

Effect of Optional Consumption of Iranian Black and Green Tea on Lipid Profile of Serum and Liver in High-Fat Diet Rats

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Abstract

Objective: Tea, *camellia sinensis*, which is used as a popular beverage in Iran, may reduce the risk of cardiovascular diseases such as coronary heart disease (CHD). In this investigation, we studied the effect of Iranian black and green tea on serum and liver lipid profile which is closely associated with obesity and many cardiovascular diseases.

Materials and Methods: 24 male adult Wistar rats were randomly divided into 4 equal groups: Control (C), high-fat (HF), black tea (HF+BT), and green tea (HF+GT). Accordingly, serum total cholesterol (TC), triglyceride (TG), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were among the parameters determined in high fat diet rats. We also evaluated the TC, TG and total lipid (TL) content of liver.

Results: Results show that optional consumption of both of teas for 8 weeks significantly decreased serum TC by 40% and only black tea lowered serum TG and VLDL-C by 34.3%. Whereas the level of serum LDL-C decreased by 36.5% after using green tea. Consumption of green tea reduced TG and TC level at liver by 47.4% and 24.7%, respectively.

Conclusion: Based on lowering effect of Iranian black and green tea on lipid profile of serum and liver, presentation of tea together with a high fat diet reduces some of hyperlipidemia indices by significant amounts and may, therefore, reduce the risk of obesity and cardiovascular diseases.

Key Words: Iranian black and green tea, high-fat diet, hyperlipidemia, rat.

Introduction

Over nutrition and hypercaloric diets cause hyperlipidemia (1). Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease (2). Coronary heart disease, stroke,

atherosclerosis and hyperlipidemia are the primary causes of death in developed countries (3). Hyperlipidemia is characterized by elevated serum total cholesterol, low-density lipoprotein (LDL) and very low density lipoprotein cholesterol (VLDL-C) with decreased high-

density lipoprotein (HDL) levels. Hyperlipidemia-associated lipid disorders are considered to cause atherosclerotic cardiovascular diseases(4). Among these, hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (5). The main aim of the treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular diseases(6). Currently, available hypolipidemic agents drugs have been associated with a number of side effects (7). The consumption of synthetic drugs leads to hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. More than thirteen thousands plants have been studied for various pharmacological properties (8). Hyperlipidemia is classified into primary and secondary types, which indicates the complexities associated with the disease. Primary hyperlipidemia is caused rarely by genetic abnormalities and commonly by an unhealthy diet and inactivity. Primary hyperlipidemia is divided into hypercholesterolemia, hypertriglyceridemia and combined hyperlipidemia (9). Combined hyperlipidemia is typically characterized by concurrent elevations in total cholesterol and triglycerides with decreased high density lipoprotein cholesterol. Secondary hyperlipidemia may be due to diabetes, excess alcohol intake and adverse drug effects (10, 11). High-fat diet may lead to the production of extra VLDL, resulting in the formation of large amounts of LDL, which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, resulting in blockades of the normal blood flow. Therefore, improvement in human diet is highly recommended for disease prevention (12). The search for new drugs capable of reducing and regulating serum cholesterol and triglyceride levels has gained momentum over the years, resulting in numerous reports on significant activities of natural agents (13). Herbal products are frequently considered to

be less toxic than synthetic agents and so have led to the discovery of new therapeutic agents including antioxidants, hypoglycemics and hypolipidemics. It is well established that diet rich in vegetables and fruits can reduce cardiovascular diseases (14).

Tea (*Camellia sinensis*) has been utilized from time immemorial, as a beverage possessing encouraging health benefits. Green tea is rich in flavonoids and indeed epidemiological in vitro, and animal-model studies have related green tea consumption with health benefits, including decreased risk of inflammation (15, 16). These effects have largely been attributed to the most prevalent polyphenol contained in green tea, the catechin or flavanol (-) epigallocatechin-3-gallate (EGCG). There is a variation between bioactive components of plants that may be mainly attributed to the different sample extraction methods and different geographic origins of herbal tea. In addition to intrinsic factors, the flavonol content in plants is strongly influenced by such extrinsic factors as variations in plant type and growth, season, climate, degree of ripeness, food preparation and processing (17).

In Iran, tea is cultivated in northern area and same as many countries it is the most popular beverage after water. Although, there are many reports about lipid lowering effect of tea, but to the best of our knowledge there are no sufficient data about Iranian tea. So this study was conducted to evaluate the anti-obesity and lipid lowering effect of Iranian black and green tea in high-fat diet rats.

Materials and Methods

Chemical material

All chemical materials, including hexane (Merck, 822280), isopropanol (Merck, k995), chloroform (Merck, 822265), methanol (Merck, 6008), acetyl acetone (Merck, 800023), meta-periodate (Merck, 6597), glacial acetic acid (Merck, 90056), sulfuric acid (Merck, 713), ammonium acetate (Merck, 115), sodium sulfate (Merck, 6645), natrium hydroxide (Merck, 6462) and kalium

hydroxide (Merck, 5012) were purchased from Merck. Co (Tehran, Iran).

Preparation of tea

Iranian black and green tea was purchased from the market (Refah TEAMAN Co.). Tea extracts were prepared similarly to human consumption by soaking 10 g of tea leaves in 500 ml of boiling water for 30 min (18). The extracts were filtered before being given to the rats.

Animals

Male Wistar rats weighting 170-180 g were housed in groups of six at 22 ± 2 °C and $60\pm 5\%$ relative humidity with a 12 h light/dark cycle. The animals had free access to water and normal or high-fat diet ad libitum.

High-fat diet

According to Vinicius et al. (19) hypercaloric diets with energy between 3.7 Kcal/g and 5.4 Kcal/g are highly palatable and induce obesity. Diet at present study (4.2 Kcal/g), with high-fat content, was made from normal pulverized chow (83%), corn oil (11%), saccharose (5%) and casein and vitamin (1%).

Treatment of rats

After the adaptation period (7 days), the rats were divided into four groups, each containing six animals: the first group of rats (C) was fed with standard rat diet as the normal control group. The other groups of animals were fed high-fat diet. The third (HF+BT) and fourth (HF+GT) groups of rats were given, in addition to high-fat diet (HF), Iranian black and green tea ad libitum instead of water, respectively. During treatment, diet intake was measured daily and the body weights were recorded before starting the tea consumption and also after each week. At the end of 8 weeks, the overnight fasted rats were anesthetized with diethyl ether and their blood samples were collected by cardiac puncture and centrifuged at 3000 rpm, 4 °C for 15 min and then euthanized under diethyl ether anesthesia. All experiments were carried out according to the guidelines for the care and

use of experimental animals and approved by state veterinary administration of the University of Tehran. Blood and liver tissue samples were taken from the animals of all groups.

Preparation of liver extract

Lipids were extracted from the livers by the modified method of Hara and Radin (20). Briefly, 9 ml of extraction solution (hexane: isopropanol 3:2 v/v) was added to 0.5 g of liver and was homogenized by glass beads for 8 h at room temperature. After homogenization, the organic phase was separated by centrifugation at $2000 \times g$ for 10 min, dehydrated by saturated sodium sulphate and finally used for the TG (20), TC (21) and total lipid(22) assay.

Biochemical analysis of serum

TG, TC, HDL-C and LDL-C in serum were determined by enzymatic kits (Pars Azmoon, Iran) and VLDL cholesterol was calculated as TG/5.

Statistical analyses

Data were analyzed using a one-way analysis of variance (ANOVA) and the post hoc Tukey test. Values are expressed as means \pm standard deviation (S.D.) for six rats per group. Significance was accepted at the level of $p<0.05$.

Results

Effects of Iranian tea on physical parameters

The daily diet intake of the rats decreased ($p<0.05$), in groups HF, HF+BT and HF+GT, while intake of energy increased in this groups in comparison with C (Fig. 1). High-fat diet apparently caused an increase in the body weight of rats of HF despite their lower food intake. However, in Group HF+BT and HF+GT the body weights of the tea consumed rats decreased. Abdominal fat pad (inguinal, retroperitoneal and mesenteric) of rats in groups C and HF+BT was decreased ($p<0.05$), (table 1).

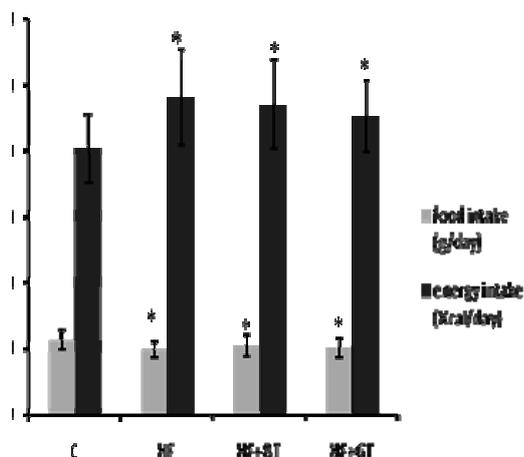


Fig. 1. Diet intake of rats fed standard laboratory diet or high-fat diet during the 8-week feeding period. C, control; HF, high-fat diet; HF+BT, high-fat diet + black tea; and HF+GT, high-fat diet + green tea. Values are mean \pm S.D., n=6.

Effect of Iranian black and green tea on serum lipid profile

The general effect of tea consumption on serum lipid levels was examined in rats fed high-fat diet. Feeding the animals a high-fat diet for 8 weeks resulted in a significant increase in the serum TC and TG levels. Both kinds of tea significantly decreased serum TC by 40% and only black tea lowered serum TG and VLDL-C by 34.3%. Whereas the level of serum LDL-C decreased by 36.5% by green tea ($p < 0.05$). Results show that consumption of Iranian black and green tea had no effect on HDL-C together with a high fat diet including corn oil (table 2).

Effect of high-fat diet on lipid content of liver

The effects of Iranian black and green tea on lipid content of liver are shown in Table 3. High-fat diet caused an increase in the TG and TC levels. Consumption of green tea lowered their TG and TC by 47.4% and 24.7%, respectively.

Discussion

Disturbances occurring in lipid metabolism are among the main causative factors for cardiovascular diseases.

Despite the presence of different hypolipidemic drugs in the market, their therapeutic application is usually associated with severe side effects (23). Hence, efforts are being made to find more safe and efficient anti-hyperlipidemic drugs.

In this regard, medicinal plants have been considered as promising resources for the discovery of new drugs. In the present study, the hypolipidemic activity of optional consumption of Iranian black and green tea was evaluated together with a high-fat diet in rats.

Based on our data, the body weight of rats of HF increased relative to group C. This is probably due to supplementation with high-fat diets. Consumption of black and green tea effectively prevented the increase in body weight to a significant extent.

Cholesterol is an essential structural element of the biological membranes. In addition, it is the precursor of many compounds such as the initiating materials for the synthesis of bile acids, steroid hormones, and vitamin D among others. Despite this knowledge, high concentration of serum cholesterol increases the risk of developing CHD (24).

The present study demonstrated that rats fed a high-fat diet showed a higher concentration of serum TC compared to rats fed a standard laboratory diet, while optional consumption of Iranian black and green tea reduced the high level of TC. It has been reported that low plasma TG levels are associated with lower risks of CHD (25). In our study, optional consumption of Iranian black tea by hyperlipidemic rats significantly lowered their serum TG levels.

Joe and Yousef (26) reported that black and green tea supplementation decreased serum TG by 20% and 48%, respectively in the hamster.

Table 1- Effect of consumption of Iranian black and green tea on physical parameters in diet-induced hyperlipidaemic rats.

	C	HF	HF+BT	HF+GT
Weight gain (g)	57.33±8.04*	76.33±11.003	59.40±8.79*	58.20±4.97*
Liver (g)	10.54±1.84	10.998±1.678	10.75±1.21	10.7±2.13
Kidney (g)	1.24±0.34	1.793±0.287	1.89±0.34	1.99±0.25
Heart (g)	0.94±0.19	0.98±0.126	0.95±0.12	0.88±0.15
Abdominal fat pad(g)	3.07±0.59*	4.186±0.769	*2.9±0.56	3.14±0.54
BMI (g/cm ²)	0.58±0.04	0.59±0.05	0.6±0.06	0.61±0.06

C, control; HF, high-fat diet; HF+BT, high-fat diet + black tea; and HF+GT, high-fat diet + green tea. Values are mean±S.D. Number of observations is six. * Significantly different with HF. ($p < 0.05$)

Table 2- Effect of consumption of Iranian black and green tea on serum lipid levels in diet-induced hyperlipidaemic rats.

	C	HF	HF+BT	HF+GT
TC	87.6±16.10	111.5±20.20	66.9±13.01*	67.07±15.31*
TG	64.68±12.30*	91.40±12.77	60.10±11.59*	77.16±14.29
LDL-C	31.85±3.42*	47.93±11.45	39.08±5.52	30.46±1.65*
HDL-C	37.56±4.96	37.38±7.20	40.40±5.24	37.66±7.16
VLDL-C	12.93±2.461*	18.28±2.554	12.02±2.11*	15.43±2.86

C, control; HF, high-fat diet; HF+BT, high-fat diet + black tea; and HF+GT, high-fat diet + green tea. Unit: mg/dl. Values are mean±S.D., Number of observations is six. * Significantly different with HF. ($p < 0.05$)

Table 3- Effect of consumption of Iranian black and green tea on lipid content of liver in diet-induced hyperlipidaemic rats.

	C	HF	HF+BT	HF+GT
TC	4.00±0.55*	5.39±1.8	4.45±0.55	4.06±0.67*
TG	8.23±1.59*	14.77±3.17	11.13±3.01	7.77±1.67*
TL	28.08±3.91	31.32±4.15	30.84±2.67	29.25±6.30

C, control; HF, high-fat diet; HF+BT, high-fat diet + black tea; and HF+GT, high-fat diet + green tea. Unit: mg/g. Values are mean±S.D. Number of observations is six. * Significantly different with HF. ($p < 0.05$)

It is widely accepted that elevation of plasma LDL levels is a major risk factor for CHD (23).

Direct correlation between LDL and atherosclerosis and also the reversibility of the related pathological events by lowering the serum level of LDL have already been reported by many research groups (27, 28). Our data indicated that, the high concentration of LDL-C in hypercholesterolemic rats was significantly reduced by optional consumption of green tea. Therefore, green tea might constitute a good candidate for the treatment of atherosclerosis by lowering serum LDL-C level.

Phytochemicals, especially the phenolics found in fruits and vegetables, have been proposed as the major bioactive compounds providing the health benefits associated with diets rich in plant-foods. Flavonoids are a class of secondary plant phenolics found ubiquitously in fruits and vegetables as well as

food products which act as pharmacological active compounds in many medicinal plants (29). Many of the biological action of flavonoids have been attributed to their powerful hypolipidemic properties (30, 31). Weggemans and Trautwein reported that flavonoids intake decreased LDL-C and increased HDL-C in hypercholesterolemic individuals (32). In present study, high-fat diet including corn oil had no effect on serum HDL-C level and subsequently effect of optional consumption of Iranian black and green tea was not significant.

Regarding the high total polyphenolics and flavonoids of tea, the results strongly suggest that the hypolipidemic activity of this popular beverage could be attributed to the presence of the valuable polyphenolic compounds specially catechins. The decrease in cholesterol balance, which indicates the total change in body pools of cholesterol, may be due to the compensatory mechanisms, such as

inhibitory effect on the intestinal absorption of lipids (33) This may prevent an excessive elevation in serum cholesterol levels and the accumulation of lipids in the liver.

Japanese Green tea has also been reported to have a preventive effect on hyperlipidemia and lipid accumulation in the liver and aorta of mice fed a cholesterol-enriched diet for 14 weeks (34).

Nobuya Shirai and Hiramitsu Suzuki (35) reported that a green tea extract intake significantly lowered the liver TC and TG concentrations in the high-fat dietary group. Our data indicated that, the high concentration of TC and TG in high-fat diet rats was significantly reduced by optional consumption of Iranian green tea.

The reduction in liver cholesterol content may also be explained by the disrupting effect of tea catechins on micelle formation (36). Bile acids can only be re-absorbed from the small intestine in the form of micelle. Tea catechins

prevent the re-absorption of bile acids by disrupting the micelle formation and thus increasing bile acid excretion. In order to replenish the loss in bile acids, the conversion of cholesterol to bile acids in the liver will be enhanced and it further reduces the content of liver cholesterol (37).

Conclusions

Based on lowering effect of Iranian black and green tea on lipid profile of serum and liver, presentation of tea together with a high-fat diet reduces some of hyperlipidemia indices by significant amounts and may, therefore, reduce the risk of cardiovascular diseases.

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