



# PROTECTIVE EFFECTS OF OLEANOLIC ACID AND VITAMINE E ON THYROID DYSFUNCTION AND LIPID PEROXIDATION IN CADMIUM- INDUCED TOXICITY IN RATS

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## ABSTRACT

The effect of oleanolic acid or vit. E on heavy metal (cadmium) -induced thyroid dysfunction and lipid peroxidation in male rats was studied. The animals divided into 3 groups each of which 6 rats. The first group were injected with 1mg/kg/day cadmium chloride, 1% solution in distilled water subcutaneously daily for 30 days. The second group were injected simultaneously with equivalent dose of cadmium chloride (1mg/kg/day) subcutaneously and oleanolic acid in a dose of 5 mg/kg/day, 2% suspension in 2% tween 80 intramuscularly for 30 days. The third group were injected simultaneously with equivalent dose of cadmium chloride (1mg/kg/day) subcutaneously and vit. E, in a dose of 100mg/kg (5% solution in saline) intramuscularly for 30 days. The control groups were divided into 3 groups. The first group was treated with distilled water, the second one was treated with tween 80 and lastly the third group was treated with saline. Cadmium chloride treatment alone led to decrease in concentrations of serum thyroid hormones, zinc and copper concentration ( $p < 0.01$ ). In addition, a significant increase in both malondialdehyde (MDA) levels and thyroid stimulating hormone (TSH) has been observed by Cd-treatment alone ( $p < 0.01$ ). Treatment with either oleanolic acid or vit.E improved the metal-induced decrease in serum thyroid function. Treatment with oleanolic acid lead to decrease in levels of blood and hepatic malondialdehyde but remain higher than normal rates ( $47.14 \pm 0.82 \mu\text{mol/L}$  and  $119 \pm 0.86 \mu\text{mol/g}$  wet tissue, respectively). However, treatment with Cd and vit. E restored blood and hepatic malondialdehyde levels toward normal values ( $34.7 \pm 0.65 \mu\text{mol/L}$  and  $100.4 \pm 1.44 \mu\text{mol/g}$  wet tissue, respectively). The effect of vit. E combined with cadmium is significant compared with the effect of oleanolic acid treatment with cadmium ( $p < 0.01$ ). The protective effect of each oleanolic acid or vit. E against cadmium-induced thyroid dysfunction is mediated through its antioxidative action.

**Key words:** Cadmium, Thyroid Dysfunction, Lipid Peroxidation, Oleanolic Acid, Vit. E.

## INTRODUCTION

Cadmium is considered one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half life of 10-30 years (Jarup *et al.*, 1998). The uptake of cadmium from the soil results in elevated concentrations in vegetables, fruits, and grains, with the highest levels in leafy greens and potatoes. High levels are also found in shellfish (up to 30 mg/kg) and organ meats (Agency for Toxic Substances and Disease Registry, 1999). The minimal risk level (MRL) for cadmium is 0.2  $\mu\text{g/kg/day}$  (Agency for Toxic Substances and Disease Registry, 1999). Iron deficiency creates a significant risk for increased cadmium exposure by increasing gastrointestinal absorption (Nordberg *et al.*, 1985). When cadmium is absorbed it circulates in erythrocytes or bound to albumin.

In the liver it can induce and bind to metallothionein, a cysteine-rich protein that can concentrate cadmium up to 3,000-fold (Klaassen *et al.*, 1999). The metallothionein/cadmium complex is slowly released over time from the liver and circulates to the kidneys where it can accumulate in renal tissue. Cadmium also accumulates in the bone, pancreas, adrenals, and placenta. The majority of accumulation, approximately 50 percent of total body stores, occurs in the liver and kidney (Pope and Rall, 1995).

Cadmium is classified as a group I human carcinogen, meaning sufficient evidence for carcinogenesis has been found in both animals and humans (Mandel *et al.*, 1995 and Sorahan and Lancashire, 1997). The mechanism of cadmium-induced damage include the production of free radicals that alter mitochondrial activity and genetic formation. The metabolism and excretion of this heavy

metal depend on presence of antioxidants and thiols that aid cadmium metallothionein-binding as cadmium exposure correlated with decreased levels of reduced glutathione (Patrick, 2003).

Cadmium (Cd) has been found to inhibit thyroxine (T<sub>4</sub>) synthesis and /or its release and to depress type 1 iodothyronine (5-DI) activity leading to decrease in serum triiodothyronine (T<sub>3</sub>) level (Gupta and Kar, 1997). Some studies suggested that membrane damage due to increased lipid peroxidation (LPO) is responsible for the depressed hepatic 5-DI activity in presence of xenobiotics (Chaurasia *et al.*, 1996).

Triterpenes are common plant compounds shown to have antioxidant (Kleijnen and Knipschild, 1992), hepatoprotective (Liu *et al.*, 1994), anti-inflammatory (Mahato *et al.*, 1992), and antitumor (Yasukawa *et al.*, 1991 and Pisha *et al.*, 1995) properties. Triterpenoids also induce metallothionein in cadmium toxicity. Oleanolic acid (OA), a triterpenoid present in many plants and one of the active constituents of *Ligustrum lucidum*, is used in China to treat hepatitis. It has also been shown to induce hepatic metallothionein in cadmium toxicity (Liu *et al.*, 1993a).

Oleanolic acid (OA) has been used to induce renal metallothionein (MT), copper and zinc in animal studies and protects against cadmium/metallothionein-induced renal injury (Liu *et al.*, 1996). Rats pretreated with zinc or copper have shown less sensitivity to cadmium toxicity, specifically in renal proximal tubule cells. Proteinuria caused by cadmium-metallothionein injections was more effectively reduced by pretreatment injections with zinc than with copper (Liu *et al.*, 1994).

Oleanolic acid (OA), is exist widely in food and some medicinal herbs (Wang and Jiang, 1992). OA treatment significantly increased liver GSH content and increased metallothionein (MT) up to 25-fold, which play an important role in OA protection against cadmium-induced liver injury (Liu *et al.*, 1993 a). MT has been proposed to function as a free radical scavenger (Sato and Bremner, 1993) and thus play a role in OA protection against radical-derived tissue damage. Along with increased MT, hepatic Zn and Cu content were also increased after OA treatment. The increased Zn and Cu can provide metals for Zn, Cu-SOD and ceruloplasmin. Both enzymes are superoxide anion scavengers. Zn itself also play a role as an antioxidant by protecting sulfhydryl groups and inhibiting reactive oxygen species produced by transition metals (Bray and Bettger, 1990). OA, also increased hepatic ascorbate concentration. Ascorbate (vit. C) is a water-soluble antioxidant that can also function as a pro-oxidant under certain conditions. Ascorbate reduces and regenerates oxidized  $\alpha$ -tocopherol and lipid peroxidation (Freeman and Crapo, 1982). Also, Liu *et al.* (2001) reported that zinc in vivo act together with metallothionein to protect against cadmium toxicity.

$\alpha$ -tocopherol (vit. E) is a lipophilic antioxidant against oxygen radical-induced toxicity (Davis and Pacht, 1991). In

addition to maintaining the integrity of cellular membranes,  $\alpha$ -tocopherol also protects against reactive oxygen species through the maintenance of cellular protein thiols (Freeman and Crapo, 1982). These increased non enzymatic components could play a role, in protecting cell from noxious insults.

In the light of these considerations, this study was designed to evaluate the protective effect of oleanolic acid and vit. E against cadmium-induced thyroid dysfunction and lipid peroxidation.

## MATERIALS AND METHODS

### Animals and treatments:

Thirty six male western rats weighing 200-300g were used. Animals were allowed water and food (laboratory chow) ad Libitum. The rats were divided into 6 groups each of 6 rats.

- Animals of group-I were injected with 1 mg/kg/day cadmium chloride (the thyrotoxic dose of cadmium chloride) (Gupta and Kar, 1998), 1% solution in distilled water subcutaneously daily for 30 days (BDH chemical Ltd Poole England).
- Rats of group-II were injected simultaneously with equivalent dose of cadmium chloride (1 mg/kg/day) subcutaneously and oleanolic acid in a dose of 5 mg/kg/day (a protective dose of oleanolic acid) (Liu *et al.*, 1995) 2% suspension in 2% tween 80 intramuscularly for 30 days (Sigma Chemical Co., USA).
- Rats of group-III were injected simultaneously with equivalent dose of cadmium chloride (1mg/kg/day) subcutaneously and vit. E (a protective dose of vit. E) (El Naser Pharmaceutical and Chemical Co., Egypt), in a dose of 100mg/kg (El-Demerdash *et al.*, 2004) (5% solution in saline) intramuscularly for 30 days.

The control groups were divided into 3 groups each of 6 rats treated likewise with the pure vehicle (0.1 ml distilled water, 0.5 ml 2% tween 80 and 2 ml saline).

### Toxicity studies:

The LD<sub>50</sub> values for cadmium chloride in rats were 37.5 mg/kg body weight (El-Demerdash *et al.*, 2004). The thyrotoxic dose of cadmium chloride which used in this study was 1 mg CdCl<sub>2</sub>/kg body weight (Gupta and Kar, 1998).

### Biochemical measurement:-

Blood samples were collected into dry clean tubes with heparin for malondialdehyde determination or without heparin for serum preparation. As quickly as possible, liver was dissected out, homogenized in distilled water and kept in ice. All biochemical investigations were carried out on fresh 20% homogenate. Measurement of malondialdehyde

(MDA), as one of the main endproduct of lipid peroxidation, will be carried out in plasma and liver homogenate according to the method of Yagi, 1998 and Uchiyama and Mihara, 1978 respectively. This is by colorimetric determination of thiobarbituric acid reactive substance (TBARS) using aliquots of 0.5 ml of 20% homogenate to which 3ml of 1% orthophosphoric acid and 1 ml of 0.6% thiobarbituric acid (2-thiobarbituric acid, Fluka, Chemica, Switzerland) were added, heated in a boiling water bath for 45 minutes, cooled and mixed vigorously after addition of 4ml of n-butanol to each tube. The tubes then centrifuged where the supernatants were separated and the absorbance of the pink layer were measured at 535 nm and 520 nm against reagent blank using a spectrophotometer. The difference in the absorbance at the two wave length was calculated and compared with that malondialdehyde (MDA) as external standard to calculate the concentration of malondialdehyde in each sample.

Copper and Zinc concentrations in serum was determined according methods of Lagesson and Andraska, (1979). They were determined by Atomic Absorption/Flame-Emission spectrophotometer Shimadzu-model AA-630-02, using an air-acetylene flame and hollow cathode lamps.

T<sub>3</sub> and T<sub>4</sub> were determined by RIA according to Chopra *et al.*, 1971 for T<sub>3</sub> and Chopra *et al.*, 1981 for T<sub>4</sub> using Elisa kit, provided by MONOBIND, INC. Costa Mesa, CA92627 (USA). T<sub>3</sub> code No: 125-300. T<sub>4</sub> code No: 225-300. Thyroid stimulating hormone (TSH) was also determined by Elisa kit, cod No: 325-3000 MONOBIND, INC. Costa Mesa, CA92627 (USA), according to Hopton and Harrap (1986).

### Statistical analysis of results:-

The variability of results was expressed as the mean  $\pm$  standard deviation ( $X \pm SD$ ). The significance of differences between mean values was determined using Student's t-test. (Snedcor, 1967).

## RESULTS

Table 1-represents the mean  $\pm$ SD showed that the treatment of rats with 1 mg/kg/day cadmium subcutaneously (s.c) for thirty days caused elevation of blood and hepatic MDA indicating that lipid oxidation had occurred ( $P < 0.001$ ). The recorded increase in blood MDA was 153.6 and hepatic MDA was 58.6.

Treatment of cadmium injected animals concomitantly with oleanolic acid 5 mg/kg/day for thirty days caused increased level in blood and hepatic MDA in comparison with the control.

Administration of vit. E 100 mg/kg/day concurrently with 1 mg/kg/day cadmium subcutaneously (s.c) for thirty days, caused decreasing in hepatic MDA toward normal level, although its blood level is still significant as compared with control one.

Treatment of cadmium injected animals concomitantly with oleanolic acid significantly decreased both blood and hepatic MDA levels compared with cadmium only-treated group ( $p < 0.01$ ).

Also, concomitant treatment with vit. E and cadmium significantly lowered both blood and hepatic MDA levels in comparison with cadmium only-treated group.

**Table 1: Effect of oleanolic acid and vit. E administration concurrently with cadmium on lipid peroxidation levels in blood (B-MDA) and liver (H-MDA) in rats.**

Treatment	Blood MDA ( $\mu\text{mol/L}$ )	Hepatic MDA ( $\mu\text{mol/g wet tissue}$ )
Control		
Range	29.92-33.5	91.5-92.92
Mean $\pm$ SD	30.95 $\pm$ 0.50	92.50 $\pm$ 0.63
Cadmium		
Range	76.90-80.5	146.0-148.6
Mean $\pm$ SD	78.50 $\pm$ 1.45	146.7 $\pm$ 1.3
P	<0.001	<0.001
L.S.D	a	a
% of difference	153.6	58.6
Cadmium + oleanolic acid		
Range	45.67-48.43	118.45-120.60
Mean $\pm$ SD	47.14 $\pm$ 0.82	119 $\pm$ 0.86
P	<0.01	<0.01
L.S.D	a,b	a,b
% of difference	52.3	28.6
Cadmium+ vit.E		
Range	34.43-35.43	96.75-102.44
Mean $\pm$ SD	34.7 $\pm$ 0.65	100.4 $\pm$ 1.44
P	<0.05	n.s
L.S.D	a,b,c	a,b,c
% of difference	12.11	8.54

Each value represent a mean  $\pm$  SD .

L.S.D. (least significant difference)

a) Significantly different from control group at  $P < 0.05$ .

b) Significantly different from group injected with cadmium alone at  $P < 0.05$ .

c) Significantly different from group injected with cadmium and oleanolic acid at  $P < 0.05$ .

Table 2 showed that injection of 1 mg/kg/day cadmium subcutaneously (s.c) for thirty days in rats, caused significant decrease in serum copper and zinc concentrations ( $P < 0.001$ ) indicating that the non enzymatic antioxidant defence mechanism is lowered. Administration of oleanolic acid 5 mg/kg/day intramuscularly in concomitant with 1 mg/kg/day cadmium subcutaneously (s.c) for thirty days to rats, elevated the copper concentration toward normal level because of its role as antioxidant agent against lipid peroxidation. While administration of vit. E 100 mg/kg/day intramuscularly in concomitant with 1 mg/kg/day cadmium subcutaneously

(s.c) for thirty days caused, significant elevation of copper concentration as compared to control rats. Administration of either oleanolic acid and vit. E to Cd-treated rats significantly increased level of zinc indicating their role in elevation the non enzymatic antioxidant deffence mechanism ( $P<0.01$ ).

Administration of 1 mg/kg/day cadmium subcutaneously (s.c) for thirty days in rats caused decreased serum  $T_3$  and  $T_4$  concentration indicating thyroid gland dysfunction (Table. 3). However, it increased the concentration of TSH significantly as compared with normal animals ( $P<0.01$ ). Administration of oleanolic acid 5mg/kg/day or vit. E 100 mg/kg/day intramuscularly in concomitant with 1 mg/kg/day cadmium subcutaneously (s.c) for thirty days improved the thyroid function as it caused significant elevation in  $T_3$  and  $T_4$  concentration, as compared with Cd-treated alone. TSH levels was significantly decreased in administration of oleanolic acid 5 mg/kg/day or vit. E 100 mg/kg/day intramuscularly in concomitant with 1 mg/kg/day cadmium subcutaneously (s.c) for thirty days (Table 3).

**Table 2: Effect of oleanolic acid and vit. E administration concurrently with cadmium on serum copper and zinc concentrations in rats.**

Treatment	Copper ( $\mu\text{g/dl}$ )	Zinc ( $\mu\text{g/dl}$ )
Control		
Range	98.7-125.6	91.98-111.69
Mean $\pm$ SD	110.1 $\pm$ 8.7	102.87 $\pm$ 5.94
Cadmium		
Range	65.2-83.8	37.8-50.49
Mean $\pm$ SD	72.6 $\pm$ 6.2	44.37 $\pm$ 3.3
P	<0.001	<0.001
L.S.D	a	a
%of difference	-34.06	-56.87
Cadmium + oleanolic acid		
Range	104.7-131	45.99-59.4
Mean $\pm$ SD	115.9 $\pm$ 8.6	51.48 $\pm$ 3.51
P	<0.01	<0.01
L.S.D	a,b	a,b
%of difference	5.27	-49.95
Cadmium+ vit.E		
Range	101.2-129.2	78.66-94.41
Mean $\pm$ SD	112.3 $\pm$ 8.8	87.48 $\pm$ 5.22
P	n.s	<0.01
L.S.D	b	a,b,c
%of difference	2.00	-14.96

Each value represent a mean  $\pm$  SD .

L.S.D. (least significant difference)

a) Significantly different from control group at  $P<0.05$ .

b) Significantly different from group injected with cadmium alone at  $P<0.05$ .

c) Significantly different from group injected with cadmium and oleanolic acid at  $P<0.05$ .

## DISCUSSION

Cadmium is a one of the most poisonous environmental contaminant, a human toxicant and carcinogen (Vivian *et al.*, 2002). The mechanism of cadmium induced damage include the production of free radicals that alter mitochondrial activity and genetic information (Partrick, 2003), and increase lipid peroxidation that cause membrane damage (Chaurrsia *et al.*, 1996). Malondialdehyde, is a toxic and mutagenic metabolites in mammalian cells and is carcinogenic in rats. Malondialdehyde reacts with DNA at physiological pH forming an adduct which is exocyclic pyrimidopurinone (Mao *et al.*, 1999).

Cadmium is known to bind to the mitochondria of the cell and is capable of inhibiting both cellular respiration (by 75%) and oxidative phosphorylation (by 100%) at low concentrations. Some of the specific changes that lead to tissue damage and death in chronic exposure have been related to oxidative stress, thiol depletion (Ereal *et al.*, 2001), and increase in MDA (Shohda *et al.*, 2001). So, cellular damage results from cadmium binding to sulfhydryl groups in tissues, the production of lipid peroxides, and the depletion of glutathione. Cadmium also has a very high affinity for glutathione and can form a complex with glutathione that is eliminated in bile. Cadmium also inhibits the activity of antioxidant enzymes, including catalase, manganese-superoxide dismutase, and copper/zinc-superoxide dismutase (Hussain *et al.*, 1987 and Casalino *et al.*, 2002). Cadmium-induced lipid peroxidation has been seen in animal studies in liver, kidney, brain, lung, heart, and testes (Ereal *et al.*, 2001). Also, cadmium enhanced membrane fluidity and reduced levels of nonprotein sulphhydryls and  $\text{Na}^+\text{K}^+\text{ATPase}$  (Yadav *et al.*, 2005). In addition, cadmium induced elevation in serum levels of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, gamma glutamyl transpeptidase and lactate dehydrogenase.

Cadmium can also substitute for zinc or selenium in metalloenzymes (Casalino *et al.*, 2002). Lowered levels of selenium as well as lowered activity of glutathione peroxidase (a selenium-dependent enzyme) have been seen in cadmium-exposed workers (Wasowicz *et al.*, 2001). Cadmium's ability to generate free radicals also leads to the expression of inflammatory chemokines and cytokines (Dong *et al.*, 1998). the oxidation of nucleic acids, the alteration of DNA repair mechanisms, eventual cell death, and the mutagenic changes involved in cadmium-induced cancers (Fowler, 1978).

In view of data obtained in the present study, administration of cadmium stimulated lipid peroxidation production. This was evident from the highly significant increase in the level of lipid peroxide (MDA) in blood and liver. Such increase confirms the previous reports (Gupta and Kar, 1998 and Abdollah *et al.*, 2003). Studies available from Xu *et al.* (2003) reported that incubation of primary hepatocytes from rats with cadmium induced oxidative

**Table 3: Effect of oleanolic acid and vit. E administration concurrently with cadmium on T<sub>3</sub>, T<sub>4</sub> and TSH levels in rats.**

Treatment	T <sub>4</sub> (µg/dl)	T <sub>3</sub> (µg/dl)	TSH (µIU/dL)
<b>Control</b>			
Range	2.87-3.29	211.5-243.2	0.422-0.67
Mean±SD	3.03±0.18	229.97±10.87	0.55±0.07
<b>Cadmium</b>			
Range	2.48-2.60	160.9-175	0.762-0.927
Mean±SD	2.53±0.04	166.75±4.44	0.83±0.06
P	<0.01	<0.01	<0.01
L.S.D	a	a	a
%of difference	-16.5	-27.49	50.9
<b>Cadmium+ oleanolic acid</b>			
Range	4.41-3.61	227.0-245.8	0.662-0.851
Mean±SD	3.55±0.05	236.64±4.9	0.76±0.06
P	<0.01	n.s	<0.05
L.S.D	a,b	a,b	a,b
%of difference	17.16	2.9	38.18
<b>Cadmium+ vit.E</b>			
Range	2.90-3.50	191.3-221	0.562-0.743
Mean±SD	3.16±0.18	204.33±10.68	0.66±0.06
P	n.s	<0.05	<0.05
L.S.D	a,b,c	a,b,c	a,b,c
%of difference	4.29	-11.14	20

Each value represent a mean ± SD .

L.S.D(least significant difference)

a)Significantly different from control group at P <0.05.

b) Significantly different from group injected with cadmium alone at P<0.05.

c) Significantly different from group injected with cadmium and oleanolic acid at P<0.05.

stress that measured by increase in cytotoxic parameters as lactate dehydrogenase (LDH) and lipid peroxidation (LPO). They found a significant increase in LDH leakage and LPO after 12 and 24 hours, respectively in the presence of 4 µM. cadmium chloride.

Metallothionein (MT), a low molecular weight, cysteine-rich protein, is also important nonenzymatic antioxidant compound (Bray and Bettger 1990 and Sato and Bremner, 1993). Oleanolic acid treatment increased MT up to 25-fold, which plays an important role in OA protection against cadmium-induced liver injury (Liu *et al.*, 1993a and Miura *et al.*, 1999). Metallothionein has been proposed to function as a free radical scavenger (Sato and Bremner, 1993), and thus may play a role, in OA protection against radical-derived tissue damage. Along with increased MT, hepatic Zn and Cu content were also increased by approximately 20% after OA treatment (Liu *et al.*, 1995). Also, Satoh *et al.* (1988) indicated the involvement of cardiac metallothionein in prevention of adriamycin induced lipid peroxidation in the heart. Metallothionein is a zinc-concentrating protein that contains 33-percent cysteine. Primarily induced and stored in the liver, it forms a complex with cadmium, sequestering it from inside the hepatic cytosol, thus reducing the amount of cadmium available to injure hepatocytes and preventing cadmium from depleting glutathione stores. Metallothionein has also been shown to

prevent acute cadmium-induced hepatotoxicity and cell death in animal studies (Klaassen *et al.*, 1999). Mice with genetically-induced high levels of hepatic metallothionein and newborn animals with naturally high levels of metallothionein are resistant to cadmium-induced hepatotoxicity (Liu *et al.*, 1996). Metallothionein also has free-radical scavenging properties and is known to function like glutathione (Klaassen and Cagen, 1981). The ability of metallothionein to scavenge hydroxyl and superoxide radicals and function like superoxide dismutase in microorganisms has been demonstrated (Thornalley and Vasak, 1985).

Metallothionein production is also induced by the presence of metals, including cadmium, mercury, copper, gold, bismuth, and most powerfully, zinc (Coyle *et al.*, 2002). Low level zinc treatments have been used in animal studies to induce metallothionein and protect against acute cadmium-induced hepatotoxicity (Leber and Miya, 1976). Similarly, oleanolic acid treatment protect against cadmium-induced hepatotoxicity as a result of metallothionein induction (Liu *et al.*, 1995).

In animals, both hepatic and intestinal metallothionein have been induced using oral zinc, and metallothionein induction using nontoxic zinc injections has been successful in reducing cadmium toxicity in animals (Onosaka *et al.*, 2002). The induction of intestinal metallothionein in

humans, using zinc acetate, is the mechanism for the FDA-approved treatment of Wilson's disease, an inherited condition where accumulation of copper in the liver, brain, and other organs leads to copper toxicosis (Brewer, 2000).

The increased Zn and Cu can provide metals for Zn, Cu-SOD and ceruloplasmin. Both enzyme are superoxide anion scavengers. Zinc itself also plays a role as an antioxidant by protecting sulfhydryl groups and inhibiting reactive oxygen species produced by transition metals (Bray and Bettger 1990).

In the present study, administration of oleanolic acid (5 mg/kg/day) in combination with cadmium (1mg CdCl<sub>2</sub>/kg body weight/day) for 30 days, reduced significantly both blood MDA and hepatic MDA levels near normal values ( $P < 0.01$ ) (when compared with that group treated with cadmium alone). These results are in agreement with previous reports. OA enhance the protection through MT and non MT (Patrick, 2003). Oleanolic acid suppress the mouse liver P<sub>450</sub> approximately by 40% and that oleanolic acid protection against some chemical require P<sub>450</sub> activation to produce tissue injury (Lindamood, 1991). Also, OA pretreatment significantly increase GSH content by approximately 20% (Liu *et al.*, 1993a). In addition, OA pretreatment decrease the activity of catalase by 20% and increase the hepatic ascorbate concentration (nonenzymatic component) (Liu *et al.*, 1995 and Pillai and Gupta, 2005). In addition, Doses of 100 mg/kg of oleanolic acid were given to mice for three days prior to cadmium injections in doses known to induce acute liver injury. Oleanolic acid resulted in a 30-fold increase in hepatic metallothionein and a significant increase in the mobilization of cadmium, preventing cadmium-binding to intracellular proteins. Liver injury was also significantly reduced, as indicated by reductions in ALT and sorbitol dehydrogenase levels. Specific triterpenoids, including oleanolic acid, have been found to be effective in reducing the hepatotoxicity of cadmium (Pisha *et al.*, 1995).

Vitamine E ( $\alpha$ -tocopherol) is a lipophilic antioxidant against oxygen radical- induced toxicity (Davis and pacht (1991), residing mainly in cell membranes. It is thought to interrupt the chain reactions involved in lipid peroxidation, and to scavenge ROS generated during the univalent reduction of molecular oxygen (Gupta *et al.*, 2003). The present data showed that the treatment of cadmium-treated group with vitamine E, restored hepatic malondialdehyde level (marker of lipid peroxidation) toward normal value ( $P < 0.05$ ), while blood malondialdehyde remain high. These results are in accordance with (Gupta and Kar, 1999 and El-Demerdash *et al.*, 2004), who detected that vitamine E in a dose of 100 mg/kg BW decrease LPO in the kidney, liver and serum of cadmium-treated rats. The mechanism involved in the vitamine E-induced decrease in LPO in cadmium-treated rats may be due to fact that vitamine E terminates the chain reaction of lipid peroxidation in membranes and lipoproteins (Dieber-Rotheneder *et al.*, 1991).

In this study, serum copper and zinc concentrations have been decreased significantly through cadmium

treatment alone by 34% and 56% respectively. On the other hand, their concentrations were elevated by treatment with cadmium in combination with either oleanolic acid and vitamine E. This result is in agreement with previous reports that showed significant decrease in the concentration of copper by cadmium treatment (Daly and Pfander, 1975; Julshamn, 1977; Paksy *et al.*, 1992; Petering, 2001 and Berger, 2006). Smith *et al.* (1991) proved that feeding 1ppm cadmium to gestating cow was sufficient to reduce liver copper store by 40%. Also, some reports indicated that cadmium treatment in a dose of 1mg CdCl<sub>2</sub>/kg animal/day for 5 weeks, significantly decreased the level of zinc in serum (Kadrabova *et al.*, 1993). The nuclear zinc concentration was significantly decreased in response to cadmium treatment and increase in response to oleanolic acid treatment. The results suggested that the liver responds differently to cadmium and oleanolic acid treatment. The difference in response to such treatment may reflect different mechanisms of cadmium transport and metabolism in the liver. Oleanolic acid did not reduced the amount of cadmium in the liver, but significantly altered the hepatic subcellular distribution of CD, with more CD in hepatic cytosol bound to MT, and with less CD in other organelles and proteins. Thus oleanolic acid protects against cadmium hepatotoxicity by inducing metallothionein (Liu *et al.*, 1993). The difference in binding affinity of MT may suggest the involvement of MT in the metabolism and transport of cadmium, an effect, which may be modified by treatment (Bataineh and Al-Alami, 2002).

Cadmium administration is known to be followed by deleterious effects on the endocrine system although its mechanism of action is not well understood (Lafuente *et al.*, 1997). (Yoshizuka *et al.*, 1991) indicated that accumulated cadmium in the mitochondria of thyroid follicular epithelial cells might disturb the oxidative phosphorylation of this organelle and the loss of energy supply possibly caused the inhibition of the synthesis and release of thyroid hormones. The present study indicate that, administration of cadmium as (1 mg/kg body wt./day) for thirty days, decreased serum T<sub>3</sub> and T<sub>4</sub> levels. On the other hand, serum thyroid stimulating hormone (TSH) has been increased by cadmium administration. Furthermore cadmium treatment resulted in increased peroxidative reactions involving membrane components. Simultaneous administration of either oleanolic acid or vitamine E ( $\alpha$ -tocopherol) restored thyroid function in rats by maintaining serum thyroid hormones concentrations. It also prevented the increase in LPO (MDA) in blood and liver. It appears that the protective effect of either oleanolic acid vitamine or E against cadmium-induced thyroid dysfunction, is mediated through their antioxidative action (Gupta and Kar, 1998).

It could be concluded that, the protective effect of either oleanolic acid or vitamine E against cadmium-induced thyroid dysfunction may be mediated through their antioxidative action. Accordingly, to avoid the risk assessment of cadmium intake, special consideration could be given to an adequate intake of either oleanolic acid or vitamine E ( $\alpha$ -tocopherol).

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