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Comparative Study Between Therapeutic Effects of Melatonin and Zinc in Lead induced Toxicity in Rats

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الخلاصة:

صممت هذه الدراسة لتحديد التأثيرات العلاجية للميلاتونين وكذلك الزنك, أما كل على حدا أو بصورة مجتمعة على التأثيرات السمية بواسطة الرصاص على الكبد والكلية عند الجرذان الذكور.

تم تعريض مجاميع الحيوانات الخمسة التي تمت دراستها إلى جرعة يومية مقدارها 100ملغم/كغم من مادة خلات الرصاص تحت الجلد لمدة شهر واحد. تم استخدام (30ملغم /كغم) من مادة الميلاتونين او الزنك عن طريق البريتون بعد استمرار التسمم للرصاص لمدة شهر آخر.

تمت متابعة التغيرات الحاصلة في معايير فرط الأكسدة ، محتوى الدم من الهيموغلوبين ،فعالية الكبد والكليتين إضافة إلى التغييرات النسيجية التي طرأت على الأعضاء التي تمت دراستها مقارنة مع المجموعة القياسية (السلبية والايجابية).

يمكن الأستنتاج من هذه النتائج أن الميلاتونين والزنك لهم القابلية على منع الضرر النسيجي نتيجة للتعرض الى جرع متعاقبة من خلات الرصاص وأن الحيوانات المختبرية لها القدرة على أستعادة فعالية أعضاءها الحيوية نتيجة للعلاج بالميلاتونين والزنك معا بعد التعرض للرصاص.

Abstract

The present study was designed to investigate the therapeutic effects of melatonin as well as zinc in combination or each one alone against the hepatic and renal toxicity of lead in male rats.

Five groups of animals were allocated as follows: Group I, includes 12 rats injected subcutaneously with 0.2 ml physiological saline for 60 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 30mg/kg/day for the next 30 days. Group IV, include 12 rats, injected with lead acetate 100 mg/kg/day s.c for 30 days, followed by treatment with intraperotoneal injection of zinc sulphate 1.5 mg/kg/day for the next 30 days. Group V, includes 12 rats injected with 100mg/kg lead acetate s.c for 30 days followed by treatment with intraperitoneal injection of 30 mg/kg/day melatonin and 1.5mg/kg/day zinc sulphate for the latter 30 days. At the end of treatment period, the rats were sacrificed by an

Accepted 7 October 2008

overdose (100mg/kg) of thiopental (twenty-four hour after the last injection). Laparotomies were performed to obtain the livers and kidneys for the assessment of tissue damage. Oxidative stress parameter (MDA), 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 or zinc reverses the damage induced by lead in many organs and tissues through the reduction of MDA levels in RBCs, liver and kidneys; in addition to the improvement in the indices of the functions of the organs studied.

These findings demonstrated that addition of zinc to melatonin are capable of further reversing damage of rat tissues caused by successive doses of lead acetate, and animals had restored their organ functions due to combined treatment.

Introduction:

Lead (pb) is a pervasive environmental toxicant known to induce a broad range of pathological changes in tissues in both laboratory animals and humans[1]. A recent study indicates that lead suppresses human transferrin synthesis and affect iron metabolism [2]. In addition lead was shown to inhibit the uptake of transferrin bound iron by erythrocytes [3]. Recent study indicated that lead suppresses human trasferrin synthesis and affects iron metabolism by interfering with transferrin levels [4]. Lead interfere with biosynthesis of porphyrins and heme, and several screening test for lead poisoning make use of this by monitoring either inhibition δ-aminolevulinic acid dehydratase or appearance of aminolevulinic acid and coproporphyrin [3].

Chronic exposure to lead leads to its accumulation in vital organs with maximum concentration reported in kidneys [5]. Oxidative stress has been reported as one of the important mechanism of toxic effect of lead [6]. It's suggested that the changes in glutathione as well as antioxidant enzyme activities implicate oxidative stress in the toxicity of lead. Previous studies indicate that the disruption of reducing status of tissue leads to the formation of reactive oxygen species (ROS), which may damage essential bio-molecules such as protein, lipids and DNA [7,8]. Lead exposure may results in systemic mobilization and depletion of the cells intrinsic antioxidant defenses. At high levels, these reactive oxygen species can be toxic to cells and may contribute to cellular dysfunction and poisoning. Recent reports suggested beneficial role of antioxidants in the prevention and treatment of acute and chronic lead poisoning[9]. The proposed mechanism of action of these antioxidants is believed to shield the cells from influence of oxidative stress by scavenging the free radicals generation and halting lipid peroxidation chain reactions that may cause damage to DNA. Several anti-oxidative strategies may be therapeutically useful

Accepted 7 October 2008

including supplementation with antioxidant and up-regulation of endogenous anti- oxidative defense system [10].

Administration of melatonin, the pineal hormone N-acetyl-methoxytryptamine, participates in many physiological functions and exhibit antioxidant activity [11].Also; melatonin was reported to be more effective than glutathione in scavenging the highly toxic hydroxyl radical and more efficient than vitamin E in neutralizing the peroxy radical. Furthermore, this indole was shown to be an efficient protector of nuclear DNA, protein, and lipids in cellular membranes against free radical attack [12].

Although the antioxidant effects of zinc are manifested through stabilization of sulfhydryls by protecting certain enzyme sulfhydryls from oxygen inactivation, preventing enzyme thiol oxidation and disulfide formation. The most enzyme that has been effected is δ- aminolevulinate dehydratase which is sulfhydryl dependent, and there is a strong correlation between thiol oxidation state and enzyme activity [13].

The aim of the present study was to examine the effect of both melatonin and zinc on lead-induced changes in liver and kidney in relation to lipid peroxidation and their antioxidant effects on male rats.

Materials and Methods:

Sixty male rats (Rattus norvegicus) are used in the present study, weighing 200-250 g, housed in the animal house of the Drug Monitoring and Research Center. The animals were maintained at controlled temperature (25 ± 2ºC) from February 2008 to May 2008, allowed free access to water, and fed standard rat chow add libitum. The therapeutic effects of melatonin and Zinc on lead-induced toxicity in rats were evaluated using 60 rats, which were allocated into 5 groups and treated as follows: Group I, includes 12 rats injected subcutaneously with 0.2 ml physiological saline for 60 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 30mg/kg/day for the latter 30 days; group IV, includes 12 rats injected with 100mg/kg lead acetate s.c daily for 30 days, followed by treatment with intraperitoneal injection of zinc sulphate 1.5 mg/kg/day for the latter 30 days; group V, includes 12 rats injected with 100mg/kg lead acetate s.c for 30 days followed by treatment with intraperitoneal injection of 30 mg/kg/day melatonin and 1.5mg/kg/day zinc sulphate 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). Laparotomies were performed to obtain livers and kidneys for the assessment of tissue damage. After animals were sacrificed, blood samples were obtained by

Accepted 7 October 2008

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], MDA, Hb and PCV 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. 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 4^{o}C, using metal head tissue homogenizer which was adjusted at set 3 for one minute. All samples were kept frozen at (-18 ^{o} C) unless analyzed immediately. Specimens from the liver and kidneys were prepared for histopathological examination according to the method of Bauer [14], using paraffin sections technique. During treatment period, 1, 4, 2, 2 and 3 animals were died from the groups I, II, III, IV and V respectively.

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:

Malondialdehyde (MDA) levels in the RBCs, liver and kidney tissues were significantly elevated ($p<0.05$) after exposure of animals to 100mg/kg lead acetate compared with saline treated animals. Therapeutic treatment with 30 mg/kg melatonin resulted in significant decrease in MDA levels in studied tissues ($p<0.05$) compared with animals challenged with 100 mg/kg lead acetate and saline only, while those treated with 1.5 mg/kg zinc sulphate resulted in significant decrease in MDA levels in studied tissues ($p<0.05$) compared with animals challenged with 100 mg/kg lead acetate and saline only, except in kidneys tissues the MDA levels were non significant. Addition of 1.5 mg/kg zinc sulphate to the therapeutic treatment of 30 mg/kg melatonin simultaneously resulted in significant ($p<0.05$) reduction compared with animals in all remaining groups (table-1).

Administration of 100 mg/kg lead acetate to the rats result in significant decrease in Hb levels and PCV %($p<0.05$), when compared with animals in other groups. Therapeutic uses of melatonin and zinc in other groups resulted in significant elevation in both Hb and PCV (table-2).

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)($p<0.05$) compared with those animals received saline only. Therapeutic treatment of melatonin and zinc

Accepted 7 October 2008

either alone or in combination resulted in significant reduction in the serum levels of hepatic enzymes activity (table -3).

However, therapeutic treatment of animals with melatonin and zinc sulphate either alone or in combination, one month after lead acetate challenge, significantly reduces serum levels of urea and creatinine, 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-4).

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, and lead levels in liver and kidney of there animals were also significantly elevated compared with animals received saline only. Melatonin and zinc sulphate either alone or in combination produces further significant reduction in all studied compartments compared with lead acetate and saline treated animals (table-5).

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 cells infiltration (figure -1).

Treatment of rats with 1.5 mg/kg zinc sulphate previously intoxicated with 100mg/kg lead acetate for one month, the liver sections still showed the same histological changes in group of rats treated with 100 mg/kg lead acetate and saline.

Meanwhile, treatment of rats with 30 mg/kg melatonin either alone or concomitantly with zinc sulphate previously intoxicated with 100 mg/kg lead acetate for one month, the liver sections showed normal structure appearance with few discrete degenerative changes (figure -2).

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). Treatment of rats with 1.5 mg/kg zinc sulphate previously intoxicated with 100mg/kg lead acetate for one month, the kidney sections still showed the same histological changes in group of rats treated with 100 mg/kg lead acetate and saline.

Meanwhile, administration of 30 mg/kg melatonin to group of rats previously intoxicated with lead acetate 100 mg/kg, the kidney sections showed normal histology but still there is slight dilatation of the renal tubules (figure-4).

Accepted 7 October 2008

Treatment groups	Malondialdehyde (MDA)		
	RBC (nmol/g Hb)	Liver (nmol/g tissue)	Kidney (nmol/g tissue)
Saline (n=11)	5.3 ± 0.12^{a}	52.7 ± 1.31^{a}	28.4 ± 1.21^{a}
Lead acetate (100mg/kg)+ Saline (n=8)	31.2 ± 2.48^{b}	144.8 ± 5.56^{b}	49.1 ± 2.17^{b}
Lead acetate (100mg/kg)+ Melatonin (30mgkg) (n=10)	13.9 ± 0.83^{c}	66.5 ± 2.13^{c}	37.8 ± 1.87^{c}
Lead acetate (100mg/kg)+ Zinc sulphate 1.5mg/kg (n=10)	24.8 ± 0.89^{d}	101.8 ± 4.63^{d}	44.1 ± 2.13^{b}
Lead acetate (100mg/kg)+ Melatonin (30mgkg), Zinc sulphate 1.5mg/kg (n=9)	9.4 ± 0.14^{d}	61.3 ± 2.18^{e}	30.7 ± 2.42^{d}

Table-1: Effects of therapeutic uses of 30 mg/kg melatonin and 1.5mg/kg zinc sulphate on the malondialdehyde (MDA) in erythrocytes, liver and kidney in rats previously intoxicated with 100 mg/kg lead acetate.

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

Accepted 7 October 2008

Treatment Groups	Hb (mg/dl)	PCV %
Saline (n=11)	14.6 ± 0.20[a]	44.3 ± 0.81[a]
Lead acetate (100mg/kg) +Saline(n=8)	12.2 ± 0.29 [b]	37.3 ± 0.87[b]
Lead acetate (100mg/kg) +Melatonin (30mgkg) (n=10)	13.6 ± 0.15 [c]	40.7 ± 0.72[c]
Lead acetate (100mg/kg) +Zinc sulphate 1.5mg/kg (n=10)	13.0 ± 0.11 [c]	39.6± 0.72[c]
Lead acetate (100mg/kg) +Melatonin (30mgkg), Zinc sulphate 1.5mg/kg (n=9)	14.1± 0.29 [d]	44.3 ± 0.81[d]

Table-2: Effects of therapeutic uses of 30 mg/kg melatonin and 1.5mg/kg zinc sulphate on the hematological parameters of rats previously intoxicated with 100 mg/kg lead acetate for one month.

Treatment groups	Liver enzymes level (U/L)		
	AST	ALT	ALP
Saline (n=11)	55.0 ± 1.53 [a]	36.0 ± 1.30 [a]	95.6 ± 2.27 [a]
Lead acetate (100mg/kg) + Saline (n=8)	144.2 ± 3.87 [b]	119.8 ± 3.23 [b]	192.9 ± 3.44 [b]
Lead acetate (100mg/kg) + Melatonin (30mgkg) (n=10)	87.7 ± 2.71 [c]	56.8 ± 2.14 [c]	112.5 ± 3.33 [c]
Lead acetate (100mg/kg) + Zinc sulphate 1.5mg/kg (n=10)	104.5 ± 2.11 [d]	98.8 ± 2.13 [d]	127.3 ± 1.73 [d]
Lead acetate (100mg/kg) + Melatonin (30mgkg), Zinc sulphate 1.5mg/kg (n=9)	79.5± 1.91 [e]	49.8 ± 1.27 [e]	108.6 ± 3.77 [c]

Table-3: Effects of therapeutic uses of 30 mg/kg melatonin and 1.5mg/kg zinc sulphate on the liver enzymes (AST, ALT, and ALP) of rats previously intoxicated with 100 mg/kg lead acetate for one month.

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

Accepted 7 October 2008

Treatment groups	Serum urea (mmol/L)	Serum creatinine (µmol/L)
Saline (n=11)	5.4 ± 0.13 [a]	72.8 ± 2.61 [a]
Lead acetate (100mg/kg) + Saline (n=8)	11.9 ± 0.62 [b]	190.7 ± 11.39 [b]
Lead acetate (100mg/kg) + Melatonin (30mgkg) (n=10)	8.5 ± 0.24 [c]	142.2 ± 4.73 [c]
Lead acetate (100mg/kg) + Zinc sulphate 1.5mg/kg (n=10)	10.77 ± 0.43 [d]	182.2 ± 4.33 [d]
Lead acetate (100mg/kg) + Melatonin (30mgkg), Zinc sulphate 1.5mg/kg (n=9)	7.6 ± 0.12 [e]	133.1 ± 3.61 [e]

Table-4: Effects of therapeutic uses of 30 mg/kg melatonin and 1.5mg/kg zinc sulphate on serum urea & creatinine of rats previously intoxicated with 100 mg/kg lead acetate for 1 month.

Treatment groups	Lead level		
	Blood (µg/dl)	Liver (µg/gm)	Kidney (µg/gm)
Saline (n=11)	12.98 ± 0.29 [a]	2.18 ± 0.1 [a]	8.23 ± 0.26 [a]
Lead acetate (100mg/kg) + Saline (n=8)	79.54 ± 3.51 [b]	105.43 ± 2.98 [b]	242.69 ± 2.28 [b]
Lead acetate (100mg/kg) + Melatonin (30mgkg) (n=10)	57.48 ± 2.15 [c]	63.38 ± 1.88 [c]	141.57 ± 2.1 [c]
Lead acetate (100mg/kg) + Zinc sulphate 1.5mg/kg (n=10)	66.71 ± 3.0 [d]	88.64 ± 2.3 [d]	190.17 ± 3.5 [c]
Lead acetate (100mg/kg) + Melatonin (30mgkg), Zinc sulphate 1.5mg/kg (n=9)	49.33 ± 1.84 [e]	55.64 ± 1.22 [e]	116.23± 1.9 [e]

Table-5: Effects of therapeutic uses of 30 mg/kg melatonin and 1.5mg/kg zinc sulphate on lead levels in blood, liver and kidney of rats previously intoxicated with 100 mg/kg lead acetate for one month.

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

Accepted 7 October 2008

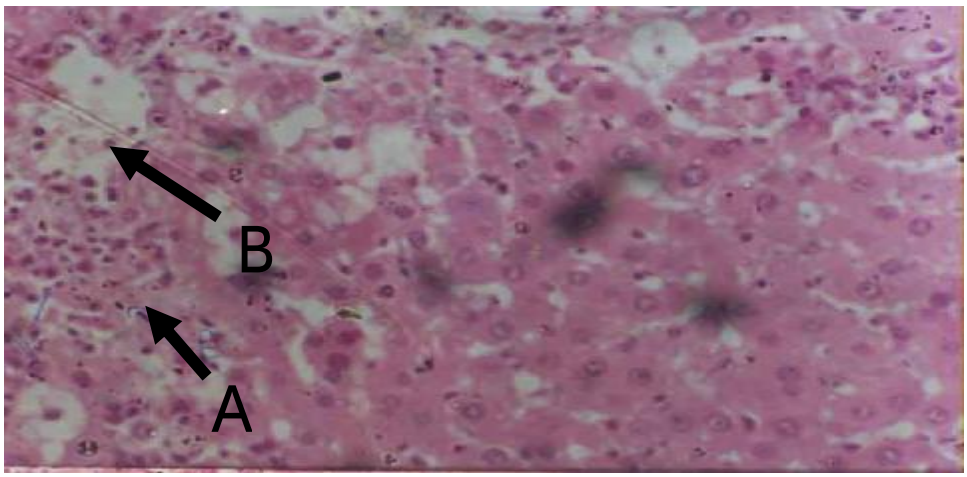

Fig-1: Section of liver tissue showing a wide area of normal appearance with presence of small area of degeneration and necrosis (arrow A) with inflammatory cells infiltration (arrow B) in rats treated with saline previously intoxicated with 100mg/kg lead acetate for one month. Magnification: 200X (hematoxylin and eosin stain).

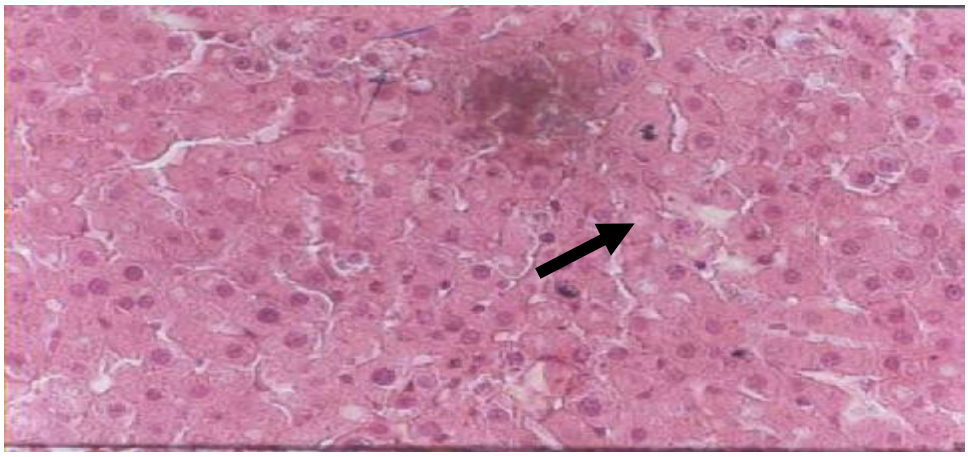

Fig-2: Section of liver tissue showing normal histology with appearance of few discrete degenerative changes (arrow) in rats treated with 30 mg/kg melatonin either alone or in combination with 1.5 mg/kg zinc sulphate previously intoxicated with 100mg/kg lead acetate for one month. Magnification: 200X (hematoxylin and eosin stain).

Accepted 7 October 2008

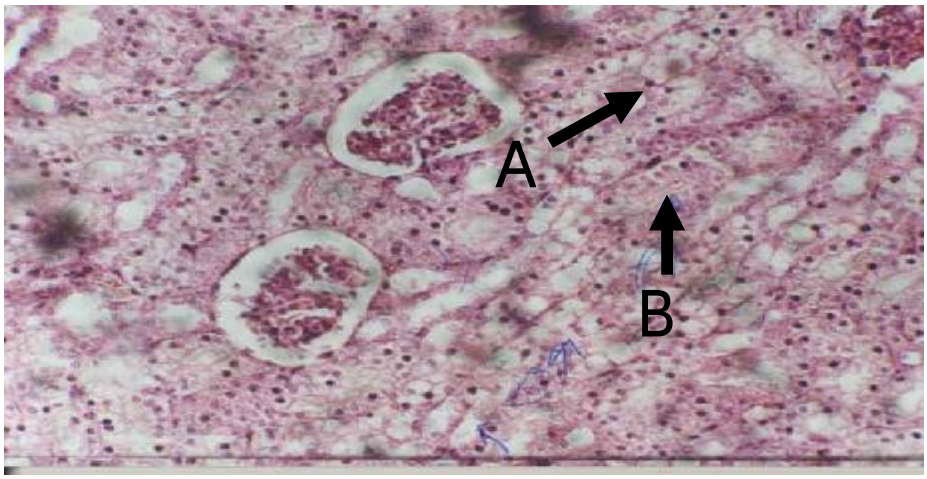

Fig-3: Section of kidney tissue showing mild degenerative changes (arrow-A) and necrosis (arrow B) in the kidney tubules in rats treated with saline previously intoxicated with 100mg/kg lead acetate for one month. Magnification: 200X (hematoxylin and eosin stain).

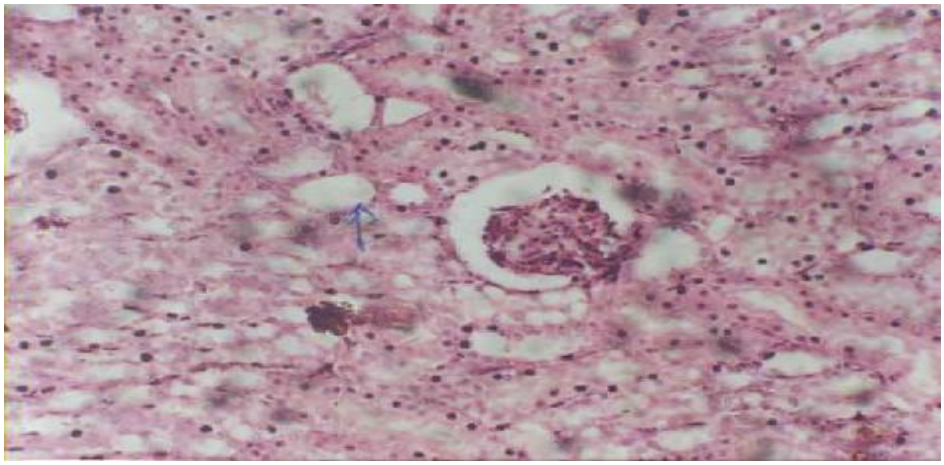

Fig-4: Section of kidney tissue showing normal histology but still there is slight dilatation of renal tubules (arrow) in rats treated with 30 mg/kg melatonin either alone or in combination with 1.5 mg/kg zinc sulphate previously intoxicated with 100mg/kg lead acetate for one month. Magnification: 200X (hematoxylin and eosin stain).

Discussion:

Exposure to lead decreased the endogenous antioxidants in the liver and kidney and attenuated haem biosynthesis [15, 16]. Lead administration also exerted toxic actions in other organs [17]. The toxic action produced by lead might be attributed to its ability to generate reactive oxygen species (ROS) which induce

Accepted 7 October 2008

oxidative damage in several tissues by enhancing lipid peroxidation through Fenton reaction [18].

In the current study the levels of the oxidized lipids, liver enzymes, serum urea and creatinine were increased while hemoglobin and PCV were decreased relative to those of controls. Our results are in agreement with other studies[19,20].

Administration of melatonin after intoxication with lead markedly hampered lead-induced toxicity on all studied parameters of blood, liver and kidneys. The protective effect of melatonin against lead-induced changes for maintaining normal measured parameters is probably complex and may be related to its lipophilic and hydrophilic nature as well as to localize manly in a superficial position in the lipid bilayer near the polar heads of membrane phospholipids [21]. The protective action of melatonin is modifying membrane organization, may due to melatonin's ability to scavenge free radicals which produced during peroxidation of lipids [22]. Since membrane functions and structure are influenced by proteins in membranes, and lead is known to damage thiol proteins, it is possible that the protective action of melatonin to membrane damage induced by lead may be related partially to the ability of the indole to prevent protein damage [23]. Besides the antioxidative properties of melatonin which serve to protect both DNA and lipids from oxidative abuse due to detoxification of free radicals, it also enhances the activity of antioxidatve enzymes. It has been shown that melatonin stimulates superoxide dismutase mRNA levels in several tissues. Melatonin was shown to prevent the loss of important dietary antioxidants including vitamins C and E, bind iron and participate in maintaining iron pool at appropriate level resulting in control of iron haemostasis, thereby providing tissue protection [24]. Thus, the spectrum of melatonin antioxidative actions is broad and it seems to be capable of efficiently counteracting oxidative stress induced by lead on cell progenitors.

Administration of zinc after intoxication with lead also shows reduction in oxidative stress and basically there are two mechanisms have been described: sulfhydryl stabilization and a reduction in the formation of $^{\bullet}OH$ from H_2O_2 and $O_2^{\bullet-}$ through the antagonism of redox-active transition metals, like iron and copper [25] and this is an acute effects, while chronic effects involve the exposure of an organism to zinc on a long term basis, resulting in an induction of some other substances that are the ultimate antioxidants. On the other hand, chronic zinc deprivation generally results in an increased susceptibility to some oxidative stress [26].

In this regard, the most studied effectors are the metallothioneins. The metallothioneins are group of low molecular weight (6000- 7000 KDa) metal binding proteins containing 60- 68 amino acid residues, of which 25- 30% are cysteine. They contain no aromatic amino acids or disulfide bonds and can bind 5- 7gm zinc (mol/ protein). Numerous studies have demonstrated that the chronic administration of zinc induces metallothionein synthesis in different

Accepted 7 October 2008

organs such as the liver, Kidney and intestine .The metallothioneins have been shown to have antioxidant effects under a variety of conditions, including radiation exposure, toxicity from anticancer drugs such as doxorubicin, ethanol toxicity and oxidatively mediated mutagenesis [27].

Based on the present results, it seems that lead levels in blood and tissues became significantly elevated when compared with control and treated groups whether melatonin alone or zinc alone or in combination which agreed with other previous studies [28].

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. In addition the uses of zinc in combination with melatonin have the ability to further improvement in the liver and kidneys from the damaging effects of exposure to lead through inhibition of lipid peroxidation and stimulation of endogenous atioxidative defense system.

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