.5 ± 3.33 [c]

Data are expressed as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same variable considered significantly different (P<0.05).

Table 6. Effects of therapeutic use with 10 or 20 mg/kg melatonin on serum urea and creatinine of rats previously intoxicated with 100 mg/kg lead acetate for one month.

Treatment groups	Serum urea (mmol/L)	Serum creatinine (µmol/L)
Normal saline + Melatonin (20mg/kg) (n=12)	5.4 ± 0.13 [a]	72.8 ± 2.61 [a]
Lead acetate (100mg/kg) + Saline (n=7)	11.9 ± 0.62 [b]	190.7 ± 11.39 [b]
Lead acetate (100mg/kg) + Melatonin (20mgkg) (n=10)	8.5 ± 0.24 [c]	142.2 ± 4.73 [c]

Data are expressed as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same variable considered significantly different (P<0.05).

Table 7. Effects of therapeutic use of 10 or 20 mg/kg melatonin on lead levels in blood, brain, liver and kidney of rats previously intoxicated with 100 mg/kg lead acetate for one month.

Treatment groups	Lead level			
	Blood (µg/dl)	Brain (µg/gm)	Liver (µg/gm)	Kidney (µg/gm)
Saline + Melatonin (20mg/kg) (n=12)	12.98 ± 0.29 [a]	0.9 ± 0.05 [a]	2.18 ± 0.1 [a]	8.23 ± 0.26 [a]
Lead acetate (100mg/kg) + Saline (n=7)	79.54 ± 3.51 [b]	35.19 ± 1.33 [b]	105.43 ± 2.98 [b]	242.69 ± 2.28 [b]
Lead acetate (100mg/kg) + Melatonin (20mgkg) (n=10)	57.48 ± 2.15 [c]	18.92 ± 0.83 [c]	63.38 ± 1.88 [c]	141.57 ± 2.1 [c]

Data are expressed as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same variable considered significantly different (P<0.05).

Sections prepared from livers of rats, previously intoxicated with lead acetate 100 mg/kg, treated with saline for one month, showed a wide area of normal appearance with presence of small area of degeneration and necrosis with inflammatory cell
(figure 1). Meanwhile, treatment
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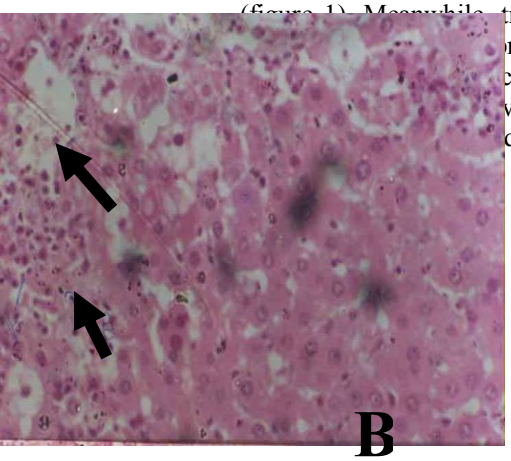

Figure (1). Section of liver tissue showing a wide area of normal appearance with presence of small area of degeneration and necrosis (arrow A) with inflammatory cells in rats treated with icated with 100mg/kg nonth. Magnification: d eosin stain).

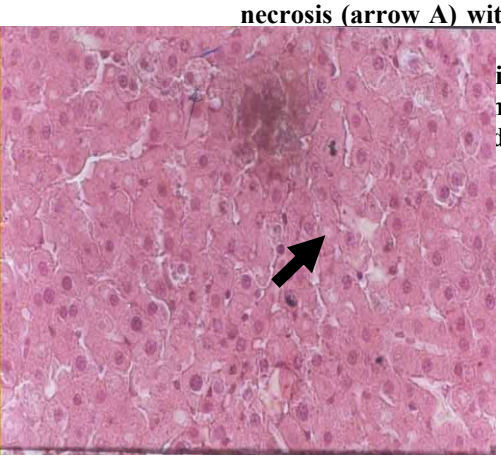

Figure (2). Section of liver tissue showing normal histology with appearance of few discrete degenerative changes (arrow) in rats treated with 20 mg/kg melatonin previously intoxicated with 100mg/kg lead acetate for one month. Magnification: 200X (hematoxylin and eosin stain).

Sections prepared from kidneys of rats treated with saline and previously intoxicated with 100 mg/kg lead acetate for one month, showed mild degenerative changes and necrosis in the kidney tubules (figure 3). Meanwhile, kg melatonin to group cated with lead acetate ey sections showed still there is slight ules (figure 4).

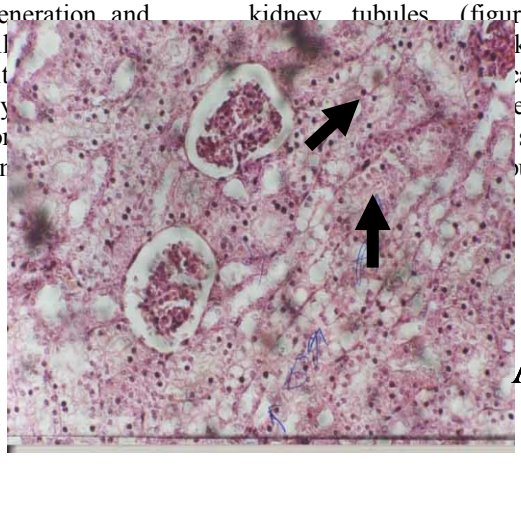

Figure (3). Section of kidney tissue showing mild degenerative changes (arrow A) and necrosis (arrow B) in the kidney tubules in rats treated with saline previously intoxicated with 100mg/kg lead acetate for one month. Magnification: 200X (hematoxylin and eosin stain).

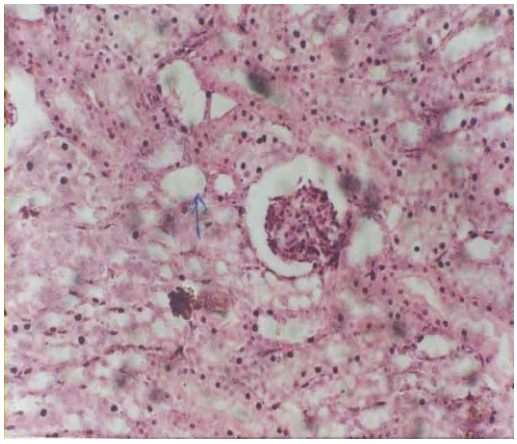

Figure (4). Section of kidney tissue showing normal histology but still there is slight dilatation of renal tubules (arrow) in rats treated with 20 mg/kg melatonin previously intoxicated with 100mg/kg lead acetate for one month. Magnification: 200X (hematoxylin and eosin stain).

Discussion

Daily administration of 100 mg/kg lead acetate to rats, reduce their total body weights compared with control animals with subsequent elevation of organ/body weight ratios, and treatment with melatonin restores body weights and the impaired organ/total body weight ratio. Lead poisoning is very well known to affect numerous organ systems, and is associated with a number of morphological, biochemical and physiological changes that include kidney dysfunction, impaired glucose metabolism, CNS disturbances, impairment of liver function and hematological disorders [13]. Among their effects, the impaired glucose metabolism is considered as a major pathway that may be followed by changes in total body or organ weights; in this respect intoxication with lead reduces the rate of glucose metabolism, with consequent reduction of the required energy for many anabolic process, and the profound decrease in serum glucose level which is reported in rabbits intoxicated with lead, might also be a cause for tissue wasting due to inappropriate availability of energy. The findings of the present study are found compatible with those reported by others [14], where loss of total body weight is found parallel with the increase in blood lead levels; furthermore, the increase in oxidative stress exhibited contributing factor, where lipid peroxidation might predispose to perturbation in the content of lipids in many organs and tissues. Exposure to lead acetate significantly elevates MDA levels in erythrocytes, brain, liver and kidney; while therapeutic use of melatonin results in significant reduction in the MDA levels in all compartments compared with control groups; their results are found compatible with those reported previously [15]. In this respect also, lead depletes the natural antioxidant molecule, the glutathione in the erythrocytes, brain, liver and kidney, and the use of melatonin therapeutically improves the levels of this antioxidant thiol in their compartments; their results are in agreement with those reported by others [16]. Lead-induced enhancement of lipid peroxidation is a major mechanism for some of the toxic effects of lead in different organ and tissues have certainly been suggested earlier [17]. Lead crosses the blood brain barrier and causes immediate effects by altering the metabolism and physiology of the brain and other organs like liver and kidney. One likely molecular mechanism involved in lead toxicity is the disruption of the pro-oxidant/ antioxidant balance [18] which leads to tissue injury via oxidative damage to critical biomolecules such as lipids, proteins, and DNA. After absorption of lead into the blood, 99% of lead is bound to erythrocytes and the remaining 1% stay in plasma to be carried to other tissues. Decreased hematocrit and hemoglobin levels might arise from reduction in serum copper as well as reduced iron metabolism and consumption induced by lead [19]. Development of anemia in lead toxicity may be attributed to the decreased red blood cell survival because of the increased membrane fragility, reduced RBC count, decreased hemoglobin production, or summation of all these factors [20]. The activities of serum enzymes AST, ALT, and ALP showed significant elevation in rats exposed to lead, administration of melatonin reduces these activities but remain significantly elevated when compared with control groups, these findings are compatible with other previous studies [21]. Increasing the activities of AST, ALT and ALP in serum was most likely a consequence of the hepatotoxic effect of lead, the accumulated lead in the liver directly damaging the hepatocytes, primarily by destroying the permeability of the cell membrane, which results in the increased release of cytosolic enzymes AST, ALT and ALP into the circulation. The results of the present study demonstrated significant increase in both urea and creatinine levels in the serum of rats exposed to 100mg/kg lead acetate daily for one month, indicating renal damage. Lead poisoning causes renal dysfunction and such type of toxicity might be due its ability to cause oxidative damage to the renal tissue, which includes enhanced lipid peroxidation, DNA damage and the oxidation of protein sulfhydryl groups[22].Lead is a pervasive environmental pollutant known to induce a broad range of physiological, biochemical and behavioral dysfunction in human and laboratory animals. Based on the present results, it seems that lead levels in blood and tissues became significantly elevated when compared with control and melatonin treated groups, and in agreement with other previous studies [23]. The results of the present study enables the conclusion that melatonin, attenuates and reverses the tissue damage induced in experimental animals by lead acetate, and the therapeutic use of this pleiotropic hormone support the idea of the oxidative stress-induced damage due to lead toxicity.

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