The Effects of Oral Administration of Green Tea and Ginger Extracts on Serum and Hepatic Lipid Content in Rats Fed a Hyperlipidemic Diet

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Abstract

The aim of this study was to investigate the effect of water extracts of green tea, ginger, or a combination of both on serum and hepatic total cholesterol (TC), low density lipoprotein-cholesterol (LDL-c), high density lipoprotein-cholesterol (HDL-c), triglycerides (TG), and total phospholipids in induced-hyperlipidemic Wistar albino rats. A group of 30 male albino rats was divided into two groups. The first group was fed a basal diet as a normal control (NC), while the second group was fed a hyperlipidemic diet for five weeks to induce hyperlipidemia. The latter group was then subdivided and administered a hyperlipidemic diet, or a hyperlipidemic diet supplemented with green tea extract, ginger extract, or both. In groups whose diets were supplemented with the extracts, we found that total body weight was reduced as compared to positive control (PC) animals. Likewise, a significant reduction in serum TC, LDL-c, TG, and total phospholipids was observed, accompanied by an increase in HDL-c levels. In the liver, a slight reduction in TC and TG was observed, though total phospholipid levels remained relatively similar. Importantly, we observed no synergism between the two extracts. Together, our data suggest that consumption of green tea or ginger could aid in the treatment of obesity and other diseases related to cardiovascular disease (CVD).

Key words: hyperlipidemia, total cholesterol, atherogenic diet, green tea and ginger.

Introduction

Hyperlipidemia is defined as the condition in which the level of plasma lipids, primarily cholesterol and triacylgycerol, are higher than the normal range. Hyperlipidemia is an important risk factor in atherosclerosis, which can lead to CVD [1]. In Libya, the mortality rate due to CVD is high, likely because of the fact that the population consumes a traditional diet rich in carbohydrates and animal fats, especially red meat, and relatively low in plant foods.

A wide variety of therapeutic agents in modern medicine are available for the treatment of hyperlipidemia. However, most hypolipidemic drugs cause potentially serious side effects, and include digestive disturbances, nausea and vomiting. Regular usage of many herbs has been recommended in the management of hyperlipidemia [2], such as garlic, onions, and cinnamon.

Consumption of green tea (*Camellia sinensis*) and ginger (*Zingiber officinale*) beverages are among the most widespread habits in Libya, such that their effects may be highly relevant for public health. Ginger extract reduces the level of cholesterol and lipid peroxidation[3], though no reports are available on the potential synergetic action of green tea and ginger in hyperlipidemic-induced rats.

The objective of this study was to investigate the effects of the consumption of water extracts of green tea, ginger, or both, after administration of a hyperlipidemic diet in rats.

Materials and Methods

Animals

Thirty healthy adult male Wistar albino rats weighing between 120 and 150 g were bred in the central animal house of al-Arab Medical University (Benghazi, Libya). The animals were housed in an air-conditioned room in stainless steel cages at room temperature (22 ± 2ºC) and 60% relative humidity with 12-h light/dark cycles. The animals had free access to a nutritionally adequate standard pellet diet (Benghazi Animal Feed Company, Benghazi, Libya) and tap water for 1 week prior to the experiment.

Experimental protocol

Rats were divided into two groups. The first group (n = 6) was fed a standard pellet diet with tap water to drink as a normal control (NC), while the second group (n = 24) was fed a hyperlipidemic diet for 5 weeks to induce hyperlipidemia. This diet consisted of standard pellets supplemented with 20% sucrose and 10% coconut oil, with tap water to drink. After the acclimatization period this group was randomly divided into subgroups. All of the subgroups remained on the hyperlipidemic diet for the nine week experiment, but their drink was substituted for green tea extract, ginger extract, or a combination of both (Table 1).

Tea extracts were prepared in a similar fashion as is typically done for humans in Libya. Fifteen grams of tea leaves or ginger rhizome slides were soaked in 500 mL of boiling water for 30 min and were then filtered. Extracts were prepared fresh daily.

Rats were euthanized by decapitation under light anesthesia with diethyl ether following an overnight fast. Blood was collected from the trunk and serum was separated by centrifugation at 4000 *x g* for 15 min at room temperature. Whole livers were quickly removed from each animal and rinsed in cold physiological saline. Tissue portions were stored at -70ºC prior to analysis.

Biochemical assays

The level of triglycerides and total cholesterol in the serum were assayed using kits from BioMeriecux, France, according to previously described methods [4,5]. Serum HDL-c was estimated after the LDL and VLDL fractions were precipitated by heparin – manganese chloride as described [6].

Serum LDL-c concentration was calculated using the Friedewald formula [7]. Liver cholesterol and triglycerides were extracted according to the method described by Folch

[8]. Briefly, liver samples were homogenized in nine volumes of physiological saline and extracted with a chloroform - methanol mixture (2:1 v/v).

Table 1. Dietary treatments

Group	**n[a]**	**Diet treatment**
Normal control (NC)	6	standard diet + tap water
Positive control (PC)	6	hyperlipidemic diet + tap water
Green tea extract (GT)	6	hyperlipidemic diet + green tea extract
Ginger extract (GI)	6	hyperlipidemic diet + ginger extract
Green tea and ginger extract (GG)	6	hyperlipidemic diet + green tea and ginger extract

[a] number of animals in each group.

The aqueous layer from each sample was extracted a second time, after which the aqueous layers were pooled. The organic phase from each sample was dried under nitrogen gas and suspended in physiological saline. The cholesterol and triglyceride contents in each suspension were determined with a diagnostic kit from BioMeriecux, France. The serum and liver total phospholipids were estimated by the method of Barlett [9].

Statistical analysis

All values are expressed as mean ± SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). P values less than 0.05 were considered statistically significant.

Results and discussion

Cholesterol is an important constituent of cellular membranes, and is a precursor of steroid hormones and bile acids. However, high cholesterol levels in the blood is the primary cause of CVD, and can result in atherosclerosis, myocardial infarction, and coronary heart disease [10]. As the incidence of CVD-related deaths in Libya is on the rise, research concerning the management of hyperlipidemia and associated diseases is warranted. Therefore, this study investigated the contribution of green tea and ginger, traditionally consumed by the Libyan population, to hyperlipidemia.

As expected, administration of a hyperlipidemic diet induced significant weight gain in our experiment (Table 2). However, consumption of either green tea extract, ginger extract, or both after the induction of hyperlipidemia, prevented the weight gain observed for the PC group. As excessive weight gain has been implicated as a risk factor for development of hypertension, ischaemic heart diseases and heart failure [11], these data

suggest that administration of these extracts may have benefits on those suffering from obesity.

It is possible that the loss in body weight observed in the hyperlipidemic rats fed the green tea extract was due to the catechins present in tea, as they are known to inhibit intestinal absorption of dietary lipid, And interfere with the emulsification, digestion, and micellar solubilization of lipids, which are critical steps involved in the intestinal absorption of dietary fat, cholesterol, and other lipids [12]. Importantly, we observed no differences in food consumption between the treatment groups, suggesting that differences in caloric intake cannot explain the results seen here, and further supports the traditional claim for the usefulness of these teas to treat obesity and weight loss.

Table 2. Body weight after dietary treatments

Group	Initial body weight (g)	Final body weight (g)	Body weight gain (g day^{-1})
Normal control (NC)	140.32 ± 2.64	278.52 ± 5.02	2.19 ± 1.06
Positive control (PC)	140.37 ± 3.93	394.37 ± 7.63	4.03 ± 2.13 [a]
Green tea extract (GT)	140.47 ± 4.10	277.46 ± 5.10	2.17 ± 1.23 [b]
Ginger extract (GI)	140.49 ± 4.23	277.87 ± 5.76	2.18 ± 0.89 [b]
Green tea and ginger extract (GG)	140.38 ± 3.07	276.98 ± 4.73	2.17 ± 0.93 [b]

Values are expressed as the mean ± S.D. for 6 animals in each group.
Body weight gain (g day^{-1}) = [Final body weight (g) - Initial body weight (g)] ÷ 63.
$p<0.05$. [a] Significantly different from NC group. [b] Significantly different from PC groups. [c] Significantly different from GT group. [d] Significantly different from GI group

We observed a reduction in TC, TG, LDL-c, and phospholipids in the serum of rats administered green tea, ginger, or both (Table 3). However, no change in HDL-c was observed. Importantly, others have observed that diets high in carbohydrates and saturated fat cause increases in serum LDL-c and hepatic triglycerides without excessive energy intake [13,14], and that consumption of sucrose increases plasma triglyceride [17]. VLDL, and LDL-c [16], and decreases the HDL-c concentration. We also observed an increase in serum TG in the PC animals, who only were fed a hyperlipidemic diet with tea supplements. This increase could be due to increased secretion of TG from the liver, or from decreased TG removal from the blood [18]. Previous studies have shown that a high-fat diet in rats increases the long-chain acyl-CoA content in red muscle and liver [19]. Therefore, it is expected that a diet high in carbohydrates and fat would increase the acyl-CoA pool, and lead to increased triglyceride storage, as seen in this study (Table 3).
We next assayed for changes in the lipid content in the liver, because high liver lipid content represents potential risk factors for cardiovascular disease. We observed that green tea, ginger, or the combination of both decreased liver TC and TG (Table 4).

However, the hyperlipidemic diet (PC group) did not induce a change in liver phospholipids.

The concentration of plasma triglycerides, total cholesterol, and phospholipids decreased significantly in the GT, GI, and GG groups. It has been reported that polyphenolic compounds present in black and green tea lowers the concentration of lipids in hyperlipidemic rats through the reactivation of lipoprotein lipases, and increased fecal excretion of cholesterol and bile acids [20,21]. The lipid-lowering effect of tea consumption in rats has also been reported and is assumed to be due to increased intestinal fermentation and formation of volatile fatty acids and acetates in the caecum and colon [22]. These alterations stimulate the secretion of humoral factors from the large intestine or central nervous system to modify cholesterol metabolism [23]. Likewise, a comprehensive study in rats found that ginger extract reduced cholesterol, inhibited lipid peroxidation, and reduced the development of atherosclerosis [3]. Consumption of ginger extract may be beneficial in attenuation of the development of atherosclerosis, since it is associated with reduced macrophage-mediated oxidation of LDL, reduced uptake of oxidized LDL by macrophages, reduced oxidative state of LDL, and reduced LDL aggregation. All of these effects lead to a reduced cellular cholesterol accumulation and foam cell formation, the hallmark of early atherosclerosis (3).

Atherogenic indices were 0.91 ± 0.05, 0.74 ± 0.13, and 0.73 ± 0.07 in the NC, GT and GI groups, respectively, but the differences were not statistically significant. The atherogenic index was significantly higher in the PC group than the other groups (2.12 ± 0.44), where in the GG group it was 0.67 ± 0.04, which was slightly lower than that in the GT and GI groups, but was significantly lower than in both the NC and PC groups (Table 5). Administration of green tea, ginger, or both increase the HDL-c / TC ratio and decreased the LDL-c / TC ratio. These observations are important, as they predict a lower risk of developing heart disease. Likewise, a high HDL-c / LDL-c raio has been shown to be beneficial and is indicate of a lower risk of CVD [24], and is what was observed here (Table 5).

Together our data suggest that green tea and ginger extracts, either alone or in combination, are effective at treating weight-gain and hyperlipidemia in rats. These extracts may be ideal beverages in retarding the development of CVD and obesity in the general population.

Table 3. Changes in serum total-cholesterol, triglycerides, HDL-c, LDL-c and phospholipids after dietary treatments

Group	**total cholesterol (mmol L^{-1})**	**triglycerides (mmol L^{-1})**	**HDL-c (mmol L^{-1})**	**LDL-c (mmol L^{-1})**	**phospholipids (mmol L^{-1})**
Normal control (NC)	1.83 ± 0.04	0.61 ± 0.03	1.10 ± 0.04	0.75 ± 0.03	2.87 ± 0.17
Positive control (PC)	3.32 ± 0.19 [a]	0.87 ± 0.05 [a]	1.09 ± 0.17	1.84 ± 0.12 [a]	5.90 ± 0.10 [a]
Green tea extract (GT)	2.31 ± 0.23 [a, b]	0.64 ± 0.03 [b]	1.33 ± 0.06 [a, b]	0.69 ± 0.18 [b]	2.57 ± 0.15 [a, b]
Ginger extract (GI)	2.21 ± 0.15 [a, b]	0.63 ± 0.02 [b]	1.28 ± 0.13 [a, b]	0.64 ± 0.03 [a, b]	2.59 ± 0.22 [a, b]
Green tea and ginger extract (GG)	2.03 ± 0.10 [a, b, c, d]	0.59 ± 0.03 [b, c, d]	1.22 ± 0.07 [a, b, c]	0.54 ± 0.04 [a, b, c, d]	2.47 ± 0.08 [a, b]

Values are expressed as the mean ± S.D. for 6 animals in each group.
$p<0.05$. [a] Significantly different from NC group. [b] Significantly different from PC groups. [c] Significantly different from GT group. [d] Significantly different from GI group.

Table 4. Changes in liver total cholesterol, triglycerides, and total phospholipids after dietary treatments

Group	**total cholesterol (mmol g^{-1})**	**triglycerides (mmol g^{-1})**	**total phospholipids (mmol g^{-1})**
Normal control (NC)	0.14 ± 0.02	0.11 ± 0.01	0.56 ± 0.04
Positive control (PC)	0.55 ± 0.03 [a]	0.21 ± 0.01 [a]	0.50 ± 0.07 [a]
Green tea extract (GT)	0.40 ± 0.05 [a, b]	0.16 ± 0.02 [a, b]	0.55 ± 0.06 [b]
Ginger extract (GI)	0.39 ± 0.03 [a, b]	0.18 ± 0.02 [a, b, c]	0.54 ± 0.05 [b]
Green tea and ginger extract (GG)	0.38 ± 0.04 [a, b]	0.16 ± 0.01 [a, b]	0.56 ± 0.06 [b]

Values are expressed as the mean ± S.D. for 6 animals in each group.
$p<0.05$. [a] Significantly different from NC group. [b] Significantly different from PC groups. [c] Significantly different from GT group. [d] Significantly different from GI group.

Table 5. Changes in the atherogenic index and the serum HDL-c / total cholesterol, LDL-c / total cholesterol, and HDL-c/LDL-c ratios after dietary treatments

Group	Atherogenic index	HDL-c / total cholesterol	LDL-c / total cholesterol	HDL-c/LDL-c
Normal control (NC)	0.91 ± 0.05	0.52 ± 0.02	0.36 ± 0.02	1.47 ± 0.11
Positive control (PC)	2.12 ± 0.44 [a]	0.33 ± 0.04 [a]	0.56 ± 0.04 [a]	0.59 ± 0.11 [a]
Green tea extract (GT)	0.74 ± 0.13 [b]	0.58 ± 0.04 [a, b]	0.30 ± 0.05 [a, b]	2.03 ± 0.55 [a, b]
Ginger extract (GI)	0.73 ± 0.07 [b]	0.58 ± 0.02 [a, b]	0.29 ± 0.02 [a, b]	1.99 ± 0.22 [a, b]
Green tea and ginger extract (GG)	0.67 ± 0.04 [a, b]	0.60 ± 0.02 [a, b]	0.26 ± 0.02 [a, b]	2.27 ± 0.22 [a, b]

Values are expressed as the mean ± S.D. for 6 animals in each group.
$p<0.05$. [a] Significantly different from NC group. [b] Significantly different from PC groups. [c] Significantly different from GT group. [d] Significantly different from GI group.

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