Effects of green tea extract on prevention and treatment of Dyslipidemia in cholesterol-fed male rabbits

Naiel Abbass K. Alkhafaji,
Dr. Abdul Razzak A. Latif
Babylon University, College of Medicine
Department of Pharmacology

الخلاصة:
الشاي الاخضر (اوراق نبات الكاميليا) له دور في تقليل خطر الاصابة بامراض القلب والشرايين من خلال منع الاكسدة للدهون بفضل المركبات البوليفينولية الموجودة في الشاي الاخضر. هذه الدراسة صممت لاجل اختبار فعالية مستخلص الشاي الاخضر في خفض نسب الدهون الضارة في الدم وزيادة نسبة البروتينات الدهنية المفيدة في الجسم . اجريت هذه الدراسة على ذكور الارانب باعطائها غذاء غني بالكولستيرول (بنسبة ١%) واستمرت الدراسة لمدة شهرين . الارانب التي استلمت غذاء غني بالكولستيرول قورنت نتائجها مع التي اعطيت غذاء طبيعي والمجاميع التي عولجت باستخدام مستخلص الشاي الاخضر والمجاميع التي اعطيت الشاي الاخضر كوقاية من ارتفاع الدهون وتصلب الشرايين . النتائج اظهرت انخفاض معنوي كبير في نسب الكوليسترول الكلي(TC) والدهون واطئة الكثافة (LDL) والكليسيريدات الثلاثية(TG) وكذلك وجد ارتفاع معنوي كبير في نسب الدهون المفيدة للجسم عالية الكثافة(HDL).
ألاستنتاج:استخدام مستخلص الشاي الاخضر المائي له دور مهم في خفض نسبة الدهون بالدم وتقليل خطر الاصابة بامراض تصلب الشرايين.

Abstract :
Green tea (leaves of *camellia sinensis*) has been associated with a reduced risk of cardiovascular disease.The mechanism of this risk reduction involves inhibition of lipoprotein oxidation by antioxidant polyphenolic compounds derived from tea .This Study was designed to test the hypolipidemic activity of aqoueous green tea extract in cholesterol-fed rabbits for a period of 2 months.
The rabbits were received a cholesterol-enriched diet(1% cholesterol) for 2 months and the results were compared to the control, treatment and prevention groups. The results showed that there is a significant decrease in total serum cholesterol(TC), triglycerides(TG),Low density lipoproteins (LDL)($P < 0.01$) and a significant increase in high density lipoprotein (HDL) ($P < 0.01$).
Conclusion : aqueous green tea extract has a hypolipidemic and antiatherosclerotic potentials.

Introduction :

Atherosclerosis involves multiple processes including endothelial dysfunction, inflammation, vascular proliferation and matrix alteration. Vascular proliferation contributes to the pathology of atherosclerosis and is linked to other cellular processes such as inflammation, apoptosis and matrix alterations.

Recent studies have emphasized the involvement of inflammation in mediating all stages of atherosclerosis(1,2). However,in addition to inflammation, a key process of at[herosclerosis involves the proliferation of vascular smooth muscle cells (VSMCs)(3,4). One precursor of lesion development in humans may be focal accumulation of VSMCs within the intima. However, VSMCs may also be important in maintaining the stability of the plaque through the formation of a firm fibrous cap.

Indeed, in lipid-laden lesions in which the fibrous cap is thin and weak, there is evidence of VSMC apoptosis, associated with inflammation(5).

Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea (6).

Green tea catechins such as epigallocatechin gallate significantly improve the serum lipid profile. These lipid-altering effects of green tea catechins occur because of their influence at various points in cholesterol metabolism(7,8). For instance, green tea extracts inhibit the absorption of lipids as well as cause inhibition of cholesterol synthesis. Cholesterol absorption is decreased by as much as 24% (9). Besides this, green tea extracts also cause upregulation of the LDL receptor. In fact, green tea consumption is associated with a decrease
in body mass index as well as body fat mass (10). Green tea catechins may have a major role to play in these weight losing effects of green tea, as they are potent inhibitors of enzymes such as glucose-6-phosphate dehydrogenase that may have a major role to play in the development of obesity (11). Similarly, green tea extracts also have a protective effect against nonalcoholic fatty liver disease (12).

Materials and methods :

Animals and Diet:

24 local domestic male rabbits were used in this study. Their weight ranged between 1.2 and 1.6 Kg, they were housed in the animal house of Babylon College of Medicine. They were put in cages with a cycle of 12 hrs. light/darkness cycle, with a room temperature of 25C and humidity kept at 50 -60 % and allowed to drink tap water and given standard chow diet. During first 7 days of adaptation, alfalfa and other vegetables were given with a small amounts, then after the end of the week, they were prevented completely to avoid the effects on lipid profile. The period of study was 8 weeks.

After the first week of acclimatization, the rabbits were randomly classified into 4 groups as follows:

1. Normal control Group: This group contains 6 male rabbits. All rabbits of this group were kept on standard chow diet and tap water through out the experiment (for 8 weeks).
2. Cholesterol – enriched diet Group (induced untreated group): This group contains 6 male rabbits. All rabbits of this group were kept on atherogenic diet (1% cholesterol-enriched diet) and tap water through all the experiment (8 weeks).
3. Green Tea Group (treatment group): This group consists of 6 male rabbits. All rabbits of this group were kept on 1% cholesterol-enriched diet and tap water for 4 weeks, then, they were treated with green tea aqueous extract 250 mg/kg body weight/ day orally for the next month and the treatment also continued with the diet (atherogenic diet).
4. hyperlipidemia prevention group: this group consists of 6 male rabbits . all rabbits of this group were kept on atherogenic diet with green tea extract 250 mg/kg/day orally and the cholesterol – enriched and green tea extract continued through all the experiment (8 weeks).

High cholesterol diet consists of 1%cholesterol(1gm for each 100mg of standard rabbit diet) (13).

Green Tea extraction:

Preparation of the extract: Distilled water was added to powdered plant (green tea) in a ratio of (1:5W/V) and boiled with continuous mixing for 10 minutes. Then was mixed for 15-20 minutes faraway from heat. The mixture was then filtered through a piece of soft cloth and filter paper to remove all the residual materials. The clear solution of the extract was dried at 45˚C by using oven and kept at 4˚C for further investigations (14). Green tea extract is used in a dose of 250mg/kg/day (15).

Blood Sampling:

From each rabbit about 4 ml of blood collected from the heart . The blood sampling was done at beginning of the study (zero time) , after 1 month and after 2 months of treatment . The blood Samples were allowed to clot at 37 C and centrifuged at 3000 rpm for 15 minutes . Sera were taken and analysed for the determination of serum total cholesterol(TC) , triglycerides(TG), high density lipoprotein(HDL-C) and low density lipoprotein(LDL-C).

Results :

At the beginning of study(day zero) ,there is no significant difference in serum total cholesterol(TC), triglycerides(TG) and low density lipoproteins(LDL) concentration in the 4 groups .At the end of the first and the second month there was significant increase in the serum concentrations of TC,TG and LDL in cholesterol group(atherosclerosis induced group) as compared with normal control group ($P < 0.01$) and there was significant increase in the TC, TG and LDL concentrations at the end of the second month as compared with the first month ($p < 0.01$).

In control and prevention groups, the serum concentration of TC,TG and LDL within the same group at different times , there was no significant changes in the serum TC,TG and LDL concentrations in normal control and prevention groups at different times.

There was significant reduction in the serum concentration of TC,TG and LDL in aqueous green tea extract treated groups as compared with cholesterol group (atherosclerosis induced group) ($P < 0.01$). Also, there was a significant reduction in TC,TG and LDL in treatment groups in the 2^{nd} month as compared to the 1^{st} month ($p < 0.01$).

Aqueous green tea extract prevention group showed a significant reduction in the serum concentration of TC,TG and LDL as compared to cholesterol group as shown in the figures 1,2 ,3 :

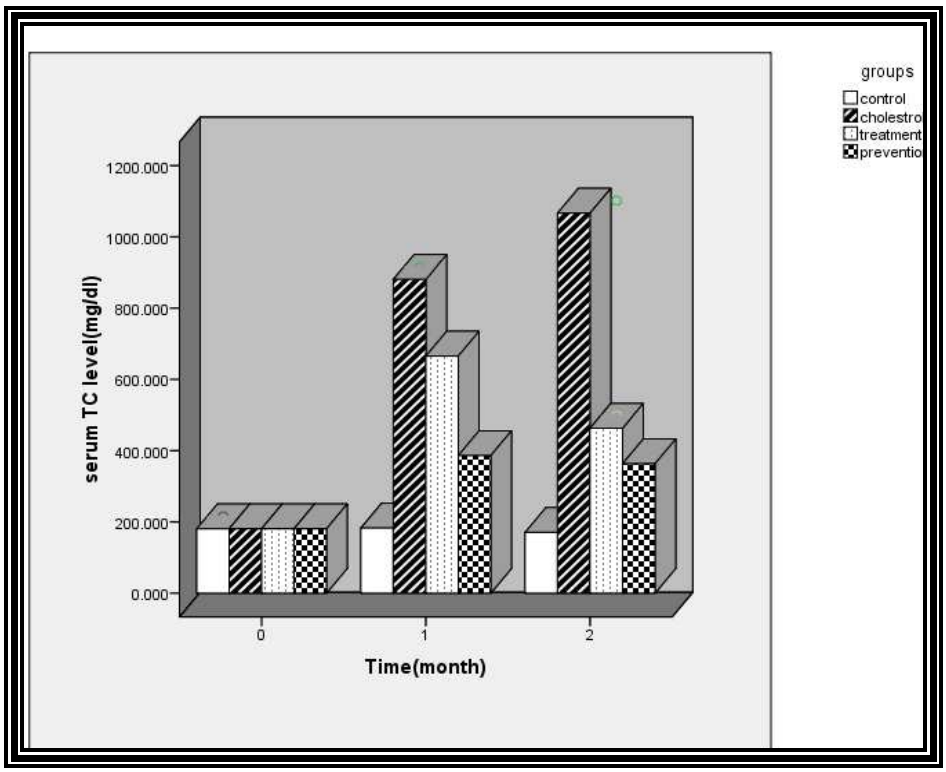

Figure -1: Mean Changes in the concentration of serum TC

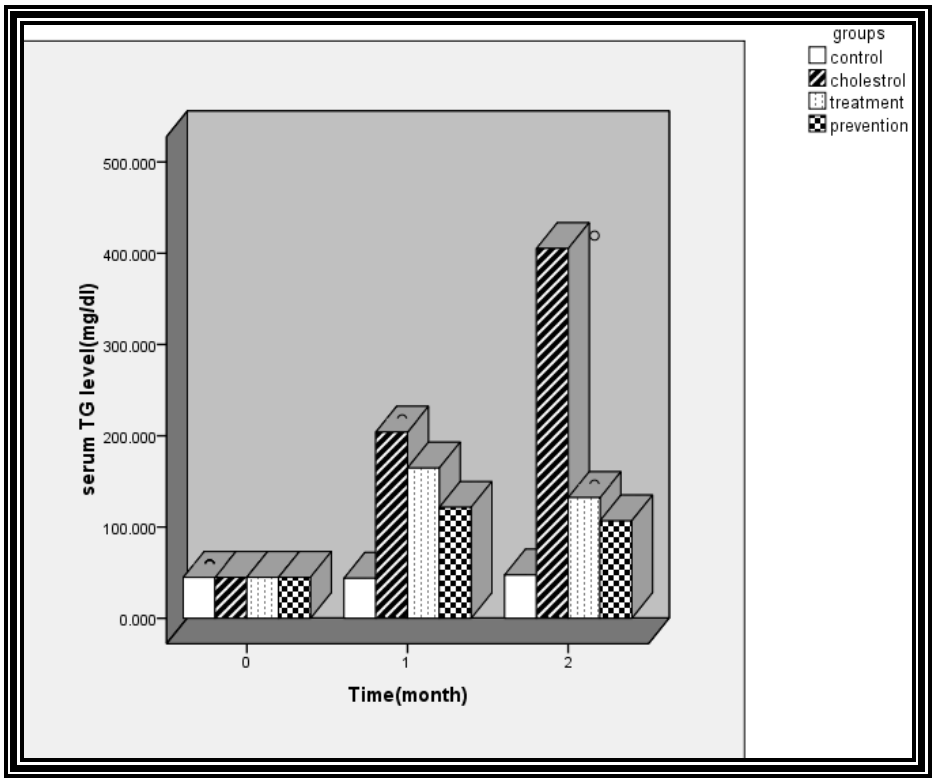

Figure-2: Mean changes in the concentration of serum TG

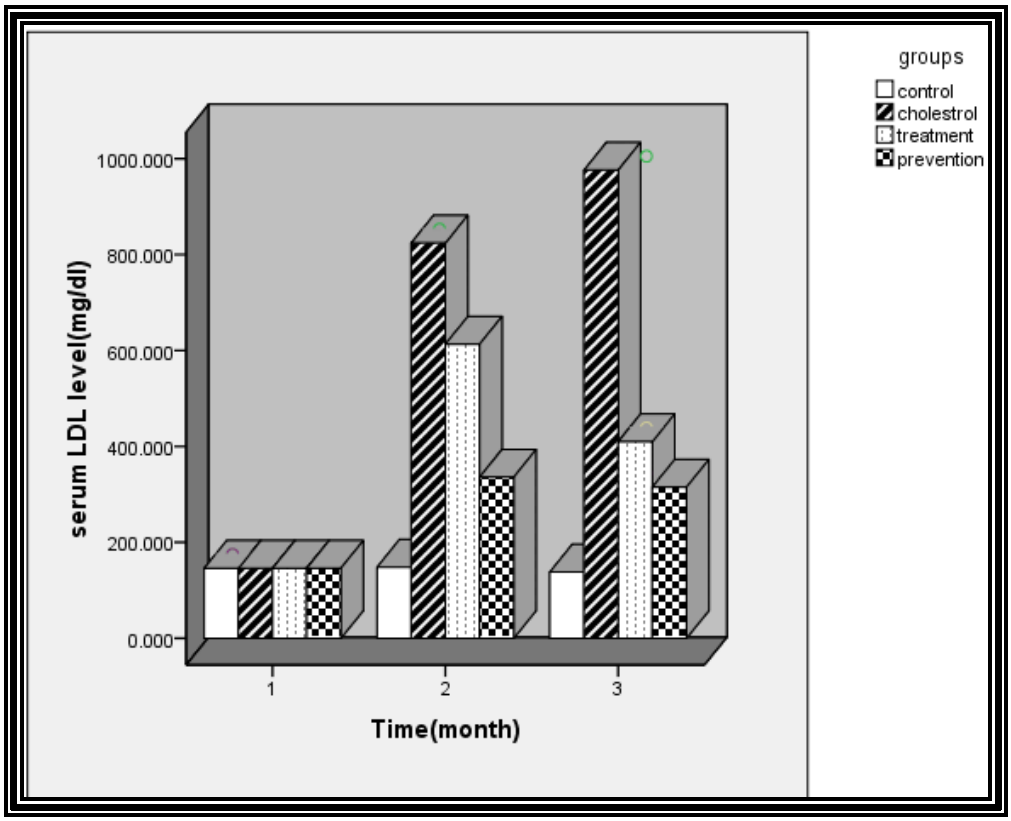

Figure-3: Mean Changes in the concentration of serum LDL-C

For high density lipoprotein(HDL), there is significant decrease in the serum concentration of HDL in cholesterol group as compared with normal control group ($P < 0.01$) and there was significant decrease in the HDL concentration at the end of the second month as compared with the first month ($p < 0.01$).

In control and prevention groups, the serum concentration of HDL within the same group at different times , there was no significant changes in the serum HDL-C concentration in control and prevention groups at different times and there is no significant changes in cholesterol induced group and treatment group in the first month (before beginning of treatment with green tea extract).

There was significant increase in the serum concentration of HDL in aqueous green tea extract treated groups as compared with cholesterol group (atherosclerosis induced group) ($P < 0.01$).Also, there is significant increase in HDL in treatment groups in the 2^{nd} month as compared to the 1^{st} month ($p< 0.01$).

In the aqueous green tea extract prevention group, there was a significant increase in the serum concentration of HDL as compared to cholesterol group and as compared to treatment group in the 1^{st} month(before the beginning of treatment)($p <0.01$) as shown in figure -4 :

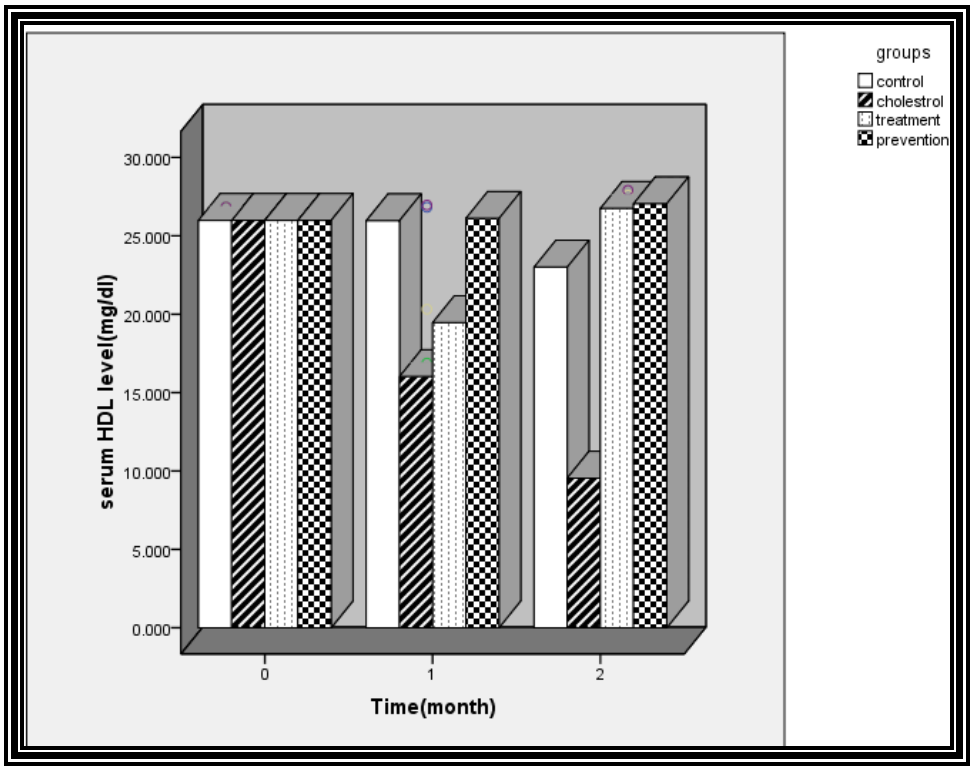

Figure-4 : mean Changes in the concentration of serum HDL.

Discussion :

The results had shown a significant decrease in serum total cholesterol(TC), triglycerides (TG), Low density lipoprotein(LDL) and a significant increase in high density lipoprotein (HDL)($P < 0.01$). These effects of green tea extract on lipid profiles had been found to be in agreement with Ramesh et al.(2008)(16). Green tea catechins affect lipid metabolism by various mechanisms and prevent the appearance of atherosclerotic plaque in various models of hyperlipidemia (17,18). In addition, catechins influence micellular solubility, luminal lipid hydrolysis and intestinal lipid absorption (8). Furthermore, green tea can up-regulate hepatic LDL receptor expression, thereby modulating biosynthesis, excretion and intracellular processing of lipids (19,7,20,21). Tea catechins were shown to reduce blood cholesterol levels and prevent the deposition and/ or accumulation of cholesterol in various tissues, including liver and heart.(21,22). The micellar solubilization of hydrolyzed lipids is the critical step for the uptake and absorption of lipids by enterocytes and this process facilitates the transfer of lipids through the unstirred water layer to the enterocyte for uptake (7). While green tea Catechins with gallate esters were shown to decrease cholesterol absorption by forming insoluble co-precipitates of cholesterol and decreasing bile acid-induced micellar solubility (23), green tea catechins may inhibit cholesterol absorption primarily by binding to cholesterol, thereby increasing fecal excretion of cholesterol (24).

Dietary flavonoids were reported to modulate the specific transport proteins located on the brush border membranes that play a significant role in the uptake of lipids by enterocytes (25,26). Based on these studies it was proposed that catechins may form complex with these transport proteins through hydrophobic interactions and hydrogen

bonding and therefore interfere with the uptake of lipids by enterocytes (7). Green tea catechins, especially , are not readily absorbed in both animals (27) and humans (28). Thus, green tea catechins may interfere with the steps involved in intestinal uptake of lipids due to their presence in high concentrations in the intestinal lumen (29). In addition, catechins may have direct inhibitory effect on cholesterol synthesis. It was recently found that green tea catechins are potent and selective inhibitors of squalene epoxidase, a likely rate limiting enzyme of cholesterol biosynthesis. The presence of galloyl moiety was suggested to be important for squalene epoxidase inhibitory activity of green tea catechins (30).

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