

The Effect of Zinc Sulfate on Oxidative Stress and Lipid Profile Parameters in Male Rabbits Fed High Cholesterol Diet

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Abstract

Atherosclerosis is a chronic oxidative inflammatory disease characterized by deposition of lipids in the artery wall and infiltration of inflammatory cells. It is initiated, in part, by the interaction of oxidized low-density lipoprotein (oxLDL) with cells of the vascular wall. Atherosclerosis is the leading cause of mortality in developed countries and it is overwhelmingly contributes to approximately half of all deaths in the Western world than any other disorder. Zinc is an essential trace element that is vital in maintaining normal physiology and cellular functions. It has a potent antioxidant and anti-inflammatory action and it is used in treatment of various diseases in different systems in the body. This study was undertaken to evaluate the effect of zinc sulfate on prevention and progression of atherosclerosis in male rabbits fed high fat diet. A 32 male rabbits were enrolled in this study, divided randomly into four groups with 8 rabbits in each one. The first group, normal control group was supplied with standard chow diet for two month. The second one, hyperlipidemia-induced group was fed additionally to the standard chow diet, with a 1% (w/w) cholesterol powder. The third and fourth group, 220 mg and 440 mg zinc sulfate-treated group, was fed as group two plus 220 mg and 440 mg zinc sulfate respectively. Blood samples were collected and used to determine the concentration serum of lipid profile parameters, serum malonodialdehyde (MDA), and serum reduced glutathione (GSH) at day 0, 30 and 60. In hyperlipidemia-induced group the serum concentration of lipid profile parameters and MDA highly significantly increased while the serum concentration of GSH was significantly decreased compared to normal control group ($p < 0.05$). Zinc sulfate did not significantly affect lipid profile ($p > 0.05$) but instead its dramatically improved oxidative stress parameters as it significantly lowered the MDA level and increased GSH level compared with hyperlipidemia-induced group ($p < 0.05$). From these results, we can conclude that the dose of 220 and 440 mg \ day of oral zinc sulfate for 60 days has a significant antioxidant effect in male rabbits fed high cholesterol diet.

تأثير كبريتات الزنك على معلمات الإجهاد التأكسدي ومعلمات الدهون في ذكور الارانب
المعتمدة على الغذاء عالي الكوليسترول
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مفاتيح البحث: ارتفاع مستوى الدهون، الكوليسترول، البروتين الدهني منخفض الكثافة المؤكسد، كبريتات الزنك، الجلوتاثيون المختزل، المالنودايدهايد.

الخلاصة

تصلب الشرايين هو مرض التهابي تأكسدي مزمن يتميز بتسرب الدهون في جدار الشريان وتسلل الخلايا الالتهابية الى داخله. يحدث تصلب الشرايين، جزئياً، من خلال تفاعل البروتينات الدهنية منخفضة الكثافة المؤكسدة مع خلايا جدار الوعاء الدموي. تصلب الشرايين هو السبب الرئيسي للوفيات في الدول المتقدمة، وهو يساهم بشكل أساسي فيما يقارب من نصف عدد الوفيات في العالم الغربي أكثر من أي مرض آخر. الزنك هو عنصر اساسي حيوي في الحفاظ على وظائف الأعضاء والوظائف الخلوية. أنه يحتوي على تأثيرات قوية مضادات للأكسدة والالتهابات وهو يستخدم في علاج أمراض مختلفة في مختلف أنظمة الجسم. أجريت هذه الدراسة لتقييم تأثير كبريتات الزنك على منع حدوث وتقدم مرض تصلب الشرايين في ذكور الأرانب ذات التغذية بنظام غذائي عالي الدسم. 32 من ذكور الأرانب تم تسجيلها في هذه الدراسة، قُسمت عشوائياً الى أربع مجموعات تضم 8 أرانب في كل واحد. المجموعة الأولى (وهي مجموعة السيطرة الطبيعية) جُهزت بالنظام الغذائي القياسي لمدة شهرين. المجموعة الثانية (وهي مجموعة تصلب الشرايين المستحدث) غُذيت بمسحوق الكوليسترول بنسبة 1% (وزن / وزن) إضافة الى النظام الغذائي القياسي. المجموعة الثالثة والرابعة (وهما المجموعة المُعالَجة بـ 220 ملغ والمجموعة المُعالَجة 440 ملغ من كبريتات الزنك) تُغذيان بنفس غذاء المجموعة الثانية إضافة الى 220 ملغ و440 ملغ من كبريتات الزنك على التوالي. عينات الدم تم جمعها واستخدمها لتحديد تركيز معلمات الدهون، المالنودايدهايد (MDA)، والجلوتاثيون المُختزل (GSH) في اليوم 0، 30 و60 من أيام الدراسة. في مجموعة تصلب الشرايين المستحدث ازداد تركيز معلمات الدهون وMDA في مصل الدم زيادة احصائية كبيرة بينما انخفض تركيز GSH في مصل الدم بشكل احصائي ملحوظ مقارنة مع مجموعة السيطرة الطبيعية ($P < 0.05$). كبريتات الزنك لم تؤثر على تركيز معلمات الدهون ($P > 0.05$) ولكن بدلاً من ذلك حسنت تركيز معلمات الاكسدة بشكل كبير حيث انها خُفّضت مستوى MDA ورفعت مستوى GSH كثيراً مقارنةً بمجموعة تصلب الشرايين المستحدث ($p > 50.0$). من هذه النتائج يمكننا أن نستنتج أن كبريتات الزنك المعطاة فموياً بجرعة 220 و440 ملغم/يوم لمدة 60 يوم لها تأثير (مُعتد به) مضاد للأكسدة في ذكور الارانب المعتمدة على الغذاء عالي الكوليسترول.

Introduction

Atherosclerosis is a chronic inflammatory disease characterized by the deposition of lipids in the artery wall and the infiltration of inflammatory cells, such as monocytes and lymphocytes. Vascular oxidative stress is thought to be an important element in its pathogenesis [1]. Indeed, the oxidative modification of low-density lipoprotein (LDL) is an important step in the development of atherosclerosis. When LDLs are oxidized, they are readily taken up into macrophages, promoting foam cell formation and the development of atherosclerotic plaque [2]. Once initiated, the atherosclerotic plaque may remain stable for many years, causing only rarely symptoms such as stable angina pectoris or claudication. However, some factors may lead to an unstable plaque, resulting in more grave or even fatal acute events such as myocardial infarction [3].

Oxidative stress is often defined as an imbalance of pro-oxidants and antioxidants, which can be quantified in humans as the redox state of plasma reduced glutathione/oxidized glutathione (GSH/GSSG). Plasma GSH redox in humans

becomes oxidized due to age, chemotherapy, smoking, and in common diseases (e.g. type 2 diabetes and cardiovascular disease [4].

Zinc is an essential trace element that is vital in maintaining normal physiology and cellular functions. It is one of the most abundant metals in the human body, second to iron. The importance of zinc is apparent from the enormous number of proteins that contain zinc ions in their structure. Zinc has catalytic and structural functions in thousands of enzymes and regulatory functions in a growing list of proteins. Ten percent of genes encode zinc-containing proteins [5].

The recommended daily allowance of zinc for adult is 11 mg for males and 8 mg for females [6]. In rats, the oral lethal dose 50 (LD50) for zinc salts is 237–623 mg/kg, the intraperitoneal injection LD50 is 28–73 mg/kg, and the inhalation LD50 for zinc chloride is 2000 mg/m³. In humans, these doses for acute toxicity may be achieved only under the most unusual circumstances [7].

From a pharmacological point of view, zinc is used in treatment of acrodermatitis enteropathica due to inherited or acquired zinc deficiency [8]; in treatment of Wilson disease as it inhibits copper absorption [9]; in treatment of diarrhea in children as it reduces the duration and severity of disease [10]; and in treatment of common cold [11].

Excessive zinc intake cause acquired copper zinc deficiency that manifested hematologically by anemia and neutropenia and neurologically by myelopathy presenting with a spastic gait and prominent sensory ataxia [12]. This study was undertaken to evaluate the effect of zinc sulfate on the oxidative stress and lipid profile parameters in male rabbits fed high cholesterol diet.

Materials and Methods

Thirty-two local domestic male rabbits had been enrolled in this study; their weight was between (2 to 2.5) kg. They were housed in the animal house in College of Medicine / University of Babylon under controlled condition of temperature 25±4°C. The rabbits were fed with standard chow diet and they had free access to drink water *ad libitum*.

The induction of hyperlipidemia and subsequent development of atherosclerosis was carried out by feeding the male rabbits with the atherogenic diet (i.e. 1% (w/w) of cholesterol powder) for 60 days [13].

After the four weeks of adaptation period, the rabbits were randomly divided into four groups (8 rabbits in each group), as the following:

Group I: Normal control group, the animals in this group were maintained on normal chow diet for two months.

Group II: Hyperlipidemia-induced group, the animals in this group were maintained on atherogenic diet (1% (w/w) cholesterol per day) besides the normal chow diet for two months.

Group III: 220 mg zinc sulfate-treated group, in addition to atherogenic and normal chow diet, the animals in this group were maintained on 220 mg (50 mg of elemental zinc) of oral zinc sulfate per day for two months.

Group IV: 440 mg zinc sulfate-treated group, in addition to atherogenic diet, the animals in this group was maintained on 440 mg (100 mg of elemental zinc) of oral zinc sulfate per day during the whole period of study.

Six ml of blood collected directly from the rabbits' heart after overnight fasting at day 0, 30, and 60 from all groups, these 6 ml of the fresh blood was placed

in gel tube, left for 15 - 20 minutes at room temperature, and used to obtain serum via centrifugation at 3000 rpm for 10 minutes. The serum was used to determine the concentration of TG, LDL, VLDL, HDL, MDA and GSH. Serum concentration of lipid parameters (TG and LDL) was measured by using Mindray, automatic analyzer, MDA was measured spectrophotometer, and GSH was measured by using ELISA reader.

Statistical analysis: The results were expressed as mean + SD. Statistical analysis was carried out using repeated measure ANOVA and one way ANOVA, repeated measure ANOVA was used to test the significant differences in the same group at different times, while one way ANOVA was used to test the significant changes among different groups. For both types of ANOVA, when it showed a statistically significant difference, further exploration for the significance of difference in mean between each pair of groups was performed by post-hoc test by applying the LSD technique. Significance differences was set at $\alpha= 0.01$. P value less than 0.01 was considered statistically significant [14].

Results

Changes in serum lipid profile parameters of the four studied groups at different times

Changes in serum concentration of TG, LDL and VLDL

At the beginning of study, there were no significant changes in serum concentration of TG, LDL and VLDL in all groups ($p>0.05$).

At day 30 of the study, while there were no significant differences ($p>0.05$) in the serum concentration of TG, LDL and VLDL among atherosclerosis-induced, 220 mg and 440 mg zinc sulfate-treated group, there was a highly significant increase ($p<0.05$) in serum concentration of lipid profile parameters between all of these groups compared with normal control group.

At day 60 of the study, there were no significant changes ($p>0.05$) in serum concentration of TG, LDL and VLDL among all groups on atherogenic diet (i.e. II, III and IV). The lipid parameters of these groups at day 60 significantly increased compared to those parameters at day 30 of the same groups. As shown in figure (1), (2) and (3).

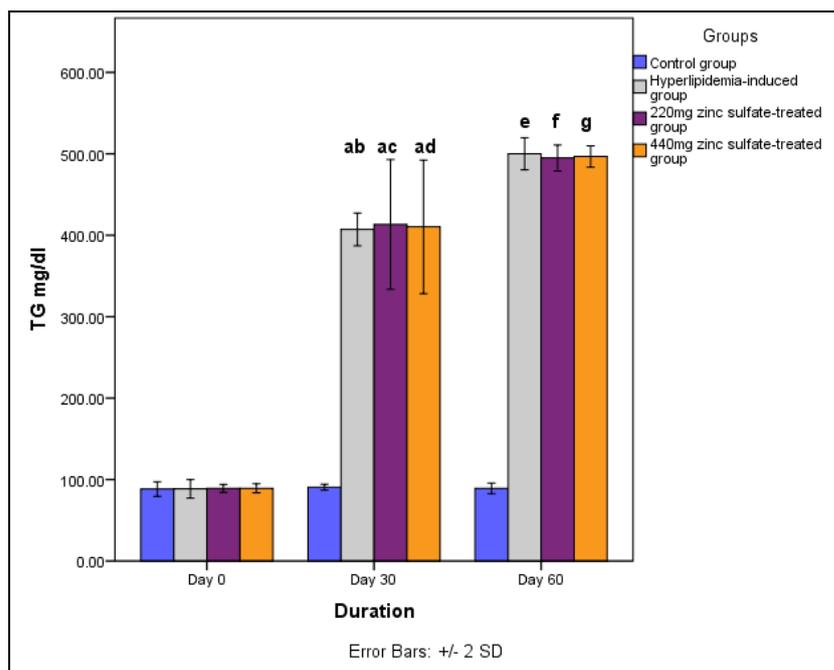


Figure (1): Changes in serum concentration of Triglyceride (TG) in (mg/dl) of the four studied groups. The results were expressed as Mean + SD.

a: significant increase as compared with normal control group at day 0 ($p < 0.05$).

b: significant increase as compared with hyperlipidemia-induced group at day 0 ($p < 0.05$).

c: significant increase as compared with 220 mg zinc sulfate-treated group at day 0 ($p < 0.05$).

d: significant increase as compared with 440 mg zinc sulfate-treated group at day 0 ($p < 0.05$).

e: significant increase as compared with hyperlipidemia-induced group at day 30 ($p < 0.05$).

f: significant increase as compared with 220 mg zinc sulfate-treated group at day 30 ($p < 0.05$).

g: significant increase as compared with 440 mg zinc sulfate-treated group at day 30 ($p < 0.05$).

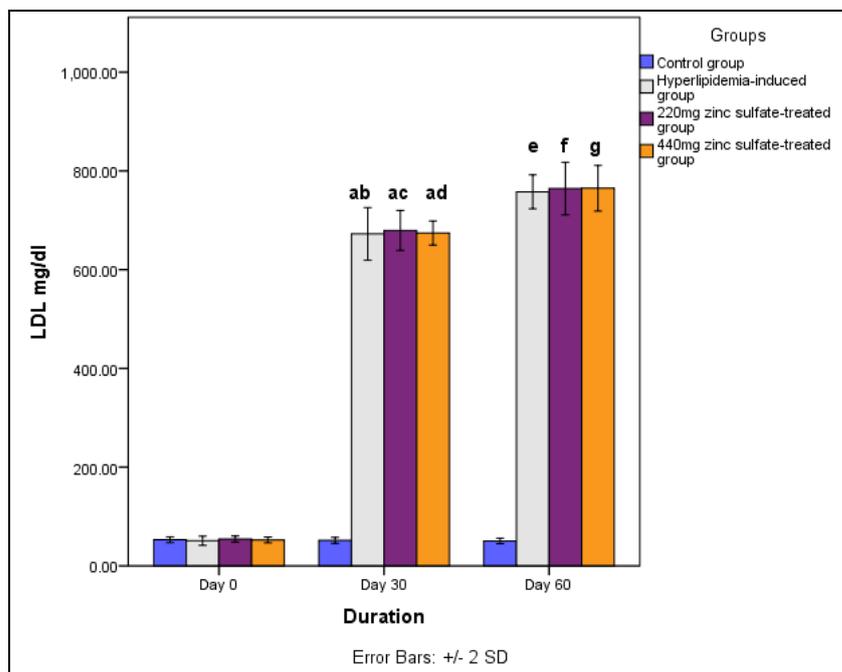


Figure (2): Changes in serum concentration of Low-density lipoprotein (LDL) in (mg/dl) of the four studied groups. The results were expressed as Mean + SD.

- a: significant increase as compared with normal control group at day 0 ($p < 0.05$).
- b: significant increase as compared with hyperlipidemia-induced group at day 0 ($p < 0.05$).
- c: significant increase as compared with 220 mg zinc sulfate-treated group at day 0 ($p < 0.05$).
- d: significant increase as compared with 440 mg zinc sulfate-treated group at day 0 ($p < 0.05$).
- e: significant increase as compared with hyperlipidemia-induced group at day 30 ($p < 0.05$).
- f: significant increase as compared with 220 mg zinc sulfate-treated group at day 30 ($p < 0.05$).
- g: significant increase as compared with 440 mg zinc sulfate-treated group at day 0 ($p < 0.05$).

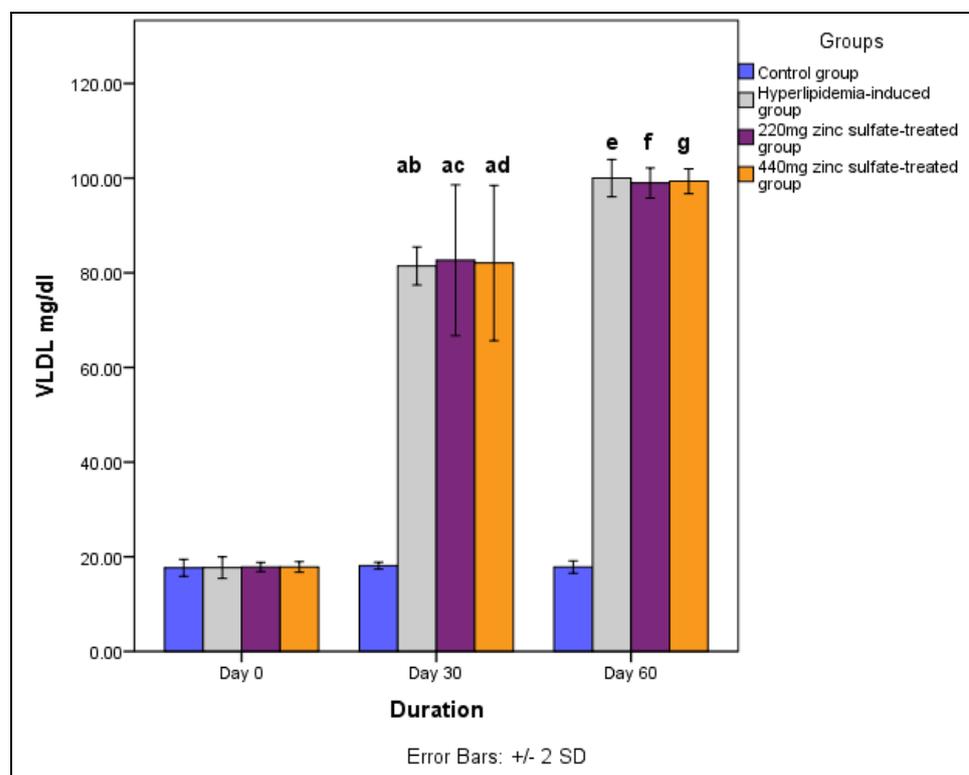


Figure (3): Changes in serum concentration of Very low-density lipoprotein (VLDL) in (mg/dl) of the four studied groups. The results were expressed as Mean + SD.

- a: significant increase as compared with normal control group at day 0 ($p < 0.05$).
- b: significant increase as compared with hyperlipidemia-induced group at day 0 ($p < 0.05$).
- c: significant increase as compared with 220 mg zinc sulfate-treated group at day 0 ($p < 0.05$).
- d: significant increase as compared with 440 mg zinc sulfate-treated group at day 0 ($p < 0.05$).
- e: significant increase as compared with hyperlipidemia-induced group at day 30 ($p < 0.05$).
- f: significant increase as compared with 220 mg zinc sulfate-treated group at day 30 ($p < 0.05$).
- g: significant increase as compared with 440 mg zinc sulfate-treated group at day 30 ($p < 0.05$).

Changes in serum concentration of HDL

As shown in figure (4), during all times of the study, there were no significant changes in HDL serum concentration among all groups ($p > 0.05$).

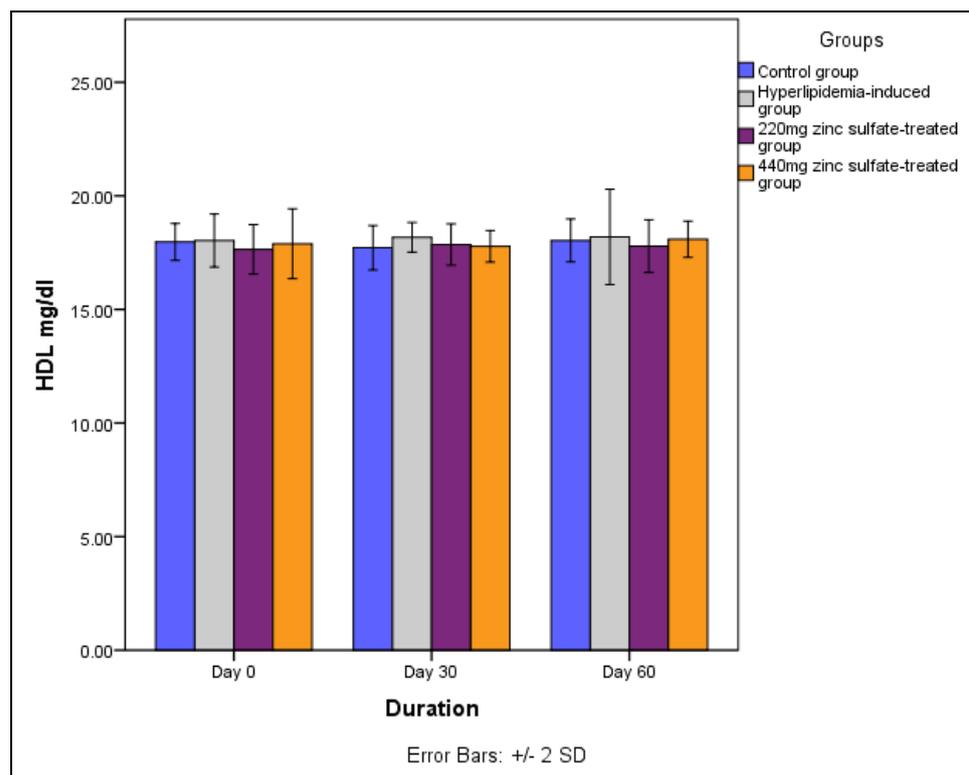


Figure (4): Changes in serum concentration of High-density lipoprotein (HDL) in (mg/dl) of the four studied groups. The results were expressed as Mean + SD.

Changes in oxidative stress parameters in four studied groups at different times

Changes in serum concentration of MDA

At the commencement of the study, there were no significant changes among all groups ($p > 0.05$).

One month thereafter, there was a highly significant increase for serum concentration of MDA of hyperlipidemia-induced group as compared with that one of normal control group ($p < 0.05$). Serum concentration of MDA of both of 220 mg and 440 mg zinc sulfate-treated group was significantly lower than that one in hyperlipidemia-induced group ($p < 0.05$). In addition, MDA serum concentration of 440 mg zinc sulfate-treated group was significant lower than that concentration of 220 mg one ($p < 0.05$).

After another month, serum concentration of MDA of hyperlipidemia-induced group increased significantly compared with its concentration at day 30 in the same group ($p < 0.05$). On the other hand, for both of groups of zinc supplementation (III and IV), there was a significant decrease in serum concentration of MDA in comparison with its concentration at day 30 ($p < 0.05$). Furthermore, MDA serum concentration of both of these groups was significantly lower than that one of hyperlipidemia-induced group ($p < 0.05$). For 440 mg zinc sulfate-treated group, MDA serum concentration it has was significantly lower than that one of 220 mg zinc sulfate-treated group ($p < 0.05$). As shown in figure (5).

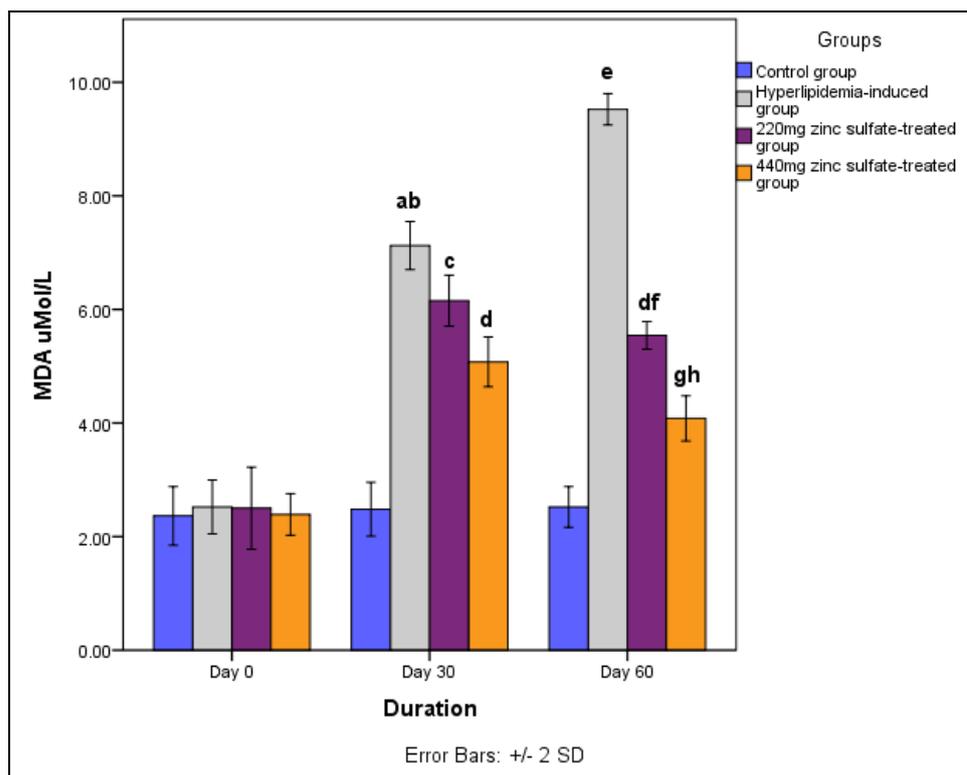


Figure (5): Changes in serum concentration of Malondialdehyde (MDA) in ($\mu\text{Mol/l}$) of the four studied groups. The results were expressed as Mean + SD.

a: significant increase as compared with normal control group at day 0 ($p < 0.05$).

b: significant increase as compared with hyperlipidemia-induced group at day 0 ($p < 0.05$).

c: significant decrease as compared with hyperlipidemia-induced group at day 30 ($p < 0.05$).

d: significant decrease as compared with 220 mg zinc sulfate-treated group at day 30 ($p < 0.05$).

e: significant increase as compared with hyperlipidemia-induced group at day 30 ($p < 0.05$).

f: significant decrease as compared with hyperlipidemia-induced group at day 60 ($p < 0.05$).

g: significant decrease as compared with 440 mg zinc sulfate-treated group at day 30 ($p < 0.05$).

h: significant decrease as compared with 220 mg zinc sulfate-treated group at day 60 ($p < 0.05$).

Changes in serum concentration of GSH

There were no significant changes among all groups at zero time ($p > 0.05$).

After one month, serum concentration of GSH of hyperlipidemia-induced group highly significantly decreased compared with that concentration in normal control group ($p < 0.05$). On the other hand, serum concentration of GSH in both of groups on zinc supplementation (III and IV) was significantly higher than that one of hyperlipidemia-induced group ($p < 0.05$). In addition, GSH serum concentration of 440 mg zinc sulfate-treated group was significantly higher than its concentration of 220 mg one ($p < 0.05$).

After another month, serum concentration of GSH of hyperlipidemia-induced group decreased significantly compared with its concentration at day 30 in the same group ($p < 0.05$). Serum concentration of GSH in 220 mg and 440 mg zinc sulfate treated-group was significantly higher than that concentration at day 30 in the same groups ($P < 0.05$). Moreover, GSH serum concentration in both of these groups was significantly higher than its concentration in hyperlipidemia-induced group ($p < 0.05$). In addition, GSH serum concentration of 440 mg zinc sulfate-treated group was significantly higher than its concentration of 220 mg one ($p < 0.05$). As shown in figure (6).

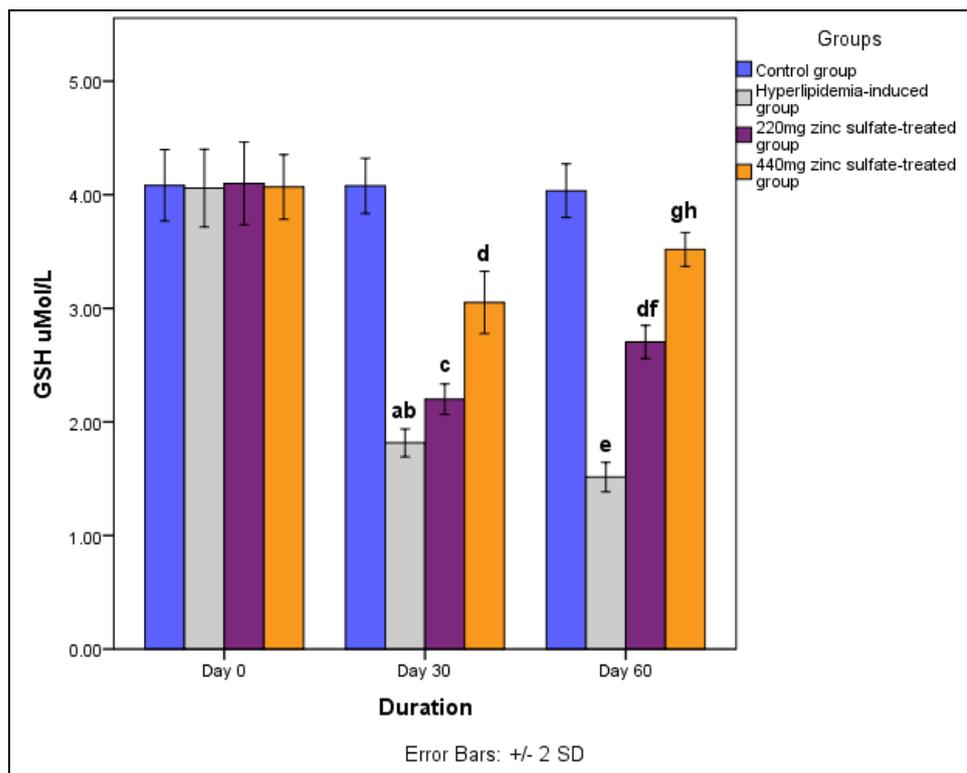


Figure (6): Changes in serum concentration of reduced Glutathione (GSH) in ($\mu\text{Mol/l}$) of the four studied groups. The results were expressed as Mean \pm SD.

a: significant decrease as compared with normal control group at day 0 ($p < 0.05$).

b: significant decrease as compared with hyperlipidemia-induced group at day 0 ($p < 0.05$).

c: significant increase as compared with hyperlipidemia-induced group at day 30 ($p < 0.05$).

d: significant increase as compared with 220 mg zinc sulfate-treated group at day 30 ($p < 0.05$).

e: significant decrease as compared with hyperlipidemia-induced group at day 30 ($p < 0.05$).

f: significant increase as compared with hyperlipidemia-induced group at day 60 ($p < 0.05$).

g: significant increase as compared with 440 mg zinc sulfate-treated group at day 30 ($p < 0.05$).

h: significant increase as compared with 220 mg zinc sulfate-treated group at day 60 ($p < 0.05$).

Discussion

Effects on lipid profile parameters

Effects of atherogenic diet on lipid profile parameters: Rabbits have become the most largely used experimental model to evaluate the development of atherosclerosis because they are very sensitive to cholesterol rich diet and accumulate large amounts of cholesterol in their plasma, their use as experimental models is highly relevant and brings information on factors that contribute to the progression and regression of this condition that can be applied to humans [15].

In present study, feeding of atherogenic diet (1% (w/w) cholesterol) to rabbits in group II, III, and IV for 8 weeks resulted in marked hyperlipidemia with a high significant increase in serum concentration of TG, LDL, and VLDL ($p < 0.05$), while HDL serum concentration was not significantly changed compared with normal control ($p > 0.05$). These results are in agreement with those reported earlier [16, 17].

The dramatic increase in lipid profile parameters is attributed to the way in which rabbits respond to a high cholesterol diet. As cholesterol intake increase, bile acids reabsorption increase too which leads to increase its uptake by the liver. The

consequence is an inhibition in the conversion of cholesterol to bile acids by the liver due to greatly decreased levels of mRNA encoding 7 α -hydroxylase, which catalyzes the rate-limiting step in bile acid synthesis. The resultant elevation in liver cholesterol content leads to an increase in VLDL production, a decrease in lipoprotein receptor activity, and an accumulation of CE-rich VLDL and LDL in the plasma. This change in plasma lipoproteins leads to the development of advanced atherosclerotic lesions [18, 19].

Effects of zinc supplementation on lipid profile parameters: From the present study, it was clear that the treatment of groups on atherogenic diet with zinc sulfate brings no significant changes in lipid parameters compared to hyperlipidemia-induced group ($p > 0.05$). These findings are in basic agreement with that of Rashtchizadeh, et al, 2008 [20].

Hughes and Samman, 2006 [21] reported that the plasma concentrations of TG and LDL are unaffected in subjects taking up to 150 mg of zinc sulfate per day. Furthermore, AREDS 2002 [22] showed that the 5 years of daily oral supplementation with 80 mg of zinc oxide and 2 mg of copper did not influence the laboratory parameters of lipid metabolism as determined from TG, LDL and HDL levels.

Effects on oxidative stress parameters

Effects of atherogenic diet on oxidative stress parameters: In current study, along the time of the study, hypercholesterolemia was associated with significant increase in serum concentration of MDA ($p < 0.05$) and significant decrease in serum concentration of GSH ($p < 0.05$). The observed increase in MDA level and decrease in GSH level in rabbits fed high cholesterol diet is consistent with several experimental studies like Fayed, et al, 2010 [23]; Solanki and Bhatt, 2010 [24] and Khan, et al, 2011 [25]. Yang, et al, 2008 [26] reported in their clinical study that serum concentration of MDA of hyperlipidemic subjects is significantly higher than that of hypolipidemic ones.

Lipid peroxidation was initiated by free radical attack on membrane polyunsaturated fatty acids leading to their transformation and fragmentation to alkanes and reactive aldehyde compounds [27]. MDA is a product and a most frequently used indicator of lipid peroxidation, it is a measure of free radical generation and an elevated the level of the same in high fat diet rabbits suggests that hypercholesterolemia could enhance the process of lipid peroxidation [28].

In fact, hypercholesterolemia could increase free radicals production through several ways, it increases activity of three major oxidant-producing enzyme systems; NADPH oxidases, xanthine oxidase, and myeloperoxidase. NADPH oxidase acts to transfer an electron to an oxygen molecule, forming superoxide ultimately H₂O₂. Xanthine oxidase forms superoxide and H₂O₂ during the reduction of oxygen, while myeloperoxidase is produced by neutrophils and monocytes and produces a toxic hypochlorous acid [29].

Furthermore, hypercholesterolemia could increase the release of platelet activating factor (PAF), which in turn could increase the synthesis and release of IL-1 and TNF. PAF, IL-1, and TNF are known to stimulate polymorph nuclear leukocytes to produce free radicals, which would increase the lipid peroxidation products [30]. Moreover increased lipid peroxidation lead to inactivation in the antioxidant enzymes by crosses linking with MDA, this will cause an increased accumulation superoxide, H₂O₂ and hydroxyl radicals, which could further stimulate lipid peroxidation, and a vicious cycle ensues [31].

Therefore, as blood level of lipid increase, free radical production increase too, which will increase lipid peroxidation leading to elevation in the serum concentration of MDA.

GSH is a low molecular weight, water-soluble tripeptide; it is an important antioxidant and plays a major role in the detoxification of endogenous metabolic products, including lipid peroxides, and xenobiotic compounds including pollutants, heavy metals, and drugs. Intracellular glutathione exists in both the oxidized disulfide form (GSSG) or in reduced (GSH) state [32]. GSH, by the catalytic action of glutathione peroxidase, detoxifies peroxides and hydroxyl radicals into nontoxic forms. As a result, it converts into oxidized GSSG, and then by the action of glutathione reductase it recycles from GSSG to GSH [33].

Therefore, the greater the production of free radical, the greater the consumption of GSH molecules.

Effects of zinc supplementation on oxidative stress parameters: In comparison with hyperlipidemia-induced group, our present study showed that treating the rabbits with the oral zinc sulfate supplementation was associated with significant improvement in oxidative stress parameters ($p < 0.05$). There was a positive relationship between the dose of zinc from one side and the decrement in MDA and the increment in GSH serum concentration from the other side. The rabbits fed 440 mg zinc sulfate (100 mg of elemental zinc) revealed statistically lower level of MDA ($p < 0.05$) and higher level of GSH ($p < 0.05$) than those fed 220 mg zinc sulfate (50 mg of elemental zinc).

Various groups have investigated the effects of zinc supplementation on level of MDA and GSH and their data were consistent with our current findings like Alabbassi, et al, 2006 [34]; Al-Kaisy, et al, 2006 [35] and Juda, et al, 2007 [36].

Alabbassi, et al, 2006 studied the effect of administration of zinc sulfate on plasma and erythrocyte level of MDA and GSH in lead exposed workers. In their study, they found that zinc significantly lowers level of MDA and elevates level of GSH. Al-Kaisy, et al, 2006 found that addition of 15 mg of elemental zinc to the usual treatment protocol to burned patients significantly reduce MDA level and increase GSH level which lead to decrease healing time, the incidence of eschar formation, and the mortality rate. While in study of Juda, et al, 2007 they showed a negative correlation between level of zinc and MDA, and a positive one between level of zinc and GSH in patients with urinary bladder cancer.

Zinc has well documented antioxidant properties. It has not been shown to interact directly with an oxidant species but exerts its effects in an indirect manner [37]. In general, the antioxidant mechanism of zinc is divided into acute and chronic [38].

Furthermore, Zhou, et al, 2005 [39] proved in their study that zinc supplementation protects GSH pool, at least, by increasing the activity of GSH reductase thus enhancing GSSG reduction to GSH; also, they demonstrated that zinc supplementation prevents ethanol-induced decrease in GSH peroxidase activity, which is one of the primary antioxidant enzymes. Moreover, the study of Ha, et al, 2006 [40] provides evidence that zinc also stimulates GSH synthesis through upregulation of glutamate-cysteine ligase, which is the rate limiting step enzyme in de novo synthesis of GSH in retinal pigment epithelial cells line.

Conclusion

We can conclude that the dose of 220 and 440 mg \ day of oral zinc sulfate for 60 days has a significant antioxidant effect in male rabbits fed high cholesterol diet.

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