Effect of vitamin E on Some Blood Parameters Related to Cardiovasacular Diseases in Cadmium Chloride- Treated Rabbits

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Abstract

This study was designed to study the effect of cadmium as an oxidant agent on cardiovascular system and some blood parameters and the possible preventive role of vitamin E on deleterious effects of cadmium in adult male rabbits. Twenty adult male rabbits were divided randomly into 4 groups (5 animals /group) and treated daily for 84 days. The first group were received ordinary tap water, serving as control (group C); the second group (T1) received ad libitum supply of drinking water containing (50ppb) cadmium chloride; the third group T2 received (50ppb) of cadmium chloride in drinking water in addition to intubation of vitamin E (40mg/Kg B.W.) orally, while the fourth group (T3) were intubated daily with 40mg/Kg B.W of vitamin E. Fasting blood samples were collected at 0, 21, 42, 63 and 84 days to determine: platelet count, partial thromboplastin (PTT), prothrombin time (PT), serum concentration of total cholesterol TC, and glutathione (GSH). Sections of heart & aorta were also assessed for histopathological changes. The results revealed that administration of 50 ppb CdCl2 in drinking water (T1) for 84 days caused a significant increase (p<0.05) in platelet count and serum TC, with a significant decrease(p<0.05) in PT, PTT and serum concentrations of GSH as compared to control and T2 and T3 groups which showed significant (p<0.05) elevation in GSH concentration. Histological sections of heart and aorta of Cd treated (T1) group revealed congestion of blood vessels. Neutrophils and cells vacuolation of cardiac muscle were also seen. Atheromatus lesions characterized by hyperplasia of intima, vacuolation in subintima and proliferation of fibrous connective tissues with the appearance of foamy cells in the subintima layer, were seen in aorta. In conclusion, this study approved the deleterious effect of Cadmium on some aspect of cardiovascular system and the cardioprotective role of vitamin E as antioxidant.

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Keywords: Cardiovascular Diseases, Cadmium chloride, Vitamin E, Antioxidant.

Introduction

Cadmium is a natural element in the earth's crust. It is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chloride (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide) (ATSDR, 2008). Food and cigarette smoke are the biggest sources of cadmium exposure for people in the general population (Willers *et al.,* 2005). For nonsmokers, food constitutes the principal environmental source of cadmium. Acute toxicity may be resulted from the ingestion of cadmium through contaminated foods and beverages (Pratap *et al.,* 2007; Yoon *et al.,* 2008). Workers of industries exposed to cadmium in air from the smelting and refining of metals, batteries products, coatings, plastics or when soldering and welding metal that contains cadmium at high risk for inhalation exposure (ATSDR,1999; Hogervorst *et al.,* 2007). Apart from smoking, inhalation and pollution sources such as coal-fired power plants and municipal waste incinerators, shellfish, liver, and kidney meats are other routes of Cd entry, causing cardiovascular anomalies (ATSDR, 1999; Navas-Acien *et al.,* 2005). Cadmium's influence on the cardiovascular system remains controversial; studies investigating cardiovascular effects in humans after oral exposure to cadmium have concentrated on the relationship between blood pressure and biomarkers of cadmium exposure such as cadmium levels in the blood, urine or other tissues (WHO, 2000; Varoni *et al.,* 2003). Recently, peripheral arterial disease has been reported to might be associated with cadmium, thus suggesting that cadmium is involved in arterial dysfunction (Navas-Acien *et al.,* 2004; Navas-Acien *et al.,* 2005). Vitamin E is an important natural antioxidant, and protects biologic membranes from lipid peroxidation and its most common and biologically active form is α-tocopherol (Horvath *et al.,* 2006; Nusret, 2009). The different isoforms of vitamin E possess important physiological roles beyond their antioxidant activities including hypocholesterolemic, antithrombotic, anti-inflammatory, and anti-proliferative effects (Fuchs *et al.,* 2003). This study was designed to study the effect of chronic exposure of adult male rabbits to cadmium chloride on antioxidant status and some parameter related to cardiovascular disease, as well as, the protective role of vitamin E.

Materials and Methods

Twenty male rabbits were randomly and equally divided into four groups (each group consist of five rabbits) and were treated for 84 days as follow:

Group I (control), Group II: rabbits of this group were received *ad libitum* supply of drinking water containing (50ppb) cadmium chloride, Group III: rabbits of this group were received *ad libitum* supply of drinking water containing(50ppb) cadmium chloride and 40 mg/kg B.W. of vitamin E ((RRR-a-tocopherol) orally.

Blood samples were collected from fasting animals (8-12 hrs) by cardiac puncture technique using disposable medical syringes (5ml). Blood sample were divided into 3 parts: 1-Part of Whole blood was kept in EDTA tube for platelet count. 2-A portion of blood sample was mixed 9 parts of freshly collected blood with 1 part of sodium citrate (0.11mol/L), centrifuged immediately for 10 minutes at 3000 rpm and plasma was stored in capped plastic test tubes at 2 to $4C^0$ for measurement of Prothormbin time (PT) and Partial Thromboplastin time (PTT). Tests were done within 3 hours after blood collection

(Biggs, 1972). 3-Serum was isolated from another part of blood by centrifugation of blood sample (3ml) at 2000-2500rpm for 15 minutes and frozen at -18 C^0 till use. The following biochemical tests were done at different intervals (0, 21, 42, 63 and 84 days): Total Platelets Count (platelet / m.m^3.) as described by (Becton-Dickinson,1996), Prothormbin Time (PT) as described by (Loeliger *et al.,*1985). Partial Thromboplastin Time (PTT) according to (Biggs,1972) and (Hoffmann and Neulendijk, 1978).Serum Total Cholesterol (TC) Concentration (mg/dl), using enzymatic kits (Allain *et al.,*1974) and (Richmond,1992) and Serum Reduced glutathione concentration (GSH) according to (Burtis and Ashwood, 1999).Besides ,histopathological changes were studied in heart and aorta and several tissues section were prepared and stained with Henatoxyllin-Esoin (H&E) stains according to (Bancroft and Stevens,1982) method. Statistical analysis of data was performed on the basis of two- way analysis of variance (ANOVA) depending on the experimental design at each time specific group differences were determined using least significant differences (LSD) test (Steel and Terrie, 1980).

Results

During treatment period (after 21,42,63 and 84 days), the exposure of rabbits to 50 ppb of cadmium chloride in drinking water group (T1) caused significant (P<0.05) elevation in the mean values of platelet count (table-1) and significant depression in prothrombine time (table-2) and thromboplastin time (table-3) as compared to control group and treated group (T2 and T3). While, treatment of male rabbits with vitamin E alone (group T3) or in combination with cadmium (group T2) for 84 days caused correction of previous parameters with significant elevation in prothrombine and thromboplastin time and significant depression in platelets count (table 1 and 2).

Table.1: Effect of cadmium chloride and vitamin E on Platelets count (cells/ mm3) in blood of male rabbits

Groups	C	T1	T2	T3
Day	Control group	50 ppb CdCl2	50ppb CdCl2 + 40mg Vit.E.	40 mg Vit.E.
Zero	62.0±1.20 A a	66.0±1.90 A a	64.4±3.60 A a	62.0±4.30 A a
21	60.0±1.10 A a	110±6.30 B b	64.0±2.90 A a	63.0±1.00 A a
42	61.4±1.09 A a	148±3.80 B c	62.0±1.70 A a	63.2±1.02 A a
63	66.0±1.90 A a	174±3.20 B d	67.6±1.90 A a	68.0±1.20 A a
84	65.6±2.23 A a	176±4.01 B d	65.6±1.80 A a	64.8±2.20 A a

Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups<0.05 vs. control., Small letters denote differences within group, P< 0.05 vs. control.

Table. 2: Effect of cadmium chloride and vitamin E on Prothrombin time (seconds) in plasma of male rabbits.

Groups Days	C Control group	T1 50 pp CdCl2	T2 50ppb CdCl2 + 40mgVit.E.	T3 40 mg Vit.E.
Zero	8.54±0.60 A a	8.22±0.30 A a	8.39±0.40 A a	8.52±0.40 A a
21	8.08±0.40 A a	7.19±0.40 B b	8.53±0.30 A a	8.06±0.30 A a
42	8.52±0.20 A a	5.61±0.40 B c	8.29±0.30 A a	8.25±0.24 A a
63	8.09±0.20 A a	4.04±0.20 B d	7.81±0.40 A a	7.73±0.20 A a
84	7.95±0.10 A a	3.97±0.20 B d	7.59±0.20 A a	7.67±0.20 A **a**

Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups, P<0.05 vs. control, Small letters denote differences within group, P< 0.05 vs. control.

Table. 3: Effect of cadmium chloride and vitamin E on Partial thromboplastin time (seconds) in plasma of male rabbits.

Groups Days	C Control group	T1 50 pp CdCl2	T2 50ppb CdCl2 + 40mgVit.E.	T3 40 mg Vit.E.
Zero	24.45±0.60 A a	24.42±0.60 A a	24.72±0.80 A a	23.85±0.90 A a
21	24.30±0.60 A a	22.28±0.70 B b	24.10±0.70 A a	24.61±0.60 A a
42	24.03±0.40 A a	19.33±0.30 B c	23.92±0.60 A a	24.47±0.50 A a
63	23.96±0.70 A a	17.40±1.20 B d	24.44±0.70 A a	23.95±1.50 A a
84	24.04±0.20 A a	14.64±0.20 B e	23.71±0.30 A a	23.85±0.20 A a

Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups, P<0.05 vs. control. Small letters denote differences within group, P< 0.05 vs. control.

Serum Total Cholesterol (TC) Concentration (mg/dl)

Table (4) showed a significant increase (P<0.05) in serum TC concentration at days 21, 42, 63 and 84 of the treatment in T1 group compared with control group and treated groups (T2 andT3).   The result also indicated that vitamin E treated groups (T2 and T3) normalized the values near that of the control.

Serum Reduced Glutathione (GSH) Concentration (µmol/l)

During the treatment period (after 84 days) a significant (P<0.05) reduction in serum GSH concentration were detected in Cd treated group (T1) at day 84 comparing to control group in the same period (table-5) The results have also clarified that vitamin E oral intubation to rabbits in T2 and T3 treated groups caused a significant (P<0.05) elevation in serum GSH.

Table .4: Effect of cadmium chloride and vitamin E total Cholesterol concentration (mg/dl) in serum of rabbits.

Groups Days	C Control group	T1 50 pp CdCl2	T2 50ppb CdCl2 + 40mgVit.E.	T3 40 mg Vit.E.
Zero	129.6±2.3 A a	127.8±1.60 A a	130.5±1.7 A a	127.2±5.2 A a
21	130.1±1.8 A a	153.4±3.70 B b	128.8±3.3 A a	126.7±1.7 A a
42	132.6±0.7 A a	170.4±2.10 B c	124.8±7.3 A a	129.1±1.2 A a
63	126.2±2.5 A a	187.1±4.90 B d	128.1±2.2 A a	129.4±2.7 A a
84	128.6±2.0 A a	217.4±7.14 B e	136.1±3.2 A a	132.2±2.5 A a

Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups, P<0.05 vs. control, Small letters denote differences within group, P< 0.05 vs. control.

Table .5: Effect of cadmium chloride and vitamin E on   GSH concentration (µmol/l) in serum of rabbits.

Groups Days	C Control group	T1 50 pp CdCl2	T2 50ppb CdCl2 + 40mgVit.E.	T3 40 mg Vit.E.
Zero	11.9±0.50 A a	11.6±o.40 A a	12.4±0.40 A a	12.1±0.40 A a
21	11.9±0.40 A a	10.1±o.20 B a	16.0±0.60 C b	16.8±0.80 C b
42	12.1±0.70 A a	5.8±0.30 B b	20.9±0.40 C c	19.9±0.90 C c
63	12.4±0.40 A a	5.2±0.10 B bd	27.2±0.80 C d	31.8±0.30 D d
84	12.4±0.40 A a	3.8±0.50 B d	27.2±.070 C d	36.2±0.70 D e

Values are expressed as mean ± SE, n = 5 each group, Capital letters denote differences between groups,P<0.05 vs. control, Small letters denote differences within group, P< 0.05 vs. control.

Histological findings of heart and aorta

Light microscopic study of adult male rabbit heart and aorta related to T1 group which received 50 ppb of $CdCl_2$ in drinking water for 84 days showed histological changes represented by congestion of blood vessels of the heart (Figure.1), and neutrophil appeared in their lumen with muscles fiber together as well as vacuolation of muscles cell comparing to control (Figure-2) and vitamin E treated groups T2 (Figure-3), T3(Figure-4). As well as, the aorta of same group (T1) clarified hyperplasia of intima with vacuolation in subintima (Figure-5) and foamy cell appeared in subintima layer and inflammatory cell infiltration as compared to control (Figure-6), T2 group (Figure-7) and T3 group (Figure-8).
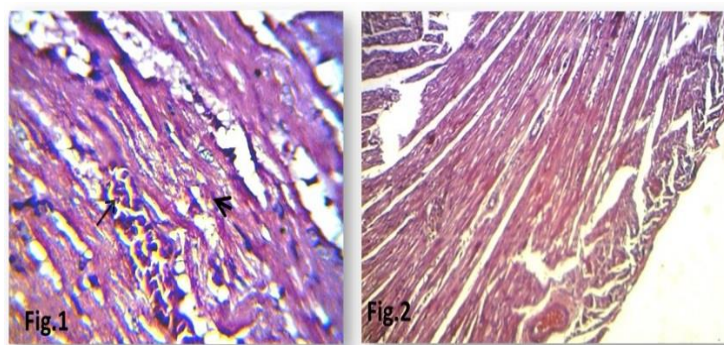

Figure (1): Histological section in the heart of animal at 84 days post treated with CdCl2 (50 ppb) in drinking water (T1) showed congestion of blood vassals between muscles fiber with inflammatory cell in their lumen(→) with vacuolation of muscles cell(➤).**Figure (2):** Histological section in the heart of animal belong to control group showed normal structure of heart (H & E 40 X)
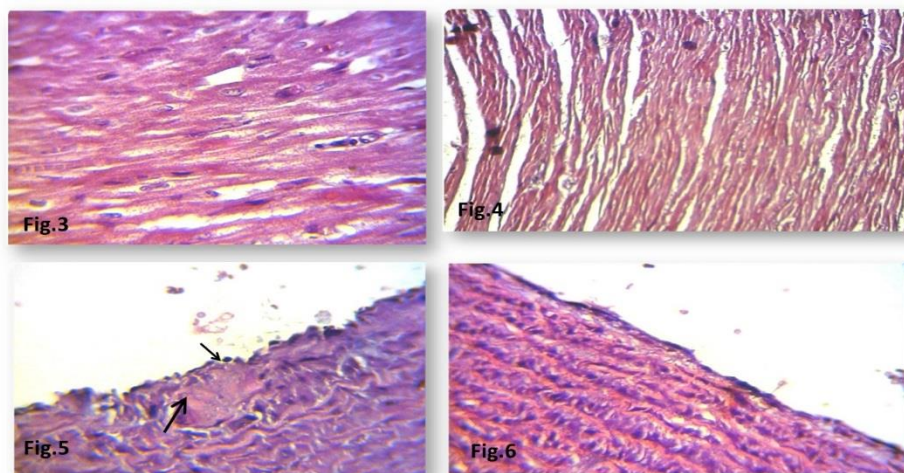

Figure (3): Histological section in the heart of animal belongs to group post treated with CdCl2 (50 ppb) in drinking water plus vitamin E (40 mg/kg B.W) (T2) showed no clear pathological lesion (H & E 40 X).

Figure (4): Histological section in the heart of animal belong to group post treated with vitamin E (40 mg/kg B.W) (T3)   showed no clear pathological lesion (H & E 40 X).
Figure (5): Histological section in the aorta of animal at 84 days post treated with CdCl2 (50 ppb) in drinking water (T1). Showed opaque area in subintimal layer (➡) with inflammatory cell attachment to intimal layer (⟶) (H & E 40 X)
Figure (6): Histological section of the aorta of the control group showed normal structure of aorta.
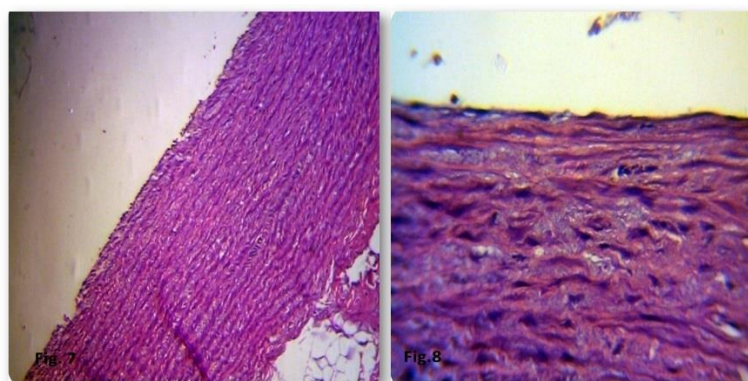

Figure (7): histological section of the aorta of the T2- group post treatment with CdCl2 (50 ppb) in drinking water plus vitamin E (40 mg/kg B.W), showed normal stature (H & E 40 X).
Figure (8): histological section in the aorta of T3- group post treatment with vitamin E (40 mg/kg B.W) showed no clear pathological lesion (H & E 40 X).

Discussion

The present study pointed to significant increase in platelets count with significant decrease in plasma PTT and PT which may be related to oxidative stress induced by cadmium. Cadmium (Cd) is a divalent transitional metal ion, which has been closely associated with oxidative stress in adult tissue (Borges *et al.,* 2008; Kaplan *et al.,* 2008; Ognjanovic *et al.,* 2008; Shukla and Kumar 2009).), and in embryonic tissue (Warren *et al.,* 2000; Paniagua-Castro *et al.,* 2008). Oxidative stress led to increase some of blood coagulation mechanisms represented by decreased prothrombin time and partial thromboplastin time with elevation in platelet count and increased risks of blood clot formation (Al-Shami, 2003). Supplementation of antioxidant vitamins including vitamin E could cause platelets aggregation (Salonen *et al.,* 1999; Mabile *et al.,* 1999).This effect might be due to its antioxidant and cardioprotective actions(Hassan and Awad, 2007; Nusret, 2009) .

Significant increase in serum cholesterol concentration in T1 treated group indicated the hypercholesterolemic effect of cadmium. In Wistar rats' subcutaneous and intra-peritoneal (i.p.) injection of cadmium (Cd) caused significant changes in lipid profile in serum (Murugavel and Pari, 2007) and brain microsomes (Modi and Katyare,2009). Because the liver plays a pivotal role in lipid homeostasis in addition to glucose homeostasis, the accumulation of Cd in liver could be responsible for the dysfunction of the liver and the observed alterations of lipid profiles (Murugavel and Pari, 2007; Larregle

et al., 2008). Generally, heavy metals induce changes in the activity of hydroxy 3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which alters cholesterol as well as all lipid metabolisms. The inflammatory cytokines (tumor necrosis factor [TNF- α] and interleukin [IL] 1β) have been reported to increase the expression of cholesterogenic enzymes including HMG-CoA reductase (HMGR) and suppressed cholesterol 7α-hydroxylase (CYP7A1), a catabolic enzyme of cholesterol in the liver (Hardardottir *et al.,* 1994; Kojima et al,2004). Several studies have also shown that cytokines are involved in increasing serum TG levels and VLDL production by stimulating hepatic lipogenesis and suppressing fatty acid oxidation (Memon *et al.,* 1993; Nachiappan *et al.,* 1994). In agreement with these reports, Cd exposure markedly increased the levels of inflammatory cytokines such as TNF-α and IL-1β in the liver (Kayama *et al.,* 1995; Harstad and Klaassen, 2004), which might be responsible for hypercholesterolemic effect of cadmium.

Vitamin E supplementation lowered the elevated cholesterol concentration in T2 group. Vitamin E down-regulates the expression of the cholesterol scavenger receptors SR-A (Teupser *et al.,* 1999) via mechanisms that appear independent of protein kinase C and antioxidant activity. Regulation of these sterol receptors occurs at the level of transcription, suggesting that α -tocopherol acts through specific receptors or tocopherol-responsive transcription factors (Azzi *et al.,* 2001). Both α- and γ-tocopherol diminished endogenous cholesterol synthesis as well as apolipoprotein-AI-(apo-AI)-mediated cholesterol efflux. These effects were the consequence of a tocopherol-mediated down-regulation of several genes implicated in endogenous cholesterol synthesis (Landriera *et al.,* 2010).

A significant decrease in serum GSH concentration T1 group are in accordance with (El-Maraghy *et al.,* 2001) and (Eybl *et al.,* 2004) who suggested that cadmium toxicity can cause oxidative stress by an interaction with –SH groups of major intracellular defender glutathione and that lipid peroxidation is an early and sensitive consequence of acute Cd exposure. The intravenous administration of cadmium chloride (2 mg/kg bw/day) resulted in a pronounced increase of lipid peroxidation in the liver of rat accompanied by a depletion of hepatic GSH (Nemmiche *et al.,* 2007). As well as, cadmium accumulation in liver and kidney of rats due to chronic dietary intake, is associated with alteration of enzymatic (SOD, CAT and GST-px) and non-enzymatic antioxidants (GSH, vitamins C, E) (El-Sharaky *et al.,* 2007; Ognjanovic *et al.,* 2008). This may possibly be due to the excessive formation of FRs, which led to deteriorations of biological molecules (Stohs *et al.,* 2001; El-Maraghy *et al.,* 2001). Vitamin E appears to be the most effective lipid soluble antioxidant in biological systems (Nagel *et al.,* 1997). It inhibits lipid peroxidation and regenerates reduced vitamin C and glutathione (GSH) (Upston *et al.,* 1999). Cadmium induces an oxidation of cellular lipids and proteins and administration of α-tocopherol can reduce Cd-induced oxidative stress and improve the glutathione level (Satarug and Moore, 2004; Nemmiche *et al.,* 2007).

Histological changes observed after cadmium treatment may indicate disturbance in cardiovascular function. A large number of studies have suggested a possible link between exposure to Cd and the development of atherosclerosis and hypertension (Navas-Acien *et al.,* 20005; Kaji, 2004) and there is evidence suggesting that the vascular endothelium may be intimately involved in mediating these effects of Cd (Szuster-Ciesielska *et al.,* 2000). Cd can cause the release of a variety of proinflammatory mediators from endothelial cells that would facilitate the inflammatory component of the

atherosclerotic process (Szuster-Ciesielska *et al.,* 2000; Mlynek and Skoczynska, 2005; Gryg *et al.,* 2002). Vitamin E is lipophilic and has been shown to inhibit the oxidative modification of low density lipoprotein (LDL), a process thought to be of crucial importance in atherogenesis (Helzlsouer *et al.,* 2000) and prevention of cardiovascular diseases. Inhibition of monocytes adhesion (Areds, 2001), cytokine expression (Nedeljkovic *et al.,* 2003) and maintenance of vascular endothelial and smooth cells integrity (Vivekananthan *et al.,* 2003; Lonn *et al.,* 2005;64) due to vitamin E antioxidant effect could also be claimed. In conclusion, this study approved that Cadmium has a deleterious effect on some aspect of cardiovascular system while vitamin E has antioxidant cardioprotective roles.

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