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Research Article

MELATONIN PROTECTS AGAINST LEAD-INDUCED OXIDATIVE STRESS IN RAT LIVER AND KIDNEY

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ABSTRACT

Treatment of rats with lead acetate at a dose of 15 mg / kg body weight intraperitoneally (i.p) for seven consecutive days caused a significant accumulation of lead with concomitant damage in the rat hepatic and renal tissues indicated by the increase in the level of activities of SGPT and serum lactate dehydrogenase 5, levels of blood creatinine and serum bilirubin. Histological studies confirmed the damages and those were caused due to oxidative stress was evident from the changes observed in the levels of lipid peroxidation and reduced glutathione, and the alterations in the activities of hepatic and renal antioxidant and pro-oxidant enzymes. Lead acetate treatment also caused alterations in the activities of mitochondrial Kreb's cycle and respiratory chain enzymes. All these changes were ameliorated when the rats were pre-treated with melatonin at a dose of 10 mg / kg (fed orally) for a similar period of time. Melatonin is a ubiquitous indole amine present naturally in plants and animals, recognized as a neurohormone secreted from the vertebrate pineal gland which has role in maintaining biorhythm and is also a potent antioxidant nutrient. The current studies indicated that melatonin protected the rat hepatic and renal tissues against lead-induced oxidative stress possibly through its antioxidant activity. As melatonin is present in almost all organisms and in many food items including cereals, green vegetables and fruits, it may be considered as one of the important component of the regular diet. Moreover, pharmacologically administered melatonin has been reported to be well tolerated in humans with no reported side-effects. The results of the current studies seem to have relevance at places where humans are exposed to lead environmentally or occupationally and where chelation therapy has limited success.

Keywords: Antioxidant, lead acetate, melatonin, oxidative stress, nutrient.

INTRODUCTION

Lead (Pb) is a heavy metal and is one among the oldest toxins in environment and is also a recognised occupational toxin [1]. Humans get exposed to lead in a multiple way daily. Overall, six categories of products have been recognized for most lead exposures .i.e., gasoline, additives, food can soldering, lead based paints, ceramic glazes, drinking water systems and folk remedies [2]. Lead enters the body through multiple routes and through circulation it is carried to various organs and gets deposited mainly in the soft organs and bones [3]. Lead is stored in soft tissues and there it facilitates generation of free radicals. An autopsy study of humans exposed to lead revealed that from among the soft organs, liver is the largest storage site of lead i.e., 33%, kidney cortex and medulla being the next [4].

Kidneys are the prime route of excretion of lead and thus get unavoidably exposed to it. The proximal tubular cells are mainly susceptible to lead induced toxicity because of their high energy demand such as reabsorptive and secretory functions [5]. Lead accumulates in the proximal tubule and results in Hyperuricemia and gout, by inhibiting uric acid secretion, and reduced glomerular filtration rate (GFR) [5]. Lead accumulates in mitochondria and causes both structural and functional deteriorative changes. The results are mitochondrial swelling, inhibition of respiratory enzyme activities leading to reduced energy (ATP) production. The consequence of which is that the energy dependent processes which includes tubular transport, gets badly affected [6].

Melatonin (N-Acetyl-5-Methoxy-Tryptamine) was originally discovered as a neuro hormone synthesized and secreted from the mammalian pineal gland [7]. Besides, it is present in all organisms from plant to animals [8]. Melatonin is found as a food constituent and consumption of it may alter the level of melatonin in the circulation [9]. The GI tract contains several times more melatonin in comparison to that secreted from pineal gland [10]. Studies reveal that Consumption of food and stimulation by nutrients, e.g., tryptophan etc. induces secretion of melatonin from GI tract into blood [10]. Melatonin is known to be responsible for maintaining the synchrony of circadian rhythm and altering sleep patterns with day and night [8, 11, 12].

Melatonin is also a potent antioxidant that protects the body from oxidative stress induced damages [13, 14]. Synthetic melatonin has gained popularity in recent times as a nutritional supplement, and now days are also used as a commercial additive [9,14]. Melatonin has been successfully tried in medical trial and pure preparations are well tolerated by patients [15]. Till date there is no report of adverse effect of melatonin in individuals [16]. Melatonin is an amphiphilic molecule. It can pass through membrane with ease and can be into any body fluid, cell and organelle [14]. Studies suggest that melatonin do possess diverse antioxidant activity [14]. The indoleamine directly scavenges free radicals and also has ability to up regulate endogenous antioxidants and down regulate certain pro oxidant enzymes [42]. Additionally, it has been reported that melatonin can strengthen the actions of other antioxidants, e.g., Trolox and ascorbate [11]. Moreover, several of its metabolites have also been shown to scavenge free radicals [14] thereby helping the cells to avoid molecular damage due to oxidative stress.

Crops like oats, sweet corn and rice; vegetables like tomatoes; fruits like grape, banana and red cherries as well as popular beverages like coffee, tea, wine and beer have been reported to contain very high levels of melatonin [i.e., picogram to microgram quantities per gram] [9,17]. Consumption of melatonin containing food can provide healthy levels of antioxidants and improve human health [18]. Moreover, melatonin has been suggested for clinical use in a variety of pathologies including neurodegenerative diseases (Alzheimer's, Parkinson's disease), heart disease, metabolic disorders, tumours, and accidental nuclear radiation [19]. Melatonin is taken through food not as a sleep aid but for its beneficial impacts on health. Quantity, spread and time at which melatonin is consumed through food is the prime factor that decides its function as an antioxidant [20].

Herein, we provide evidence that a low pharmacological dose of melatonin provides protection to hepatic and renal tissues from

oxidative injury following exposure of the experimental rats to lead acetate. The results of the current studies indicate that this small indole provides protection against heavy metal-induced oxidative onslaught of the rat tissues possibly through its antioxidant mechanism(s) and point toward its therapeutic usefulness as a protective anti-oxidant against situations of hepatic and renal toxicity arising out of exposure of the humans to lead environmentally or occupationally and where chelation therapy has limited success.

MATERIALS AND METHODS

Chemicals

Melatonin and lead acetate were purchased from SRL Chemicals, Mumbai, India. Thiobarbituric acid (TBA) was procured from Spectro Chem., India. All other chemicals used were of analytical grade.

Animals

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 in accordance with the committee for the purpose of control and supervision of experiment on animals, 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.

Induction of oxidative stress with lead acetate and protection by melatonin

The animals were kept in quarantine for getting adapted to laboratory environment and conditions. After that they were grouped and 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 melatonin was fed, the animals of the lead acetate and the melatonin +lead acetate treated groups were intraperitoneally injected with lead acetate solution (15 milligram per kg body weight, which is 10% of LD_{50} for lead acetate) for the 7 consecutive days. At this dose of lead acetate there was no mortality of rats during the entire period of the experiment (Ghosh et al., 2012) [42]. The dose-response studies carried out earlier in our laboratory demonstrated higher rates of animal mortality with a higher dose of lead acetate and a lower dose was found to be less effective (data not shown). Animals of the control group received the vehicle only. Each day the body weight of the animals were measured and recorded.

Collection of blood and tissues, and preparation of the serum

At the end of the treatment period, the animals of each group were kept fasted overnight. The body weight of the animals of the each group were measured and recorded. The animals were sacrificed through cervical dislocation and the, abdominal cavity was carefully opened and blood was immediately collected from hepatic vein in two different sets of tubes, one used for blood analysis and the other for measurement of serum parameters. The blood in the latter tube was allowed to clot for serum to separate out and then centrifuged at 2500 rpm for 15 minutes. Serum was collected carefully with auto pipette in individual microfuge tube and stored at –20°C. The liver and the kidneys were excised carefully and washed several times in ice cold saline, and bottled dry, immediately weighed and stored at -20 ° C until analysis.

Measurement of the activities of serum glutamate pyruvate transaminase (SGPT)

Serum GPT activity was measured by standard routine methods. The activities of the enzyme were expressed as IU/L [21].

Measurement of serum total LDH and lactate dehydrogenase 5 (LDH 5) activity

Total serum lactate dehydrogenase activity was measured according to the method of Strittmatter [22] with some modifications [23].

The activity of lactate dehydrogenase 5 (LDH 5) in the serum from the same rat was measured according to the method of Strittmatter [22] with some modifications [23].The enzyme activity was expressed as IU/L.

Determination of serum bilirubin level

Serum bilirubin (total) level was measured using the Diazo method (Royden et al., 1962). The method is based on van den Bergh reaction [25].

Determination of blood creatinine level

Blood creatinine level was estimated by the method of Folin Wu with slight modification [26]. A blank and a standard were prepared using distilled water and creatinine was dissolved in acid solution.

Estimation of the lead (Pb) content in the rat liver and kidney tissues by Atomic Absorption Spectrophotometry (AAS)

The tissue samples were prepared and the lead content was measured as per the protocol mentioned in the cook book of the Varian AA 240 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 tissue samples were processed according to the method adopted by Mitra et al., 2012 [27].

Histological studies

Immediately following sacrifice of the animals, liver and the kidneys were surgically extirpated and fixed in 10% formalin and embedded in paraffin following routine procedure as described earlier [27]. Tissue sections (5 μM thick) were prepared and stained with hematoxylin-eosin.

Besides, a small portion of the hepatic tissue was fixed in 10% neutral buffered formalin acetic acid alcohol fixative and processed further for Per-iodic Acid Schiff (PAS) staining for glycogen.

Another set of the 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.

Preparation of homogenate, measurement of lipid peroxidation level and reduced glutathione content in rat liver and kidney tissues

The liver and the kidney tissues were separately homogenized (10%) in ice-cold 0.9%saline (pH 7.0) with a Potter Elvenjem glass homogenizer for 30 s and lipid peroxides in the homogenate were determined as thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust (1978) [28] with some modification as adopted by Bandyopadhyay et al. [10] & Mitra et al.,(2012) [27]. In brief, the homogenate was added to thiobarbituric acid–trichloro acetic acid (TBA–TCA) reagent with thorough shaking and was heated for 20 min at 80°C. The samples were then cooled to room temperature. The absorbance of the pink chromogen present in the clear supernatant after centrifugation at 1200 g for 10 min at room temperature was measured at 532 nm using a UV–Vis spectrophotometer (Bio-Rad, Hercules, CA, USA). Tetraethoxypropane (TEP) was used as standard. Values were expressed as nmoles of TBARS/mg protein.

Reduced GSH content (as acid soluble sulfhydryl) of the liver and kidney tissues were estimated by its reaction with DTNB (Ellman's reagent) following the method of Sedlak and Lindsey [29] with some modifications [27]. The tissues were homogenized (10%) in 2 mM ice-cold ethylenediaminetetraacetic acid (EDTA). The homogenate was

mixed with Tris–HCl buffer (pH 9.0) followed by addition of DTNB for colour development. Using a UV–Vis spectrophotometer (BIORAD, Smart Spec Plus), the absorbance was recorded at 412 nm and the values were expressed as nmoles/mg protein.

Measurement of the activities of cytosolic (Cu-Zn type) and mitochondrial (Mn-type) superoxide dismutase (SOD) and catalase (CAT)

Copper-Zinc superoxide dismutase (SOD1) activity was measured by hematoxylin autooxidation method of Martin et al. [30] with some modifications as adopted by [31]. The supernatant was collected. Inhibition of hematoxylin auto-oxidation by the cell free supernatant was measured at 560 nm using a UV–Vis spectrophotometer. The enzyme activity was expressed as U / mg of tissue protein.

Manganese superoxide dismutase (Mn-SOD or SOD2) activity was estimated by pyrogallol autooxidation method [32]. The absorbance was recorded at 420 nm for 3 min in a UV/VIS spectrophotometer. The enzyme activity was expressed as units/min/mg of tissue protein.

Catalase was assayed by the method of Beers and Sizer [33] with some modifications [27]. The enzyme activity was expressed as μm H_2O_2 consumed / min / mg protein.

Measurement of xanthine oxidase and xanthine dehydrogenase activities

Xanthine oxidase activity was estimated by the conversion of xanthine to uric acid following the method of Greenlee and Handler [34]. The enzyme activity was expressed as milli units/min/mg protein.

Xanthine dehydrogenase activity was measured by following the reduction of NAD^+ to NADH according to the method of Strittmatter [22] with some modifications [27]. The enzyme activity was expressed as milli units/min/mg tissue protein.

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

The liver and the kidney 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.The 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 according to the method of Chretien et al., [35], with some modifications as adopted by Mitra et al., 2012 [27].The enzyme activity was expressed as Units / mg protein.

Isocitrate dehydrogenase (ICDH) activity was measured according to the method of Duncan et al [36].The enzyme activity was expressed as units/mg protein.

Alpha-ketoglutarate dehydrogenase (α-KGDH) activity was measured spectrophotometrically according to the method of Duncan et. al [36]. The enzyme activity was expressed as units/mg protein.

Succinatedehydrogenase (SDH) activity was measured spectrophotometrically by following the reduction of potassium ferricyanide (K_3FeCN_6) at 420nm according to the method of Veeger et.al [37] with some modifications [27]. The enzyme activity was expressed as units/mg protein.

Measurement of some of the mitochondrial respiratory chain enzymes

The NADH-cytochrome c oxidoreductase activity was measured spectrophotometrically by following the reduction of oxidized cytochrome c at 565nm according to the method of Goyal and Srivastava [38]. The enzyme activity was expressed as Units / mg protein.

Cytochrome c oxidase activity was determined spectrophotometrically by following the oxidation of reduced cytochrome c at 550nm according to the method of Goyal and Srivastava [38]. The enzyme activity was expressed as Units / mg protein.

Measurement of tissue protein content

Protein was estimated by the method of Lowry et al., [39] using bovine serum albumin (BSA) as the standard.

Statistical analysis

Each experiment was repeated at least three times with different sets of male Wistar rats. Data are presented as means ± S.E.M. Significance of mean values of different parameters between the treatments groups were analyzed using one way analysis of variances (ANOVA) after determining 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. (A,B) demonstrates accumulation of lead in liver and kidney tissues following treatment of rats with lead acetate at a dose of 15 mg / kg bw (i.p.) for a period of seven consecutive days. 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 in liver (58.89%, p<0.001 vs. Pb-treated group), and kidneys (30.49%, p<0.001 vs. Pb-treated group).

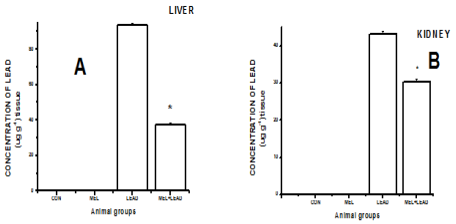


FIGURE 1

Biomarkers of organ functions

Table 1. demonstrates a 66.13% increase in the level of serum billirubin (p<0.001 vs. control) following treatment of rats with the present dose of lead acetate for seven consecutive days. Likewise, in the lead acetate treated rats, serum creatinine level was also found to be significantly increased (1.75 fold, p< 0.001 vs. control) compared to control. When the rats were pre-treated with melatonin at a dose of 10 mg /kg bw (fed orally) for the similar time period, both serum billirubin and creatinine levels were found to be almost completely prevented from being increased. Melatonin alone, however, was found to have no effect on serum level of billirubin or serum creatinine.

Biomarkers of organ damage

Table 1. further shows that treatment of rats with lead acetate caused a significant elevation in the level of activities of SGPT (nearly two-fold, p<0.001 vs. control) and serum total LDH (2.4 folds, p<0.001 vs. control) as well as LDH 5 (1.76 folds, p<0.001 vs. control; a specific marker enzyme of liver damage). However, when the rats were pre-treated with the present dose of melatonin, the activities of all the three enzymes in serum were found to be significantly protected from being increased (LDH:65.81%, p< 0.001 vs. Pb-treated group ; LDH 5: 1.76 folds, p< 0.001 vs. Pb-treated group; SGPT:63.98%, p< 0.001 vs. Pb-treated group). Melatonin alone was found to have no significant effect on the activities of these marker enzymes for hepatic damage.

Histopathological studies of liver tissue

Figure 2 A (upper panel) documents H and E stained sections of hepatic tissue (magnification 400X) showing intact portal veins, scattered dead hepatocytes, focal hepatic necrosis with dilated

sinusoids in lead acetate treated rats compared to control. However, pre-treatment of rats with melatonin protected the tissue from being damaged and found to have normal sinusoids. Melatonin alone, however, has no effect on hepatic tissue morphology.

Figure 2 A (middle panel) shows Sirius red stained hepatic tissue section (magnification 400X) with deposition of collagen around the central vein region following treatment of rats with lead acetate. Pre-treatment of rats with melatonin prevented the deposition of hepatic tissue collagen. Melatonin alone was found to have no effect on tissue collagen content.

Fig. 2 A (lower panel) shows similar images (magnification 400X) captured by confocal laser scanning microscope. Figure 2 B represents quantification of fibrosis as percent collagen volume. The results further indicate a protective effect of melatonin against Pb-induced damage in rat hepatic tissue.

Figure 2 C represents the status of hepatic glycogen studied by PAS staining of the tissue sections. Here also, pre-treatment of rats with melatonin was found to have a protective effect on tissue glycogen content compared to lead acetate treated rats. Melatonin alone, however, has no effect on tissue glycogen content.

Figure 2 D demonstrates a reduction of hepatic glycogen content following treatment of rats with lead acetate for seven consecutive days. However, pre-treatment of rats with melatonin significantly protected the tissue glycogen content from being reduced compared to lead acetate group. However, melatonin alone was found to have no effect on hepatic tissue glycogen content compared to control.

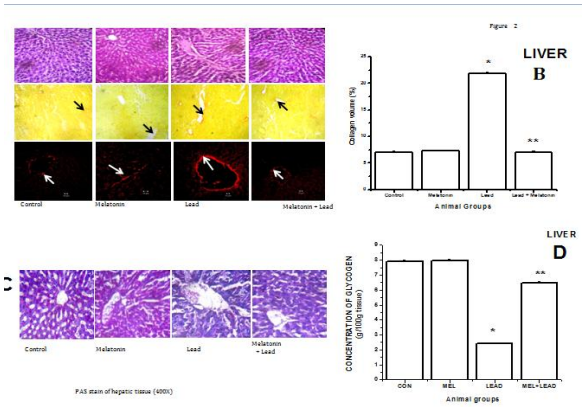
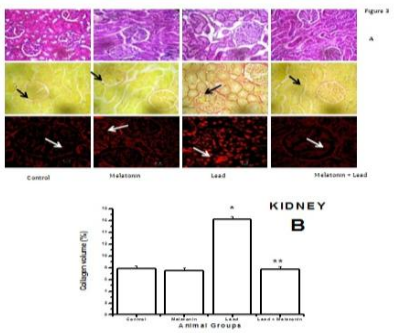


FIGURE 2

Histopathological studies of kidney tissue

Figure 3. A (upper panel) documents severe degeneration of tubular epithelial cells along with collapse of tubules indicating severe acute tubular necrosis (ATN) and increased inter tubular gap in renal tissue of lead acetate treated rats as evident from hematoxylin and eosin stained tissue sections compared to control. However, pre-treatment of rats with melatonin prevented the tissue damage to some extent. Melatonin alone, however, has no effect on renal tissue morphology.



FEGURE 3:

Furthermore, the renal tissue sections depicted in figure 3 A (middle panel, magnification 400X) show collagen deposition around glomeruli and renal tubules following treatment of rats with lead acetate for seven consecutive days. Pre-treatment of rats with melatonin prevented the deposition of renal tissue collagen. Fig. 3 A (lower panel) shows similar images of the renal tissue captured by confocal laser scanning microscope (magnification 400X). Figure 3 B depicts quantification of fibrosis as percent collagen volume. The results further indicate a protective effect of melatonin against Pb-induced damage in rat renal tissue.

Biomarkers of oxidative stress

Treatment of rats with lead acetate at a dose of 15 mg/Kg body weight for a period of 7 consecutive days caused generation of oxidative stress in rat liver and kidney tissues as evident from significantly increased level of lipid peroxidation compared to control [Table 2.] (1.3 folds in hepatic tissue and 2.4 folds in renal tissue, *P< 0.001 vs. control group for both the tissues). Pre-treatment of rats with melatonin significantly protected the lipid peroxidation level from being increased (62.58% in hepatic tissue and 39.17% in renal tissue, **P< 0.001 vs. Pb acetate-treated group for both the tissues). However, melatonin alone, has no effect on the lipid peroxidation level of both the tissues studied. Table 2. further shows that there occurred a significant increase in GSH level of liver, and kidney tissues of rat following lead acetate treatment (67.26% in hepatic tissue, 93.46% in renal tissue, *P< 0.001 vs. control group for both the tissues). Pre-treatment of rats with melatonin almost completely protected the tissue GSH level from being increased in hepatic tissue (31.85% in hepatic tissue and 26.34% in renal tissue, **P< 0.001 vs. Pb-treated group for both the tissues). However, melatonin alone has no significant effect on the GSH level of liver and kidneys.

Status of antioxidant enzymes

Table 2. reveals 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 hepatic and renal tissues (1.2 folds, 1.3 folds and 1.2 folds increase respectively in hepatic tissue vs control; 1.3 folds, 2.2 folds and 59.63% increase respectively in renal tissue, * P < 0.001 vs. control). Pre-treatment of rats with melatonin was found to protect the activities of these antioxidant enzymes from being increased in both the tissues studied (54.87%, 2.2 folds and 55.04% decrease respectively in hepatic tissue and 52.75%, 52.63% and 44.81% decrease respectively in renal tissue , **P < 0.001 vs. Pb-treated group). However, melatonin alone did not significantly alter the activity of any of the enzymes studied.

Status of pro-oxidant enzymes

The activities of hepatic and renal xanthine oxidase (XO) [Table 3] and xanthine dehydrogenase (XDH) as well as the total enzyme activity, i.e., XO plus XDH and, XO : XDH and XO / XO + XDH ratio, all increased significantly following treatment of rats with lead acetate (84.27%, 26.92%, 28.57%, 42.85% and 55% increase respectively in hepatic tissue vs. control and 2.3 folds, 50%, 44.76% and 1 fold each increase respectively in renal tissue, *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* (57.93%, 24.24%, 44.44%, 23.33% and 25.80% decrease respectively in hepatic tissue vs. lead acetate treated groups and 74.88%, 50.12%, 23.33%, 33.33% and 67.27% decrease respectively in renal tissue, **P < 0.001 vs. Lead acetate-treated group). However, melatonin alone has no effect on the activities of xanthine oxidase (XO) xanthine dehydrogenase (XDH), the total enzyme activity, i.e., XO plus XDH and, XO: XDH and XO / XO +XDH ratio.

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

Table 4 reveals that treatment of rats with lead acetate inhibits rat hepatic and renal pyruvate dehydrogenase activity (4.67 folds and 90.1% decrease respectively, *P < 0.001 vs. their respective control for both the tissues). Pre-treatment of rats with melatonin significantly protected the enzyme activity from being decreased in hepatic and renal tissues (4.33 folds and 91.3% increase respectively

in hepatic and renal tissues, **P < 0.001 vs. Lead acetate -treated group). However, melatonin alone was found to have no effect on the activity of this enzyme in any of the tissues studied.

Table 4 further reveals that treatment of rats with lead acetate significantly decreased the activity of isocitrate dehydrogenase in hepatic and renal tissues (90.91%and 94.26% respectively, *P < 0.001 vs. their respective control for both the tissues studied). Isocitrate dehydrogenase is a key enzyme in cellular defence against oxidative damage as it provides NADPH in the mitochondria, which is needed for the regeneration of mitochondrial GSH or thioredoxin. The activity of the enzyme in the liver and the kidney tissues were found to be protected significantly from being decreased when the rats were pre-treated with melatonin (1fold and 1.3 folds respectively, **P < 0.001 vs. Lead acetate -treated group). However, melatonin alone has no effect on the activity of isocitrate dehydrogenase in the hepatic and renal tissues.

Treatment of rats with lead acetate inhibits alpha keto glutarate dehydrogenase (α-KGDH) activity in hepatic and renal tissues (94.29% and 75.66% respectively, *P < 0.001 vs. respective control for both tissues) [Table 4]. This enzyme 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 the enzyme was found to be significantly protected from being decreased in both the organs studied when the rats were pre-treated with 10 mg/kg body weight of melatonin (12 folds and 3.1 folds respectively in hepatic and renal tissues, **P < 0.001 vs. Lead acetate -treated group). However, melatonin alone has no significant effect on the activity of α-KGDH in hepatic and renal tissues.

Treatment of rats with lead acetate for seven consecutive days, inhibited the activity of succinate dehydrogenase (SDH) in hepatic and renal tissues significantly (85.48%, and 93.86 % respectively, *P < 0.001 vs. their respective control for both the tissues studied) [Table IV]. This might result in interference of the metal in electron transport chain (ETC) and thus generate copious amounts of superoxide anion free radicals in the tissue mitochondria. However, pre-treatment of rats with melatonin significantly protected the SDH activity from being decreased in hepatic and renal tissues (3.7 folds and 87.52% respectively, **P < 0.001 vs. Lead acetate -treated group). Melatonin alone has no effect on the activity of this enzyme in any of the tissues studied.

Status of the activities of mitochondrial respiratory chain enzymes

Treatment of rats with lead acetate for similar period of time also decreased cytochrome c oxidase activity in hepatic and renal tissues (87.61% and 75% decrease respectively in hepatic and renal tissues, *P< 0.001 vs. respective control group for both the tissues

studied). The activity of this enzyme was found to be significantly protected from being decreased compared to lead acetate treated group when rats were pre-treated with melatonin (87.80% and 74.35% respectively in hepatic and renal tissues respectively, **P< 0.001 vs. Lead acetate -treated group). Melatonin alone, however,

has no effect on the activity of this enzyme in the hepatic and the renal tissues.

Treatment of rats with lead acetate for seven consecutive days at a dose of 15 mg / kg body weight inhibits NADH cytochrome c oxido-reductase activity (73.43% in hepatic and 72.57% in renal tissues , *P< 0.001 vs. respective control group for both the tissues). However, the enzyme activity was found to be completely protected when the rats were pre-treated with melatonin at a dose of 10 mg/kg body weight for the similar period of time (2.7 folds in hepatic and 2.6 folds in renal tissues, **P< 0.001 vs. Lead acetate -treated group for both the tissues). However, melatonin alone has no significant effect on the activity of this enzyme in hepatic as well as renal tissues.

Table1: Table shows activities of blood creatinine, serum bilirubin, LDH, LDH 5, SGPT, in lead acetate treated and melatonin protected rats.

PARAMETERS STUDIED	CON	MEL	PB	MEL+PB
Creatinine (mg %)	1.57±0.129	1.495±0.175	4.403±0.227*	1.414±0.102**
Bilirubin (mg %)	12.43±0.622	11.55±0.343	20.65±0.456*	12.21±0.591**
LDH (IU/L)	1.80±0.072	1.78±0.091	6.22±0.098*	2.12±0.088**
LDH 5(IU/L)	2.425±0.166	2.405±0.145	6.993±0.137*	3.997±0.147**
SGPT (IU/L)	10.77±0.096	9.14±1.843	31.87±1.164*	11.48±1.463**

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;**
CON = Control;Mel=Melatonin; Pb=Lead; Mel+Pb= Melatonin+Lead.

Table 2: Table shows the levels of lipid peroxodation, reduced glutathione content as well as the activities of Cu-Zn superoxide dismutase, Mn superoxide dismutase and catalase of the hepatic and renal tissues in lead acetate treated and melatonin protected rats.

Parameters Studied	LIVER				KIDNEY			
	CON	MEL	Pb	MEL+Pb	CON	MEL	Pb	MEL+Pb
LPO (nmoles of TBARS/mg protein)	0.140± 0.122	0.123± 0.010	0.316± 0.017*	0.118± 0.013**	0.316± 0.0196	0.268± 0.0139	0.611± 0.029*	0.373± 0.014**
GSH (nmoles/mg protein)	24.16± 0.316	25.84± 0.561	40.41± 0.728*	27.54± 0.605**	10.645± 0.722	12.993± 0.278	20.594± 0.833*	15.169± 1.442**
Cu-Zn SOD activity (units/min/mg protein)	4.985± 0.246	4.992± 0.256	13.56± 0.358*	4.97± 0.365**	0.967± .049	1.064± 0.065	2.252± 0.173*	1.029± 0.019**
Mn SOD activity (units/min/mg rotein)	4.528± 0.205	5.521± 0.205	10.063± 0.291*	5.688± 0.291**	1.198± 0.149	1.193± 0.251	3.925± 0.182*	1.775± 0.133**
Catalase activity(µmoles H_2O_2 consumed/min/mg protein)	172.09± 4.129	172.34± 3.208	383.89± 4.609*	172.61± 3.465**	20.431± 0.799	19.877± 1.035	35.006± 0.731*	19.318± 0.923**

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;
CON = Control;Mel=Melatonin; Pb=Lead; Mel+Pb= Melatonin+Lead.

Table 3: Table shows the activities of xanthine oxidase, xanthine dehydrogenase, XO+XDH, XO/XDH, XO/XO+XDH of the hepatic and renal tissues in lead acetate treated and melatonin protected rats.

Parameters Studied	LIVER				KIDNEY			
	CON	MEL	Pb	MEL+Pb	CON	MEL	Pb	MEL+Pb

XO (milliunits/min/mg rotein)	0.027±	0.051±	0.049±	0.021±	0.013±	0.013±	0.042±	0.014±
	0.001	0.0015	0.002*	0.003**	0.0022	0.0027	0.003*	0.003**
XDH (milliunits/min/mg protein)	0.255±	0.255±	0.322±	0.241±	0.016±	0.016±	0.031±	0.017±
	0.0089	0.0094	0.0091*	0.0093**	0.0013	0.0016	0.015*	0.0017**
XO+XDH	0.272±	0.282±	0.352±	0.223±	0.029±	0.028±	0.072±	0.031±
	0.0068	0.0071	0.0088*	0.0071**	0.0064	0.00541	0.0041*	0.0028**
XO/XDH	0.105±	0.098±	0.152±	0.115±	0.812±	0.811±	1.425±	0.823±
	0.0065	0.0046	0.0041*	0.0029**	0.061	0.072	0.056*	0.047**
XO/XO+XDH	0.097±	0.089±	0.139±	0.100±	0.448±	0.456±	0.586±	0.454±
	0.0031	0.0036	0.0064*	0.0047**	0.0091	0.0086	0.0084*	0.0097**

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;**
CON = Control;Mel=Melatonin; Pb=Lead; Mel+Pb= Melatonin+Lead.

Table 4. Table shows the activities of pyruvate dehydrogenase, isocitrate dehydrogenase, alpha-keto glutarate dehydrogenase, succinate dehydrogenase activity of the hepatic and renal tissues in lead acetate treated and melatonin protected rats.

Parameters Studied	**LIVER**				**KIDNEY**			
	CON	**MEL**	**Pb**	**MEL+Pb**	**CON**	**MEL**	**Pb**	**MEL+Pb**
PDH (units/min/mg protein)	1.713±	1.564±	0.328±	1.564±	0.891±	0.883±	0.032±	0.784±
	0.013	0.059	0.030*	0.025**	0.013	0.059	0.030*	0.025**
ICDH(units/min/mg protein)	0.055±	0.054±	0.0051±	0.033±	0.053±	0.050±	0.003±	0.046±
	0.0018	0.0017	0.0014*	0.0024**	0.0047	0.0041	0.0045*	0.0024**
α-KGDH (units/min/mg protein)	0.043±	0.048±	0.0025±	0.035±	0.035±	0.031±	0.0085±	0.034±
	0.0014	0.0031	0.0024*	0.0028**	0.0029	0.0027	0.0032*	0.0021**
SDH(units/min/mg protein)	3.151±	3.121±	0.457±	2.173±	2.212±	2.305±	0.135±	1.853±
	0.176	0.163	0.034*	0.194**	0.137	0.022	0.0051*	0.038**

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;**
CON = Control;Mel=Melatonin; Pb=Lead; Mel+Pb= Melatonin+Lead.

Table 5:Table shows the activities of cytochrome c oxidase activity, NADH cytochrome c oxidoreductase activity of the hepatic and renal tissues in lead acetate treated and melatonin protected rats.

Parameters Studied	**LIVER**				**KIDNEY**			
	CON	**MEL**	**Pb**	**MEL+Pb**	**CON**	**MEL**	**Pb**	**MEL+Pb**
Cytochrome c oxidase	1.049±	1.051±	0.132±	1.051±	0.082±	0.083±	0.022±	0.078±
activity(units/min/mg protein)	0.0067	0.0073	0.0058*	0.0054**	0.0011	0.0021	0.0011*	0.0011**
NADH cytochrome c oxidoreductase	8.166±	8.176±	2.166±	8.043±	3.503±	3.472±	0.963±	3.463±
activity(units/min/mg protein)	0.088	0.062	0.088*	0.039**	0.073	0.026	0.038*	0.027**

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;**
CON = Control;Mel=Melatonin; Pb=Lead; Mel+Pb= Melatonin+Lead.

DISCUSSION

The available literature to date demonstrates that there have been multiple studies on lead induced toxicity and its probable underlying mechanism(s). However, the exact mechanism(s) by which lead induces oxidative stress are not completely understood yet. Investigations reveal that lead induced toxicity is multifactorial and multi-mechanistic [39]. Any compound or situation that accelerates pro oxidant formation or reduces the intracellular antioxidant defence or alters both is the one that induces oxidative stress. The present study describes the protective effect of melatonin against lead - acetate induced damage in liver and kidney tissues in experimental rats.

Our AAS study revealed a highly significant content of Pb in liver and kidney tissues of the experimental rats following treatment of those with lead acetate for seven consecutive days. The high amount of lead that accumulated in the tissues might have brought about or have by some means accelerated the process of oxidative stress-induced damages in the liver and kidney tissues of the experimental rats. Oxidative stress-induced damage in the organs is probably the reason behind the deteriorative deviation of their functions from normal status. Tissues of control and melatonin only (positive control) treated rats had zero lead concentration and melatonin pre treatment reduced the concentration of metal lead in both the tissues significantly. This indicates that melatonin removes lead from the organs 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 organs by melatonin.

A molecule can induce the generation of ROS directly [40]. The other mechanism is that a molecule can indirectly induce oxidative stress by increasing the vulnerability of membranes to the attack of ROS [40]. The major constituents of biological membranes are lipids and proteins. Membrane lipid peroxidation is a deleterious process solely carried out by free radicals. Lead is known to produce oxidative damage in the tissues by enhancing the process of peroxidation of membrane lipids [41]. In our experiments, we have also found a significant increase in TBARS concentration in the hepatic and the renal tissues following treatment of rats with lead acetate. We had similar observation in our other experiment also [42]. Pre-treatment of rats with melatonin prevented the enhancement in TBARS concentration in both the tissues studied. This suggests that melatonin can protect the liver and kidney tissues of rats from lead induced oxidative damage.

Certain cytosolic enzymes are released into blood following destruction of cellular membrane integrity. The levels of such cytosolic enzymes are compared to their normal levels in serum to decide a pathological state of a particular organ. The enhanced levels of serum hepatic and renal damage marker enzymes indicate that there has been cellular leakage in the organs and also a loss of functional integrity of membrane architecture. It is evident from our

studies that there occurred a significant increase in levels of the marker enzymes due to treatment of rats with lead acetate and we did not observe any animal mortality during the entire treatment period. High levels of SGPT, total serum LDH and LDH5 indicate hepatic damages 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 these marker enzymes indicating that melatonin has the capacity to provide protection to the loss of cellular membrane integrity of rat liver and renal tissue. This protection might have been exerted through some antioxidant or radical scavenging or metal chelating role of melatonin which has protected the tissues from being damaged due to causes induced by lead acetate.

We have also studied the markers of function of the organs and found that treatment of rats with lead acetate for a period of seven consecutive days caused an elevation in the level of serum bilirubin and blood creatinine indicating functional disorder of liver and kidneys respectively. Elevated creatinine level has been considered as functional evidence of lead induced nephrotoxicity. Increase in creatinine concentration has been reported to be due to loss of 50 % of kidney function [43]. Pre-treatment of rats with melatonin prevented the elevation of serum bilirubin and blood creatinine. Thus, our observation suggests that melatonin can prevent lead induced oxidative damage to the organs and can help to maintain the normal structure and functions of these organs.

Histological study of the liver and kidney tissues of rats from all groups showed that lead acetate caused prominent damage to these organs and also altered the levels of collagen deposition in the organs and pre-treatment of rats with melatonin protected the organs from being damaged. Thus, our observation further strengthen the fact that melatonin can protect liver and kidney from lead induced oxidative damages and can thus prevent situations of lead acetate induced pathogenesis related to these organs.

Earlier studies also suggest that lead causes hepatotoxicity decreases the glycogen content of liver [4]. Our study also establishes the fact that lead acetate deplete the glycogen reserve of liver and we have observed for the first time that the depletion of glycogen content of liver following lead acetate treatment can be prevented with pre-treatment of the rats with melatonin.

Exposure of rats to lead acetate resulted in an increase in tissue lipid peroxides and an elevation in tissue glutathione (GSH) concentration in both the tissues studied, which can be related to peroxidative damage of cell membranes in Pb-exposed animals.The concentrations of GSH in the liver, heart and kidneys were found to be significantly increased after lead-acetate feeding in male wistar rats [44]. In our experiments, pre-treatment of rats with melatonin protected the level of GSH from being altered in both the tissues. This indicates that melatonin is capable of maintaining the tissue antioxidant levels in the liver and kidneys in the face of oxidative stress.

To defend oxidative damage, organisms have developed antioxidative mechanisms with evolution. The antioxidative defence system is comprised of enzymes and metabolites in the subcellular compartments. These mechanisms are thought to be triggered by increased ROS production [27]. In stress conditions, however, normal capacities of these antioxidative mechanisms are compromised which triggers cells to increase and expand their antioxidative network. In this context, catalase and SOD are the two prime antioxidant enzymes that are affected by lead acetate induced oxidative stress situation. Superoxide dismutase (SOD) and catalase (CAT) act mutually and provide defence against reactive oxygen species (ROS) [27]. Superoxide dismutase (SOD) catalyzes the conversion of superoxide anion free radical to H_2O_2 through dismutation reaction [27]. Catalase catalyzes the conversion of hydrogen peroxide to water and oxygen. Thus, catalase protects the tissues from oxidative damage brought about due to H_2O_2.

We, in our experiments, have found that the activities of SOD and CAT increased in liver and kidney tissues following treatment with lead acetate. Increase in the activities of these two vital antioxidant enzymes were also reported by Uzun et al. in 2010 [45] in chlorpyrifos-induced lung toxicity in male rats. Pre-treatment of rats with melatonin protected the activities of these antioxidant enzymes from being altered. Studies by Rodriguez et al [46] suggested regulatory role of melatonin on antioxidant enzymes.

The current studies clearly reveal that following lead acetate treatment, the activities of XO and XDH are highly significantly increased compared to control with a concomitant increase in the XO plus XDH, XO/XDH ratio, XO/XO + XDH ratio in rat liver and kidney tissues. Metabolic reactions involving these two enzymes do serve as the source of superoxide anion free radical generation. Earlier workers have also indicated the involvement of XO in free radical production [22, 33] .Thus, it may be logical to conclude that enhancement in the activities of these two pro-oxidant enzymes in both liver and kidney tissues stimulates generation of superoxide anion free radicals. The enhancement of XO and XDH activities following cadmium-induced oxidative damage of heart in rats has also been recently reported [27].

Mitochondria are the prime site as well as the primary target of oxidative stress. We have studied the effect of lead acetate on the mitochondrial enzymes related to energy metabolism. Some heavy metals have been reported to affect respiratory chain complexes and there is clear cut substrate specificity [47]. Studies suggest that impairment of electron transfer through NADH:ubiquinone oxidoreductase (complex I) and ubiquinol:cytochrome c oxido-reductase (complex III) may induce superoxide formation [48]. This finding is supported by the observations made by Mitra et al., 2012 [27] in respect of cadmium induced myocardial damage in rat model. Mitochondrial production of ROS is considered to be significantly involved in many pathologic states of organs. Mukherjee et al. [31] reported involvement of mitochondrial oxidative stress in isoproterenol induced myocardial damage. Our studies has investigated the status of the activities of pyruvate dehydrogenase and some of the mitochondrial tricarboxylic acid cycle's enzymes , mainly Isocitrate dehydrogenase, alpha keto glutarate dehydrogenase and succinate dehydrogenase related to ATP production in mitochondria following treatment of rats with lead acetate for seven consecutive days. The activity of each of the enzyme is highly significantly decreased in lead acetate treated rats. 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. Decrease of mitochondrial TCA cycle enzymes increases free radical generation. Mitochondrial oxidative stress has been reported to be the underlying mechanism of pathogenesis of ageing, cancer, cirrhosis of liver, myocardial infarction, renal necrosis, and other disorders and diseases [27,46]. A reduction in the activity of NADH-cytochrome c reductase and cytochome 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 protected the activity of these enzymes from being altered indicating that melatonin is capable of ameliorating oxidative stress generated following treatment of rats with lead acetate.

Thus, alteration in mitochondrial redox metabolism and respiratory functions may lead to the increased production of ROS in cells, which in our case, was found to be effectively alleviated by melatonin. Moreover, melatonin has been reported to possess the ability to protect and improve mitochondrial functions [16, 47, 49] .

These observations which is presented in a summarized version in scheme 1 suggest that melatonin may be used as a potential clinical agent for providing protection against lead acetate induced oxidative stress and damages of the vital organs i.e., liver and kidney. Pharmacological doses of melatonin possess very low or no cytotoxicity and hence minimum or no side effects [31]. Parmer et al., [50] reported protective potential of melatonin against copper induced oxidative stress. There is a general agreement that 'classical' antioxidants are electron donors and are regenerated by redox reactions which can in turn promote the formation of other oxidized

species. On the contrary, melatonin seems to interact with free radicals by addition reactions, whose products are stable and excreted as such. Thus melatonin would be a 'suicide' antioxidant, which is not regenerated and does not promote further oxidation reactions. Melatonin can also regulate the activity and expression of antioxidant and pro-oxidant enzymes and could have a synergistic effect with other antioxidants, such as resveratrol [51]. Since melatonin is found in many foods specially the fruits (like grape skins, tart cherries and bananas) and vegetables [52] (like tomatoes), nuts and cereals (like walnuts, rice etc.) [9, 17, 19] 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 oxidative damage of the vital organs like liver and kidneys. Phyto derivatives e.g., Olive oil, wine and even beer are also rich sources of melatonin [12]. Though most of these foods contain significantly smaller amounts of melatonin than the dose typically used as supplement, yet including a variety of these foods into our daily diet is easy and can supply necessary amount of the compound. An important consideration is whether the body can obtain sufficient amounts of melatonin from food alone. A recent comprehensive review cites studies that show significant increases in both melatonin levels and antioxidant status in the blood of people who consumed melatonin-rich foods [17]. Oxidative damage induced by free radicals can be compensated by antioxidant activity of melatonin. Studies suggest that the possible mode of action is through its contributions in maintaining proper internal circadian phasing, and by its improvement of mitochondrial metabolism, in terms of avoiding electron leakage and enhancing complex I and complex IV activities. Intestinal absorption of dietary melatonin should not be a particular problem because the amphiphilic molecule can easily cross biological membranes. Therefore, an efficient uptake of the indoleamine from food should be expected to influence the blood plasma concentration [11] and will be a first hand measure for combatting lead induced hepatotoxicity and nephrotoxicity.

CONCLUSIONS

Treatment of rats with lead acetate at a dose of 15 mg/kg bw i.p. every consecutive day for a period of 7 days caused oxidative stress-induced damage in the hepatic and renal tissue as reflected from the alterations in the levels of biomarkers of hepatic and renal function and oxidative stress, antioxidant and pro-oxidant enzymes, with changes in the hepatic and renal mitochondrial Kreb's cycle and respiratory chain enzymes. These biochemical alterations were supported by our histological studies of the rat hepatic and renal tissue sections. Pre-treatment of rats with melatonin at a dose of 10 mg/kg bw fed orally ameliorated all these changes brought about by lead acetate. Melatonin seems to provide protection to rat liver and kidney against lead-induced oxidative stress through its direct as well as indirect antioxidant activity and also through its possible lead mobilizing or chelating properties. The results may be of future therapeutic significance particularly in the areas where man is chronically exposed to lead environmentally or occupationally. Melatonin is present in various fruits, cereals and vegetables which can be easily included in regular diet. 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 toxicity and lead-induced severe patho-physiological symptoms.

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REFERENCES

1. Chatterjee MN, Rana S. Metabolism of minerals and trace elements. In: Textbook of medical biochemistry. 5th ed. New Delhi: Jaypee Brothers Medical Publishers; 2002 p 35-39.
2. Markowitz M. Lead Poisoning. Pediatrics in Review 2000; 21: 327-335.
3. Pocock SJ.Factors influencing household water lead: A British National Survey. Arch Environ.Health 1980;35: 45-51.
4. Mudipalli A. Lead hepatotoxicity and potential health effects. Indian J Med Res 2007;126: 518-527.
5. Gonick HC. Nephrotoxicity of cadmium and lead. Indian J Med Res 2008; 128: 335-352.
6. Khan N, Anjum N, Perveen K, Rafique M. Lead induced nephrotoxicity with special reference to proximal tubule in albino rats. PJPS 2008; 25: 29-35.
7. Gomez FJV, Raba J, Cerutti S, Silva MF. Monitoring melatonin and its isomer in Vitis vinifera cv. Malbee by UHPLC- MS / MS from grape to bottle. J Pineal Res 2012; 52: 349-355.
8. Hardeland R, Fuhrberg B. Ubiquitous melatonin – presence and effects in unicells, plants and animals. Trends Comp Biochem Physiol 1996; 2:25-45.
9. Reiter RJ, Manchester LC, Tan DX. Melatonin in walnuts: Influence on levels of melatonin and total antioxidant capacity of blood. Nutrition 2005; 21: 920-924.
10. Bandyopadhyay D, Ghosh G, Bandyopadhyay A, Reiter RJ. Melatonin protects against piroxicam-induced gastric ulceration. J Pineal Res 2004; 36:195–203.
11. Hardeland R, Pandi-Perumal SR. Melatonin, a potent agent in antioxidative defense: actions as a natural food constituent, gastrointestinal factor, drug and prodrug. Nutr. Metab (Lond) 2005; 2: 22-31.
12. Paredes SD, Korkmaz A, Manchester LC, Tan DX, Reiter RJ. Phytomelatonin: a review. J Exp Bot 2009; 60: 57-69.
13. Reiter RJ, Paredes SD, Manchester LC, Tan DX.Reducing oxidative / nitrosative stress: a newly-discovered genre for melatonin. Crit Rev Biochem. Mol Biol 2009; 44:175-200.
14. Tan D X, Manchester L C, Terron M P, et al. One molecule many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007;42: 28-42.
15. Dominiguez-Rodrguez A, Abreu-Gonzalez P, Garcia Gonzalez MJ, Kaski JC, Reiter RJ, Jimenez-Sosa A. A unicenter, randomized, double-blind, parallel-group, placebo-controlled study of Melatonin as an Adjunct in patients with acute myocardial Infarction undergoing primary Angioplasty The Melatonin Adjunct in the acute myocaRdial Infarction treated with Angioplasty (MARIA) trial: study design and rationale. Contemp. Clin Trial 2007; 13: 532-539.
16. Reiter RJ, Tan DX, Manchester LC, El-Sawi MR. Melatonin reduces oxidant damage and promotes mitochondrial respiration: implications for aging. Ann N Y Acad Sci 2002;959:238–250.
17. Reiter RJ, Tan DX, Manchester LC, Simopoulos AP, Maldonadoa MD, Flores LJ, Terrona MP. Melatonin in edible plants (phytomelatonin): identification, concentrations, bioavailability and proposed functions. World Rev. Nutr. Diet 2007; 97: 211–230.
18. Paradies G, Petrossilo G, Paradies V, Reiter R J, Ruggiero F M. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. J Pineal Res 2010; 48:297-310.
19. Reiter RJ, Tan DX, Korkmaz A, Manchester LC. The disaster in Japan: utility of melatonin in providing protection against ionizing radiation. J Pineal Res 2011; 50:357-358.
20. Iriti M, Varoni EM, Sara V. Melatonin in traditional Mediterranean diets. J Pineal Res 2010; 49:101-105.
21. Reitman S, Frankel S. Determination of serum glutamic oxaloacetic andglutamic pyruvic transaminase. Am J Clin Pathol 1957; 28: 56–63.

22. Strittmatter CF.Studies on avian xanthine dehydrogenases: properties and patterns of appearance during development. J Biol Che1965; 240: 2557–2564.
23. Varcoe JS. Clinical Biochemistry: Techniques and Instrumentation-Apractical approach, first ed. World Scientific Publishing Company 2001; 40–43.
24. Royden, N. R., di Pasqua, A. A.,1962. New Diazo Method for the Determination of Bilirubin. Clin Chem. vol 8, p. 570-578.
25. Singh M, Batish MK, Singh M, Singh K, Lochan KK, Garg R. Correlation of Plasma Color Index with Serum Bilirubin in Neonatal Jaundice. Indian Pediatr. 2001;38: 278-280.
26. Bonsnes RW,Taussky HH. On the colorimetric determination of creatinine by the Jaffe reaction. J Biol Chem1945; 158: 581-591.
27. Mitra E, Ghosh A K, Ghosh D, Mukherjee D, Chattopadhyay A, Dutta S, Pattari SK, Bandyopadhyay D. Protective effect of aqueous Curry leaf (Murraya koenigii) extract against cadmium-induced oxidative stress in rat heart. Food. Chem. Toxicol 2012; 50:1340–1353.
28. Buege JA, Aust SG. Microsomal Lipid Peroxidation. Methods Enzymol 1978;52:302–310.
29. Sedlak J, Lindsay RH. Estimation of total, protein-bound, nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968; 25: 192–205.
30. Martin Jr JP, Daily M, Sugarman E. Negative and positive assays of superoxide dismutase based on hematoxylin autooxidation. Arch Biochem Biophys 1987; 255: 326–329.
31. Mukherjee D, Roy SG, Bandyopadhyay A, Chatyopadhyay A, Basu A, Mitra E, Ghosh AK, Reiter R, Bandyopadhyay B. Melatonin protects against isoproterenol-induced myocardial injury in the rat: antioxidative mechanisms. J Pineal Res 2010; 48: 251–262.
32. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyragallol and a convenient assay for superoxide dismutase.Eur J Biochem1974; 47: 469–474.
33. Beers Jr RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952; 195: 133–140.
34. Greenlee L, Handler P. Xanthine oxidase. IV. Influence of pH on substratespecificity. J Biol Chem 1964; 239:1090–1095.
35. Chretien D, Pourrier M, BourgeronT, Séné M, Rötig A, Munnich A, Rustin P. An improved spectrophotometric assay of pyruvate dehydrogenase inlactate dehydrogenase contaminated mitochondrial preparations from humanskeletal muscles. Clin Chim Acta1995; 240: 129–136.
36. Duncan MJ, Fraenkel DG. Alpha-ketoglutarate dehydrogenase mutant of Rhizobium meliloti. J Bacteriol 1979; 137: 415–419.
37. Veeger C, Der Vartanian DV, Zeylemaker WP. Succinate dehydrogenase.Methods Enzymol 1969;13: 81–90.
38. Goyal N, Srivastava VM. Oxidation and reduction of cytochrome c bymitochondrial enzymes of Setariacervi. J Helminthol 1995. 69: 13–17.
39. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265–275.
40. Gurer H, Ercal N. Can Antioxidants Be Beneficial In The Treatment Of Lead Poisoning? Free Radic Biol Med 2000; 29: 927–945.
41. Adly AMA. Oxidative stress and disease: an updated review. Res J Immunol 2010; 3:129-145.
42. Ghosh D, Firdaus SB, Mitra E, Dey M, Bandyopadhyay D. Protective effect of aqueous leaf extract of *Murraya koenigi* against lead induced oxidative stress in rat liver, heart and kidney: a dose response study. Asian J Pharm Clin Res. 2012; 5 (4):54-58.
43. Raju DSS, Lalitha DL, Kiranmayi P. Observation of estimated GFR in the assessment of chronic kidney disease: application and practice. Asian J Pharm Clin Res. 2012; 5 (4):201-206.
44. Hsu JM. Lead Toxicity as Related to Glutathione Metabolism. J Nutr 1981; 111: 26-33.
45. Uzun F G, Demir F, Kalender S, Bas H, Kalender Y. Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food Chem Toxicol 2010; 48:1714-1720.
46. Rodriguez C, Mayo JC, Sainz RM et al. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004; 36:1-9.
47. Desco MC, Ascensi M, Marquez R, Martínez-Valls J, Vento M, Pallardó FV, Sastre J, Viña J.Xanthine oxidase is involved in free radical production in type 1 diabetes.Diabetes 2002; 51:1118–1124.
48. Belyaeva EA, Korotkov SM, Saris NE. In vitro modulation of heavy metal induced rat liver mitochondria dysfunction: a comparison of copper and mercury with cadmium. J Trace Elem Med Biol 2011; 25: 63–73.
49. Leon J, Acuna-Castroviejo D, Escames G, Tan DX, Reiter RJ. Melatonin mitigates mitochondrial malfunction. J. Pineal Res 2005; 38:1–9.
50. Parmar PJ, Limson T, Nyokong et al. Melatonin protects against copper mediated free radical damage. J Pineal Res 2002; 32: 237–242.
51. Mercolini L, Mandrioli R, Raggi MA. Content of melatonin and other antioxidants in grape-related foodstuffs: measurement using a MEPS-HPLC-F method. J Pineal Res 2012; 53: 21-28.
52. Reiter RJ, Tan DX, Burkhardt S, Manchester LC. Melatonin in plants. Nutr Rev 2001;59:286–90.