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Research Article

MELATONIN PROTECTS AGAINST LEAD-INDUCED CARDIO TOXICITY: INVOLVEMENT OF ANTIOXIDANT MECHANISM

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ABSTRACT

Objective: The objective of the present studies is to find out whether melatonin is capable of providing protection to rat heart against lead acetate induced oxidative damage.

Methods: Rats of the first group were intraperitoneally (i.p.) injected with lead acetate [15mg/kg body weight(bw)], another group was pre-treated with melatonin (10 mg / kg, fed orally), the positive control group was fed melatonin (10 mg / kg bw), and the control animals received vehicle treatment i.p. for 7 consecutive days. Concentration of lead in cardiac tissue was estimated by AAS. The alterations in the activity of the different bio-marker enzymes of cardiac damage, levels of biomarkers of oxidative stress, activities of the antioxidant and some of the mitochondrial enzymes were studied. Histomorphological changes and alteration in tissue collagen level was also studied through H-E and Sirius red stainings respectively.

Results: Treatment of rats with lead acetate at the indicated dose for seven consecutive days caused significant accumulation of lead in cardiac tissue , alterations of all the parameters studied and caused injury to the cardiac tissue. All these changes were ameliorated when the rats were pre-treated with melatonin.

Conclusion: The results of the current studies indicate melatonin's ability to mitigate lead-induced oxidative damage in cardiac tissue of experimental rats possibly through its antioxidant mechanisms, and, may have future therapeutic relevance in humans exposed to lead environmentally or occupationally and in situations where chelation therapy has limited success.

Keywords: Antioxidant, Lead acetate, Melatonin, Oxidative stress, Tissue injury.

INTRODUCTION

The toxicity of metals is often explained on the basis of their ability to cause the generation of oxygen and nitrogen-based reactants [1]. Some heavy metals including mercury (Hg), cadmium (Cd) and nickel (Ni) have the ability to deplete the important endogenous antioxidant glutathione, and also to bind to sulfhydryl groups on proteins [2]. Several of these metals participate in the Fenton reaction which produces the highly reactive •OH. The toxicity of lead (Pb), another heavy metal , has been documented in experimental animals as well as in humans [2,3].

The indoleamine, melatonin, is produced in all animals from unicells to humans [2, 4, 5, 6] and also in plants [7]. Melatonin has many functions in organisms, i.e., it helps to synchronize circadian rhythms [8], promotes sleep [9] , strengthens immune system [10], regulates blood pressure and seasonal reproduction [11]. Melatonin has also been reported to be oncostatic [12], antidepressive [13] , etc. but it seems to be unparallel as an antioxidant from plants to humans [14. 15] .

Since its discovery as a potent antioxidant in 1993 [16] , the ability of melatonin (N-acetyl-5-methoxytryptamine) to protect all cells and organs from oxidative/nitrosative damage has been studied well [6, 17] .

Melatonin's functional repertoire in terms of limiting molecular destruction by both oxygen and nitrogen-based radicals and associated metabolites is highly diverse. This indole amine functions at all levels to aid in the ability of organisms to resist the onslaught of damage normally inflicted by radicals and radical related products. Thus, melatonin reduces free radical generation at the mitochondrial level in a process generally referred to as radical avoidance [18]. It stimulates antioxidative enzymes that convert highly toxic species to innocuous products [19], it promotes the synthesis of another antioxidant, glutathione, and it inhibits at least one enzyme, nitric oxide synthase (NOS), that normally produces free radicals, in this case NO• [20]. Melatonin has been reported to directly neutralize free radicals [20]. Moreover, several of its metabolites have also been shown to scavenge free radicals [21] thereby helping the cells to avoid molecular damage in the face of oxidative stress. Melatonin is also capable of modulating the activities of the enzymes that maintain the redox balance within the cells. Thus, melatonin, through these collective mechanisms appears to be an uncommonly efficient protector of molecules, subcellular organelles, cells, tissues, and organisms from both harm as well as death via apoptosis or necrosis due to a wide variety of toxic agents [22]. There are studies suggesting protective role of melatonin against lead-induced neurotoxicity [23, 24] and hematotoxicity in rats [25] and genotoxicity in lymphocytes [26] as well as also DNA damage [27]. There is no study yet regarding the capacity of melatonin to ameliorate lead induced cardiotoxicity in rats.

Herein, we provide evidence that a low pharmacological dose of melatonin provides protection to the cardiac tissue of rats from lead-induced oxidative injury. The results of the current studies indicate that this small indole provides protection against lead induced oxidative onslaught of the rat cardiac tissue possibly through its antioxidant mechanism(s) and point toward its therapeutic usefulness as a protective anti-oxidant against situations arising out of exposure of the humans to this heavy metal environmentally or occupationally and where chelation therapy has limited success.

MATERIALS AND METHODS

Chemicals

Melatonin and lead acetate were purchased from SRL Chemicals, India.Thiobarbituric acid (TBA) from Spectro Chem., India. All other chemicals used were of analytical grade and were procured from E.Merck, Germany, India and Sigma Chemicals, USA.

Animal treatment

Male Wistar rats of body weight 160-180 gm were used throughout the experiments. The animals were handled as per the guidelines of

Institutional Animal Ethics Committee (IAEC) in accordance with the committee for the purpose of control and supervision of experiment on animal (CPCSEA), Ministry of Environment and Forest, Government of India. All the experimental protocols had the approval of Institutional Animal Ethics Committee IAEC [IAEC/PROPOSAL/DB-2/2010, APPROVAL DATE:16/11/2011] of the Department of Physiology, University of Calcutta. Prof. P. K. Samanta, M.Sc. (Vet.), Ph. D., Professor and Veterinary Surgeon and CPCSEA Nominee to Department of Physiology, University of Calcutta, acted as the advisor for animal care and handling.

Pb induced cardiotoxicity *in vivo* and cardio protection with Melatonin

After acclimatization to laboratory conditions, the rats of the melatonin and the melatonin + lead acetate group were fed melatonin dissolved in normal drinking water, at a dose of 10 mg kg^{-1} body weight for 7 consecutive days. An hour after the melatonin was fed, the animals of the lead acetate and the melatonin +lead acetate treated groups were injected with lead acetate solution, i.p., at a dose of 15 mg kg^{-1} body weight (LD_{50} for lead acetate is 150 mg/ kg bw) for the 7 consecutive days. The animals of the control group received the vehicle only. Each day the body weight of the animals were measured and recorded.

At the end of the treatment period, the animals of each group were kept fasted overnight. The body weight of the animals of each group were measured and recorded. The animals were sacrificed through cervical dislocation after subjecting them to mild ether anaesthesia. Blood was carefully collected from each rat through cardiac puncture in microfuge tube and was allowed to clot for serum to separate out and then centrifuged at 2500 rpm for 15 minutes. Serum, thus obtained, was collected carefully in individual microfuge tube and stored at −20°C. The hearts were surgically removed after opening the thoracic cavity and immersed in ice-cold 0.9% saline for proper washing, blotted dry and weighed. The degree of alteration in heart size was calculated in terms of heart (ventricular) weight/body weight (HW/BW) ratio.

Determination of Lead content of cardiac tissue

Lead content was determined as per the protocol mentioned in the cook book of the Varian AA240 Atomic Absorption Spectrophotometer, GTA 120 (Graphite tube atomizer) available at the Chemical Engineering Department of University College of Science and Technology, University of Calcutta. The calibration curves were constructed by adding known amount of lead standards. The lead content was expressed in µg/g of rat cardiac tissue (Mitra et al., 2012).

Histomorphological studies

Immediately following sacrifice of the animals, hearts were surgically extirpated and fixed in 10% formalin and embedded in paraffin following routine procedure as described earlier [29]. Tissue sections (5 µM thick) were prepared and stained with hematoxylin-eosin.

Another set of the cardiac tissue sections were stained with Sirius red (Direct Red 80) and the stained tissue sections were examined under Olympus BX51 (Olympus Corporation, Tokyo, Japan) microscope and images were captured with a digital camera attached to it. The same tissue sections were further imaged with laser scanning confocal system (Leica TCS, SP2, Germany) and the stacked images through multiple slices were captured. The digitized images were then analyzed using image analysis system (Image J, NIH Software, Bethesda, MI) and the total collagen area fraction of each image was measured and expressed as the % collagen volume.

Assessment of biomarkers of organ damage

Serum GOT and GPT activities were measured by standard routine methods. The enzyme activities were expressed as IU/L [28]. Total serum lactate dehydrogenase (LDH) activity and the activity of lactate dehydrogenase1 (LDH1) was measured according to the method of Strittmatter [29] with some modifications [28] using a UV–Vis spectrophotometer (Bio-Rad, Hercules, CA, USA).

Assessment of biomarkers of oxidative stress and activities of the antioxidant enzymes

The cardiac tissues were homogenized (10%) in ice-cold 50 mM phosphate buffer for measurement of LPO, and for GSH estimation, in 2 mM EDTA , pH 7.4, with a Potter Elvehjem glass homogenizer (Belco Glass Inc., Vineland, NJ, USA) for 30s. Lipid peroxides were determined as thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust [30] with some modification [31] . GSH content (as acid soluble sulfhydryl) of the tissues was estimated by its reaction with DTNB (Ellman's reagent) following the method of Sedlak and Lindsey [32] with some modifications [28] . Copper-zinc superoxide dismutase (Cu-Zn SOD or SOD1) activity was measured spectrophotometrically by hematoxylin auto oxidation method of Martin et al (2012) [28] . Manganese superoxide dismutase (Mn-SOD or SOD2) activity was also measured spectrophotometrically by pyrogallol autooxidation method [33] . Catalase was assayed spectrophotometrically by the method of Beers and Sizer (1952) [34] with some modifications [28].

Measurement of xanthine oxidase and xanthine dehydrogenase activities, the pro-oxidant enzymes

Xanthine oxidase (XO) was assayed by measuring the conversion of xanthine to uric acid [35] . Xanthine dehydrogenase (XDH) activity was measured by following the reduction of NAD^+ to NADH [35] with some modifications [28] .

Measurement of the activities of the pyruvate dehydrogenase and some of the key mitochondrial Kreb's cycle enzymes

The cardiac tissues were homogenized (10%) in ice-cold 50 mM phosphate buffer, pH 7.4, with a Potter Elvehjem glass homogenizer (Belco Glass Inc., Vineland, NJ, USA) for 30s. The homogenate was then centrifuged at 500 g for 10 min and the resulting supernatant was again centrifuged at 12,000 g for 15 min to obtain the mitochondrial fraction. The mitochondrial pellet, thus obtained, was re-suspended in the buffer and used for assaying the mitochondrial enzymes.

Pyruvate dehydrogenase (PDH) activity was measured spectrophotometrically [36]. Isocitrate dehydrogenase (ICDH) and alpha-ketoglutarate dehydrogenase activities were measured spectrophotometrically according to the method of Duncan et al (1979) [37].

Succinate dehydrogenase (SDH) activity was measured spectrophotometrically according to the method of Veeger et.al (1969) [38].

Measurement of some of the mitochondrial respiratory chain enzymes

The NADH-cytochrome c oxidoreductase activity and Cytochrome c oxidase activity was measured spectrophotometrically by the method of Goyal and Srivastava (1995) [39].

Measurement of tissue protein content

Protein of the different samples were estimated by the method of Lowry et al (1970) [40] using bovine serum albumin (BSA) as the standard.

Statistical analysis

Data are presented as means ± S.E.M. Significance of mean values of different parameters between the treatment groups were analyzed using one way analysis of variances (ANOVA) after ascertaining the homogeneity of variances between the treatments. Pairwise comparisons were done by calculating the least significance. Statistical tests were performed using Microcal Origin version 7.0 for Windows.

RESULTS

Status of tissue lead content

Figure 1 demonstrates accumulation of lead in the rat cardiac tissue following treatment of rats with lead acetate at a dose of 15 mg / kg bw (i.p.) for a period of seven consecutive days compared to control ($p<0.001$ Vs. control). However, when the rats were pre-treated with melatonin at a dose of 10 mg / kg bw (fed orally), the tissue lead content was found to be reduced significantly compared to lead acetate treated group (65%, $p<0.001$ vs. Pb acetate treated group).

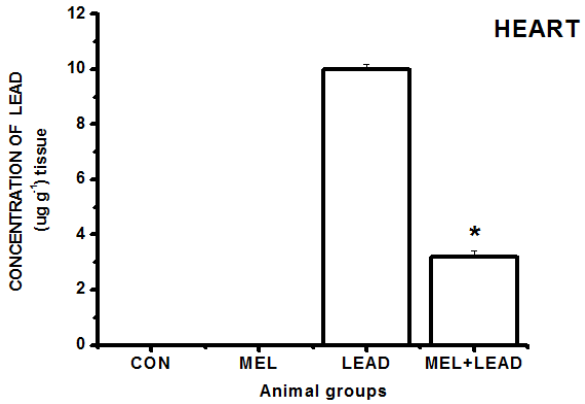


Fig. 1: Figure shows effect of melatonin on lead content of rat cardiac tissue.

CON = control, injected with vehicle; LEAD= 15 mg/kg bw Pb $(CH_3COO)_2$ injected i.p.; MEL = 10 mg/kg bw melatonin (fed orally); MEL +LEAD= 10 mg/kg bw of melatonin (fed orally) + 15 mg/kg bw Pb $(CH_3COO)_2$ injected i.p.; the values are expressed as Mean ± S.E.M. of six rats in each group; *P < 0.001 compared to control values. **P < 0.001 compared to lead acetate treated values.

Heart size and Heart weight body weight ratio

Figure 2 A reveals a reduction in heart size in the rats treated with lead acetate compared to control. However, pre -treatment of rats with melatonin protected the heart size from being reduced. Figure 2B reveals a significant decrease in heart weight: body weight ratio compared to control. Here also, pre-treatment of rats with melatonin significantly attenuated the heart weight: body weight ratio. However, melatonin alone was found to have no effect either on heart size (Figure 2A) or heart weight: body weight ratio (Figure 2B).

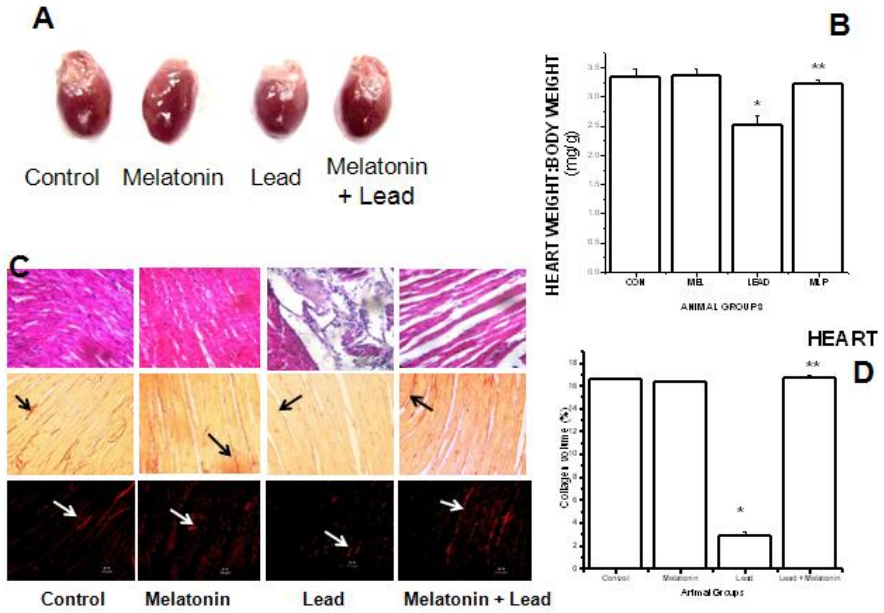


Fig. 2: Figure shows histopathological studies of Heart, effect of melatonin against lead-induced reduction in the heart size of the rats and Effect of melatonin on heart weight: body weight ratios.

(The values are expressed as Mean ± S.E.M. of six rats in each group; *P < 0.001 compared to control values. **P < 0.001 compared to lead treated values)

A. Effect of melatonin against lead-induced reduction in the heart size of the rats

B. Effect of melatonin on heart weight: body weight ratios

C. Upper panel : Protective effect of melatonin against lead acetate induced changes in the rat cardiac tissue morphology (Hematoxylin and Eosin stained , 400X magnification)

Middle panel: Protective effect of melatonin against lead acetate induced changes in the collagen status of rat cardiac tissue morphology (Sirius red stained sections, 400 X magnifications)

Lower panel: Similar images captured by confocal laser scanning microscope for quantification of fibrosis. Arrow heads indicate collagen fibres in b and c.

D. Graph showing collagen volume % of the cardiac tissue.

Con = control, injected with vehicle; Lead = 15 mg/kg bw, $Pb(CH_3COO)_2$ injected i.p.; Melatonin = 10 mg/kg bw (fed orally) melatonin; Melatonin + Lead = 10 mg/kg bw of melatonin (fed orally) + 15 mg/kg bw $Pb(CH_3COO)_2$ injected i.p.

Histomorphological studies

Figure 2A shows the photograph of rat heart which indicates a reduction in the size of the heart of lead acetate treated rats when compared to control. However, when the rats were pre-treated with melatonin, the heart was protected from being reduced in size.Here also melatonin alone was found to have no effect on heart size.

Figure 2 B demonstrates a significant reduction in the heart weight : body weight ratio in lead acetate treated rats compared to control (24.2% decrease, P<0.001 Vs control).However, when the rats were pre-treated with melatonin , the heart weight : body weight ratio was found to be protected from being reduced. Melatonin alone has no effect on heart weight: body weight ratio.

Figure 2 C (upper panel) reveals that there occurred focal ischemia and tissue damage in lead acetate treated rats as evident from hematoxylin and eosin stained tissue sections compared to control. However, in rats pre-treated with melatonin, there was no sign of ischemia or tissue damage. Melatonin alone, however, has no effect on cardiac tissue morphology. Figure 2 C (middle panel) shows a depletion of cardiac tissue collagen following treatment of rats with lead acetate. Pre-treatment of rats with melatonin protected the cardiac tissue from being depleted of tissue collagen. Melatonin alone was found to have no effect on tissue collagen content as evident from the microscopic examination of the cardiac tissue sections stained with acid Sirius. Fig. 2 C (lower panel) shows similar images captured by confocal laser scanning microscope. Figure 2 D, represents quantification of fibrosis as % collagen volume. The results further indicate a protective effect of melatonin against Pb-induced damage in rat cardiac tissue.

Biomarkers of organ damage

Table 1 shows that treatment of rats with lead acetate caused a significant elevation in the level of activity SGOT (44%, p<0.001 vs. control). However, when the rats were pre-treated with the present dose of melatonin, the activities of all the three enzymes in the serum were found to be similar to that observed in the control animals. Melatonin alone, however, was found to have no significant effect on the activities of this marker enzyme for organ damage.

A significant increase in the activity of serum LDH 1 (3.42 folds, p<0.001 vs. control; a specific marker enzyme of cardiac damage) was observed following treatment of rats with lead acetate. However, when the rats were pre-treated with the indicated dose of melatonin (fed orally), the activity of this isoenzyme of LDH was found to be significantly protected from being increased compared to lead acetate treated group (LDH 1, 42.86% p<0.001 vs. Pb acetate treated group . However, melatonin alone has no effect on the activity of the isoenzyme.

Table 1: Table shows the effect of melatonin on the activities of SGOT and the levels of serum LDH1 in heart of the experimental rats.

Parameters Studied	Control	Melatonin	Lead	Melatonin+lead
SGOT(IU/L)	9.14±0.583	8.56±0.741	13.12±0.341*	7.64±0.390**
LDH 1(IU/L)	1.113±0.046	1.098±0.096	5.238±0.097*	2.308±0.094**

Values are expressed as Mean ± SE of 6 animals in each group. Data were analyzed by using one way analysis of variances (ANOVA) using Microcal Origin version 7.0 for Windows.

*P<0.001 compared to control; **P< 0.001 compared to lead treated group;

Biomarkers of oxidative stress

Treatment of rats with lead acetate at a dose of 15 mg/Kg bw for a period of 7 consecutive days caused generation of oxidative stress in rat heart as evident from significantly increased level of lipid peroxidation (1.3 folds, *P< 0.001 vs. control group). Pre-treatment of rats with melatonin significantly prevented the lipid peroxidation level from being increased (69.09% in cardiac tissue, **P< 0.001 vs. Pb acetate-treated group) (Table 2). However, melatonin alone has no effect on the basal level of lipid peroxidation.

Table 2 further shows that there occurred a significant increase in GSH level of cardiac tissues of rat following lead acetate treatment (53.27%, *P< 0.001 vs. control group). Pre-treatment of rats with melatonin almost completely prevented the tissue GSH level from being increased (29.41%, **P< 0.001 vs. Pb-treated group). However, melatonin alone has no effect on the cardiac tissue GSH level.

Status of antioxidant enzymes

Table 2 additionally shows that treatment of rats with lead acetate at the indicated dose increased the activities of cytosolic Cu–Zn–SOD, the mitochondrial Mn–SOD and the catalase of the rat cardiac tissue (1.5 folds, 1.3 folds and 1.3 folds increase respectively in cardiac tissue vs control, * P < 0.001 vs. control). Pre-treatment of rats with melatonin was found to protect the activities of cytosolic Cu–Zn–SOD , the mitochondrial Mn–SOD and catalase of cardiac tissue from being increased (57.80%, 68.57% and 51.37% decrease respectively in cardiac tissue ,**P < 0.001 vs. Pb acetate treated group for all the three enzyme activities). However, melatonin alone has no significant effect on the activity of any of the enzymes studied.

Table 2: Table shows the effect of melatonin on the levels of LPO, GSH content as well as the activities of Cu-Zn SOD, Mn SOD and catalase of the heart of experimental rats.

Parameters Studied	Control	Melatonin	Lead	Melatonin +lead
LPO (nmoles of TBARS/mg protein)	0.129±0.029	0. 113±0.017	0.564±0.020*	0.174±0.027**
GSH (nmoles/mg protein)	12.12±0.373	12.74±0.389	18.7±0.396*	13.2±0.392**
Cu-Zn SOD activity (units/min/mg protein)	4.322±0.199	4.23±0.241	11.2±0.453*	4.71±0.221**
Mn SOD activity (units/min/mg protein)	1.52±0.052	1.512±0.063	4.958±0.051*	1.54±0.061**
Catalase activity (µmoles H_2O_2 consumed/min/mg protein)	15.11±0.874	14.69±0.537	32.22±0.991*	15.67±0.222**

Values are expressed as Mean ± SE of 6 animals in each group. Data were analyzed by using one way analysis of variances (ANOVA) using Microcal Origin version 7.0 for Windows.

*P<0.001 compared to control; **P< 0.001 compared to lead treated group;

Status of pro-oxidant enzymes

Table 3 shows that the activities of cardiac XO, XDH, the total enzyme activity, i.e., XO plus XDH, XO :XDH ratio and XO/(XO+XDH) all increased significantly following treatment of rats with lead acetate (88.11%, 2.1 folds, 1.28 folds, 19.67% and 1.16 folds increase respectively vs control, *P < 0.001 vs. control) . All these parameters were significantly protected from being increased when the rats were pre-treated with melatonin indicating melatonin's ability to neutralize free radicals *in vivo* (53.60%, 72.50%, 61.25%, 22.01% and 50% decrease respectively in cardiac tissue vs lead acetate treated group, **P < 0.001 vs. Lead acetate-treated group). However, melatonin alone has no effect on the activities of those two enzymes and their ratios.

Table 3: Table shows the effect of melatonin on the activities of XO, XDH and the values of XO+XDH, XO/XDH, XO/XO+XDH of the heart of experimental rats.

Parameters Studied	Control	Melatonin	Lead	Melatonin +lead
XO (milliunits/min/mg protein)	0.0075±0.00075	0.0075±0.0011	0.02±0.0060*	0.0075±0.0014**
XDH (milliunits/min/mg protein)	0.022±0.0022	0.021±0.0021	0.052±0.0022*	0.030±0.0020**
XO+XDH	0.027±0.0015	0.027±0.0016	0.071±0.0016*	0.031±0.0014**
XO/XDH	0.303±0.025	0.302±0.019	0.421±0.016*	0.279±0.013**
XO/(XO+XDH)	0.027±0.00048	0.019±0.00075	0.060±0.00054*	0.023±0.00066**

Values are expressed as Mean ± SE of 6 animals in each group. Data were analyzed by using one way analysis of variances (ANOVA) using Microcal Origin version 7.0 for Windows.

*P<0.001 compared to control; **P< 0.001 compared to lead treated group;

Status of the activities of pyruvate dehydrogenase and some of the mitochondrial Kreb's cycle enzymes

Table 4 shows that treatment of rats with lead acetate significantly decreases the activities of rat cardiac PDH (90% decrease, *P < 0.001 vs. control), ICDH (52.25%, *P < 0.001 vs. Control), α KGDH (75.91%, *P < 0.001 vs. control) and SDH (79.30%, *P < 0.001 vs. control). Pre-treatment of rats with melatonin significantly protected the PDH activity from being decreased (4 folds increase in cardiac tissues, **P < 0.001 vs. Lead acetate -treated group).ICDH is a key enzyme in cellular defense against oxidative damage as it provides NADPH in the mitochondria, which is needed for the regeneration of mitochondrial GSH or thioredoxin. The activity of cardiac ICDH was protected significantly from being decreased when the rat were pre-treated with melatonin (1.2 folds, **P < 0.001 vs. Lead acetate -treated group). Alpha KGDH was found to be able to generate ROS during its catalytic function, which is regulated by the NADH/NAD+ ratio (Tretter and Adam-Vizi, 2005). The activity of α KGDH was found to be significantly protected from being decreased when the rats were pre-treated with 10 mg/kg bw of melatonin (4.9 folds in cardiac tissue, **P < 0.001 vs. Lead acetate-treated group). Pre-treatment of rats with melatonin significantly protected the SDH activity from being decreased (62.47%, **P < 0.001 vs. Lead acetate-treated group). However, melatonin alone has no significant effect on the activity of any of those enzymes studied rat cardiac tissues.

Table 4: Table shows the effect of melatonin on the activities of PDH, ICDH, α-KGDH, SDH of the heart of experimental rats.

Parameters Studied	Control	Melatonin	Lead	Melatonin + lead
LDH (units/min/mg protein)	0.033±0.003	0.034±0.002	0.0066±0.002*	0.033±0.004**
PDH (units/min/mg protein)	0.913±0.013	0.843±0.059	0.022±0.030*	0.864±0.025**
ICDH(units/min/mg protein)	0.057±0.0018	0.058±0.0031	0.027±0.0026*	0.061±0.0018**
α-KGDH (units/min/mg protein)	0.059±0.0028	0.062±0.0023	0.014±0.0031*	0.083±0.0027**
SDH(units/min/mg protein)	1.605±0.105	1.554±0.043	0.336±0.062*	0.896±0.067**

Values are expressed as Mean ± SE of 6 animals in each group. Data were analyzed by using one way analysis of variances (ANOVA) using Microcal Origin version 7.0 for Windows.

*P<0.001 compared to control; **P< 0.001 compared to lead treated group;

Status of the activities of the mitochondrial respiratory chain enzymes

Table 5 shows that treatment of rats with lead acetate significantly decreased the activities of cytochrome c oxidase (85.98%,*P< 0.001 vs. control group) and NADH cytochrome c oxidoreductase (2.5 folds, *P< 0.001 vs. control group) in cardiac tissue. The activity of cytochrome c oxidase was found to be significantly protected from being decreased compared to lead acetate treated group when rats were pre-treated with melatonin (4.6 folds), **P< 0.001 vs. Lead acetate-treated group). NADH cytochrome c oxidoreductase activity was also found to be completely protected when the rats were pre-treated with melatonin at the indicated dose (2.5 folds, **P< 0.001 vs. Lead acetate -treated group). Melatonin alone, however, has no significant effect on the activity of these enzymes.

Table 5: Table shows the effect of melatonin on the activities of PDH, ICDH, α-KGDH, SDH of the heart of experimental rats.

Parameters Studied	Control	Melatonin	Lead	Melatonin + lead
Cytochrome c oxidase activity(units/min/mg protein)	0.126±0.011	0.129±0.0011	0.017±0.0028*	0.095±0.0035**
NADH cytochrome c oxido-reductase activity(units/min/mg protein)	7.121±0.036	8.072±0.045	2.0066±0.012*	7.013±0.0058**

Values are expressed as Mean ± SE of 6 animals in each group. Data were analyzed by using one way analysis of variances (ANOVA) using Microcal Origin version 7.0 for Windows.

*P<0.001 compared to control; **P< 0.001 compared to lead treated group;

DISCUSSION

Lead is a ubiquitous, non essential heavy metal and an environmental toxin. Although the mechanisms by which lead induces oxidative stress are not completely understood, evidence indicates that multiple mechanisms may be involved [2,3, 41] .

Our AAS study revealed a highly significant content of Pb in the cardiac tissue of the experimental rats following treatment of those with lead acetate for seven consecutive days. The high amount of lead in the tissues might have brought about oxidative stress-induced damages.The cardiac tissues of control and melatonin only (positive control) treated rats had zero lead concentration, Pre-treatment of rats with melatonin reduced the concentration of metal lead significantly. This indicates that melatonin removes lead from the organ either by chelating lead or by increasing the clearance of the heavy metal from organs by mechanism (s) not yet clearly known. This might also be due to accelerated mobilization and redistribution of lead from the

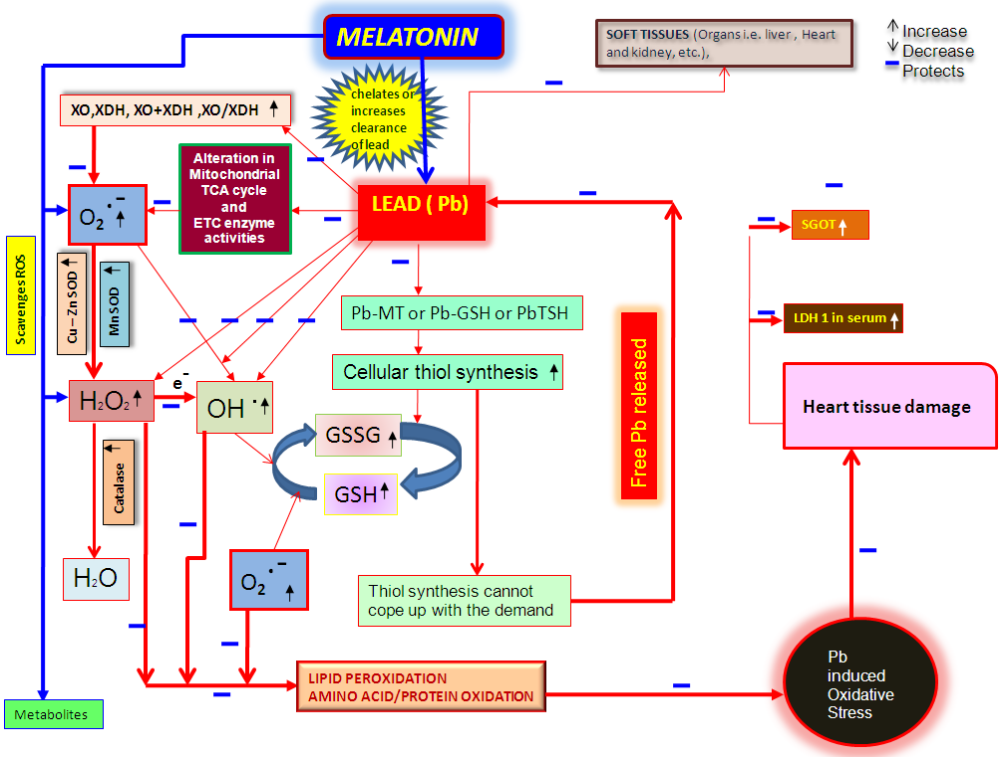
cardiac tissue by melatonin. Our finding is partially supported by the studies of Flora et al (2004) [42].

There was a significant increase in the level of TBARS in the cardiac tissue following treatment of rats with lead acetate. Lead is known to produce oxidative damage in the tissues by enhancing peroxidation of membrane lipids, a deleterious process solely carried out by free radicals. However, pre-treatment of rats with melatonin prevented the enhancement in the levels of TBARS and GSH in the cardiac tissue. Increased content of GSH is also indicative of an elevated level of oxidative stress following treatment of rats with lead acetate. Hsu J M reported in 1981, the concentrations of GSH in the erythrocytes, liver and kidney were significantly increased after 4 weeks of lead-acetate feeding in both males and females. The basis for clinical diagnosis of pathological symptoms depends on comparing the levels of a number of cytoplasmic enzymes, which are released as a result of physicochemical alterations in the tissues. It is evident from our studies that there occurred a significant increase in the levels of the marker enzymes for organ damage (SGOT and LDH1) due to lead acetate treatment in experimental rats.The present dose of lead acetate i.e., 15 mg/kg bw, for seven consecutive days not only produced significant changes in the parameters studied in comparison to control animals but also there was no animal mortality during the entire treatment period (the results of our dose-response studies are not shown). High levels of activity of SGOT and LDH 1 indicate myocardial damage in the experimental rat. Oral administration of melatonin at the present dose (i.e., 10 mg/kg bw) attenuated the lead acetate -induced elevation of the serum levels of the activities of these marker enzymes indicating that melatonin may have the capacity to provide protection against lead – induced cardiac damage.

Moreover, our histological studies showed that lead acetate caused prominent damage to cardiac tissue and also altered the levels of collagen deposition in the heart and pre-treatment of rats with melatonin protected the organ from being damaged.

Superoxide dismutase (SOD) catalyzes the conversion of superoxide anion free radical to H_2O_2 through dismutation reaction. Catalase catalyzes the conversion of H_2O_2 to water and oxygen. The activity of SOD and CAT in cardiac tissue was found to be increased following administration of lead acetate indicating elevated levels of oxidative stress. Pre-treatment of rats with melatonin protected activities of these antioxidant enzymes from being altered. The activities of the pro-oxidant enzymes , the XO and XDH are highly significantly increased compared to control with a concomitant increase in the XO plus XD, XO/XD ratio, XO/XO + XD ratio. This strongly indicates that metabolic reactions involving these two enzymes do serve as the source of superoxide anion free radical generation and increment in the activity of these enzymes indicate elevated levels of oxidative stress following treatment of animals with lead acetate. Earlier workers have also indicated the involvement of XO in free radical production [43, 44].

Mitochondria are the seat and the prime target of oxidative stress (Leon et al., 2005). We have studied the effect of lead acetate on some of the mitochondrial enzymes related to energy metabolism.The impairment of electron transfer through NADH: ubiquinone oxidoreductase (complex I) and ubiquinol: cytochrome c oxido-reductase (complex III) may induce superoxide formation. Chen et al. also explained this in their experiments studying ROS generation during the oxidation of complexI substrates.Mitochondrial production of ROS is thought to play an adverse role in many pathologic states of heart [45] .



Scheme 1: Scheme shows the mechanism by which melatonin protects against lead induced oxidative stress in heart.

The activity of the enzymes, like, PDH, ICDH, alpha KGDH and SDH is highly significantly decreased in lead acetate-treated rats, an observation that is supported by our earlier studies [46, 47, 48]. Pre-treatment of rats with melatonin significantly protected the activities of these important enzymes from being decreased and maintained their activities near normal indicating melatonin's ability to protect these enzymes either by scavenging the toxic reactants produced within the mitochondria in lead acetate treated rats or by protecting the substrate binding site of these enzymes by some mechanism yet to be explored. A reduction in the activity of NADH-cytochrome c reeducatase and cytochrome c oxidase of the respiratory chain following lead acetate treatment indicates elevated state of oxidative

stress in mitochondria. Pre-treatment of rats with melatonin, however completely restored the activity of these enzymes indicating that melatonin is capable of ameliorating oxidative stress generated in mitochondria following treatment with lead acetate. Thus, lead acetate induced alteration in mitochondrial redox metabolism and respiratory functions may lead to the increased production of ROS in cells which is effectively alleviated by melatonin. Melatonin's ability to protect and improve mitochondrial functions is supported by studies of Reiter et al., (2002) [49] and Leon et al., (2005) in some other stress models [50, 51]. These observations suggest that melatonin may be used as a potential clinical agent for providing cardioprotection against lead induced oxidative stress and damages of the heart. Pharmacological doses of melatonin possess very low or no cytotoxicity and hence minimum or no side effect as other drugs do [52]. Thus use of melatonin as a cardio protective agent in situation of lead induced oxidative cardiotoxicity is safe and devoid of any kind of risk of cytotoxic side effect. Melatonin is a ubiquitous potent antioxidant found in many foods specially fruits, vegetables, nuts and cereals. Thus, inclusion of some of these foods containing melatonin, in the daily diet of people who get regularly exposed to high concentration of lead environmentally or occupationally may serve as a first hand protection against lead- induced cardiotoxicity. We have found protective effect of melatonin in lead induced oxidative stress in rat liver and kidney tissues [53]. Melatonin, thus, may be used as a pharmaceutical agent in the form of a drug at a pharmacological dose or may also be used as a nutraceutical in the form of foods containing melatonin for protecting millions of people around the globe against lead induced oxidative cardiac damage. Scheme 1 depicts the probable mechanism by which melatonin protects against lead induced oxidative stress in rat cardiac tissue.

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