

Study the effect of green tea extract on lipid profile in nitrate treated rabbits

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Abstract

The present study was conducted to determine the effected of using an aqueous extract of green tea in lipid profile in rabbits treated with sodium nitrate. To achieve this goal, 28 local adult female rabbits, were randomly divided into four equal groups in different cages. The first group has been arranged as control group and has been drenched the distilled water daily for 60 days. While the second group (T1) has been drenched aqueous extract of green tea at a dose of 200 mg / kg of body weight for a period of 60 days, while the third group (T2) has been drenched the aqueous extract of green tea at a dose of 200 mg / kg of body weight and at the same time they have been dosed with sodium nitrate dissolved in distilled water at a dose of 300 mg / kg of body weight, while the fourth group (T3) have been drenched with sodium nitrate dissolved in distilled water at a dose of 300 mg / kg of body weight for a period of 60 days. The results of lipid profile test show significant increase in total cholesterol and low density lipoprotein in a (T3) group, as well as there was a significant decrease in a (T1) group, with no significant differences between (T2) and control group. While in the triacylglycerol and very low density lipoprotein show significant incense in the T3 group compared with the rest of the groups. In concern to the high density lipoprotein concentration significant decrease in (T3) group and significant increase in the (T1) group, while the concentration of (T2) was no significant increase close to the concentration of the control group.

Key words: Aqueous extract, green tea, lipid profile, sodium nitrate, rabbit.

دراسة تأثير المستخلص المائي للشاي الاخضر على الدهن في الارانب المعاملة بالنترات

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الخلاصة

اجريت الدراسة الحالية لمعرفة مدى تأثير المستخلص المائي للشاي الاخضر في صورة الدهن في الارانب المعاملة بنترات الصوديوم. ولتحقيق هذا الهدف استخدمت 28 انثى من الارانب المحلية البالغة قسمت عشوائيا الى اربعة مجاميع متساوية في اقصاف مختلفة بعد ذلك تركت المجموعة الاولى كمجموعة السيطرة وجرعت الماء المقطر يوميا لمدة 60 يوم. وجرعت المجموعة الثانية (T1) بالمستخلص المائي للشاي الاخضر بجرعة 200 ملغم \ كغم من وزن الجسم لمدة 60 يوم ، في حين جرعت المجموعة الثالثة (T2) المستخلص المائي للشاي الاخضر بجرعة 200 ملغم \ كغم من وزن الجسم وفي نفس الوقت جرعت بمادة نترات الصوديوم المذابة في الماء المقطر بجرعة 300 ملغم \ كغم من وزن الجسم ، بينما جرعت المجموعة الرابعة (T3) مادة نترات الصوديوم المذابة في الماء المقطر بجرعة 300 ملغم \ كغم من وزن الجسم لمدة 60 يوم. وكانت نتائج فحوصات الدهن حصول ارتفاع معنوي في الكوليسترول الكلي و البروتين الدهني واطنة الكثافة في مجموعة (T3)، كذلك حصول انخفاض معنوي في مجموعة (T1) ، مع عدم وجود فروق معنوية بين (T2) ومجموعة السيطرة. في ما يخص الكليسيريدات الثلاثية و البروتين الدهني واطنة الكثافة جدا حصول ارتفاع معنوي في مجموعة (T3) بالمقارنة مع بقية المجاميع اما فيما يخص البروتين العالي الكثافة لوحظ وجود انخفاض معنوي في مجموعة (T3) وارتفاع معنوي في مجموعة (T1) في حين كان التركيز في مجموعة (T2) ارتفاع غير معنوي قريبا من تركيز مجموعة السيطرة.

الكلمات المفتاحية: المستخلص المائي ، الشاي الخضر ، الدهن ، نترات الصوديوم ، الارانب

Introduction

Pollution issue is now among the most important issues, that the world countries are interested in, since this issue has a great effect on all life aspects and causes great threaten to animals, plants, environment, and human health. The pollution problems rose up after the industrial evolution. Nitrate is the most common chemical contaminant in the world's ground water aquifers (1). Excessive use of nitrogenous fertilizers leads to ecosystem pollution by the accumulation of nitrates in vegetables and fodder as well as the contamination of surface and ground water (2). Nitrates and nitrites are of great importance and concern to man and animals because they possess mutagenic, carcinogenic, teratogenic and embryo toxic activities (3). Nitrates and nitrites are likely to exert harmful effects on the respiratory function as a consequence of acute intoxication while being responsible for methaemoglobinemia (4), (5). In a human, consumption of water with nitrate levels was associated with thyroid hypertrophy (6), increased blood pressure (7), and acute respiratory tract infections (8). Adverse reproductive outcomes of nitrates in drinking water have been reviewed (9). Green tea is natural dried leaves of the tea plant, (*camellia sinensis*). Tea has been one of the most consumed beverages in all over the world. Tea is used not only as fresh drink but also as traditional herb which has many benefits for human health. Recently tea has attracted scientific attention for its anticancer and antioxidant activities (10).

Materials and methods

Twenty eight adult female local breed rabbits (1250-1600gm), aged between 6-8 months were used in this investigation. Animals in all stages of the experiment housed in cages in conditioned room (22-25°C) in the animal house of department of physiology and pharmacology at the College of Veterinary Medicine-University of Al-Qadisiya for the period from beginning of November 2012 to the end of December 2012 with providing daily light of twelve hours (7.00 to 19.00) and twelve hours night cycle. They were left for two weeks for

acclimation with the experimental conditions. Animals had free access to water and standard pellet diet along the experiment. Animals were randomly assigned into four groups (7 rabbits in each group) and treated for 60 days as follows: Group C was administered distal water 5ml/kg. B.W. and served as control. Group T1 were administered green tea extract (GTE) (True nutrition-USA) 200 mg / kg .B .W orally by gavage needle (11) dissolved in (5 ml distal water/kg. B.W). Group T2 were administered sodium nitrate (300mg/kg. BW) plus (200mg/kg. B.W) green tea extract orally by gavage needle dissolved in (5 ml distal water /kg. B.W.). Group T3 were administered sodium nitrate 300mg/kg. B.W orally by gavage needle (12) dissolved in (5 ml distal water /kg. B. W.). Blood samples were collected by cardiac puncture from anesthetized rabbits (by injection of Ketamine 40mg/Kg B.W. and xylazine 5mg/kg B.W.) at the 60th day of the experiment. Blood sample was kept in tube followed by centrifugation for 15 minutes at 4000rpm. Serum was isolated and frozen at -20°C until analysis (13). Serum biochemical measurements like, total cholesterol determination, determination of serum triglyceride, measurement of serum lipoprotein cholesterol, high-density lipoprotein (HDL) very low density lipoprotein (VLDL), and Low-density lipoprotein (LDL) were performed.

Results

1. Serum cholesterol (CT), low density lipoprotein (LDL) concentration:

There were a significant decrease ($p<0.05$) in serum CT, LDL concentration in T1 group at 60 days of experiment when compared with T2, T3 and control groups. While, results of T3 group showed, a significant increase ($p<0.05$) in serum CT, LDL concentration in comparable to T1, T2 and control groups. At the same time, the protective effect of GTE against hypercholesterolemia, and increase LDL induced by sodium nitrate was clarified in group T2 and recorded no significant changes ($p>0.05$) in serum CT, LDL concentration in T2at 60 day of experiment

as compared to control group, while, showed significant increase in T1 and significant decrease in T3 (Table 1).

2. Serum triglyceride (TGA), very low density lipoprotein (VLDL) concentration:

Within the results of serum TGA, VLDL concentration clarified in table (1) revealed that the average of sodium nitrate treated group (T3) recorded higher significant ($p < 0.05$), than other groups, where (T1), (T2) and control groups showed no significantly ($p > 0.05$) differences between each other.

3. Serum high density lipoprotein concentration (HDL):

Table (1) showed a significant increase ($p < 0.05$) in serum HDL concentration in

group (T1) at 60 day of experiment in comparable to T2, T3 and control groups. Reversely group (T3) showed a significant decrease ($p < 0.05$) in serum HDL concentration compare with (T1), (T2) and control groups. Currently, HDL concentration in serum of group (T2) revealed no difference when compared with control group at 60 days of experiment, on the other hand, results revealed significant decrease in HDL concentration in serum for group (T1) when compared with control group and significant increase in serum HDL concentration for group (T3) when compared with control group (Table 1).

Table (1): Effect of green tea extract on lipid profile test in sodium nitrate treated adult female rabbits in mg/dl.

Groups Parameter	C	T1	T2	T3
Chol. mg/dl	77 ±1.51A	70.8±1.24B	76.20±1.77A	90.40±2.42C
Trig mg/dl	70.20±2.35A	68.6± 2.37A	71.60±3.23A	87.20± 2.47B
HDL mg/dl	20.38±1.06A	25.54±1.50B	20.48±1.02A	15.56±1.80C
LDL mg/dl	42.58±2.5A	31.54±1.38B	41.40±1.78A	55.40± 2.61C
VLDL mg/dl	14.04±0.47A	13.72±0.47A	14.32±0.64A	17.44± 0.49B

Values express as mean ± SE, Number of animals per each group = (7). C: control group which received distilled water for 60 days. T1: Animals received 200mg/kg. B.W of green tea for 60 days. T2: Animals received 300mg/kg. B.W of sodium nitrate and 200 mg/kg. B.W. of green tea extract for 60 days. T3: Animals received 300mg/kg. B.W of sodium nitrate for 60 days. Different capital letters denote significant differences between groups, $P < 0.05$.

Discussion

The results of the present study pointed to significant changes in lipid profile system after green tea extract administration in T1. These changes are manifested by a decrease in TC, TAC, LDL, VLDL and increase in serum HDL-C concentration. Dietary supplementation of green tea extract decrease serum concentrations of total cholesterol and malondialdehyde and increase serum concentrations of high density lipoprotein in humans, As such, tea polyphenols are beneficial for the treatment of coronary heart disease and hypertension (14). In a variety of in vitro and in vivo studies, green tea polyphenols were found to scavenge NO, H₂O₂, OH⁻ and O₂⁻ and reduce damage caused by oxygen free radicals (15, 16) and (17). It has been proposed that catechin polyphenols reacts with peroxy radicals

involving termination of radical chain reaction (18). However, results of the present study are in agreement with (19, 20) and (21) at their study on rabbits, mice, hamster and rats. Also, the study done by (22) improves the effective role of polyphenols catechins as anticholesterolemic agent. Tea flavonoids are potent antioxidant that is absorbed from the gut after consumption, the fact that, catechins are rapidly and extensively metabolized during absorption in the small intestine, colon and liver: Emphasizes its importance role as antioxidant agents in vivo (23, 24), as well as, the results of (25) showed decrease the solubility of cholesterol micelles in animal models after treated with the green tea catechins. Interestingly, catechins epigallocatechin (ECG) and epigallocatechin gallate (EGCG) were the more effective

compounds because inhibiting of cholesterol absorption (26, 27). An increase in fecal bile acids and cholesterol excretion (28) as well as inhibition of dietary fat and cholesterol absorption (29). Aucamp, (30) reported pancrelipases inhibition after EGCG treatment in vitro. However, EGCG inhibits the activity of the xanthine oxidase enzyme lead to improve endothelial vasodilation in hypercholesterolemic persons (31). Green tea polyphenols, such as epigallocatechin gallate (EGCG), significantly increases plasma antioxidant activity in vivo (32). Frei and Higdon, (33) referred the ability of EGCG to recycle vitamin E as an antioxidant, the recycling of antioxidants by free radical electron transfer between antioxidants allows antioxidants to protect cells against lipid peroxidation in both membranes and LDL, because LDL oxidation increases the risk of atherogenic process, antioxidant protection of LDL particles is fundamental for preventing cardiovascular disease. In animal model of atherosclerosis, green tea administration has resulted in modest improvement in the resistance of lipoproteins to ex vivo oxidation. Vita, (34) mentioned that one mechanism might explain a beneficial effect of green tea on the cardiovascular system is that it improves the vascular endothelium function could be suggested mechanism of hypolipidemic effect of green tea polyphenols. Reduction in non-esterified fatty acids and leptin in serum of rabbit fed green tea extract 200 mg / kg. B.W. for 60

days was also documented (35). Besides, green tea polyphenol may exert an antiatherosclerotic action by virtue of its antioxidant properties and increasing HDL-cholesterol levels (36). Present study pointed to significant changes in lipid profile system after nitrate intubation T3. These changes were manifested by an increase in TC, TAC, LDL, VLDL and decrease in serum HDL-C concentration, which is in agreement with other studies (37, 38). Hypothyroidism was well established cause of hyperlipidemia and elevated LDL-C in animals (39) and human (40). Thyroid hormone (Triiodothyronine) influence hepatic cholesterol metabolism and play an important role in the regulation of cholesterol 7-hydroxylose, the rate limiting enzyme in bile acid synthesis (41). Accordingly, the postulated induced hypothyroidism following NaNO₃ exposure (38, 42) may be responsible for such hyperlipidemia. Since the liver play a pivotal role in lipid homeostasis in addition to glucose homeostasis the development of liver injury by administration of nitrosamine compounds is associating with a significant increase in liver lipid peroxidation content (increasing TBARS), oxidative stress and altering the antioxidant status in several tissues and the observed alteration of lipid profile (43, 44, 45, 46). Hassan et al., (47) suggested that the nitrosamine compounds that occurred after nitrate intubation may be responsible for hyperlipidemia observed in this experiment.

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