PROTECTIVE EFFECT OF VITAMIN E ON ACETAMINOPHEN–INDUCED HYPERLIPIDEMIA IN FEMALE RABBITS

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Abstract

The protective effect of vitamin E against acetaminophen (paracetamol) induced hyperlipidemia in female rabbits was tested. Twenty female rabbits were randomly divided into four groups (five animals in each group). The first group was dosed with acetaminophen at 200 mg/kg (BW). The second group was dosed with 50 mg/kg. BW of vitamin E and the third group was treated with 200 mg/kg (BW) of acetaminophen and 50 mg/kg (BW) of vitamin E, while the last was administered with distilled water and considered as a control group. These animals were orally dosed using a micropipette for 14 days.

The results showed that there was a significant ($P<0.05$) increase in serum cholesterol, triglycerides, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) concentrations and atherosclerosis index except high-density lipoprotein (HDL) which decreased significantly in the treated group with acetaminophen compared with the control group. On the other hand, these above-mentioned parameters decreased significantly ($P<0.05$) except HDL concentration in the group treated with vitamin E compared with the control group. Concerning the animals treated with acetaminophen and vitamin E, the results also showed that these above mentioned parameters decreased significantly ($P<0.05$) except HDL concentration compared with the group treated with acetaminophen and no significant ($P\geq0.05$) difference in the above mentioned parameters compared with the control group.

In conclusion, the antioxidant vitamin E may reduce the oxidative modification of LDL by acetaminophen and may be used as a therapeutic agent in preventing the development and progression of atherosclerosis.

Key words: Acetaminophen, vitamin E, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein.

التأثير الوقائي لفيتامين E على فرط الدهون المستحدث بالأسيتامينوفين في إناث الأرانب

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الخلاصة

اختبر التأثير الوقائي لفيتامين E تجاه عقار الأسيتامينوفين (الباراسيتامول) المستحدث لفرط الدهون في إناث الأرانب. قسمت الحيوانات بصورة عشوائية إلى أربع مجموعات بواقع خمس حيوانات في المجموعة الواحدة. جرعت المجموعة الأولى بعقار الأسيتامينوفين بجرعة مقدارها 200 ملغم/ كغم من وزن الجسم فيما جرعت المجموعة الثانية بجرعة مقدارها 50 ملغم/ كغم من وزن الجسم من فيتامين E، أما المجموعة الثالثة فقد جرعت 200 ملغم/ كغم من وزن الجسم من عقار الأسيتامينوفين و 50 ملغم/ كغم من وزن

الجسم من فيتامين E فيما جرعت المجموعة الأخيرة بالماء المقطر وأعتبرت كمجموعة سيطرة. جرعت هذه المجاميع بالتراكيز المذكورة فمويا باستخدام الماصة الدقيقة ولمدة 14 يوما.

أظهرت النتائج أن هناك ارتفاعا معنويا ($P<0.05$) في معدل تركيز الكوليستيرول والشحوم الثلاثية والبروتين الدهني الواطئ الكثافة (LDL) والبروتين الدهني واطئ الكثافة جدا (VLDL) ودليل تصلب الشرايين ماعدا البروتين الدهني عالي الكثافة (HDL) الذي انخفض معنويا في المجموعة المعاملة بعقار الأسيتامينوفين مقارنة بمجموعة السيطرة. وفي الجانب الآخر، فان المتغيرات المذكورة أعلاه قد انخفضت معنويا ($P<0.05$) ماعدا HDL في المجموعة المعاملة بفيتامين E مقارنة بمجموعة السيطرة. أما بخصوص الحيوانات المعاملة بعقار الأسيتامينوفين وفيتامين E، فقد بينت النتائج بأن المتغيرات المذكورة أعلاه قد انخفضت معنويا ($P<0.05$) ماعدا HDL مقارنة بالمجموعة المعاملة بعقار الأسيتامينوفين ولم يظهر هناك فرقا معنويا ($P\geq0.05$) في المتغيرات المذكورة مقارنة بمجموعة السيطرة.

يمكن الاستنتاج من هذه الدراسة بان مضاد الأكسدة فيتامين E يمكن أن يقلل من التحوير التأكسدي لـ LDL المسبب بالأسيتامينوفين وربما يستعمل كعامل علاجي لمنع تطور وتقدم تصلب الشرايين.

الكلمات المفتاحية: الأسيتامينوفين، فيتامين E، الكوليستيرول، الشحوم الثلاثية، البروتين الدهني عالي الكثافة، البروتين الدهني واطئ الكثافة.

Introduction

Paracetamol or acetaminophen is an effective, well-tolerated and popular domestic analgesic and antipyretic for adults and children [1, 2, 3]. It is a major metabolite of the now obsolete phenacetin, with an analgesic effect, which is similar to that of aspirin. Although generally considered a safe drug, it continues to be a cause of death through overdose, idiopathic reaction, or synergism with alcoholic liver disease. Death from acetaminophen overdose is thought to be secondary to liver failure, which is caused by massive hepatic necrosis, the hallmark pathological feature of acetaminophen toxicity [4]. Acute liver failure caused by acetaminophen has been attributed to the metabolic activation of acetaminophen to a toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) in the liver by cytochrome p450 isoenzymes especially CYP2E1. It was found that NAPQI depletes liver glutathione thereby inducing oxidative stress. It also binds to vital cellular and mitochondrial proteins leading to cellular necrosis, and activates cells of the immune system leading to the release of pro-inflammatory cytokines [5]. Acetaminophen-induced hepatic injury is mediated through an increased lipid peroxidation in hepatic tissues [3]. In addition to liver, however, many organ systems may fail under acute overdose such as renal, cardiac and central nervous systems [6].

Acetaminophen, easy available as well as ease of acquisition to the public even without prescription, has led to the increase in reported cases of toxicity caused by paracetamol [2]. Furthermore, it has been reported that the toxic effects of acetaminophen are the result of oxidative reactions that take place during its metabolism [1].

On the other hand, antioxidants play an important role in inhibiting and scavenging free radicals and thus providing protection against infections and degenerative diseases [6]. Literature have shown that the most abundant and effective antioxidants in the human body are vitamins. In view of the intrinsic antioxidant activity of vitamins, the present study was designed to investigate the protective effect of single, and daily oral dose of vitamin E in high dose acetaminophen-treated rabbits for 14 days [3].

Materials and methods

Experimental animals

Twenty female local rabbits with an average age of about 3-3.5 months and weight between 1150 – 1600 g were used. They were bred in special cages at Al- Nahrain University Research Center for Biotechnology, fed pellets (contain 20 % crude protein and 11% crude fibre, rich in protein and energy) and given tap water *ad libitum* during the experimental period which lasted for 14 days. Concerning conditions of the laboratory, average temperature was about 21 - 24° C. and the light cycle was divided into 12 hours light: 12 hours dark [7].

Doses and design of the experiment

The animals were randomly divided into four groups (five animals in each group). The first group was dosed orally with acetaminophen

(Samara Drug Factory, Iraq) using micropipette. The acetaminophen dose (200 mg/kg) is reported to relevant for analgesic effect to rabbits by oral route [8]. Acetaminophen solution in water was prepared daily and the volume of administered dose was 1ml/ day for 14 days. The second group was dosed with 50 mg/kg of vitamin E (The Arab Company for Manufacturing Veterinary and Agricultural Products, Jordan). The dose was prepared by dissolving 0.5 ml of stock solution (100 mg/ml) with 1 ml of distilled water [9]. The used form of vitamin E is "water soluble" in which certain compounds during a manufacturing process were added that made it more efficiently absorbed through the intestinal wall. The third group was treated with 200 mg/kg (BW) of acetaminophen and 50 mg/kg (BW) of vitamin E while the last group was considered as control and daily administered with 1 ml of distilled water.

Blood sample collection

At the end of treatment, blood samples were collected by heart puncture. The volume of collected blood was 3 ml. The blood sample was slowly expressed into the vial to reduce the risk of hemolysis after removing of the needles from syringes [10].

Serum was separated by putting the tubes in the centrifuge at 3000 rpm for 15 min at 37°C. Serum samples were stored at -4°C until assayed for lipid profile [9, 10].

Lipid profile

Total cholesterol, HDL- cholesterol and triacylglycerides concentrations were determined using assay kits (Biomaghreb Company, Tunis) for *in vitro* diagnosis use [10, 11]. The kits for total cholesterol and triglycerides determination depend on enzymatic hydrolysis, while the kit for HDL-cholesterol determination depend on the precipitation reaction and supernatant formation.

Friedewald equation was used to calculate the concentration of LDL-cholesterol as below:

[LDL-chol]= [Total-chol] - [HDL-chol] - [VLDL- chol]

Concerning the VLDL-chol concentration, it was calculated by dividing triglycerides value on 5 as below:

[VLDL-chol] = TG/5, while the atherosclerosis index was obtained by dividing the LDL-cholesterol on the HDL- cholesterol [11].

Statistical analysis

The results were analyzed statistically using analysis of variance (ANOVA) applicable to a completely randomized design. Then, the significance among means was tested depending on Duncan multiple range test using SPSS program [12].

Results

The results showed that there was a significant ($P<0.05$) increase in cholesterol and triglycerides concentrations in the group treated with 200 mg/kg (BW) of acetaminophen compared with control animals. In this group treated with acetaminophen, the cholesterol and triglycerides concentration means were 125.37 and 146.82 mg/dl, respectively, while they were 106.59 and 114.76 mg/dl, respectively in control group (Figure 1). The results also showed that there was a significant ($P<0.05$) decrease in cholesterol and triglycerides concentrations in the animals treated with 50 mg/kg (BW) of vitamin E compared with control group. The cholesterol and triglycerides means were 92.65 and 97.58 mg/dl, respectively. In the group treated with acetaminophen and vitamin E, the results showed that there was a significant ($P<0.05$) decrease in cholesterol and triglycerides concentrations compared with the group treated with acetaminophen and no significant ($P\geq0.05$) difference in cholesterol and triglycerides concentrations compared with control group. The means of cholesterol and triglycerides were 108.36 and 118.57 mg/dl, respectively (Figure 1).

On the other hand, the results revealed that there was a significant ($P<0.05$) decrease in serum HDL-C concentration and a significant ($P<0.05$) increase in serum LDL and VLDL concentrations in the group treated with acetaminophen compared with the control animals (Figure 2). The HDL-C, LDL-C, VLDL-C means were 29.22, 66.78 and 29.36 mg/dl respectively in animals treated with acetaminophen. While, the means of the mentioned parameters in control group were 44.50, 39.15 and 22.95 mg/dl, respectively

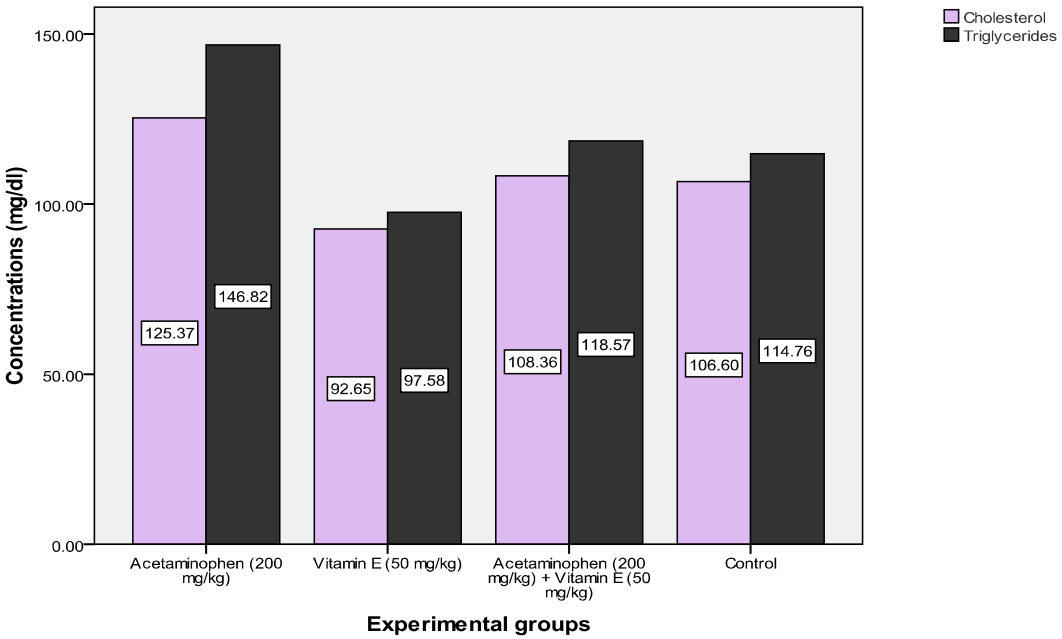

Figure 1: Effect of vitamin E on serum cholesterol and triglycerides concentrations in female rabbits treated with acetaminophen.

Concerning the group treated with acetaminophen + vitamin E, the results revealed that there was a significant ($P<0.05$) increase in HDL-C concentrations and a significant ($P<0.05$) decrease in LDL and VLDL-C concentrations compared with the group treated with acetaminophen. Furthermore, there was no significant ($P\geq0.05$) difference in the means of HDL-C, LDL-C and VLDL-C compared with control group. The means of these parameters were 41.03, 43.61 and 23.71 mg/dl, respectively. (Figure 2) explains the effect of acetaminophen and vitamin E on lipoprotein concentrations in female rabbits.

Concerning the effect of these treatments on atherosclerosis index, the results revealed that there was a significant ($P<0.05$) increase in atherosclerosis index in the group treated with acetaminophen compared with control (Figure 3). The index mean in this group was 2.305, while it was 0.89 in the control group. In addition, the results showed that there was a significant ($P<0.05$) decrease in atherosclerosis index mean in the group treated with vitamin E compared with control group. In the group treated with acetaminophen + vitamin E, there was also a significant ($P<0.05$) decrease in atherosclerosis index mean compared with the group treated with acetaminophen and no significant ($P\geq0.05$) difference in atherosclerosis index mean compared with the control group. The means of this index were 0.334 and 1.076. (Figure 3).

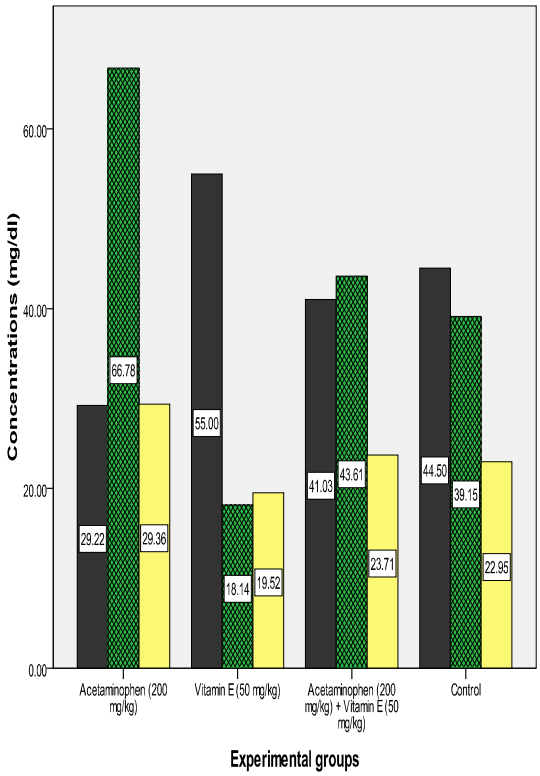

Figure 2: Effect of vitamin E on serum HDL, LDL and VLDL concentrations in female rabbits treated with acetaminophen. (Dark bars represent HDL concentration, shadow bars represent LDL concentration and light bars represent VLDL concentration)

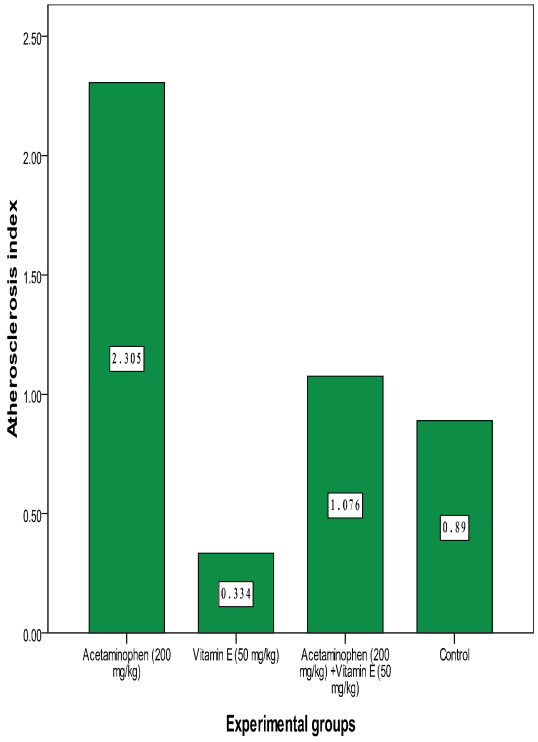

Figure 3: Effect of vitamin E on atherosclerosis index in female rabbits treated with acetaminophen.

Discussion

The results above are come into agreement with the result of [13] in that paracetamol at 2 g/kg has enhanced the cholesterol levels and reduced the serum levels of HDL. They are also similar with the results of [14] in that the acetaminophen-treated animals showed an elevation in the concentrations of total lipids, cholesterol, triglycerides and serum LDL-cholesterol with depletion in the levels of serum HDL-cholesterol and tissue phospholipid. It has proposed that O_2^- or its dismutation production, H2O2 generated during the cytochrome P450-mediated microsomal metabolism of acetaminophen, was responsible for the increase in lipid peroxidation [15]. The increase in the lipid peroxidase enzyme (LPO) activity in liver induced by acetaminophen suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals [6]. Furthermore, a number of studies demonstrated indirectly and directly the presence of reactive oxygen species in cells following acetaminophen administration [6]. This may be due to the depletion of cellular glutathione, a natural antioxidant, which leaves the cell particularly vulnerable to oxidative insults [5]. The depletion of glutathione leading to accumulation of a highly toxic metabolite of acetaminophen; N–acetyl–p–benzoquinamine (NAPQI) which is normally conjugated with glutathione and excreted in urine [6].

On the other hand, the protective effect of vitamin E against acetaminophen is in accordance with the result of [4] in that several antioxidants have shown to protect against acetaminophen toxicity such as β-carotene and α-tocopherol. Furthermore, in animal studies, a diet containing 0.125% vitamin E increased its levels in plasma two-fold and prevented formation of early atherosclerotic lesions in the thoracic aorta of hypercholesterolemic rabbits [16]. In addition, there was an increase in the HDL-C component and HDL/LDL ratio and a decrease in the LDL-C component and triglycerides in the group treated with vitamin E [17]. In another study, the effect of 6 months treatment of vitamin E on the susceptibility of low-density lipoproteins (LDLs) to oxidative modification and on atherosclerotic lesions in rabbits was studied. Vitamin E levels in plasma and LDL increased threefold in the course of treatment with this antioxidant [18]. Dose-response studies in humans have reported that 400 IU/day vitamin E increased its levels in plasma two-fold and prolonged the lag time before LDL oxidation. This might be beneficial in decreasing the individual risk of coronary heart diseases (CHD) [16].

From the results above, it was concluded that the efficacy of vitamin E as antioxidant, was dependent on its capacity of either reducing the harmful effect or restoring the normal lipid profile and its ability to reduce the oxidative modification of LDL that has been distributed by acetaminophen. Thus, vitamin E may be used as a therapeutic agent in preventing the development and progression of atherosclerosis.

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