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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 present study was carried to evaluate the protective effects of alpha-lipoic acid and melatonin against cadmium (Cd) induced oxidative stress to erythrocytes in rats. 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^{-1} b.wt. of 1/20th of LD_{50}) orally and once per day over a period of 10 weeks. Group III: (Cadmium+Alpha-lipoic acid treated group) received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with alpha-lipoic acid (54 mg kg^{-1} b.wt./i.p). Group IV: (Cadmium+Melatonin treated group) received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with melatonin (10 mg kg^{-1} b.wt./orally). Group V: (Cadmium+alpha-lipoic acid and melatonin treated group). Heparinized blood used for Glucose-6-phosphate dehydrogenase and hemoglobin determination. Plasma used for determination of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Gamma-glutamyltransferase (γ-GT) activities and urea, creatinine, total cholesterol and phospholipids concentrations. Moreover, erythrocyte hemolysate were processed for the determination of L-Malondialdhyde (L-MDA), Catalase (CAT), Superoxide Dismutase (SOD), Glutathione-s-transferaseand (GST) and reduced Glutathione (GSH). Also, liver and kidney specimens were excised for histopathological examination and for cadmium residues determination. The obtained results revealed that, a significant increase in plasma ALT , AST and GGT activities, urea, creatinine, total cholesterol and phospholipids concentrations and erythrocyte L-MDA level, SOD activity ,Liver and kidney cadmium residue were observed in cadmium intoxicated rats. However, administration of alpha-lipoic acid, melatonin and their combination in cadmium intoxicated rats 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 and hemoglobin concentrations were observed in cadmium intoxicated rats. Meanwhile, alpha-lipoic acid and melatonin administrations alone and in combination in cadmium intoxicated rats resulted in significant increase in all mentioned parameters. The histopathological studies in the liver and kidney of rats also supported that alpha-lipoic acid and melatonin markedly reduced the Cd induced pathological changes and preserved the normal histological architecture of the liver and kidney tissues.It could be concluded that, the potential 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. Also, these compounds have a protective antioxidant effect and could be also applicable as a cytoprotective against oxidative stress of tissue damage mediated by heavy metals intoxication.

Key words: Antioxidant enzymes, nephrotoxicity, histopathology of liver and kidney, cadmium, oxidative stress, alpha-lipoic acid, melatonin

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 (Tiran *et al*., 1995), skeletal damage (Brzoska *et al*., 2008), severe damage in nervous, endocrine and immune system, linked to enhanced aging process as well as cancer (Jarup *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 (Ognjanovic *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 (liver and 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. Also, histopathological changes of renal and hepatic tissues were investigated.

MATERIALS AND METHODS

One hundred white male albino rats of 8-10 weeks old and weighing 160-200 g 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.

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^{-1} b.wt. (1/20 of LD_{50}). Oral rat LD_{50} for Cadmium Chloride anhydrous is 88 mg kg^{-1} b.wt. (Onwuka *et al.*, 2010)
- **Alpha-Lipoic acid (Thiotacid)^{R}:** Thiotacid was obtained as pack of five ampoules of 10 mL 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^{-1} b.wt. (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^{-1} b.wt. (Kim *et al.*, 1998). Propylene glycol was manufactured by El-Nasr Pharmaceutical Chemicals Co. Abu zaabal, Egypt

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^{-1} b.wt.) 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^{-1} b.wt.) and treated daily with alpha-lipoic acid (54 mg kg^{-1} b.wt./i.p)
- **Group IV (Cadmium chloride+melatonin treated group):** Rats received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with melatonin (10 mg kg^{-1} b.wt./orally)
- **Group V (Cadmium Chloride+alpha-lipoic acid+melatonin treated group):** Rats received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with alpha-lipoic acid (54 mg kg^{-1} b.wt./i.p) in combined with melatonin (10 mg kg^{-1} b.wt./orally) for 10 weeks

Sampling

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 min. The clean clear plasma was separated by Pasteur pipette and kept in a deep freeze at -20°C untile used for subsequent biochemical analysis. Moreover, after plasma separation, erythrocytes were washed three times with an equal volume of cold saline, then 1 mL RBCs lysed with 4 mL distilled water in dry sterile caped tubes. The samples were kept at -20°C for subsequent biochemical analysis.

Tissue specimen (Liver and kidney):

- **For cadmium residue determination:** Liver and 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)
- **For histopathological examination:** Liver and kidney specimen of rats were carefully examined by naked eyes for detection of any abnormalities. Small specimen of each organ were taken and immediately fixed in 10% neutral phosphate buffered formalin solution for histopathological examination

Biochemical analysis: Plasma ALT and AST, GGT, Urea, Creatinine, total cholesterol and Phospholipids were determined according to the method described by Schumann *et al.* (2002), Szasz *et al.* (1974), Kaplan *et al.* (2003), Tietz (1995), Meiattini *et al.* (1978) and Zilversmit and Davies (1950), respectively. Moreover, blood haemoglobin, G-6-PDH, erythrocyte MDA, CAT, SOD, GST and GSH were determined according to the method described by Stadie (1920), Sood *et al.* (1981), Esterbauer *et al.* (1982), Sinha (1972), Packer and Glazer (1990), Habig *et al.* (1974) and Beutler *et al.* (1963), respectively.

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 $p<0.05$.

RESULTS

Plasma ALT, AST and γ-GT activities: The obtained results demonstrated in Table 1 revealed that, cadmium intoxicated rats showed significant increase in plasma ALT, AST and γ-GT activities,

Table 1: Effect of alpha-lipoic acid, melatonin alone and their combination on plasma ALT, AST and γ-GT activities, Urea and Creatinine concentrations in cadmium intoxicated male rats

	ALT (U L^{-1}) (weeks)			AST (U L^{-1}) (weeks)			(γ-GT) (U L^{-1}) (weeks)		
Animal groups	2	4	10	2	4	10	2	4	10
Control normal	43.58±0.63^{e}	48.40±2.33^{d}	50.43±2.00^{d}	74.23±1.36^{e}	115.75±1.71^{d}	138.08±2.63^{e}	11.15±0.16^{b}	11.53±0.11^{c}	12.98±1.09^{d}
Cadmium chloride	63.03±0.85^{a}	95.93±2.02^{a}	114.28±1.61^{a}	117.60±1.75^{a}	171.80±3.74^{a}	276.28±3.08^{a}	15.19±1.25^{a}	22.72±1.93^{a}	38.75±1.23^{a}
Cadmium chloride+ α-lipoic acid	57.58±0.49^{b}	82.32±1.01^{b}	93.68±3.17^{b}	106.15±2.37^{b}	171.80±3.74^{a}	230.35±2.51^{b}	15.10±0.20^{a}	21.57±0.28^{a}	31.97±0.40^{b}
Cadmium chloride +melatonin	53.85±1.47^{c}	67.67±2.96^{c}	71.90±4.19^{c}	90.23±0.39^{c}	149.53±0.75^{b}	198.68±2.38^{c}	12.38±0.28^{b}	17.40±0.48^{b}	26.27±0.51^{c}
Cadmium chloride+ α-lipoic acid+ melatonin	49.95±0.36^{d}	62.47±1.04^{c}	64.28±0.67^{c}	84.55±1.30^{d}	131.14±1.17^{c}	175.42±3.46^{d}	11.39±0.31^{b}	12.63±0.48^{c}	13.73±0.99^{d}

	Urea (mg dL^{-1}) (weeks)			Creatinine (mg dL^{-1}) (weeks)		
Animal groups	2	4	10	2	4	10
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+ α-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+ α-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±SE SE: Standard error. Mean values with different superscript letters in the same column are significantly different at $p \leq 0.05$

urea and creatinine concentrations when compared with normal control group. Treatment with alpha-lipoic acid, melatonin alone and in combination to cadmium intoxicated rats caused significant decrease plasma ALT, AST and γ-GT activities, urea and creatinine concentrations when compared with cadmium intoxicated group.

Plasma total cholesterol and phospholipids concentrations: Plasma total cholesterol and phospholipids concentrations increased significantly in cadmium exposed rats all over the periods of the experiment when compared with normal control group. Treatment with Alpha-lipoic acid, Melatonin alone and in combination to cadmium intoxicated male rats caused significant decrease in plasma total cholesterol and phospholipids concentrations when compared with cadmium exposed group (Table 2).

Erythrocytes L-MDA and GSH concentrations: The results illustrated in (Table 3) revealed that, cadmium intoxicated rats showed significant increase in erythrocytes L-MDA and significant decrease in erythrocytes GSH concentrations allover the periods of the experiment when compared with normal control group. Treatment with Alpha-lipoic acid, Melatonin alone and in combination to cadmium intoxicated male rats caused significant decrease in erythrocytes L-MDA and significant increase in erythrocytes GSH concentrations when compared with cadmium exposed group.

Table 2: Effect of alpha-lipoic acid, melatonin alone and their combination on plasma total cholesterol and phospholipids concentrations in cadmium intoxicated male rats

	Total cholesterol (mg dL^{-1}) (weeks)			Phospholipids (mg dL^{-1}) (weeks)		
Animal groups	2	4	10	2	4	10
Control normal	69.12±3.32^{a}	76.40±0.40^{b}	108.33±0.49^{b}	280.63±13.97^{d}	337.67±13.43^{e}	406.63±5.22^{e}
Cadmium chloride	69.60±0.30^{a}	83.48±0.80^{a}	129.83±1.83^{a}	638.54±30.95^{a}	811.83±16.66^{a}	1050.40±11.53^{a}
Cadmium chloride+α-lipoic acid	67.61±0.49^{a}	77.00±0.63^{b}	93.10±0.81^{c}	568.58±6.65^{b}	690.08±17.72^{b}	845.21±34.20^{b}
Cadmium chloride+melatonin	66.00±0.17^{a}	69.67±1.87^{c}	85.08±4.92^{d}	461.13±8.89^{c}	589.75±15.90^{c}	762.75±26.13^{c}
Cadmium chloride+A-lipoic acid+melatonin	61.37±1.08^{b}	69.08±0.37^{c}	73.42±0.99^{e}	426.33±6.10^{c}	443.54±10.74^{d}	503.58±17.64^{d}

Data are presented as Mean±SE, SE: Standard error. Mean values with different superscript letters in the same column are significantly different at $p \leq 0.05$

Table 3: Effect of alpha-lipoic acid, melatonin alone and their combination on erythrocytes L-MDA and GSH concentrations, CAT, SOD and GST activities in cadmium intoxicated male rats

	L-MDA (nmol mL^{-1}) (weeks)			GSH (mg dL^{-1}) (weeks)			Catalase (U L^{-1}) (weeks)		
Animal groups	2	4	10	2	4	10	2	4	10
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}
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}
Cadmium chloride+α-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}
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}
Cadmium chloride+α-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}

	SOD (U L^{-1}) (weeks)			GST (U g^{-1} Hb) (weeks)		
Animal groups	2	4	10	2	4	10
Control normal	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}
Cadmium chloride	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}
Cadmium chloride+α-lipoic acid	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}
Cadmium chloride+Melatonin	351.08±52.41^{a}	348.65±7.29^{bc}	189.08±50.14^{bc}	43.18±0.91^{c}	26.61±0.29^{b}	19.40±0.08^{b}
Cadmium chloride+α-lipoic acid+melatonin	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}

Data are presented as Mean±SE, SE: Standard error. Mean values with different superscript letters in the same column are significantly different at $p \leq 0.05$

Erythrocytes CAT, SOD and GST activities: The obtained data revealed that, erythrocytes Catalase (CAT) and Glutathione-S-transferase (GST) activities decreased significantly in cadmium exposed rats. However, erythrocytes Superoxide Dismutase (SOD) activity was 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 and GST activities with significant decrease in SOD activity when compared with cadmium exposed group (Table 3).

Table 4: Effect of alpha-lipoic acid, melatonin alone and their combination on blood haemoglobin concentrations and G-6PDH activity in cadmium intoxicated male rats

	Haemglobin (g dL^{-1}) (weeks)			G-6-PDH($U g^{-1}$ Hb) (weeks)
Animal groups	2	4	10	10
Control normal	7.500±0.026^{a}	10.800±0.052^{a}	14.433±0.067^{a}	72.65±2.64^{a}
Cadmium chloride	5.600±0.037^{e}	9.317±0.108^{d}	10.333±0.061^{e}	45.05±0.45^{c}
Cadmium chloride+α-lipoic acid	6.717±0.048^{b}	9.650±0.034^{c}	11.650±0.043^{d}	51.45±0.76^{bc}
Cadmium chloride+melatonin	7.637±0.200^{a}	10.232±0.093^{b}	12.125±0.044^{c}	52.05±5.42^{bc}
Cadmium chloride+α-lipoic acid+melatonin	7.314±0.113^{a}	10.417±0.119^{b}	13.417±0.048^{b}	57.63±5.32^{b}

Data are presented as Mean±SE, SE: Standard error. Mean values with different superscript letters in the same column are significantly different at $p \leq 0.05$

Table 5: Effect of alpha-lipoic acid, melatonin alone and their combination on liver and kidney cadmium residue concentrations in cadmium intoxicated male rats

	Kidney cadmium (ppm g^{-1} wet tissue) (weeks)		Liver cadmium (ppm g^{-1} wet tissue) (weeks)	
Parameters				
Animal groups	4	10	4	10
Control normal	21.44±0.97^{e}	20.94±1.61^{d}	18.43±0.60^{e}	18.66±0.60^{e}
Cadmium chloride	653.07±86.90^{a}	722.71±68.60^{a}	202.93±39.29^{a}	359.71±4.99^{a}
Cadmium chloride+α-lipoic acid	281.16±9.00^{b}	383.43±22.46^{b}	120.29±10.73^{b}	275.28±10.25^{b}
Cadmium chloride+melatonin	209.62±49.70^{b}	321.54±62.75^{bc}	83.32±27.40^{bc}	231.54±7.93^{c}
Cadmium chloride+α-lipoic acid+melatonin	60.73±1.69^{e}	206.39±62.14^{c}	42.60±28.96^{bc}	115.09±15.03^{d}

Data are presented as Mean±SE, SE : Standard error. Mean values with different superscript letters in the same column are significantly different at $p \leq 0.05$

Blood haemglobin concentration and G-6-PDH activity: Blood haemglobin concentration and glucose-6-Phosphate dehydrogenase (G-6-PDH) activity were significantly decreased in cadmium exposed rats when compared with normal control group. Treatment with alpha-lipoic acid and melatonin and their combination to cadmium intoxicated rats caused a significant increase in blood haemoglobin concentration and G-6-PDH activity when compared with cadmium exposed group (Table 4).

Kidney and liver cadmium residues concentration: The obtained results presented in Table 5 revealed that, the mean value of kidney and liver 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 and liver cadmium residues concentrations when compared with cadmium exposed group.

Histopathological findings: As shown in Fig. 1-10:

DISCUSSION

Cadmium intoxicated rats showed significant increase in plasma ALT, ASTandγ-GT activities, urea and creatinine concentrations when compared with normal control group. It is well known that long-term exposure to Cd causes various toxic effects in various organ systems such as

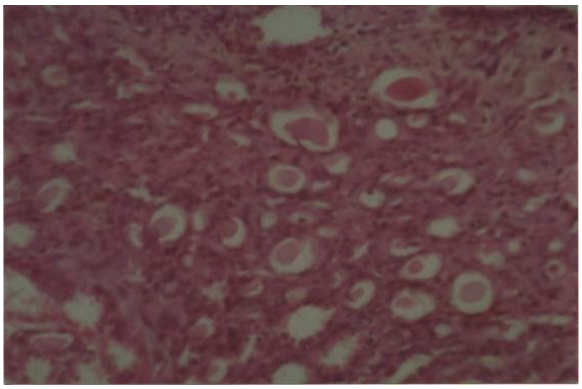

Fig. 1: Kidney of rats of group II, that received cadmium chloride (4.4 mg kg^{-1} b.wt. of 1/20th of LD_{50}) orally and once per day over a period of 10 weeks. The renal tubules were completely destructed and showing loss of the lining epithelium with the presence of hyalinized eosinophilic cast in their lumina

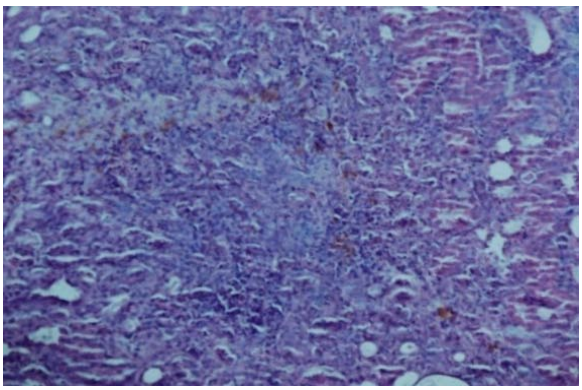

Fig. 2: Liver of rats of group II, that received cadmium chloride (4.4 mg kg^{-1} b.wt. of 1/20th of LD_{50}) orally and once per day over a period of 10 weeks. Showing severe congestion and dilatation of the hepatic sinusoids. Focal area of necrosis infiltrated with mononuclear cells, H and E x300

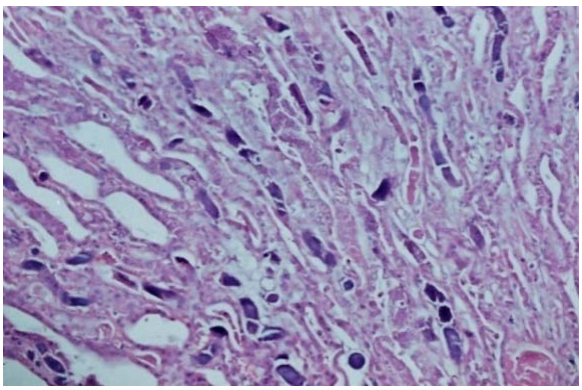

Fig. 3: Kidney of the rats of group III, that received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with alpha-lipoic acid (54 mg kg^{-1} b.wt./i.p). Showing complete destruction and calcification of the renal tubules particularly in medulla H and E x300

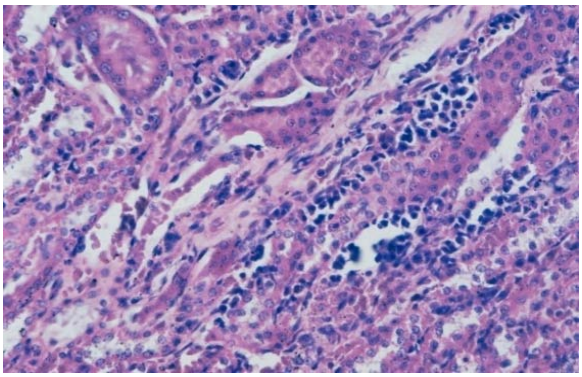

Fig. 4: Kidney of rats of group III, that received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with alpha-lipoic acid (54 mg kg^{-1} b.wt./i.p). Showing focal interstitial mononuclear cellular aggregation and infiltration in the renal medulla by H and E x600

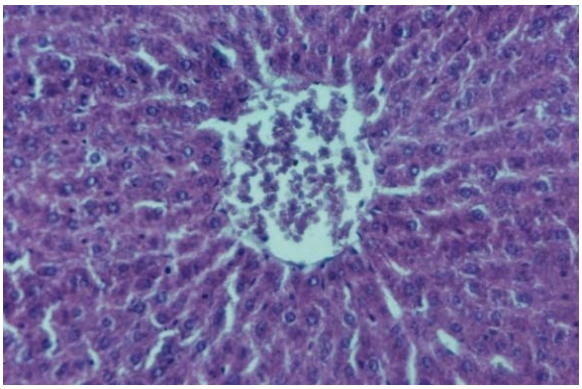

Fig. 5: Liver of rats of group III, that received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with alpha-lipoic acid (54 mg kg^{-1} b.wt./i.p). Showing severe congestion of the central vein with vacuolar degeneration of hepatocytes by H and E x800

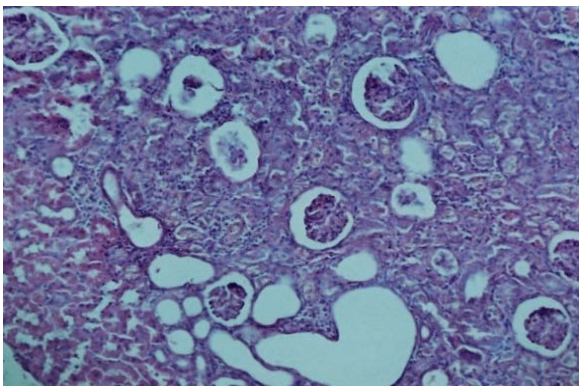

Fig. 6: Kidney of rats of group IV, that received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with melatonin (10 mg kg^{-1} b.wt./orally). Showing shrinkage of glomerular tuft with cystic dilatation of renal tubules by H and E x300

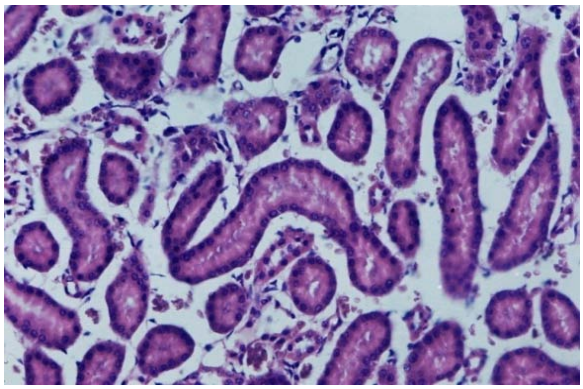

Fig. 7: Kidney of the rats of group IV, that received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with melatonin(10 mg kg^{-1} b.wt./orally). Showing coagulation necrosis of the renal epithelium with pyknotic nuclei-more eosinophilic cytoplasm by H and E x400

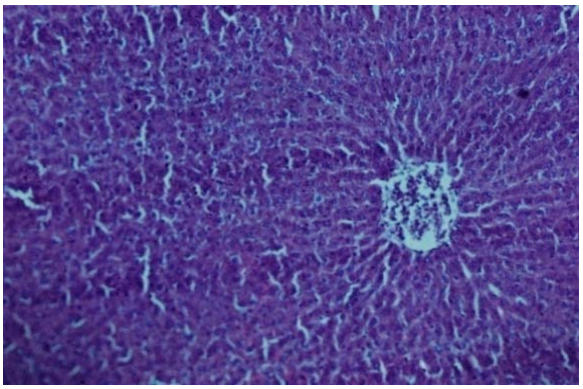

Fig. 8: Liver of the rats of group IV, that received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with melatonin(10 mg kg^{-1} b.wt./orally). Showing severe congestion of the central vein of liver by H and E x300

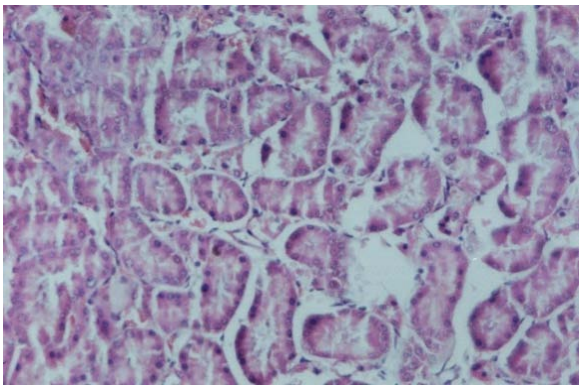

Fig. 9: Kidney of rats of group V, that received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with alpha-lipoic acid (54 mg kg^{-1} b.wt./i.p) and melatonin(10 mg kg^{-1} b.wt./orally) for 10 weeks. Showing mild desquamation of the epithelial cells lining the renal tubules in the medulla by H and E x600

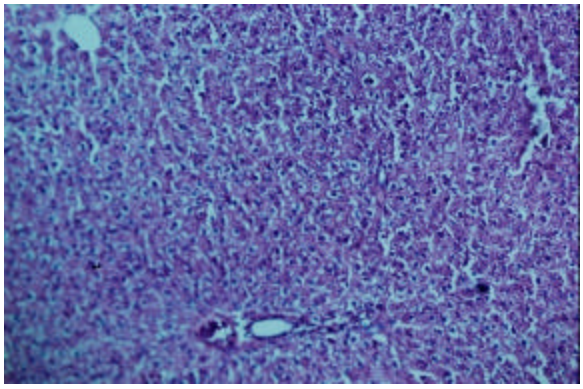

Fig. 10: Liver of rats of group V, that received cadmium chloride (4.4 mg kg^{-1} b.wt.) and treated daily with alpha-lipoic acid (54 mg kg^{-1} b.wt./i.p) and melatonin (10 mg kg^{-1} b.wt./orally) for 10 weeks. Showing mild degenerative changes manifested by hydropic degenerative of the hepatocytes by H and E x300

cardiovascular, kidneys, liver, lung, bones, immune/haemopoietic, endocrine and reproductive systems (Fowler, 2009; Satarug *et al*., 2010). These results came in accordance with the recorded data of Ibrahim (2013) she reported that, hepatic and nephritic pathological changes included significant increases of serum alanine transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) activities and creatinine and urea concentrations were observed in cadmium exposed rats. Also, Goncalves *et al*. (2010) reported that, urea and creatinine levels were increased in serum of Cd-intoxicated rats. Moreover, Preet and Dua (2011) reported that, exposure to cadmium in rats induced a significant increase in serum urea and creatinine concentrations. Furthermore, Kumar *et al*. (2010) who reported that, rats injected with cadmium chloride showed significant increase in the activities of serum AST, ALT, Lactate Dehydrogenase (LDH) and GGT when compared to the values in control rats. Liver damage induced by cadmium was clearly shown by the increased activities of serum hepatic marker enzymes namely AST, ALT, Alkaline Phosphatase (ALP), LDH and GGT (Renugadevi and Prabu, 2010). These results may be related to that, aminotransferases (ALT and AST) being an important class of enzymes linking carbohydrate and amino acid metabolism, have established a relationship between the intermediates of the citric acid cycle. These enzymes are regarded as markers of liver injury since; liver is the major site of metabolism (Liss *et al*., 1985). Additionally, elevated serum levels of these variables may be due to hepatocellular necrosis which causes increase in the permeability of the cell membrane resulting in the release of transaminases in the blood stream. The increase in the liver AST and ALT activities may be due to liver dysfunction and disturbance in the synthesis of these enzymes. Therefore, such increase in the enzymes activities in plasma indicating the hepatotoxic effect of cadmium chloride and is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Sarkar *et al*., 1998). 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). These results may be related to that, as a result of long-term exposure to cadmium there may come to grade I urethras impairment and then to glomerular filtration impairment (Uriu *et al*., 2000) which might justify the elevation of urea and creatinine concentrations. 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). This suggestion was supported by Lall *et al.* (1997) who mentioned that, rise in creatinine value is an indication of renal tubular damage due to cadmium-induced nephrotoxicity. As confirmed by Skoczynska and Smolik (1994) who reported that, cadmium intake increased lipoperoxide concentration. This indicated that, the renal toxicity induced by cadmium involved superoxide radicals. Who concluded that, the toxicity of cadmium involves oxidative reactions such as cadmium-induced lipid peroxidation. Superoxide radical is an important toxic intermediate in the development of renal damage induced by cadmium.

Treatment with alpha-lipoic acid, melatonin alone and in combination to cadmium intoxicated rats caused significant decrease plasma ALT, AST and γ-GT activities, urea and creatinine concentrations when compared with cadmium intoxicated group. Similarly, Rashwan *et al.* (2012) reported that, treatment with α-lipoic acid in cadmium intoxicated rats resulted in decrease in serum AST, ALT and ALP activities, urea and creatinine concentrations compared to cadmium group. Also, Alabbassi *et al.* (2008) reported that, therapeutic administration of melatonin at a dose of 20 mg kg^{-1} in lead acetate treated rats significantly reduces serum ALT and AST activities, urea and creatinine concentrations. Moreover, Preet and Dua (2011) reported that, administration of dietary nutrients i.e., N-acetyl cystiene, methionine, melatonin, Vit-B1 and their combination in cadmium chloride exposed rats resulted in significant decrease in serum AST and ALT activities, urea and creatinine levels compared to animals receiving cadmium chloride alone. So the decreased in plasma ALT and AST activities in cadmium exposed rats treated with alpha-lipoic acid and melatonin could be attributed to decreased leakage of AST and ALT resulted from protective effect of alpha-lipoic acid or melatonin to liver against injury produced by cadmium toxicity. 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. Also, dietary supplementation with antioxidants may be such as a new strategy to reduce the destructive effects caused by free radicals and ROS (Frank and Biesalski, 1997). Furthermore, the decreased in plasma creatinine concentration observed in alpha-lipoic acid and melatonin treated cadmium intoxicated rats could be attributed to the protective effect of alpha-lipoic acid and melatonin to kidney against damage produced by cadmium toxicity.

Plasma total cholesterol and phospholipids concentrations increased significantly in cadmium exposed rats and treatment with alpha-lipoic acid and Melatonin alone and in combination caused significant decrease. Nearly similar results were recorded by Abdel-Mageid and El-Shawarby (2006) who reported that, serum total cholesterol concentration was significantly increased in lead intoxicated rabbit. Also, Rashwan *et al.* (2012) reported that, cadmium exposed rats group showed significant increase in serum total cholesterol, triacylglycerols, LDLc and VLDLc and significant decrease in serum HDLc compared to normal control group. Who added that, treatment with α-lipoic acid in cadmium exposed rats showed significant decrease in serum total cholesterol and LDLc and significant increase in serum HDLc levels compared to cadmium non treated group. Moreover, Anwar and Meki (2003) reported that, treatment of diabetic animals with melatonin (200 μg kg^{-1}) for 15 days significantly decreased serum total cholesterol levels.

The obtained results revealed that, cadmium intoxicated rats showed significant increase in erythrocytes L-MDA concentration when compared with normal control group. 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. Also, Sinha *et al.* (2008) reported that, increased production of intracellular ROS, elevated lipid peroxidation and protein carbonylation were observed in the erythrocytes of the $CdCl_2$-intoxicated experimental mice. The recorded significant increase in plasma L-MDA in cadmium intoxicated rats may be due to cadmium induced production of reactive oxygen species may contribute to the tissue damaging effects of this metal ion (Stohs and Bagchi, 1995). This suggestion was confirmed by the findings of Sumathi *et al.* (1994) who reported that, cadmium may induced oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and by inhibiting the enzymes involved in the utilization of some of the activated oxygen species. Moreover, all products of LPO inactivate cell constituents by oxidation or cause oxidative stress by undergoing radical chain reaction ultimately leading to loss of membrane integrity (Maiti *et al.*, 1995). Additionally, 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 (Ognjanovic *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^{-1} day^{-1}) for 4 weeks in cadmium intoxicated rats significantly diminished the levels of oxidative stress markers, lipid peroxidation and protein carbonyls in brain. Also, Alabbassi *et al.* (2008) reported that, treatment with 20 mg kg^{-1} melatonin in lead acetate treated rats resulted in significant decrease in MDA levels in the RBCs, brain, liver and kidney tissues. 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). Moreover, melatonin was shown to be an efficient free radical scavenger against the toxic actions of the extremely reactive hydroxyl radical which abstracts a hydrogen atom, i.e., initiates lipid peroxidation, from cellular membrane (Reiter *et al.*, 1995). Moreover, in the inner mitochondrial membranes, melatonin has been shown to reduce the free radical generation by increasing the efficiency of the electron transport chain (Acuna-Castroviejo *et al.*, 2001).

Cadmium intoxicated rats showed significant decrease in erythrocytes GSH concentrations. These results came in accordance with the recorded data of Renugadevi and Prabu (2010) who found that, reduced glutathione level was depressed and its dependent enzymes in Cd-intoxicated rats. Also, Dallak (2009) reported that, oral administration of cadmium chloride induced free radicals caused a significant decrease in RBCs reduced glutathione (SGH). The depletion in GSH defense system after Cd exposure may allow free radicals such that $OH^{\bullet}$ and $O^{\bullet}_2{}^{-}$ to cause oxidative

damage via lipid peroxidation, protein oxidation and DNA damage, leading to various pathological conditions (Stohs and Bagchi, 1995). 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) and Newairy *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 to be 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). As supported by Kim *et al.* (1998) who reported that, a decrease in hepatic GSH level was produced by Cd treatment and was accompanied by a decrease in the activity of hepatic GSSG-reductase which is capable of reducing GSSG to GSH.

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, Preet and Dua (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 (Ognjanovic *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 can 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. Moreover, many studies reported the hepato-protective effects of melatonin via increasing the GSH concentration that depleted by different toxic agents, such as endosulfan (Omurtag *et al.*, 2008), mercury (II) chloride (Sener *et al.*, 2003a), acetaminophen (Sener *et al.*, 2003b) and methotrexate (Jahovic *et al.*, 2003). Thus, melatonin is important not only in promoting high levels of intracellular GSH (Urata *et al.*, 1999) but also in ensuring that it is maintained primarily in the reduced state, i.e., as 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 3).

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^{-1} day^{-1}) for 4 weeks. Also, Dallak (2009) reported that, oral administration of cadmium chloride induced free radicals and caused significant decrease in erythrocyte CAT activity. Moreover, Caylak *et al.* (2008) reported that, an increase in SOD activity of erythrocytes was observed in lead treated rats. Cadmium induces increased Reactive Oxygen Species (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). Furthermore, it inhibits the activities of many enzymes by binding to their sulfhydryl groups or by inhibiting the protein synthesis (Waisberg *et al.*, 2003). 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). Also, 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 (Ognjanovic *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 group. These obtained results are in accordance with the results of Rashwan *et al.* (2012) she 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 decreases 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^{-1} day^{-1}) for 4 weeks in cadmium intoxicated rats significantly elevated the activities of enzymatic antioxidants SOD, CAT, GPx and GST in brain. It was shown previously that GST activity in erythrocytes, as well as liver and kidney tissues, dropped slightly during diabetes and significantly increased in melatonin-treated diabetic rats (Anwar and Meki, 2003). Melatonin administration induced an increase in the mRNA levels of both isoforms of SOD i.e., manganese and copper/zinc (Antolin *et al.*, 1996). Moreover, Pal and Chatterjee (2006) recognized that, besides melatonin ability to scavenge ROS, this substance has been demonstrated to activate the antioxidative enzymes such as CAT and GPx.

Erythrocyte glucose-6-Phosphate dehydrogenase (G-6-PDH) activity and blood haemglobin concentration were significantly decreased in cadmium intoxicated rats when compared with normal control group (Table 4). These results came in accordance with the recorded data of Renugadevi and Prabu (2010) who reported that, a significant decrease in the activity of hepatic G-6-PDH was observed in cadmium-treated rats. Also, El-Demerdash *et al.* (2004) recorded that, treatment with cadmium chloride in rats caused a significant decrease in Blood Hemoglobin (Hb) concentration. Moreover, Preet and Dua (2011) reported that, exposure to cadmium induced a decrease in haematocrite value and haemoglobin concentration in blood of rats.

Glucose-6-Phosphate dehydrogenase is the first enzyme in the pentose phosphate pathway that provides most of the extra mitochondrial nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) to cells. The pathway is more important for RBCs because RBCs lack mitochondria. Within the RBCs, NADPH-reducing equivalents are necessary for keeping GSH in its reduced form through the enzyme glutathione reductase. NADPH is also important for catalase activity, albeit as a co-factor and not as a substrate (Kirkman *et al*., 1987). The most important output of the pathways regulation has been suggested to be the NADPH/$NADP^+$ ratio (Zubay, 1993). The turnover of the pathway is shown to increase under oxidative stress conditions where demand for NADPH increases (Brigelius, 1986). Under oxidative stress conditions, formation of GSSG would be expected to increase during consumption of hydrogen peroxide via glutathione peroxidase. Glutathione disulfide will then be reduced to GSH by glutathione reductase using NADPH as a substrate. Blood hemoglobin concentration may be diminished because of hemolysis or because of impaired blood formation in bone marrow in rats administrated cadmium chloride compared with hemoglobin levels in blood of control group rats receiving normal saline. Additionally, cadmium inhibits the bone marrow to make hemoglobin by interfering with several enzymatic steps in the heme synthesis. Also, cadmium has been found to have direct effect on blood hemoglobin by decreasing its formation as results from two basic red cell defects, shortened life span and impaired heme synthesis. The mechanisms by which synthesis of the red cell pigment heme is inhibited by cadmium involves at least two enzymes, a cytoplasmic one (delta-aminoleuvinic acid) at the beginning of heme synthesis and a mitochondrial one, ferrochelatase, at the end of heme synthesis (Moore *et al*., 1987). 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 and blood haemoglobin concentration when compared with cadmium exposed group These results are nearly similar with those of Sudnikovich *et al*. (2007) who reported that, melatonin treatment of diabetic rats increasing liver glucose-6- phosphate dehydrogenase activity. Also, Caylak *et al*. (2008) reported that, lipoic acid administrations was found to be very effective in increasing Hb levels when compared to those in the lead-group.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 and liver cadmium residues concentrations increased significantly in cadmium exposed rats when compared with normal control. Similarly, Lakshmi *et al*. (2012) reported that, significantly higher levels of cadmium were found in liver and kidney of cadmium treated rats group compared to control. Also, Preet and Dua (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 and liver cadmium residues concentrations

when compared with cadmium exposed group. 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, Preet and Dua (2011) reported that, administration of dietary nutrients i.e., N-acetylcystiene, methionine, melatonin and Vit-B1 to cadmium chloride treated rats resulted in decreased Cd accumulation in liver and kidney. Moreover, Chwelatiuk *et al.* (2006) reported that, 8-week melatonin co-treatment with orally administered cadmium chloride is referred to decrease renal, hepatic and intestinal cadmium concentrations. Furthermore, El-Sokkary *et al.* (2010) reported that, melatonin administration to cadmium-treated rats reduced the liver cadmium residue concentrations compared to those of cadmium intoxicated rats.

Kidney and liver cadmium residues concentrations increased significantly in cadmium exposed rats. Treatment with alpha-lipoic acid, melatonin alone and in combination to cadmium intoxicated male rats resulted in significant decrease in kidney and liver cadmium concentrations (Table 5). These results came in accordance with the recorded data of Preet and Dua (2011) who reported that, a significant increase in cadmium level was observed in the liver, kidney and blood with higher amount in the kidney which showed maximum accumulation of cadmium after 21 days. Also, Katsuta *et al.* (1993) recorded that, the hepatic and renal Cd concentrations increased in female rats after intravenous administration of cadmium chloride. Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the liver and kidneys, as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis (Waisberg *et al.*, 2003).

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; Masters *et al.*, 1994). 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). In addition to, cadmium provided orally to rats induces the synthesis of a cadmium-binding protein, metallothionein, in liver (Sabbioni and Girardi, 1977). 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). This suggestion was confirmed by Chan *et al.* (1992) indicated that, the liver was the primary organ for accumulation of Cd salts while kidney for Cd-metallothionein (Cd-MT).

Administration of melatonin and/or alpha-lipoic acid (ALA) together offsets 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). Also, 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 ther 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 peroxynitrite. 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; Jones *et al*., 2002). 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 liver marker enzymes and 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.

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