PROTECTIVE EFFECTS OF ALPHA-LIPOIC ACID AND MELATONIN AGAINST CADMIUM-INDUCED OXIDATIVE STRESS IN ERYTHROCYTES OF RATS

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ABSTRACT

Cadmium (Cd) is a well-known human carcinogen and a potent nephrotoxin. The protective effects of alpha-lipoic acid and melatonin against cadmium (Cd) induced oxidative stress to erythrocytes in rats were evaluated. One hundred male albino rats were divided into five groups containing 20 rats each. Group I: (control) administered distilled water. Group II :(Cadmium exposed group) received cadmium chloride (4.4 mg/kg body weight of 1/20th of LD50 / day) orally for 10 weeks. Group III :(Cadmium +alpha-lipoic acid) received cadmium chloride (4.4 mg/kg body weight) and treated daily with alpha-lipoic acid (54 mg/kg body weight/ i.p). Group IV :(Cadmium +Melatonin) received cadmium chloride (4.4 mg/kg body weight) and treated daily with melatonin (10 mg/kg body weight/orally). Group V :(Cadmium +alpha-lipoic acid and melatonin). Urea and creatinine concentrations were determined in plasma. However, erythrocyte hemolysate were processed for the determination of L-Malondialdhyde (L-MDA), catalase (CAT), superoxide dismutase (SOD), Glutathione- S-transferase (GST) and reduced Glutathione (GSH) in addition to erythrocytes Glucose -6-phosphate dehydrogenase (G-6-PDH) activity. Also, kidney specimens were used for cadmium residues determination. The obtained results revealed that, a significant increase in plasma urea, creatinine concentrations and erythrocyte L-MDA level, SOD activity in addition to kidney cadmium residue concentrations were observed in cadmium intoxicated rats. However, administration of alpha-lipoic acid, melatonin and their combination exhibited a significant decreased in all mentioned parameters. On the other hand, a significant decreased in erythrocyte CAT, GST and G-6-PDH activities, GSH concentration were observed in cadmium intoxicated rats. Meanwhile, treatment with alpha-lipoic acid and melatonin resulted in significant increase in all mentioned parameters. It could be concluded that, the potential protective effect of alpha-lipoic acid and melatonin as a powerful agents and may be useful as an antioxidants in combating free radical-induced oxidative stress and tissue injury that is a result of cadmium toxicity.

Keywords: Antioxidant enzymes, cadmium, oxidative stress, alpha-lipoic acid, Melatonin

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1. INTRODUCTION

Cadmium (Cd) is one of the most toxic heavy metals. This metal is a serious environmental and occupational contaminant and may represent a serious health hazard to humans and other animals. Exposure to Cd can produce both acute and chronic tissue injury and can damage various organs and tissues, including liver, kidney, lung, bone, testis and blood depending on the dose, route and duration of exposure (Tarasub et al., 2011). In humans, chronic Cd exposure leads mainly to the nephrotoxicity (Trian and Trian, 1995), skeletal damage (Brzoska et al., 2008), severe damage in nervous, endocrine and immune system, linked to enhanced aging

process as well as cancer (J¨arup et al., 1998), whereas acute Cd exposure primarily affects the liver, inducing hepatocyte swelling and fatty change, with focal, zonal or massive necrosis (Habeebu et al., 1998).

The oxidative stress induced by Cd in a biological system may be due to increased lipid peroxidation, which may be attributed to alterations in the antioxidant defense system (Newairy et al., 2007). The renal impairment is the main effect observed upon chronic Cd exposure and the proximal tubules of the kidney are the primary target (Goyer and Clarkson, 2001).

It has been reported that, chronic treatment with cadmium induced oxidative damage in erythrocytes of rats, causing destruction of cell membranes and increased lipid peroxidation, as well as alteration of the oxidative enzyme system, energy metabolism and the appearance of anemia (Ognjanović et al., 2000). The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) induced by Cd could be responsible for its toxic effects in many tissues and organs (Waisberg et al., 2003).

Antioxidants are substances, which inhibit or delay oxidation of a substrate while present in minute amounts. By other words, Antioxidants are substances those are easily oxidized by ROS in a biological system, decreasing the rate at which the ROS react with cellular components like lipid membranes, DNA, or proteins. The most important source of antioxidants is provided by nutrition (Flora, 2002).

Alpha-lipoic acid is naturally occurring compound that is synthesized by plants and animals, including humans (Self et al., 2000). Moreover, alpha-lipoic acid acts as an antioxidant in fat and water soluble tissue in both its oxidized and reduced forms (Kagan et al., 1992). A number of studies suggest that alpha-lipoic acid is able to recycle other natural antioxidants specially is capable of reducing the oxidized forms of vitamin C, α-tocopherol, glutathione and coenzyme-Q (Smith et al., 2004).

Melatonin is powerful antioxidant that can easily cross cell membranes and the blood brain barrier (Hardeland, 2005). Moreover, melatonin apparently stimulates several antioxidant enzymes, including glutathione reductase, glutathione peroxidase and superoxide dismutase, promoting quick disposal of H_2O_2 from rat brain cortical cells (Kotler et al., 1998), enhances the production of enzymes that are involved in the synthesis of glutathione (Reiter et al., 1999), prevents the reduction of membrane fluidity caused by lipid peroxidation, and helps in scavenging free radicals (Garcia et al., 1997). Accordingly, the purpose of this study to elucidate the harmful effects of cadmium toxicity on several biochemical blood parameters in male rats exposed to cadmium chloride. Also, the possible protective effects of alpha-lipoic acid and melatonin alone and in combination on biomarkers of oxidative stress and antioxidant enzymes in erythrocytes and vital organs (kidney) were also assessed to evaluation whether alpha-lipoic acid and melatonin would ameliorate the toxic effect of cadmium induced oxidative tissue damage in male rats.

2. MATERIALS AND METHODS

2.1. Experimental animals:

One hundred white male albino rats of 8-10 weeks old and weighing 160 – 200 gm were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied ad-libitum. All rats were acclimatized for minimum period of two weeks prior to the beginning of study.

2.2. Chemicals and drugs

All chemicals were of analytical grade and obtained from standard commercial suppliers. The drug and chemicals used in the present study were:

Cadmium chloride: Cadmium chloride has molecular weight 218.41. Each one gram of cadmium chloride 72% contains 515 mg of cadmium. It was manufactured by Riedel-Dehoen Ag Seelze- Hannover, West Germany. Cadmium chloride was dissolved in distilled water, freshly prepared and administered orally and daily at a dose level of 4.4 mg/kg body weight (1/20 of L.D.50). Oral rat LD_{50} for Cadmium Chloride anhydrous is 88 mg/kg body weight (Onwuka et al., 2010).

Alpha- Lipoic acid (Thiotacid) ®*:* Thiotacid was obtained as pack of five ampoules of 10ml solution. Each ampoule contains thioctic acid (alpha lipoic acid) 300 mg. Alpha-lipoic acid (Thioctic acid) manufactured by EVA pharma for pharmaceuticals and Medical Apliances, Egypt. Alpha lipoic acid was injected intraperetinoel in a daily dose of 54 mg/kg body weight (Gruzman et al., 2004).

Melatonin (N-acetyl-5-methoxytryptamine): Melatonin was obtained as packs of 120 tablets. Each tablet contains melatonin 3 mg. Melatonin purchased from puritan's pride, inc. (Oakdale, NY 11769 U.S.A.).The tablets were dissolved in warm saline solution (0.9%NaCl) contained 40% volume of propylene glycol freshly prepared and administered orally and daily at a dose level of 10 mg/kg body weight (Kim et al., 1998).Propylene glycol was manufactured by El-Nasr Pharmaceutical Chemicals Co. Abu zaabal, Egypt.

2.3. Experimental design

After acclimatization to the laboratory conditions, the animals were randomly divided into five groups (twenty rats each) placed in individual cages and classified as follow:

Group I (control normal group): Rats received no drugs, served as control non-treated for all experimental groups.

Group II (Cadmium chloride exposed group): Rats received cadmium chloride 1/20 of LD_{50} (4.4 mg/kg body weight) orally and once per day over a period of 10 weeks.

Group III (Cadmium Chloride+ Alpha-lipoic acid treated group): Rats received cadmium chloride (4.4 mg/kg body weight) and treated daily with alpha-lipoic acid (54 mg/kg body weight/ i.p).

Group IV (Cadmium Chloride +Melatonin treated group): Rats received cadmium chloride (4.4 mg/kg. body weight) and treated daily with melatonin (10 mg/kg body weight/orally).

Group V (Cadmium Chloride +Alpha-lipoic acid + melatonin treated group): Rats received cadmium chloride (4.4 mg/kg body weight) and treated daily with alpha-lipoic acid (54 mg/kg body weight/i.p) in combined with melatonin (10 mg/kg body weight/orally) for 10 weeks.

2.4. Sampling:

2.4.1. Blood samples:

Blood samples were collected by ocular vein puncture from all animal groups 3 times along the duration of experiment in dry, clean and screw capped heparinized tubes and plasma were separated by centrifugation at 3000 r.p.m for 10 minutes. The clean clear plasma was separated by Pasteur pipette and kept in a deep freeze at -20°C until used for subsequent biochemical analysis Moreover, after plasma separation, erythrocytes were washed three times with an equal volume of cold saline, then 1ml RBCs lysed with 4 ml distilled water in dry sterile caped tubes. The samples were kept at -20 ºC for subsequent biochemical analysis.

2.4.2. Tissue specimens (kidney):

Kidney specimen were taken from each group of rats after had been sacrificed at 4 and 10 weeks of the experiment. The specimens were quickly removed and washed several times with saline, weighed and processed for determination of cadmium residues by using Atomic Absorption Spectrophotometer as described by Al Ghais (1995).

2.5. Biochemical analysis

Plasma Urea and Creatinine were determined according to the method described by Kaplan, et al. (2003) and Tietz (1995) respectively. Moreover, blood G-6-PDH, erythrocyte MDA, CAT, SOD, GST and GSH were determined according to the method described by Sood et al. (1981); Esterbauer et al., (1982); Sinha, (1972); Packer and Glazer, (1990); Habig and Pabst (1974) and Beutler et al., (1963), respectively.

2.6. Statistical Analysis

The results were expressed as mean ± SE and statistical significance was evaluated by one way ANOVA using SPSS (version 10.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when $p<0.05$.

Table (1) Effect of alpha- lipoic acid, melatonin alone and their combination on plasma Urea and creatinine concentrations in cadmium intoxicated male rats.

Parameters	Urea (mg/dl)			Creatinine (mg/dl)		
Animal groups	2 weeks	4 weeks	10 weeks	2 weeks	4 weeks	10 weeks
Control normal	15.35 ± 0.28 ^{d}	25.52 ± 0.17 ^{e}	25.65 ± 0.42 ^{c}	0.50 ± 0.256 ^{a}	0.57 ± 0.021 ^{c}	0.62 ± 0.018 ^{c}
Cadmium chloride	29.03 ± 0.30 ^{a}	38.32 ± 0.65 ^{a}	35.45 ± 2.63 ^{a}	0.84 ± 0.034 ^{b}	0.95 ± 0.019 ^{a}	0.98 ± 0.060 ^{a}
Cadmium Chloride + Alpha-lipoic acid	28.45 ± 0.78 ^{ab}	36.33 ± 0.31 ^{b}	35.88 ± 0.66 ^{a}	0.73 ± 0.036 ^{b}	0.73 ± 0.042 ^{b}	0.74 ± 0.020 ^{b}
Cadmium Chloride + Melatonin	27.63 ± 0.34 ^{b}	32.63 ± 0.44 ^{c}	30.78 ± 0.16 ^{b}	0.68 ± 0.031 ^{b}	0.70 ± 0.037 ^{b}	0.76 ± 0.037 ^{b}
Cadmium Chloride + Alpha-lipoic acid + Melatonin	25.12 ± 0.14 ^{c}	28.27 ± 0.48 ^{d}	27.83 ± 0.48 ^{bc}	0.67 ± 0.025 ^{b}	0.68 ± 0.031 ^{b}	0.70 ± 0.015 ^{bc}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P\leq0.05$).

3. RESULTS

3.1. Plasma urea and creatinine concentrations

The obtained results demonstrated in (Table 1) revealed that, cadmium intoxicated rats showed significant increase in plasma urea and creatinine concentrations when compared with normal control group. Treatment with alpha-lipoic acid, melatonin

Table (2) Effect of alpha- lipoic acid, melatonin alone and their combination on erythrocytes L-MDA and GSH concentrations, CAT, SOD , GST and G-6-PDH activities in cadmium intoxicated male rats.

Parameters	L-MDA (nmol/mL)			GSH (mg/dl)			Catalase (U/L)			SOD(U/L)			GST (U/g Hb)			G-6-PDH (U/g Hb)
Animal groups	2 weeks	4 weeks	10 weeks	2 weeks	4 weeks	10 weeks	2 weeks	4 weeks	10 weeks	2 weeks	4 weeks	10 weeks	2 weeks	4 weeks	10 weeks	10 weeks
Control normal	20.36 ± 1.307 d	19.45 ± 1.251 d	12.26 ± 0.315 e	5.61 ± 0.15 a	2.28 ± 0.09 a	5.49 ± 0.06 a	1.21 ± 0.023 a	0.86 ± 0.003 a	0.68 ± 0.006 a	127.77 ± 19.26 b	416.19 ± 10.23 a	142.93 ± 9.99 c	45.81 ± 0.46 b	25.99 ± 0.49 b	18.83 ± 0.55 bc	72.65±2.64 a
Cadmium chloride	34.48 ± 1.671 a	30.58 ± 0.174 a	30.09 ± 0.473 a	1.43 ± 0.07 e	0.86 ± 0.06 e	1.62 ± 0.06 e	1.04 ± 0.011 c	0.79 ± 0.004 d	0.65 ± 0.006 bc	335.98 ± 27.79 a	404.11 ± 33.05 ab	272.20 ± 21.94 a	38.24 ± 0.94 e	23.99 ± 0.27 c	17.79 ± 0.25 d	45.05±0.45 c
Cadmium Chloride + Alpha-lipoic acid	29.63 ± 0.322 b	25.95 ± 0.253 b	23.77 ± 0.505 b	2.26 ± 0.11 d	1.27 ± 0.03 d	2.55 ± 0.07 d	1.14 ± 0.005 b	0.81 ± 0.006 c	0.64 ± 0.005 c	136.98 ± 15.13 b	329.43 ± 7.85 c	177.89 ± 14.32 bc	40.86 ± 0.70 d	25.68 ± 0.08 b	18.35 ± 0.37 cd	51.45±0.76 bc
Cadmium Chloride + Melatonin	26.42 ± 0.070 c	23.32 ± 0.084 c	20.35 ± 0.239 c	2.84 ± 0.04 c	1.51 ± 0.03 c	3.26 ± 0.01 c	1.14 ± 0.005 b	0.82 ± 0.004 b	0.65 ± 0.004 bc	351.08 ± 52.41 a	348.65 ± 7.29 bc	189.08 ± 50.14 bc	43.18 ± 0.91c	26.61 ± 0.29 b	19.40 ± 0.08 b	52.05±5.42 bc
Cadmium Chloride + Alpha-lipoic acid + Melatonin	24.43 ± 0.211 c	20.90 ± 1.287 d	15.14 ± 0.103 d	3.55 ± 0.08 b	1.74 ± 0.04 b	4.18 ± 0.02 b	1.16 ± 0.005 b	0.83 ± 0.004 b	0.66 ± 0.004 b	375.45 ± 37.98 a	265.72 ± 27.32 d	235.00 ± 11.79 ab	49.38 ± 0.84 a	32.71 ± 0.33 a	24.42 ± 0.09 a	57.63±5.32 b

Data are presented as (Mean ± S.E) S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (*P≤0.05*).

alone and in combination to cadmium intoxicated rats caused significant decrease plasma urea and creatinine concentrations when compared with cadmium intoxicated group.

3.2. Erythrocytes L-MDA and GSH concentrations ,CAT, SOD , GST and blood G-6-PDH *activities*

The obtained data revealed that, erythrocytes catalase (CAT) and

Table (3): Effect of alpha- lipoic acid, melatonin alone and their combination on kidney cadmium residue concentrations in cadmium intoxicated male rats.

Parameters	Durations of treatment Kidney cadmium (ppm/gm wet tissue)	
Animal groups	4 weeks	10 weeks
Control normal	21.44 ± 0.97 [c]	20.94 ± 1.61 [d]
Cadmium chloride	653.07 ± 86.90 [a]	722.71 ± 68.60 [a]
Cadmium Chloride + α-lipoic acid	281.16 ± 9.00 [b]	383.43 ± 22.46 [b]
Cadmium Chloride + Melatonin	209.62 ± 49.70 [b]	321.54 ± 62.75 [bc]
Cadmium Chloride + α-lipoic acid + Melatonin	60.73 ± 1.69 [c]	206.39 ± 62.14 [c]

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$)

Glutathione-S-transferase (GST), blood glucose-6-Phosphate dehydrogenase (G-6-PDH) activities and GSH concentration were significantly decreased and erythrocytes L-MDA concentration and superoxide dismutase (SOD) activity were significantly increased in cadmium intoxicated rats when compared with normal control group. Treatment with alpha-lipoic acid and melatonin and their combination to cadmium intoxicated rats caused a significant increased in erythrocytes CAT , GST and blood G-6-PDH activities and GSH level with significant decrease in SOD activity and L-MDA concentration when compared with cadmium exposed non treated group (Table 2).

3.3. Kidney cadmium residue concentrations

The obtained results presented in (Table 3) revealed that, the mean value of kidney cadmium residues concentrations increased significantly in cadmium exposed rats when compared with normal control group. Treatment with alpha-lipoic acid, melatonin alone and in combination to cadmium intoxicated male rats resulted in significant decrease in kidney cadmium residues concentrations when compared with cadmium exposed group.

4. DISCUSSION

Cadmium intoxicated rats showed significant increase in plasma urea and creatinine concentrations when compared with normal control group These results came in accordance with the recorded data of Ibrahim (2013) who, reported that, nephritic pathological changes included significant increases of serum creatinine and urea concentrations were observed in cadmium exposed rats. Urea is the first acute renal marker which increases when the kidney suffers any kind of injury. Otherwise, creatinine is the most trustable of them (Borges et al. 2008). The increase of plasma urea and creatinine concentrations in cadmium exposed rats may be attributed to the toxic effect of cadmium on the renal tubules and glomeruli lead to nephrotoxicity and renal tubular damage Aisha and Elham (2000).

Treatment with alpha-lipoic acid, melatonin alone and in combination to cadmium intoxicated rats caused significant decrease plasma urea and creatinine concentrations

when compared with cadmium intoxicated group. Similarly, Rashwan and Anfenan (2012) reported that, treatment with α-lipoic acid in cadmium intoxicated rats resulted in decrease in serum urea and creatinine concentrations compared to cadmium group. Also, Al Abbassi et al., (2008) reported that, therapeutic administration of melatonin at a dose of 20 mg/kg in lead acetate treated rats significantly reduces serum urea and creatinine concentrations. This suggestion was confirmed by the findings of Shaikh et al., (1999) who indicated that, free-radical scavengers and antioxidants are useful in protecting against cadmium toxicity.

The obtained results revealed that, cadmium intoxicated rats showed significant increase in erythrocytes L-MDA concentration when compared with normal control group. Likewise, Kowalczyk et al., (2002) observed that, long-term intoxication with cadmium chloride elevated serum and erythrocytes TBARS concentrations. These results may be related to that, Cd inhibits the activity of majority of enzymes involved in AOS (Casalino et al., 2002) inducing an increased production of free radicals, lipid peroxidation, and destruction of cell membranes (Ognjanović et al. 2003). Since Cd causes lipid peroxidation in numerous tissues both in vivo and in vitro (El-Demerdash et al. 2004), it has been suggested that Cd may induce oxidative stress by producing hydroxyl radicals (O'Brien and Salasinski 1998), superoxide anions, nitric oxide and hydrogen peroxide (Waisberg et al., 2003). Furthermore, Cd induces increased ROS formation, which, in turn causes lipid peroxidation, DNA damage and oxidatively modified proteins, and eventually leads to cellular dysfunction and necrotic cell death (Thevenod, 2009). On the other hand, as reported by Nemmiche et al. (2007) the mechanism of Cd-induced LPO is still not fully understood. Available data indicate that the mechanism is multidirectional and may involve a decrease in the level of glutathione and the total pool of sulphydryl groups and changes in the activities of antioxidant enzymes (Nemmiche et al. 2007).

Treatment with Alpha-lipoic acid, Melatonin alone and in combination to cadmium intoxicated male rats caused significant decrease in erythrocytes L-MDA concentration. Similarly, Shagirtha et al., (2011) reported that, administration of melatonin (10 mg/kg/day) for 4 weeks in cadmium intoxicated rats significantly diminished the levels of oxidative stress markers, lipid peroxidation and protein carbonyls in brain. These results may be related to that, the protective action of melatonin against LPO as a factor modifying membrane organization, may due to melatonin's ability scavenge the LPO initiating agents, which produced during the peroxidation of lipids (El-Sokkary et al., 2003).

Cadmium intoxicated rats showed significant decrease in erythrocytes GSH concentrations. These results came in accordance with the recorded data of Renugadevi and Prabu (2009) who found that, reduced glutathione level was depressed and its dependent enzymes in Cd-intoxicated rats. Oxidative stress was generated as results of the inhibition of antioxidant enzymes (G6PD, CAT and SOD) and the depletion of GSH content due to cadmium toxicity which accompanied by excess generation of free radicals, came in accordance with (Jemai et al., 2007) who demonstrated that, the oxidative stress induced by Cd in a biological system may be due to increased lipid peroxidation, which may be attributed to alterations in the antioxidant defense system.

Glutathione are considered the first line of cellular defense against Cadmium-mediated oxidative damage. GSH functions by

detoxifying various xenobiotics as well as scavenging free radicals and is consequently converted to its oxidized form, glutathione disulfide (GSSG). However, conditions of marked toxicity or oxidative stress elevate intracellular levels of GSSG, which brings GSSG-reductase into play to reduce GSSG to GSH (Plummer et al. 1981). Moreover, a decreased GSH level was associated with the increased LPO process in rats intoxicated with Cd (Nemmiche et al. 2007).

Treatment with Alpha-lipoic acid, Melatonin alone and in combination to cadmium intoxicated male rats caused significant increase in erythrocytes GSH concentrations when compared with cadmium exposed group. Similarly, Gaurav et al., (2011) reported that, administration of some dietary nutrients i.e. N-acetyl cystiene, methionine, melatonin, Vit-B1 alone or their combination with cadmium chloride resulted increase in GSH level in blood. Also, Stohs et al., (2000) reported that, since cadmium is well-documented as an intracellular GSH deplete in some organs the stimulatory effect of melatonin on GSH homeostasis may in part account for its protective actions against oxidative stress. These results may be related to that, the antioxidants such as Vit E, Vit C and GSH protect the erythrocyte membrane from oxidative damage (Ognjanović et al. 2003). Lipoic acid (LA) has the ability to generate endogenous antioxidants, such as GSH (Biewenga et al. 1997), but the data indicate that cadmium was removed from the hepatocytes by LA/DHLA compounds. Antioxidant activity of LA/DHLA was reflected in terms of decreased Cd^{2+}-depleted intracellular glutathione (GSH) (Muller and Menzel 1990). LA could either mitigate GSH consumption by acting as an alternate ROS scavenger or increase GSH levels by stimulating its biosynthesis with an unknown mechanism. A recent hypothesis to explain how LA stimulates GSH biosynthesis came from Packer (1998). They suggested that LA administration could induce increases in GSH levels by facilitating transport of cystine, the limiting factor in GSH synthesis, into the cells. Once LA is taken up by the cell, it is immediately reduced to DHLA that is then released. The released DHLA induces a chemical reduction of extracellular cystine to cysteine. Cysteine can be taken up rapidly (10 times more) by the cells than cystine and can then be used in the biosynthesis of GSH.

A significant decrease in erythrocytes catalase (CAT) and glutathione-S-transferase (GST) activities were observed in cadmium exposed rats. However, erythrocytes superoxide dismutase (SOD) activity was significantly increased when compared with normal control group (Table 2). These results came in accordance with the recorded data of Shagirtha et al., (2011) who recorded that, a significant decrease in the activities of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) were observed in rats intoxicated with cadmium (5 mg/kg/day) for 4 weeks. Also, Caylak et al., (2008) reported that, an increase in SOD activity of erythrocytes was observed in lead treated rats. These results may be related to that, the GST enzyme has an important role in detoxification of xenobiotics, drugs and carcinogens and thus protects the cells against redox cycling and oxidative stress (Casalino et al. 2004). In addition, Sinha et al., (2008) reported that, Cd intoxication decreased the activities of other thiol-based antioxidant enzymes (GST and G-6-PDH) through modification of the −SH (thiol) groups. Catalase is an inducible cytosolic enzyme, which serves to protect the biological system against reactive oxygen species, converting hydrogen peroxide (formed in excess in the process of the dismutation reaction of the superoxide radical anion) to non-toxic oxygen and water

at a rapid rate. It has been shown that various antioxidants and antioxidant defense systems protect cells from Cd-induced toxicity (Ognjanović et al. 2006). In this sense, it has been shown that ROS formation induced by Cd is inhibited by catalase, superoxide dismutase, and by hydroxyl radical scavengers (Pourahmad and O'Brien 2000). So the antioxidant enzymes are considered to be the second line of cellular defense in prevention of biological macromolecules from oxidative damage.

Treatment with alpha-lipoic acid and melatonin and their combination to cadmium intoxicated rats caused a significant increased in erythrocytes CAT and GST activities with significant decrease in SOD activity when compared with cadmium intoxicated group. These obtained results are in accordance with the results of Rashwan and Anfenan (2012) who reported that, α-lipoic acid treatment in cadmium-intoxicated rats showed increase in SOD, CAT and GPX activities compared to cadmium group. Also, Caylak et al., (2008) reported that, a significant decrease in erythrocytes SOD activity was observed in lipoic acid treated lead exposed rats. Moreover, Shagirtha et al., (2011) reported that, administration of melatonin (10 mg/kg/day) for 4 weeks in cadmium-intoxicated rats significantly elevated the activities of enzymatic antioxidants SOD, CAT, GPx and GST in brain.

Erythrocyte glucose-6-Phosphate dehydrogenase (G-6-PDH) activity was significantly decreased in cadmium-intoxicated rats when compared with normal control group (Table 2). These results came in accordance with the recorded data of Renugadevi and Prabu (2009) who reported that, a significant decrease in the activity of hepatic G-6-PDH was observed in cadmium-treated rats.

These results may be related to that, the formation of lead sulfydryl complex was suggested as a plausible mechanism behind G-6-PDH inhibition (Lachant et al., 1984).Where,G-6-PDH supplies the cells with most of the extra mitochondrial NADPH through oxidation of glucose-6-phosphate. This NADPH keeps GSH at a constant level by providing NADPH for GR, which mediates the reduction of GSSG to GSH. G-6-PDH is known to contain many SH groups, which play a crucial role in maintaining its tertiary structure (Yoshida and Huang 1986).

Treatment with alpha-lipoic acid and melatonin and their combination to cadmium intoxicated rats caused a significant increase in erythrocyte G-6-PDH activity when compared with cadmium exposed group These results are nearly similar with those of Elena et al., (2007) who reported that, melatonin treatment of diabetic rats increasing liver glucose-6- phosphate dehydrogenase activity. Lipoic acid treatment of animals receiving lead for 5 weeks returned G6PD activity to control levels that can be explained by the decreased need for NADPH (Gurer et al., 1999). LA may achieve this by acting as an alternative sulfhydryl nucleophile to GSH, thereby preventing its oxidation to GSSG in detoxification reactions against ROS.

The obtained results revealed that, kidney cadmium residues concentrations increased significantly in cadmium-exposed rats when compared with normal control (Table 3). Similarly, Gaurav et al., (2011) reported that, a significant increase in cadmium level was observed in liver, kidney and blood with higher amount of cadmium accumulation in the kidney in Cd-treated rats after 21 days as compared with controls.

Treatment with alpha-lipoic acid, melatonin alone and in combination to cadmium intoxicated male rats resulted in significant decrease in kidney cadmium residues concentrations when compared with

cadmium-exposed group (Table 3). These results came in accordance with the recorded data of Biewenga et al., (1997) who reported that, Lipoic acid (LA) has the ability to generate endogenous antioxidants, such as GSH but the data indicate that cadmium was removed from the hepatocytes by LA/DHLA compounds. Also, Gaurav et al., (2011) reported that, administration of dietary nutrients i.e. N-acetyl cystiene, methionine, melatonin and Vit-B1 to cadmium chloride treated rats resulted in decreased Cd accumulation in liver and kidney.

Cadmium induces production of metallothionine (MT), a low molecular weighed protein that has high affinity for the metal (Nordberg and Nordberg, 2000). This seems to provide a mechanism by which the metal can be sequestered in a relatively inert and thus nontoxic state (Liu et al., 1995). When the amount of Cd exceeds the binding capability of MT, the non-MT-bound Cd ions are believed to cause toxic to the organ systems such as hepato- and nephrotoxicity (Nordberg and Nordberg, 1987). Hepatic metallothionein also binds zinc (Webb, 1972). Possibly, in cadmium supplemented rats, hepatic concentration of cadmium and zinc increased because these elements bound to an induced metallothionein (Meyer et al., 1982).

Administration of melatonin and/or alpha-lipoic acid (ALA) together offset the cadmium-induced changes in antioxidant defense, biochemical parameters and tissue accumulation of cadmium. The combination of Cd+melatonin +ALA was more effective than either of these protectants compared with the value of control. This can be explained according to action of this dual antioxidants where, Melatonin is a lipophilic molecule that freely crosses cell membranes and enters cells (Menendez-Pelaez et al., 1993), where it has been reported to alter redox balance, i.e., by increasing glutathione levels (Urata et al., 1999) and via radical scavenging (Reiter et al., 1994). In addition, melatonin has been shown to be five times superior to glutathione in scavenging free hydroxyl radicals. Moreover, Melatonin prevents the reduction of membrane fluidity caused by lipid peroxidation and thereby helps in scavenging free radicals (Garcia et al., 1997).On Other hand, Alpha-lipoic acid and its metabolites can scavenge many other reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hypochlorous acid (HOCl), hydroxyl radicals, peroxyl radicals, superoxide and peroxynitrire. In addition to, both alpha-lipoic acid and DHLA may chelate or bind metal ions that prevent them from generating free radicals (Biewenga et al., 1997). Moreover, A number of studies suggest that, alpha-lipoic acid is able to recycle other natural antioxidants specially is capable of reducing the oxidized forms of vitamin C, α-tocopherol, glutathione and coenzyme-Q (Smith et al., 2004). Thus, in combination, these protectants seem to complement each other leading to complete quenching of free radicals (Sumathi et al., 1996). The obtained biochemical changes of plasma Kidney damage correlated well and confirmed with the histological findings in the present study.

It could be concluded that, melatonin has ameliorating effect and may be more efficient than ALA in cadmium toxicity. Also, this study provides novel evidence that treatment with melatonin or/and ALA exert modulator effects in cadmium toxicity and decrease cadmium destructive effect as revealed by marked improvement in biomarkers of oxidative stress and antioxidant enzymes in rats erythrocytes with distinct decrease of cadmium residues in liver and kidney. This study indicate that, the potential of alpha-lipoic acid and melatonin as a cytoprotective and powerful agents against cadmium -induced oxidative stress of tissue and erythrocytes of rats.

5. REFERENCES

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التأثيرات الوقائية لحمض ألفاليبويك و الميلاتونين ضد الإجهاد التأكسدى لخلايا الدم الحمراء المحدث بالكادميوم في الفئران.

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الملخص العربي

يعتبر التلوث بالعناصر الثقيلة من أخطر أنواع التلوث التي يتعرض لها الإنسان والحيوان على حد سواء لما يحدثه من تدمير خلايا وأنسجة وأعضاء الجسم الحيوية ذلك لما تنتجه داخل الخلايا من الشوارد الحرة. وحيث أن مضادات الأكسدة تلعب دورا هاماً في السيطرة والتقليل من خطورة الشوارد الحرة على الأنسجة والأعضاء المختلفة فقد جاءت فكرة هذا البحث لدارسة الآثار الضارة للتسمم بعنصر الكاديميوم وتقييم التأثيرات الوقائية لحامض الألفاليبويك والميلاتونين ضد الإجهاد التأكسدى لخلايا الدم الحمراء المحدث بالكادميوم في الفئران حيث أستخدم لإجراء تلك الدراسة عدد مائه من ذكور الفئران البيضاء قسمت إلى خمس مجموعات كلاً منها يحتوي على عشرون. المجموعة الأولى (المجموعة الضابطة): أعطيت ماء مقطر، المجموعة الثانية (المجموعة المسممة بالكادميوم): تم تجريعهم للكادميوم فقط يوميا عن طريق الفم بجرعة مقدارها 4.4 مليجرام لكل كيلوجرام من وزن الجسم لمدة 10 أسابيع. المجموعة الثالثة (مجموعة الكادميوم + الألفاليبويك): تم تجريعهم للكادميوم يوميا عن طريق الفم بجرعة مقدارها 4.4 مليجرام لكل كيلوجرام من وزن الجسم وحقنهم بالألفاليبويك فى الغشاء البريتونى بجرعة مقدارها 54 مليجرام لكل كيلوجرام. المجموعة الرابعة (مجموعة الكادميوم + الميلاتونين): اشتملت على20 فأر تم تجريعهم للكادميوم يوميا عن طريق الفم بجرعة مقدارها 4.4 مليجرام لكل كيلوجرام من وزن الجسم وعولجت بالميلاتونين بجرعة مقدارها 10 مليجرام لكل كيلوجرام من وزن الجسم عن طريق الفم لمدة 10 أسابيع. المجموعة الخامسة (مجموعة الكادميوم + الألفاليبويك + الميلاتونين): استخدم الدم في قياس أنزيم جلوكوز -6 فوسفات دى هيدروجينيز.استخدمت البلازما في قياس نسبة اليوريا، الكرياتينين. استخدم الهيموليزات فى قياس المالون داى ألدهيد، جلوتاثيون مختزل، الكتاليز، السوبر أكسيد دسميوتيز وجلوتاثيون إس ترانسفيراز.أيضا تم استئصال الكلى واستخدمت لقياس نسبة بقايا الكادميوم بها. أظهرت النتائج وجود زيادة معنوية فى نسبة اليوريا، الكرياتينين بالإضافة إلى تركيز المالون داى ألدهيد ونشاط السوبر أكسيد دسميوتيز في كرات الدم الحمراء لدى الفئران التي تعرضت للتسمم بالكادميوم، فى المقابل أظهرت المعالجة بحامض الألفاليبويك والميلاتونين كلاً بمفرده ومجتمعين وجود انخفاض معنوى فى جميع تلك القياسات المذكورة. من ناحية أخرى أظهرت النتائج وجود نقص معنوي فى نشاط الكتاليز، جلوتاثيون إس ترانسفيراز، جلوكوز -6 فوسفات دى هيدروجينيز في كرات الدم الحمراء لدى الفئران التي تعرضت للتسمم بالكادميوم ، في حين أظهرت المعالجة بحامض الألفاليبويك و الميلاتونين كلاً بمفرده و مجتمعين وجود زيادة معنوية فى جميع تلك القياسات المذكورة.
يستخلص من تلك النتائج أن كلاً من حامض الألفاليبويك والميلاتونين يعتبر عامل قوى مضاد للأكسدة فى مكافحة الذرات الطليقة المسببة للإجهاد التأكسدى ودمار الأنسجة المحدث في حالات التسمم بالكادميوم وكذلك العناصر الثقيلة. ولذلك ننصح بإعطاء حامض الألفاليبويك والميلاتونين بالجرعة العلاجية الآمنة وذلك للتقليل من الأثار الضارة غير المرغوب فيها التي يحدثها التعرض للتسمم بالعناصر الثقيلة.

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