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Prophylactic Role of Vitamin E and Olive Oil on Some Physiological and Histological Alterations of Male Rabbits Liver Exposed to Cadmium

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Abstract: This experiment pertains to the protective role of vitamin E and olive oil against Cadmium (Cd)-induced toxicity on glucose, total cholesterol, triglycerides and bilirubin, enzyme activities of alanine Amino Transaminase (ALT), Aspartate Amino Transaminase (AST) and Alkaline Phosphatase (ALP) and on liver tissue of New Zealand rabbits. New Zealand white male rabbits weighing 800-1000 g were used in the present study. Rabbits divided into 4 groups. The 1st group is the control group and received only deionized water orally for 8 weeks. The 2nd group received a dose of (2 mg kg^{-1} b.wt.) of $CdCl_2$ daily. The 3rd group received a dose of (150 mg kg^{-1} b.wt.) vitamin E prior $CdCl_2$ administration daily. The 4th group received a dose of (1 mL kg^{-1} b.wt.) olive oil prior $CdCl_2$ administration daily. Animals of both control and experimental subgroups were decapitated weekly. Administration of $CdCl_2$ showed highly significant increases in serum glucose, cholesterol, bilirubin, triglyceride concentration and ALT, AST and ALP activities all over the experimental periods ($p \leq 0.01$). However, pretreatment with vitamin E and olive oil decreased serum glucose and total cholesterol all over the experimental periods to become near normal. The histopathological alterations were manifested in the liver of the $CdCl_2$ treated rabbits. Hepatocellular damage as congestion in the central and portal veins with dilation of these veins and congestion in sinusoids degenerative hepatocytes showed with cytomegaly clearly observed at 4th week. Also, diffuse Kupffer cells between the degenerative hepatocytes showed obviously in the 1st and 4th weeks. The histopathological studies in the liver of rats also showed that vitamin E and olive oil markedly reduced the toxicity of cadmium and preserved the normal histological architecture of the tissue. The present study suggested that vitamin E and olive oil may be beneficial in ameliorating the cadmium-induced oxidative damage in the liver of rabbits.

Key words: Cadmium chloride, vitamin E, olive oil, liver, biochemical parameters, histology

INTRODUCTION

Cadmium (Cd) is an inorganic toxicant of great environmental and occupational concern which was classified as a group 1 human carcinogen (Waalkes, 2000). The toxicity of cadmium as an industrial pollutant, a food contaminant and as one of the major components in cigarette smoke has been well established (Morselt, 1991). The most important sources of cadmium in agricultural soils are atmospheric deposition and direct inputs through, for example, the application of phosphate fertilizers and other soil amendment products. Among the various effects induced by Cd in biological systems, the oxidative destruction of membrane polyunsaturated fatty acids, a phenomenon termed Lipid Peroxidation (LPO) has been observed in numerous tissues both *in vitro* and *in vivo* (Jamall and Smith, 1985; Muller, 1986). It exerts its toxic effect by causing specific cell membrane lesions. Reports suggested that lipid peroxidation may be involved in the cell membrane lesion with subsequent altered membrane permeability (Sumathi *et al*., 1994).

Cd-induced cytotoxicity is known to be intimately associated with the induction of oxidative stress (Hart *et al*., 1999; Pathak and Khandelwal, 2006). These evidences indicate that apoptosis probably play an important role in acute and chronic intoxication with Cd (Li *et al*., 2000).

Tandon *et al*. (1992) concluded that the antioxidant properties of vitamin E seemed to be responsible for protection from cadmium toxicity.

Vitamin E (α-tocopherol) are naturally occurring antioxidants that play important roles in animal health by inactivating harmful free radicals produced through the normal cellular activity and from various stresses. The antioxidant function of these micro-nutrients could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune

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cells (El-Demerdash *et al*., 2004; Yang *et al*., 2006). Because the health problems induced by many environmental pollutants, much effort have been expended in evaluating the relative antioxidant potency of vitamin E (Beytut and Aksakal, 2002; Pillai and Gupta, 2005).

Olive oil is a natural juice which preserves the taste, aroma, vitamins and properties of the olive fruit. Olive oil is the only vegetable oil that can be consumed as it is freshly pressed from the fruit. The beneficial health effects of olive oil are due to both its high content of monounsaturated fatty acids and its high content of antioxidative substances. According to Viola (1997), the ratio of vitamin E to polyunsaturated fatty acids in olive oils is better than other edible oils. Olive oil, as a high monounsaturated oil is resistant to oxidation. In addition, the presence of phenols, tocopherols and other natural antioxidants prevent lipid oxidation within the body, eliminating the formation of free radicals which may cause cell destruction.

MATERIALS AND METHODS

Experimental animals and dosing: A total number of 192 New Zealand white male rabbits were used in the present study weighing 800-1000 g. Rabbits were housed in the usual metal cage at room temperature and fed on a commercial balanced diet prepared specially for rabbits (Anbar 590). The diet and tap water were offered *ad libitum* all over the experimental period of 8 weeks. Animals were divided into four groups, each of 48 rabbits and treated as follows:

Group 1 (G1): Animals of this group (48 rabbits) were considered as control and were given orally deionized water daily for 8 weeks.

Group 2 (G2): Each animal of this group (48 rabbits) was orally given 2 mg kg^{-1} b.wt. ($CdCl_2$) daily for 8 weeks according to El-Sharaky *et al*. (2007) using a stomach tube.

Group 3 (G3): Animal of this group (48 rabbits) were orally given 150 mg kg^{-1} b.wt. of vitamin E dissolved in a few drops of corn oil (El-Nahas *et al*., 1993) for 8 weeks prior to administration of 2 mg kg^{-1} b.wt. ($CdCl_2$).

Group 4 (G4): Animals in this group (48 rabbits) were given 1 mL kg^{-1} b.wt., olive oil (Abd El-Aziz, 2000) prior oral administration of $CdCl_2$ (2 mg kg^{-1} b.wt.) daily for 8 weeks. Then animals of each subgroup were decapitated weekly for 8 weeks.

Cadmium chloride ($CdCl_2$) was purchased from Sigma chemical company (USA). All other chemicals used were of analytical grade.

Blood sampling and processing: Animals of both control and experimental groups were decapitated weekly. While 5 mL blood was collected and clear serum samples were separated by centrifugation at 3000 r.p.m. For 20 min and then transferred into Eppendorf tubes and stored in a deep freeze (-20°C) for biochemical analysis. However, the determination of glucose and enzymes were carried out on fresh serum samples.

Biochemical parameters and enzyme activities: Serum glucose, triglycerides, total cholesterol and bilirubin were determined using the method described by Trinder (1969), Allain *et al*. (1974) and Perry *et al*. (1983), respectively. The kits were purchased from Biotech Laboratories, UK. The activities of serum (AST) and (ALT) were determined according to the method of Reitman and Frankel (1957). The measurement of serum (ALP) activity was based on the method of Bessey *et al*. (1946).

Histopathological study: For qualitative analysis of liver histology, the tissue samples were fixed for 48 h in 10% formalin-saline and dehydrated by passing successfully in different mixture of ethyl alcohol, water, cleaned with xylene and embedded in paraffin. Sections of the tissue (5-6 mm thick) were prepared by using a rotary microtome and stained with haematoxylin and eosin (H&E) dye which was mountedin a neutral deparaffinated xylene medium for microscopical observations and histopathological examinations.

Statistical analysis: The results were analyzed for statistical significance by independent student t-test using the SPSS statistical program version 15 (SPSS, Chicago, IL). MS excel program was also used for figure design. Data were expressed as Mean±SD, $p \leq 0.05$ were considered significant and highly significant at $p \leq 0.01$.

RESULTS

Physiological studies: The mean values of rabbit's serum glucose were summarized in Table 1. Daily oral administration of $CdCl_2$ for 8 weeks increased serum glucose level ($p \leq 0.01$) from 2nd week by 34.83% as compared to control. The rate of change, elevated week by week to become 77.6% at the end of 8th week. However, it was decreased in G3 and 4 commencing from the 1st week to become 30.64 and 22.76% in G3 and 4, respectively.

Table 1: Effect of daily administration of $CdCl_2$ (2 mg kg^{-1} b.wt.) on serum glucose concentrations (mg dL^{-1}) of male rabbit possible prophylactic role of antioxidant vitamin E (150 mg kg^{-1} b.wt.) and olive oil (1 mL kg^{-1} b. wt.) for 2 months

			Experimental groups		
Weeks	Statistics	Control group (G1)	Pure $CdCl_2$ (G2)	$CdCl_2$+vit. E (G3)	$CdCl_2$+olive oil (G4)
1	Mean±SD	94.0±5.65	115.67±0.52**	100.50±4.89*	113.67±10.61**
	% of change		23.05	6.91	20.92
2	Mean±SD	88.5±0.70	119.33±3.93***	111.33±0.82***	115.50±6.63***
	% of change		34.83	25.79	30.50
3	Mean±SD	91.5±0.70	122.67±3.88***	112.17±0.41***	115.17±5.08***
	% of change		34.06	22.59	25.86
4	Mean±SD	91.0±1.41	130.50±4.72***	112.33±0.52***	114.27±3.49***
	% of change		43.40	23.43	25.57
5	Mean±SD	91.5±0.70	139.67±4.55***	113.83±0.75***	114.83±2.93***
	% of change		52.64	24.40	25.49
6	Mean±SD	93.0±1.41	151.17±5.27***	115.17±0.41***	113.00±3.16***
	% of change		62.54	23.83	21.50
7	Mean±SD	94.0±2.82	154.50±5.47***	115.67±2.16***	116.17±10.09***
	% of change		64.36	23.05	23.58
8	Mean±SD	93.0±1.41	165.17±4.12***	121.50±1.87***	114.17±5.04***
	% of change		77.60	30.64	22.76

Table 2: Effect of Daily Administration of $CdCl_2$ (2 mg kg^{-1} b.wt.) on serum cholesterol concentrations (mg dL^{-1}) of male rabbit possible prophylactic role of antioxidant vitamin E (150 mg kg^{-1} b.wt.) and olive oil (1 mL kg^{-1} b.wt.) for 2 months

			Experimental groups		
Weeks	Statistics	Control group (G1)	Pure $CdCl_2$ (G2)	$CdCl_2$+vit. E (G3)	$CdCl_2$+olive oil (G4)
1	Mean±SD	205.0±7.07	245.00±14.83***	219.83±5.15*	219.00*±9.01
	% of change		19.51	7.23	6.82
2	Mean±SD	204.0±1.41	237.83±14.15***	218.33±2.66**	227.17±1.17***
	% of change		16.58	7.02	11.35
3	Mean±SD	201.5±0.70	241.00±9.72***	223.33±1.97***	230.33±1.21***
	% of change		19.60	10.83	14.30
4	Mean±SD	205.0±7.07	239.00±4.00***	225.00±1.10***	232.83±5.42***
	% of change		16.58	9.75	13.57
5	Mean±SD	188.5±0.70	244.83±1.60***	225.83±1.72***	221.33±4.46***
	% of change		29.88	19.80	17.41
6	Mean±SD	196.0±7.07	248.67±1.63***	230.83±0.98***	220.83±2.04***
	% of change		26.87	17.77	12.66
7	Mean±SD	199.5±0.70	253.50±1.38***	230.67±0.82***	224.83±5.49***
	% of change		27.06	15.62	12.69
8	Mean±SD	205.0±7.07	257.50±6.92***	231.50±0.84***	210.67±8.04*
	% of change		25.60	12.92	2.76

SD = Standard Deviation; *,**Non-significant change at $p>0.05$, $p\leq0.05$; ***Highly significant change at $p\leq0.01$

Treatment with $CdCl_2$ caused a highly significant ($p\leq0.01$) increase in serum cholesterol level of the 1st week by a rate of 19.51 and 25.60% at the end of 8th week (Table 2). However, this increment was reduced in G3 and 4 with percentage change of 12.92 and 2.76%, respectively as compared to control. The result of the effects of daily administration of $CdCl_2$ (2 mg kg^{-1} b.wt.) on serum total bilirubin concentrations (mg dL^{-1}) of male rabbit possible prophylactic role of antioxidant vitamin E (150 mg kg^{-1} b.wt.) and olive oil (1 mL kg^{-1} b.wt.) for 2 months is presented in Table 3. There was a highly significant increase ($p\leq0.01$) in bilirubin concentration from the 4th week to the end of the experiment as compared to control.

Vitamin E pretreatment generally ameliorate bilirubin concentration but olive oil pretreatment is better in keeping bilirubin concentration near to normal, since the rate of increment became 37.64, 21.17 and 7.05% in G2 and 3. A highly significant increase ($p\leq0.01$) in serum Triglyceride (TG) concentration was demonstrated throughout the experimental periods in all $CdCl_2$ intoxicated group at the rate of 27.03 and 42.92% at the end of the 1st and 8th week, respectively. On vitamin E pretreatment, the rate of increment was decreased generally to become 19.90 and 26.01% at the end of 1st and 8th week, respectively but olive oil pretreatment is best in keeping (TG) concentration near normal during all week of experiment so the rate of increment became 4.47 and 2.11% at the end of 1st and 8th week, respectively, compared to control (Table 4).

The obtained results in Table 5 revealed that $CdCl_2$ treatment elevated AST activity highly significant ($p\leq0.01$) from the end of the 2nd to the end of 8th week. The toxic effect of $CdCl_2$ was reduced by vitamin E and olive oil pretreatment, compared to control where olive oil is better than vitamin E in keeping the AST activity near to normal, so the rates of increase were 57.89, 5.26 and 3.08% in G2-G4 as compared to the G1.

Table 3: Effect of daily administration of $CdCl_2$ (2 mg kg^{-1} b.wt) on serum total bilirubin concentrations (mg dL^{-1}) of male rabbit possible prophylactic role of antioxidant vitamin E (150 mg kg^{-1} b.wt.) and olive oil (1 mL kg kg^{-1} b.wt.) for 2 months

			Experimental groups		
Weeks	Statistics	Control group (G1)	Pure $CdCl_2$ (G2)	$CdCl_2$+vit. E (G3)	$CdCl_2$+olive oil (G4)
1	Mean±SD	0.85±0.07	0.93±0.06*	0.92±0.03*	0.88±0.07*
	% of change		9.41	8.23	3.952
2	Mean±SD	0.85±0.07	0.93±0.01*	0.94±0.03*	0.88±0.08*
	% of change		9.41	10.58	3.52
3	Mean±SD	0.90±0.07	0.96±0.01*	0.92±0.01*	0.92±0.02**
	% of change		6.66	2.22	2.22
4	Mean±SD	0.80±0.14	0.96±0.00***	0.92±0.01***	0.92±0.02**
	% of change		20.00	15.00	15.00
5	Mean±SD	0.85±0.07	0.97±0.01***	0.96±0.02***	0.91±0.01***
	% of change		14.11	12.94	7.05
6	Mean±SD	0.80±0.14	1.01±0.04***	0.98±0.01***	0.94±0.02***
	% of change		26.25	22.50	17.50
7	Mean±SD	0.80±0.07	1.07±0.08***	1.02±0.04***	0.93±0.03***
	% of change		33.75	27.5	16.25
8	Mean±SD	0.85±0.07	1.17±0.05***	1.03±0.12***	0.91±0.02*
	% of change		37.64	21.17	7.05

Table 4: Effect of daily administration of $CdCl_2$ (2 mg kg^{-1} b.wt.) on serum triglycerides concentrations (mg dL) of male rabbit possible prophylactic role of antioxidant vitamin E (150 mg kg^{-1} b.wt.) and Olive Oil (1 mL kg^{-1} b.wt.) for 2 months

			Experimental groups		
Weeks	Statistics	Control group (G1)	Pure $CdCl_2$ (G2)	$CdCl_2$+vit. E (G3)	$CdCl_2$+olive oil (G4)
1	Mean±SD	100.5±0.70	127.67±1.37***	120.50±0.55***	105.00±1.79***
	% of change		27.03	19.90	4.47
2	Mean±SD	100.5±0.70	127.50±1.05***	123.17±0.98***	111.67±1.03***
	% of change		26.86	22.55	11.11
3	Mean±SD	101.5±0.70	119.17±2.04***	124.50±0.55***	113.83±1.33***
	% of change		17.40	22.66	12.14
4	Mean±SD	100.5±6.36	119.00±3.52***	125.33±0.52***	117.33±3.88***
	% of change		18.40	24.70	16.74
5	Mean±SD	101.5±0.70	129.50±2.51***	125.83±0.41***	116.33±4.18***
	% of change		27.58	23.97	14.61
6	Mean±SD	102.5±0.70	138.50±3.94***	126.83±0.68***	105.33±5.50*
	% of change		35.12	23.73	2.76
7	Mean±SD	101.0±2.82	142.83±1.47***	127.67±0.52***	102.33±1.75*
	% of change		41.41	26.40	1.31
8	Mean±SD	102.5±0.70	146.50±1.38***	129.17±0.41***	104.67±2.16*
	% of change		42.92	26.01	2.11

Table 5: Effect of daily administration of $CdCl_2$ (2 mg kg^{-1} b.wt.) on serum (AST) activity (U/L) of male rabbit possible prophylactic role of antioxidant vitamin E (150 mg kg^{-1} b.wt.) and Olive Oil (1 mL kg^{-1} b.wt.) for 2 months

			Experimental groups		
Weeks	Statistics	Control group (G1)	Pure $CdCl_2$ (G2)	$CdCl_2$+vit. E (G3)	$CdCl_2$+olive oil (G4)
1	Mean±SD	30.50±0.70	31.17±0.75*	31.00±0.63*	33.50±2.43**
	% of change		2.19	1.63	9.83
2	Mean±SD	28.50±0.70	33.83±0.93***	31.50±0.55***	31.35±1.70***
	% of change		18.70	10.52	10.00
3	Mean±SD	28.05±0.07	35.33±0.82***	30.33±1.37*	31.82±0.66***
	% of change		25.95	8.14	13.44
4	Mean±SD	29.00±1.41	37.50±0.84***	30.50±0.55*	30.70±0.47***
	% of change		29.31	5.17	5.86
5	Mean±SD	29.50±0.70	37.50±4.76**	31.67±0.52*	30.58±0.49*
	% of change		27.11	7.35	3.66
6	Mean±SD	30.00±0.70	41.83±1.21***	32.00±0.45***	31.14±0.08***
	% of change		39.43	6.66	3.8
7	Mean±SD	29.00±2.82	44.00±0.63***	31.00±0.52**	30.18±0.95***
	% of change		51.72	6.89	4.06
8	Mean±SD	28.50±0.70	45.00±0.89***	30.00±0.55***	29.38±0.38***
	% of change		57.89	5.26	3.08

SD = Standard Deviation; *,**Non-significant change in $p>0.05$, $p\leq0.05$; ***Highly significant change at $p\leq0.01$

Table 6: Effect of daily administration of $CdCl_2$ (2 mg kg^{-1} b.wt.) on serum (ALT) activity (U/L) of male rabbit possible prophylactic role of antioxidant vitamin E (150 mg kg^{-1} b.wt.) and Olive Oil (1 mL kg^{-1} b.wt.) for 2 months

			Experimental groups		
Weeks	Statistics	Control group (G1)	Pure $CdCl_2$ (G2)	$CdCl_2$+vit. E (G3)	$CdCl_2$+olive oil (G4)
1	Mean±SD	28.50±0.70	31.67±0.82***	31.33±0.52***	33.33±2.07***
	% of change		11.12	9.92	16.94
2	Mean±SD	29.00±1.41	33.00±0.89***	31.33±0.52***	31.08±1.28**
	% of change		13.79	8.03	7.17
3	Mean±SD	30.00±0.70	34.67±0.52***	31.67±0.52*	31.00±0.63*
	% of change		15.56	5.56	3.33
4	Mean±SD	29.5±1.41	36.67±0.82***	31.50±0.55*	30.33±0.52***
	% of change		24.30	6.77	2.81
5	Mean±SD	30.00±0.70	38.17±0.75***	33.17±0.98**	31.00±0.40*
	% of change		27.23	10.56	3.33
6	Mean±SD	29.00±0.70	41.00±1.67***	33.67±0.52***	29.92±0.92***
	% of change		41.37	16.10	3.17
7	Mean±SD	29.50±0.70	44.67±2.25***	33.33±0.82***	30.5±0.90**
	% of change		51.42	12.98	3.38
8	Mean±SD	30.05±0.70	46.83±1.47***	34.83±0.41***	30.83±0.68***
	% of change		55.84	15.90	2.59

SD = Standard Deviation; *, **Non-significant change in $p>0.05$, $p\leq0.05$; ***Highly significant change at $p\leq0.01$

As shown in Table 6, ALT activity increased highly significantly ($p\leq0.01$) and gradually from the 1st to the 8th week of $CdCl_2$ treatment by the rate of 11.12, 13.79, 15.56, 24.30, 27.23, 41.37, 51.42 and 55.84%, respectively compared to correspondent controls. While, pretreatment with vitamin E and olive oil ameliorated ALT activity since olive oil in more effective in keeping their activity near to normal. On the other hand, treatment with $CdCl_2$ caused highly significant ($p\leq0.01$) increase in the activity of ALP from the 4th to the 8th week. The ALP activity was improved in G3 and 4 but olive oil is more effective in keeping (ALP) activity near to normal during all periods of the experiment. The rates of increase at the end of 8th week were 51.71, 14.10 and 2.17% in G2-4 as compared with the G1.

Histopathological studies: Histopathological studies showed that the treatment with cadmium caused severe liver damage, including congestion in the central vein associated with diffuse kupffer cell proliferation in between the hepatocytes, dilatation and congestion in the portal vein and accumulation of monocytes cells (Fig. 1 and 2). With the advance of time, sections of liver after the 6th week of the study showed congestion in the portal vein with fibrosis and inflammatory cell infiltration while the hepatocytes had degenerative change (Fig. 3). Sections of liver after the 8th week of the study showed inflammatory cell infiltration in the portal area and between the degenerated hepatocytes associated with fibrosis and congestion in the portal vein in the portal area (Fig. 4) when compared with control liver which showed normal histological structure of the Central Vein (CV) and surrounding hepatocytes (h) (Fig. 1).

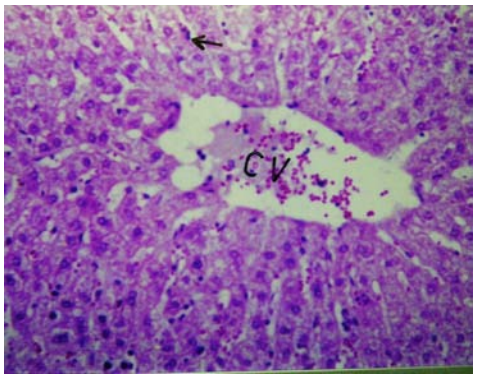

Fig. 1: Section of liver after the 1st week of study showing congestion in the Central Vein (CV) associated with diffuse kupffer cell proliferation (←) in between the hepatocytes (H&E×64)

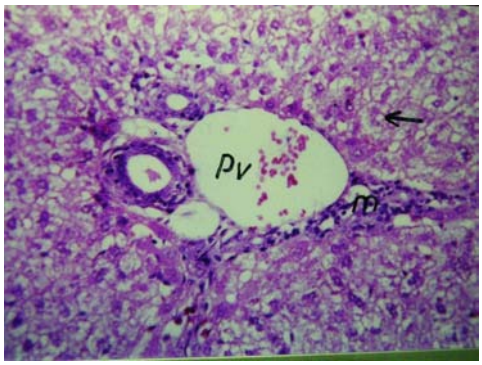

Fig. 2: Section of liver after the 3rd week of study showing degenerative change in the hepatocytes (←) associated with dilatation and congestion in the Portal Vein (PV), monocytes cells are seen (m) (H&E×64)

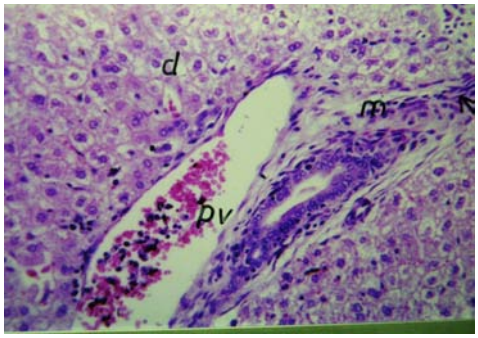

Fig. 3: Section of liver after the 4th week of the study showing congestion in the Portal Vein (PV), inflammatory cell infiltration (m↑) and fibroblastic cell proliferation and degenerative hepatocytes (d) (H&E×64)

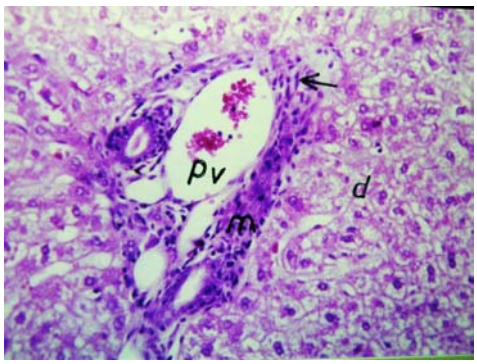

Fig. 4: Section of liver after the 5th week of a study showing congestion in the Portal Vein (PV) with fibrosis and inflammatory cell infiltration (m) while the hepatocytes had a degenerative change (d) (H&E×64)

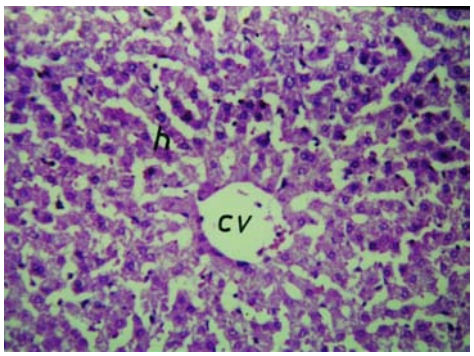

Fig. 5: Section of control liver showing normal histological structure of the Central Vein (CV) and surrounding hepatocytes (h) were recorded (H&E×64)

A group of rabbits administrated cadmium chloride andvitamin E: Histological examination of liver sections

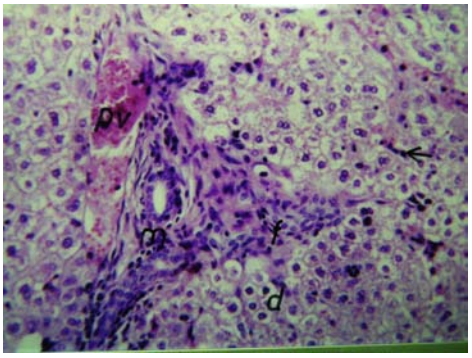

Fig. 6: Section of liver after the 8th week of a study showing inflammatory cell infiltration (f) were observed in the portal area, as well as between the degenerated hepatocytes (d) associated with fibrosis (←) and congestion in the Portal Vein (PV) at the portal area(H&E×64)

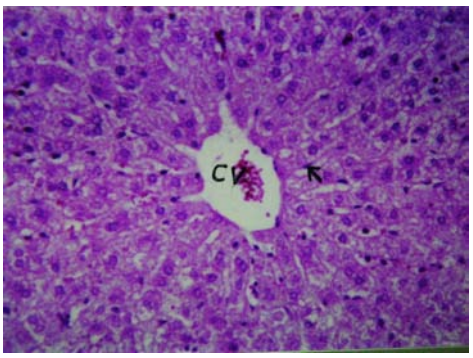

Fig. 7: Section of liver after 1st week showing diffuse kupffer cell proliferation in between the hepatocytes (↑) (H&Ex64)

after being maintained on $CdCl_2$ and vitamin E for 1 week revealed a few improvements of liver tissues compared with sections treated with cadmium. It showed few diffuse Kupffer cell proliferation in between the hepatocytes (Fig. 6). After the 3rd and the 6th week, the microscopic examination showed dilation of central vein and vacuolar degeneration surrounding and congestion in the central vein (Fig. 7 and 8) while the surrounding hepatocytes showed degenerative changes (Fig. 9). After the 8th week of study, sections studied showed congested of central vein associated with vacuolar degeneration in the surrounding hepatocytes (Fig. 10).

A group of rabbits administrated cadmium chloride and olive oil: Histological examination performed 1 week after the administration of $CdCl_2$ and olive oil revealed degenerative change in the hepatocytes (Fig. 11). Sections after the 3rd week after administration cadmium chloride and olive oil showing inflammatory cells

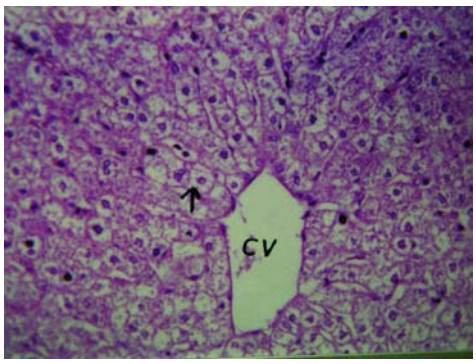

Fig. 8: Section of liver after the 3rd week showing a central vein was dilated while the surrounding hepatocytes showed vacuolar degeneration (↑) and cytomegaly (H&E×64)

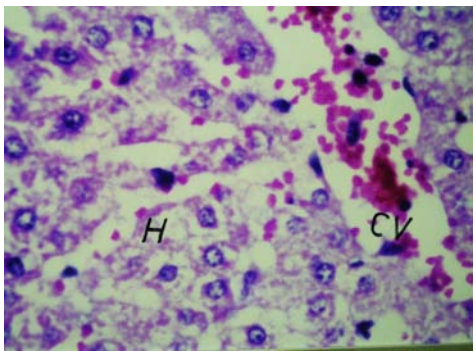

Fig. 9: Section of liver after the 6th week showing congestion in the Central Vein (CV) while the surrounding hepatocytes showed degenerative changes (H) (H&E×100)

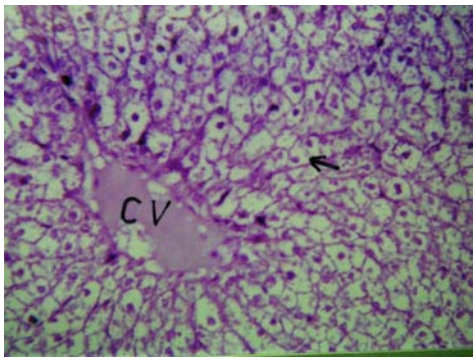

Fig. 10: Section of liver after the 8th week showing a Central Vein was congested (CV) associated with vacuolar degeneration in the surrounding hepatocytes (←) (H&E×64)

infiltration and congestion in the portal vein were detected in the portal area while the hepatocytes

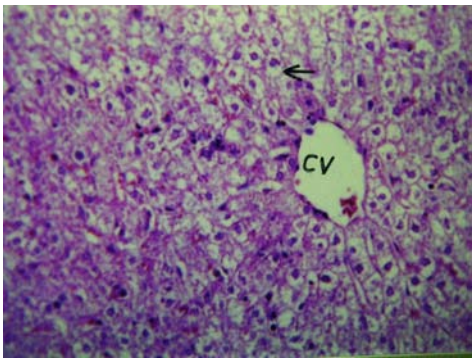

Fig. 11: Section of liver after the 1st week of administration cadmium chloride and olive oil showing degenerative change in the hepatocytes (←) (H&E×64)

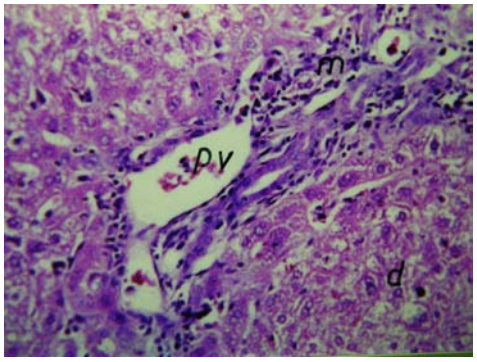

Fig. 12: Section of liver after the 3rd week after administration cadmium chloride and olive oil showing inflammatory cell infiltration (m) and congestion in the portal vein were detected in the portal area while the hepatocytes showed degenerative changes (d) (H&E×64)

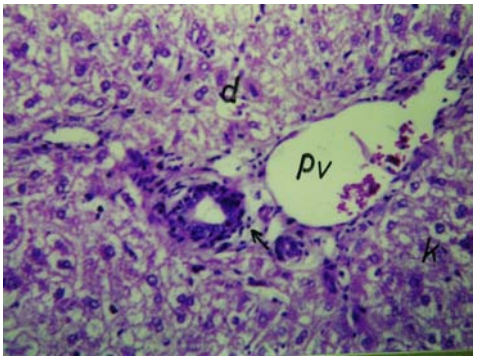

Fig. 13: Section of liver after the 6th week of a study showing diffuse kupffer cells (k) proliferation in-between the degenerated hepatocytes (d) while the portal area showed few inflammatory cell infiltration (↑) and congestion in the Portal Vein (PV) (H&E×64)

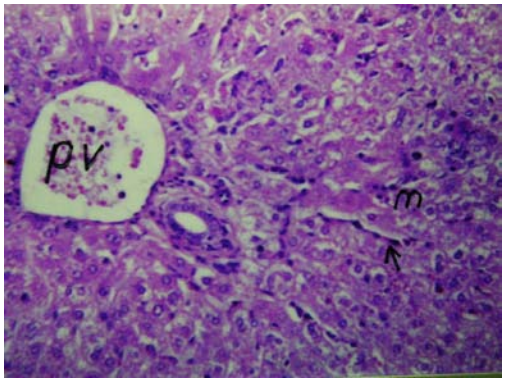

Fig. 14: Section of liver after the 8th week showing congestion in the Portal Vein (PV) associated with diffuse kupffer cell proliferation between the hepatocytes (↑) (H&E×64)

showed degenerative changes (Fig. 12). Sections of livers examined after 6 weeks perceived diffuse kupffer cell proliferation in-between the degenerated hepatocytes while the portal area showed few inflammatory cell infiltration and congestion in the portal vein (Fig. 13). Sections of liver after the 8th week showed congestion in the portal vein associated with diffuse kupffer cell proliferation in between the hepatocytes (Fig. 14).

DISCUSSION

The present study evaluates the protective effect of vitamin E and olive oil against toxicity induced by cadmium in rabbits. The results revealed that serum glucose concentrations were highly significant increase in rabbits treated with $CdCl_2$ as compared with the control group. This increase agrees with the findings of Massanyi *et al.* (1995) and Ognjanovic *et al.* (2005) who attributed the increase in glucose levels to the decrease in glucose utilization and/or the disruption in insulin and glucagon hormones. Pretreatment of rabbits with vitamin E and olive oil diminished the negative effect on serum glucose level. These findings were in accordance with Ognjanovic *et al.* (2000) and El-Demerdash *et al.* (2004).

Concerning lipid metabolism, results demonstrated that total cholesterol and triglyceride levels were highly significant increase in response to $CdCl_2$ oral administration to the rabbits. Similar results were obtained previously by Larregle *et al.* (2008) and Koriem *et al.* (2009). The possible explanation of these observed increments may be residing in direct or indirect action of $CdCl_2$ on lipid metabolism or lipid peroxidation (Berne and Levy, 1998). From the previous results, one can say that this increment was associated with a decrease of lipoprotein lipase activity in post heparinic plasma and the high triglyceride mass was related to the increased glycerol-3-phosphate acyltransferase mRNA expression (Larregle *et al.*, 2008).

The present study findings showed a protective role of vitamin E and olive oil may be by increasing the antioxidant potential of the animals or decreasing lipid peroxidation (Bansal *et al.*, 2005).

Beytut *et al.* (2003) demonstrated that the effectiveness of vitamin E in reducing oxidative stress in $CdCl_2$-treated rabbits and suggested that reductions in increased Thiobarbituric Acid Reactive Substances (TBARS) due to $CdCl_2$ toxicity may be an important factor in the action of vitamin E.

The present study revealed an increase in serum bilirubin levels in rabbits due to treatment with $CdCl_2$, this result is in agreement with the finding of Rana *et al.* (1996) and El-Demerdash *et al.* (2004) who reported that the increase in plasma bilirubin (hyper-bilirubinimia) may result from decreased liver uptake, conjugation or increased hemolysis. Pretreatment with vitamin E and olive oil diminished the toxic effect of $CdCl_2$ on serum bilirubin levels may be due to decrease in lipid peroxidation (Meydani, 1995) or the quenching of hydroxyl radicals (Boldyrev *et al.*, 1995) revealed.

A large number of enzyme activities have been reported to be differently influenced by cadmium. Transaminases (AST, ALT and ALP) show functional activity of the liver, as the agent causes hepatotoxic effects. The present study showed that there was a highly significant increase in the activities of these enzymes as compared to control. These results are in accordance with the findings obtained by Ognjanovic *et al.* (2000), Koriem *et al.* (2009) and Rhman *et al.* (2011). The increase in the activities of these enzymes may be due to damage of liver and disturbed carbohydrate and protein metabolism. These enzymes have an important role in the processes of amino acids and protein metabolism.

Alkaline Phosphatase (ALP) belongs to a group of enzymes that catalyze the hydrolysis of phosphomonoesters at alkaline pH. ALP is present in cell surface in most human tissues (Moss and Handerson, 1999). The increase in ALP activities of the liver (Table 7) may be attributed to either *de novo* synthesis of enzyme molecules or loss of other proteins from the tissues (Wright and Plummer, 1994). The increased enzyme activity might have resulted from increased functional activity of the tissues caused by cadmium. Such, increase in ALP activities can constitute a threat to the life of the cells that are dependent on the variety of phosphate esters for their vital process, since there may be indiscriminate hydrolysis of phosphate ester of the tissues.

Table 7: Effect of daily administration of $CdCl_2$ (2 mg kg^{-1} b.wt.) on serum (ALP) activity (U/L) of of male rabbit possible prophylactic role of antioxidant vitamin E (150 mg kg^{-1} b.wt.) and Olive Oil (1 mL kg^{-1} b.wt.) for 2 months

			Experimental groups		
Weeks	Statistics	Control group (G1)	Pure $CdCl_2$ (G2)	$CdCl_2$+vit. E (G3)	$CdCl_2$+olive oil (G4)
1	Mean±SD	38.00±1.41	41.08±0.60*	41.17±0.62*	40.67±0.52*
	% of change		8.10	8.34	7.02
2	Mean±SD	38.50±2.12	42.50±1.05*	42.00±0.33*	41.17±0.61*
	% of change		10.38	9.09	6.93
3	Mean±SD	39.00±1.41	44.83±1.47*	42.37±0.42**	40.23±0.15***
	% of change		14.94	8.64	3.15
4	Mean±SD	39.50±0.70	47.83±1.17***	42.67±0.52	40.15±0.05***
	% of change		21.08	8.02	1.64
5	Mean±SD	39.00±3.53	50.67±0.82***	43.00±0.63	40.12±0.08***
	% of change		29.92	10.25	2.87
6	Mean±SD	38.00±7.07	52.50±1.05***	43.67±0.52***	39.67±0.41**
	% of change		38.15	14.92	4.39
7	Mean±SD	39.50±0.70	54.50±1.22***	44.32±0.74*	39.62±0.93***
	% of change		37.97	12.20	0.30
8	Mean±SD	39.00±1.41	59.17±3.97***	44.50±1.05***	39.85±1.06*
	% of change		51.71	14.10	2.17

SD = Standard Deviation; *, **Non-significant change in $p>0.05$, $p\leq0.05$; ***Highly significant change at $p\leq0.01$

Pretreatment of rabbits with vitamin E caused a decrease in the activity of serum AST, ALT and ALP, to become nearer to normal activity as compared with control. These results coincide with those obtained by Tandon *et al.* (1992) and El-Demerdash *et al.* (2004). Moreover, the activities of AST, ALT and ALP in rabbits which receiving olive oil prior $CdCl_2$ drinking were improved to become near to normal. Also, the result of Ognjanovic *et al.* (2000, 2005) showed that olive oil pretreatment diminished the harmful effects of $CdCl_2$ on the activities of ALT and AST enzyme.

Liver histology and histopathology: In the present study, the hepatic histoarchitecture of the Cd-treated rabbits resulted severe necrotic changes, inflammatory cell infiltration, fatty degeneration sinusoidaldilation and vacuolization (Fig. 2-7) when compared with control liver (Fig. 5). These results are hepatocytes vacuolation and infiltration of lymphocytes around the central veins agreed with that obtained by Mason (2009).

The process of cellular necrosis involves disruption of the membranes structural and functional integrity. It might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by Cd. The accumulated hydroperoxides can cause cytotoxicity which is associated with the peroxidation of membrane phospholipids by lipid hydro per-oxides, the basis for hepatocellular damage. Desia *et al.* (1994) suggested that tissue necrosis could be due either to the direct effect of the compound on the cells or to an accumulation of acetylcholine in the tissues. Accordingly, the role in metabolic conversions is its susceptibility to chemical injury (Shakoori *et al.*, 1992).

El-Banhawy *et al.* (1986) pointed out that cellular degeneration might be attributed to liberation of acid hydrolases released from the destructed lysosomes to facilitate the process of autolysis. In addition, the cellular degeneration may be probably due to the direct effect of cadmium as a cytotoxic material. Kawakita *et al.* (1993) stated that cadmium is a noxious chemical with cytotoxic effect on mitochondria. Moreover, El-Banhawy *et al.* (1986) added that there is a relationship between the pathological alterations in the liver and the reduction of the activities of the oxidative enzymes which are present in the mitochondria, such as cytochrome oxidase, succinic dehydrogenase and cytochrome P-450. In the present study, it was evident that the degenerative changes appeared earlier than in the cytoplasm in the nuclei of hepatocytes. This result is consistent with the findings of El-Banhawy *et al.* (1986) who suggested that the nuclear damage is a sequence of cytoplasmic damage.

The lymphocytic infiltration showed various intensities with the treated animal. This indicates signs of irritability, inflammation and hypersensitivity to the chemical used. The dense lymphocytic infiltrate is confined to portal tracts and where there was no erosion of hepatic architecture, this abnormally progress rarely to cirrhosis (Shakoori *et al.*, 1992).

Hemolysis observed in the present study could be responsible for damage and destruction of hepatic cells because this consequently led to hypoxia of cells as oxygen supply was prohibited from reaching hepatocytes. Robbins and Angella (1999) considered hypoxia as the serious cause of cellular injury where it might lead to anaerobic cellular oxidation as a result of absence of enough oxygen.

In this study, there was congestion in the central vein. This result corresponded with that observed by Shakoori *et al.* (1992). The present study showed also histopathological changes, such as parenchymal swelling,

congestion, pyknosis, karyorrhexis and karyolysis of the nuclei. This result coincides with the result obtained by Dudley *et al.* (1982) and Dudley and Klaassen (1984).

The present histological study showed that pretreatment of rabbits with $CdCl_2$ and vitamin E or olive oil was effective for liver tissues. These tissues became gradually normal with the progress of time (for the 1st week to the last week of study). These data matches with the results which obtained by Moharram and Hafiez (2001). They observed normal hepatic lobule with granulated cytoplasmic hepatocytes after treating rats with vitamin E.

Vitamin E significantly inhibited hepatic glutathione depletion and lipid peroxidation induced by cadmium. Furthermore, this antioxidant provided partial protection against lethality produced by the insecticides. It is counteracting the entry of the metal induced free radicals in the cell and ultimately gets transformed into a tocopheroxyl radical (Shukla and Chandra, 1989). It inhibits oxidation by an effect on calcium metabolism (Stohs *et al*., 2001) protein kinase C. In the study of Obianime and Roberts (2009) vitamin C, E and selenium, individually and collectively caused an inhibition of cadmium-induced increases or changes in the phosphates, urea, creatinine and hormonal parameters, thus reversing histologically distortions in the liver, kidney and testes of the male Wistar rats. The necrotic conditions coincide with our biochemical observations which showed the increased level of lipid peroxidation. Administration of vitamin E reduced the histological alterations provoked by cadmium quite appreciable. It can be attributed to the antiradical/antioxidant and metal-chelating efficacy of vitamin E which significantly reduced the oxidative stress leading to the reduction of histopathological alterations and restoration of normal physiological state of an organism.

Several studies have demonstrated the ability of olive oil to inhibit oxidative stress in the liver through various mechanisms (Kyle *et al*., 1987). The beneficial properties of olive oil have been mainly attributed to its high content of monounsaturated oleic acid which is reported to affect the serum lipid profile (Riccardi and Rivellese, 1993) and to decrease, both *in vivo* and *ex vivo*, LDL susceptibility to oxidation (Scaccini *et al*., 1992). However in recent years, converging evidence indicates that olive oil nonglyceride fraction, rich in polyphenols, significantly contributes to its benefits to human health (Manna *et al*., 1999).

The increasing popularity of olive oil is mainly attributed to its antioxidant and anti-inflammatory effects which may help prevent disease in humans (Tuck and Hayball, 2002; Covas, 2007). In the current study, an attempt has been made to assess the hepatoprotective potential of olive oil in animals subjected to cadmium chloride. As a toxicological agent, it is conceivable that cadmium chloride might interact primarily with the liver resulting in structural damage and changes in enzyme leakage and in the metabolism of the constituents.

Robbins *et al.* (1994) reported that hepatic failure might be a consequence of toxic damage by drugs or any toxic substance. Other pathological changes of the liver included cellular degeneration of hepatocytes and dilation of central veins. Degenerative changes have been reported to result in cell death which is of 2 types, namely apoptotic and necrotic cell death (Cohen, 1993). Degenerative changes of hepatic parenchymal cells are well corroborated by a significant increase in serum activity of AST and ALT. These increases in serum level of these enzymes agree with that result obtained by Bhat *et al.* (1998) on minocydine and Aubrecht on hygromycin B.

The histological pattern was almost normal in rabbits treated with vitamin E and cadmium and olive oil and calcium (Fig. 6).

Hepatoprotective nature of olive oil and vitamin E against cadmium were further supported by the improvement in the histopathological changes occasioned by cadmium. In view of the present study, it can be concluded that olive oil and vitamin E played a role of an antioxidant which includes free radical scavenging and metal-chelating property and thereby improved the detrimental state of liver cells which unraveled its use as a possible mitigator/attenuating agent in cadmium- induced hepatotoxicity.

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