

Role of Vitamin E-Selenium in ameliorating sub chronic cadmium sulfate toxicity in rabbits

May J. Abd

Coll. of Med. / Univ. of Al-Qadisiya

email: Lecturermay79@gmail.com

(Received 2 November 2014, Accepted 22 February 2015)

Abstract

The study aim to evaluate the ameliorating role of vitamin E-selenium supplementation on serum chemistry and histopathological alterations of liver and heart in rabbits caused by sub chronic cadmium sulfate toxicity. Twenty white newzealand rabbits of six month old were used and they divided in to two groups' ten animals of each. G1 given cadmium sulfate 250mg/L in drinking water, G2 were supplemented with cadmium sulfate 250 mg/L plus vitamin E-selenium 500mg/L daily for 60 days. The results show vitamin E-selenium were significantly improved ($P \leq 0.01$) serum ALT, AST, bilirubin, alkaline phosphate, GGT and total protein, while total cholesterol, TG, HDL, LDL, VLDL had some improvement but they didn't reach significant value ($P \geq 0.01$), where no changes in the serum myoglobin, troponin and Ck-MP, and the histopathological examinations of liver were confirm these results. in conclusion supplementation of vitamin E-selenium to the feed of rabbits exposed to sub chronic toxicity of cadmium sulfate reduce the effect of toxicity on the bio indicators of liver in this study.

Key words: Vitamin-E-selenium, cadmium poisoning, cardiovascular disease, liver disease, lipids.

دور فيتامين هـ - سيلينيوم في تحسين حالات التسمم تحت المزمن بكبريتات الكادميوم في الأرانب

مي جليل عبد
كلية الطب / جامعة القادسية

الخلاصة

اجريت هذه الدراسة لمعرفة مدى تأثير اضافة فيتامين هـ - سيلينيوم على بعض المعايير الحيوية المعتمدة والتغيرات النسيجية للكبد والقلب في الارانب المصابة بالتسمم بكبريتات الكادميوم تحت المزمن. استخدم 20 من الارانب النيوزلاندية بعمر ستة اشهر بواقع عشر ارانب لكل مجموعة ، المجموعة الاولى: اعطيت كبريتات الكادميوم 250ملغم/لتر في ماء الشرب ، والمجموعة الثانية اعطيت كبريتات الكادميوم 250ملغم/ لتر مع اضافة فيتامين هـ - سيلينيوم 500ملغم/لتر يوميا لمدة 60 يوما. بينت النتائج وجود فروقات معنوية ($P \leq 0.01$) في اختبارات الالنين امينو ترانسفيريز ، الاسبارتيت امينو ترانسفيريز، البليروبين ، الالكلاين فوسفاتيز ، الكاما كلوتاميل ترانسامينيز والبروتين الكلي. اما اختبارات الشحوم والتي شملت الكوليسترول الكلي ، الكلسريدات الثلاثية ، البروتين الشحمي عالي الكثافة ، البروتين الشحمي واطى الكثافة ، والبروتين الشحمي واطى الكثافة جدا فقد اشارت الى بعض التحسن الغير معنوي ($p \geq 0.01$) وفيما يخص اختبارات الجهاز القلبي الوعائي والتي شملت المايوكلوبين والتروبونين والكايينيز القلبي فلم يلاحظ وجود اي تغير. كما ان التغيرات النسيجية للكبد تتوافق مع هذه النتائج. نستنتج ان اضافة فيتامين هـ - سيلينيوم قد ادى الى تقليل التأثيرات السلبية الناجمة عن التسمم المزمن بكبريتات الكادميوم في الكبد.

الكلمات المفتاحية: فيتامين هـ - سيلينيوم ، التسمم بالكادميوم ، امراض الجهاز القلبي الوعائي ، امراض الكبد ، الدهون.

Introduction

Increased industrialization all over the world has been associated with the extraction and distribution of minerals from their natural deposits, many of them have been subject to chemical changes process and finally passed in waters, earth and thus the

food chains, this include heavy metals, some of them include cadmium, which is cumulative toxin. The risk of chronic toxicity is the biggest problem, include disorders of different enzymatic system due to injure of the tissues and main organs including nervous system, endocrine system, lungs, liver, kidneys, reproductive organs etc., The impact heavy metals can induce the change of certain genes expression (1). Cadmium acts as a catalyst in forming reactive oxygen species. It increases lipid peroxidation, in addition it depletes glutathione and protein-bound sulfhydryl groups. It also promotes the production of inflammatory cytokines (2), and Increase concentrations of urinary beta-2 microglobulin can be an early indicator of renal dysfunction in persons chronically exposed to low but excessive levels of environmental cadmium (3). Under some circumstances, Inhaling cadmium-laden dust quickly leads to respiratory tract and kidney problems which can be fatal (often from renal failure), Ingestion of any significant amount of cadmium causes immediate poisoning and damage to the liver and the kidneys (2). Compounds of cadmium are also carcinogenic. The bones become soft (osteomalacia), lose bone mineral density (osteoporosis) and become weaker, this causes the pain in the joints and the back, and also increases the risk of fractures. Extreme the cases of cadmium poisoning, the mere body weight causes a fracture (4). Vitamin E is the best fat soluble antioxidant known forte protective effect on lipid membranes and unsaturated fatty acids, and well documented to prevent atherosclerosis may also prevent Alzheimer's disease, protective effect include the heart, brain, skin, eyes, liver, breast and prostate, it is stabilized the blood fats so the blood vessels and heart are protective from free radicals induced injury. Selenium benefits treat or prevent some health conditions like heart disease, HIV and ADIS, miscarriages, arthritis, muscular degeneration, strokes, gray hair and different type of cancer (5). Vitamins E and selenium are known to be protective anti-oxidants (6). They cause the inhibition of peroxidation, mopping up of free oxygen radicals and disorganization and

breakage of peroxidation chain reactions by an inhibition of glutathione peroxide, protein kinase C (PKC) inhibition and calcium metabolism, thus resulting in the blockade of oxidative mechanisms (7). Therefore the aim of study to seek and establish the detailed correlation between biochemical parameters, and damage in heart, liver and kidney organs using cadmium overloading on one hand, and the effect of anti-oxidants vitamin E selenium on the other hand as ameliorating agent and to evaluate the antioxidant role of vitamin E selenium in correcting sub chronic cadmium toxicity of affected laboratory rabbits.

Materials and methods

Animals

Twenty clinically healthy white New Zealand rabbits six month olds, weighing 2500 ± 115 gm., randomly divided into two groups ten animals of each. They were kept in warm place 28°C in individual cages, and all animals were given basic diet. G1 was giving tap water with 250 mg/L cadmium sulfate and, G2 was given tap water and cadmium sulfate 250 mg /L plus vitamin E - selenium 500 mg/L per day by oral administration for 60 days.

Sample collection

At the day sixty of experiment, (5 ml) of blood was collected in anticoagulant tubes via cardiac puncture from anesthetized animal by (Ketamine 50mg/kg, Xylazine 10mg/kg), Rabbits were then euthanized with a single over dose cardiac injection of pentobarbital sodium. The heart, kidney and liver tissue samples were collected for histopathological studies.

Biochemistry tests

Serum ALT, AST, bilirubin, alkaline phosphatase, GGT, total protein, Troponin, myoglobin, CK-MP, cholesterol, triglyceride, HDL, LDL, VLDL concentration in plasma were determined using commercially available kits (Sigma).

Statistical analysis

Means \pm SE were carried. All data were analyzed using Duncan's multiple range tests (8) to determine the significances ($P \leq 0.01$) between readings.

Results

A result of vitamin E treated group refers significant differences in the values of liver profile test and cardiovascular parameters. Table (1) illustrates a significant ($p \leq 0.01$) reduction in the level of serum aminotransferase and alanine aminotransferase in G2 as compared with G1. Also the situation was same with the levels of bilirubin, alkaline phosphatase and GGT. The cardiovascular profile tests were refers a significant ($P \leq 0.01$) improvement of creatine kinase and total protein level in G2 compared with G1. The lipid tests indicate a slight non-significant improvement in cholesterol, TG, HDL, LDL and VLDL levels in G2, and no

differences in CK-MP, troponin and myoglobin level between the two groups (table 2). The histopathological feature of liver in G1 showed vacuolation of hepatocytes greater than normal (Fig. 1), and clusters of aggregations of inflammatory cells in different parts of liver parenchyma without limited line (Fig. 1,2). While the liver in G2 show mild or less cellular degeneration, and less significant residue of different types of inflammatory cells in some locations of liver tissue with irregular and renewing cells (Fig. 3,4). In contrast there were no any pathological signs in cardiac tissue, which appears like normal (Fig. 5,6).

Table (1): Explain the correcting role of vitamin E-selenium on liver profile test in sub chronic cadmium toxicity. Values are means \pm SE.

Liver profile test	G1; Cd only	G2; Cd + Vitamin E-Selenium
SALT	60.7 \pm 2.51 a	32.86 \pm 2.162 b
SAST	35.75 \pm 1.319 a	29.33 \pm 0.47 b
Bilirubin	0.923 \pm 0.025 a	0.31 \pm 0.026 b
Alkaline phosphatase	175.33 \pm 23.366 a	91.4 \pm 2.736 b
GGT	16.6 \pm 0.76 a	7.66 \pm 0.672 b

Table (2): The effect of vitamin E-Selenium on cardiovascular profile test and total protein in sub chronic cadmium toxicity. Values are means \pm SE.

Biochemical indicator	G1; Cd only	G2; Cd + Vitamin E-selenium
Cholesterol	92 \pm 0.76 a	0.99 a
TG	60.1 \pm 1.97 a	58.9 \pm 1.8 a
HDL	17 \pm 2.31 a	17.5 \pm 1.7 a
LDL	68 \pm 1.39 a	66.8 \pm 1.357 a
VLDL	12.8 \pm 0.326 a	12.2 \pm 0.61 a
Creatinin kinase	278 \pm 0.006 a	72.1 \pm 10.436 b
CK-MP	-ve	-ve
Troponin	-ve	-ve
Myoglobin	-ve	-ve
Total protein	9.45 \pm 0.53 a	13.5 \pm 1.06 b

Different letters between groups refer to significant variation under ($p < 0.01$). Degree of freedom :1, 9

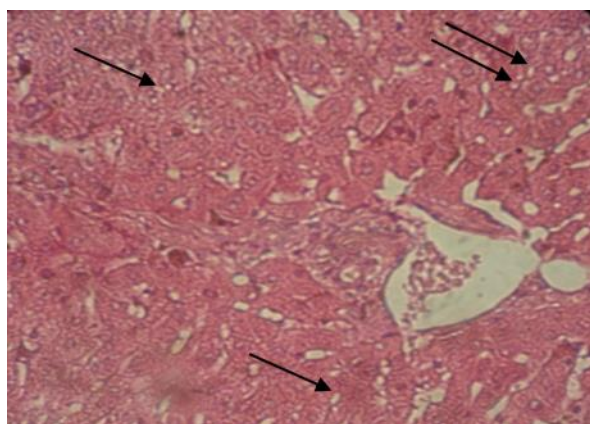


Fig. (1): Cross sections photomicrograph in liver of rabbit (G1) show presences of vacuolation of hepatocyte (arrows) (H&E X40).

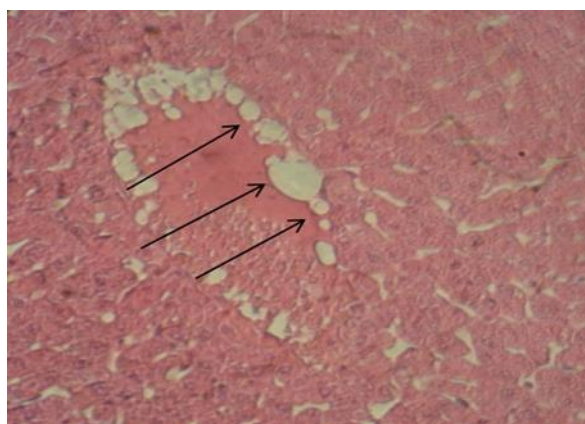


Fig. (2): Cross sections photomicrograph in liver of rabbit (G1) show presences of abnormal vacuolation of hepatocyte (arrows) (H&E X40).

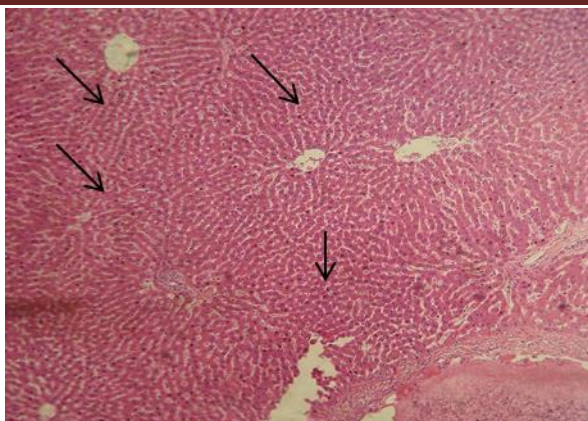


Fig. (3): Cross sections photomicrograph in liver of rabbit (G2) show less inflammatory cells in liver parenchyma, and renewing cells. Reduction in size of inflammation and size of vacuolation (arrows) (H&E X10).

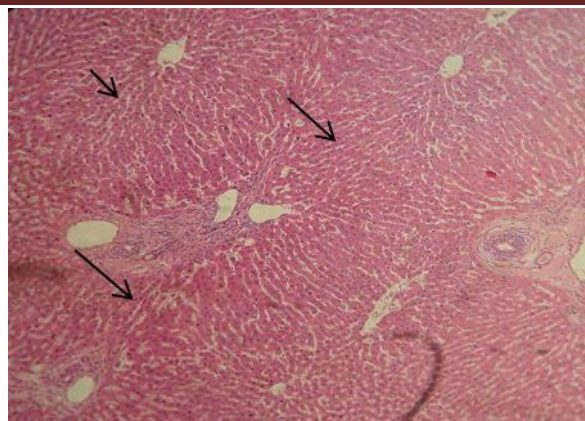


Fig. (4): Cross sections photomicrograph in liver of rabbit (G1) show more degeneration damage of liver parenchyma, more residual inflammatory cells than G2 (arrows) (H&E X10).

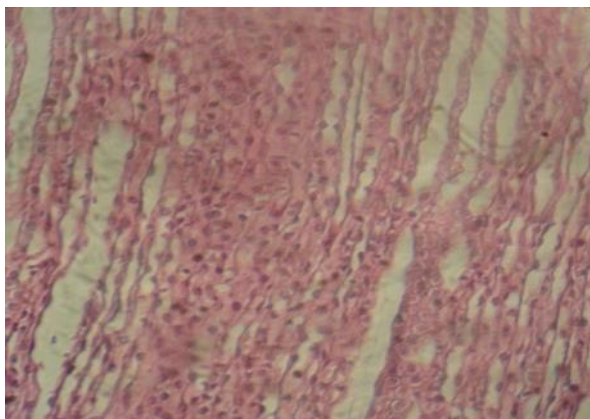


Fig. (5): Photomicrograph of rabbit heart (G1) show normal cardiac tissue (H&E X40).

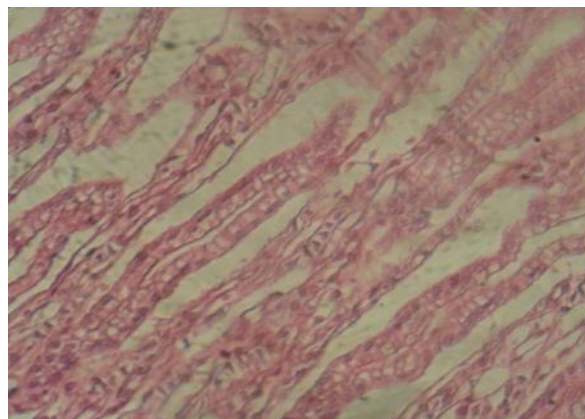


Fig. (6): Photomicrograph of rabbit heart (G2) show normal cardiac tissue (H&E X40).

Discussion

Vitamin E - Selenium is nutritional supplement and offered to fight against free radicals in a natural way. Results showed that vitamin E - selenium cause significant reduction in the level of hepatic function tests in rabbits exposed to sub chronic cadmium toxicity, as present in the level of alanine amino transferase (ALT), Aspartate aminotransfrase (AST) of G2 compared with G1, these enzymes are involved in intermediary metabolism and stored in hepatocytes cells. They released when these cells are actually damaged, their increment in the serum of G1 animals mean hepatocytes injury; they also used to evaluate liver function test (9), decrease the level of these two enzymes in the serum of G2 animals may be due to antioxidant activity in reduction oxidative damage resulting from

cadmium toxicity (10). Our results also refer to a significant increment in the level of serum bilirubin, alkaline Phosphatase, and gamaglutamyle transaminase (GGT) of G1 animals as compared with G2, cadmium sulfate considered as potent hepatotoxic agent, and these indicators are sensitive to liver injury. Generally they released after treatment with cadmium sulfate as consider the main causes of lipid peroxidation for hepatocytes membrane by the action of free radicals. Furthermore it plays a role in antioxidant enzyme depletion which acts as free radical scavengers. Hepatocellular injury leads to excretion of large quantity of these enzymes where seen elevated in serum of G1 animals. Vitamin E-selenium in turn acts to reserve the structural integrity of liver from toxic cadmium effect (9). Results also

refer to slight improvement in the levels of cholesterol, triglyceride, HDL, LDL, VLDL of G2, although these improvement did not reach to significant value. Oxidative stress that resulted from chronic cadmium exposure stimulate hormone sensitive lipase that act in lipid destruction of fatty tissue and converted it to free fatty acids. The later was further converted to phospholipid and LDL. These lipids deposit in the body blood vessels that may be affect cardio vascular system integrity (11). Results related to CK-MP, myoglobin and troponin in G1 are did not affected, these parameters may be not gain sufficient time to elevated that reach to significant variation. This could be concluded by the significant elevation of creatinine kinase in G1 animals that refer to early damage for renal and cardiac tissue. Creatinine kinase mostly present during muscle damage as occurred by the action of free radicals resulted from chronic cadmium exposure (12). Vitamin E in turn affect lipoprotein enzyme that act to increase lipolysis activity also increase paraoxonase (PON1), the later play important role as antioxidant and anti-atherosclerotic activity of HDL component (13). Selenium also act as component part of Se-cysteine which is an active site of glutathione peroxidase, glutathione peroxidase in turn has antioxidant activity since it catalyze the conversion of hydro peroxides to stable non radical product (14). Our results also illustrate significant decrement in the level of total protein in G1

more than G2 animals; low total protein value may be refer to liver or kidney disorder, as these organ firstly affected by chronic heavy metal poisoning (15). Vitamin E-Selenium play important role in maintain muscle integrity, absence of these elements may lead to muscle paralysis, and improved indirectly body performance by affection thyroid hormone metabolism. Selenium is needed for hepatic conversion of T4,3,3,5-T3 and that type Iodithyroninedeiodinase identified as elenocysteine containing enzyme catalases deiodination of T4 to biologically action thyroid hormone T3(16). Hisopathological alteration of liver and cardiac tissues in G1 refers to vacuolation of hepatocytes greater than normal, aggregation of clusters of inflammatory cells in different part of liver parenchyma present without limited lines between different groups, these may be due to the action of free radicals, while histopathological alteration of G2 animals, reveals mild or less degeneration damage than G1, residual inflammatory cells in some location of liver parenchyma, presence of small area characterized by irregular cells and have renewing cells may be due to vitamin E-selenium in reduction the toxic effect of cadmium. While results related with cardiac tissue belong to G1 and G2 reveal no histopathological differences represented by normal cardiac tissue and did not present any pathological signs where agreed with (17).

References

- 1-Albana M Dashamir, X Dritan L and L lirDova (2010) Cadimuilation in the main organs during the chronic exposure. *Naturamontenegrina, podgorica*, 9(3): 719-730.
- 2-Maret Wolfgang, Moulis Jean-Marc (2013) "Chapter 1. The Bioinorganic Chemistry of Cadmium in the Context of its Toxicity". In Astrid Sigel, Helmut Sigel and Roland K O Sigel. *Cadmium: From Toxicology to Essentiality. Metal Ions in Life Sciences 11*. Springer.
- 3-Shannon LM, Winchester JF (1998) *Clinical Management of Poisoning and Drug Overdose, Third Edition*,
- 4-Nordberg GF (2010) Biomarkers of exposure, effects and susceptibility in humans and their application in studies of interactions among metals in China. *Toxicol. Lett.* 192: 45-49.
- 5-Attia HF, Soliman MM, Ismail TA (2012) Protective effect of vitamin E and selenium on the liver, heart and aorta. *J. Vet. Ana.* 5 (1) 17-29.
- 6-Stohs SJ, Bagchi D, Hassoun E, Bagchi M (2001) Oxidative mechanisms in the toxicity of chromium and cadmium ions. *J. Environ. Pathol. Toxicol. Oncol.*, 20: 77-88.
- 7-Das EN, King GL (2007) The role of protein kinase C activation and the vascular complications of diabetes. *Pharmacol Res.* 55:498-510.
- 8-Murray KM, Granner DK, Mayer PA, Rodwell VW (2000) *Harpers Biochemistry 25th Edition* 8: 83-85, 130-136.
- 9-Duncan B D (1955) Multiple range and multiple F test. *Biometrics.* 11:1-42.
- 10-Kattwinkel J, Taussig LM, Statland B E (1973) The effects of age on alkaline phosphatase and other serologic liver function tests in normal

- subjects and patient with cystic fibrosis. *J. Pediatr.* 82:234-42.
- 11-Kagan VE, Serbinova EA, Forte T, Scita G, Packer L (1992) Recycling of vitamin E in human low density lipoproteins. *J. lipid research*, 33:385-397.
- 12-Puthongsiriporn U, Scheideler S E, Sell J L, Beck M M (2001) Effect of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation and antioxidant status of laying hens during heat stress.
- 13- Bela DS, Lilton FA, Martinez RC, Marcio HM, Mda EP (2008) Clinical presentation, laboratory values and coronary heart disease risk in marked HDL deficiency states. *National Lipid Association*, Vol. 2, Issue 4; 237-247.
- 14-Gursu M F, Muhittin O, Funda G, Kazim S (2004) Effect of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress in vivo. *J. Neutr.* 24: 157- 164.
- 15-Ursini F, Bindoli A (1987) The role of selenium peroxidases in the protection against oxidative damage of membranes, *Chem. Phys. Lipids* 44, 255–279.
- 16-Warren Buffetts (2013) Comprehensive panel of tests. WWW.ehoe.com facts-5522875-low total protein indicat. Html.
- 17-Shahat KH E, Abdel Monem UM (2011) Effects of dieter supplementation with vitamin E and /or selenium on metabolic and reproductive performance of Egyptian Baladi ewes under subtropical conditions. *World Applied Sciences Journal* 12 (9): 1492-1499.