Cholesterol/HDL-c ratio lowering effect of green tea in rats exposed to depot medroxyprogesterone acetate

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ABSTRACT

Objective: This study aimed to investigate whether an extract of green tea is able to decrease low density lipoprotein-cholesterol (LDL-c) and increase high density lipoprotein (HDL)-c in rats exposed to depot medroxyprogesterone acetate (DMPA). Methods: Twenty five female Wistar rats were divided into five groups (n = 5 each): (1) untreated negative control; (2) positive control which received DMPA only; (3) DMPA plus green tea extract at the dose of 10.8 mg/kg/day; (4) DMPA plus green tea extract at the dose of 21.6 mg/kg/day; (5) and DMPA plus green tea extract at the dose of 43.2 mg/kg/day. The treatment with green tea was performed for four weeks. Serum lipid profile and atherogenic index were analyzed by automated enzymatic technique. Results: DMPA significantly changed the serum lipid profile marked by decreased HDL, increased LDL and cholesterol/HDL-c ratio compared with the untreated group. The increase in LDL was significantly attenuated by all treatment doses of green tea. Similarly, the decrease in HDL level was significantly attenuated by all doses of the extract. On the other hand, increased levels of cholesterol/HDL-c ratio in the DMPA group was significantly reduced by lowest and highest doses of green tea extract. Conclusion: Green tea extract inhibits the increase in LDL-c and cholesterol/HDL-c ratio induced by DMPA. This may provide a natural therapeutic alternative for normalizing the lipid profile found in subjects exposed to DMPA.

KEY WORDS: Catechin, lipid, progestin, tea

INTRODUCTION

Previous studies showed inconsistent results regarding lipid profile in women who received depot medroxyprogesterone acetate (DMPA) treatment; this may be attributable to race, duration of treatment and intake behavior. The high density lipoprotein-cholesterol (HDL-c) level was reported to be not significantly different in women after 2, 6, 12 months of DMPA injection [1]. In another study, DMPA significantly decreased HDL-c compared with the control group [2]. Other study compared the new acceptors and chronic users of DMPA: a moderate increase in serum total triglyceride at the expense of the very low density lipoprotein (VLDL) fraction was reported in the group of chronic DMPA users; in new acceptors DMPA insignificantly decreased total cholesterol and HDL-c [3]. After 3 months of DMPA treatment, the HDL-c levels significantly decreased and a significant increase in LDL-c, but no change in total lipids, total cholesterol and triglycerides were reported. In women who received DMPA for 15 months, the total lipids, total cholesterol and LDL-c levels were significantly increased and HDL-c significantly decreased. It was suggested that the possibility risk of atherosclerosis appears as there are significant changes in serum lipid levels [4].

Animal and epidemiological studies suggest that green tea catechins may reduce the risk of cardiovascular diseases [5]. Green tea intake induces significant reductions in total and LDL-c levels [6]. Green tea consumption as drink significantly decreased cholesterol, LDL-c, ratio of cholesterol/HDL-c, and increased the HDL-c level [7]. The normalization of hypertriglyceridemia was better with green and black tea drink compared with oolong tea extract, but plasma HDL-c concentrations were not affected by any tea extracts [8].
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As far we know, there is no study focusing on the effect of green tea extract on lipid profiles in rats received DMPA treatment. Accordingly, in the present study, we investigated the effect of DMPA treatment on the lipid profile and the potential ameliorative action of green tea extract in experimental rats.

MATERIAL AND METHODS

Animals

A total of 25 female Wistar rats, weighing 100-125 g, provided by the Animal Facility of Physiology Laboratory, Faculty of Medicine, University of Brawijaya, Malang, Indonesia, were housed in standard laboratory conditions. All animals received food and water ad libitum during the experimental period. They were divided into following groups (n = 5 rats for each group): (1) untreated control group; (2) positive control group which received DMPA; (3) DMPA plus green tea extract at the dose of 10.8 mg/kg/day; (4) DMPA plus green tea extract at the dose of 21.6 mg/kg/day; and (5) DMPA plus green tea extract at the dose of 43.2 mg/kg/day. The green tea treatment was performed at subchronical level for 28 days. The study protocol has been approved by the local research ethics committee, Faculty of Medicine, University of Brawijaya, Malang, Indonesia.

Green tea extraction

Green tea plants were harvested from Wonosari Tea Plantation, Lawang, Malang, East Java, Indonesia. The protocol for preparation and extraction were followed according to the method described by Fard et al [9] with minor modification. The cleaned leaves of green tea dried at 40°C in a dark room for 3 days and grounded into fine powder using a miller. Then, the powder (200 g) was stirred for 8 h at room temperature. Finally, the extract was then filtered and concentrated under negative pressure at 40°C and 70°C for 1 h, respectively. The extract was oven dried (at 40°C) overnight to produce powdered extracts. The residue was then dissolved in 3000 ml of distilled water and stirred for 5 h at room temperature. Finally, the extract was then filtered and concentrated under negative pressure at 40°C and 70°C for 1 h, respectively. The extract was oven dried (at 40°C) overnight to produce powdered extracts. Finally, the extract was stored at -20°C in airtight containers until the application in the experiment.

DMPA treatment

DMPA (Depo Progestin®) was injected intramuscularly every single week for four weeks at a dose of 2.7 mg/kg. This drug was mixed with 0.2 ml of saline. DMPA dose is calculated according on the conversion of the human dose for rats.

Lipid profile and atherogenic index assay

After the experimental period, rats in all groups were anesthetized by ether. Blood samples were collected by cardiac puncture. The serum lipid profiles, including total cholesterol, triglycerides (TG), HDL-c and LDL-c were assayed using the method reported earlier by Lu et al [10]. The atherogenic index (AI) was defined by following formula: “TC − HDL-c / HDL-c”

Statistical analysis

Data are presented as the mean ± standard deviation (SD) and differences between groups were analyzed using one way analysis of variance (ANOVA) with SPSS software (version 15.0). If the ANOVA was significant, post hoc test was applied; P values less than 0.05 were considered statistically significant.

RESULTS

Serum LDL-c levels were significantly higher in the DMPA group compared to the untreated control group (P < 0.05). Only the highest dose of the green tea extract achieved the LDL-c level in the untreated group (P > 0.05). First and second doses of the extract significantly prevented DMPA-induced increase in LDL-c level, but can’t reach the control level. Besides, there was no significant difference between the effects of these two lowest doses.

The HDL-c levels were significantly lower in the DMPA group compared to the untreated group. These decreased HDL-c levels were significantly increased by all doses of the green tea extract. Among the three doses, administration of 10.8 mg/kg (lowest dose) extract to the DMPA-treated rats elevated HDL-c levels to comparable values with the untreated group. Besides, the second and third doses of the extract significantly increased HDL-c levels to higher values than the untreated group.

The cholesterol/HDL-c ratio was found to be significantly greater in the DMPA group compared to the untreated controls. The ratio was significantly reduced by the lowest and highest doses of the tea extract, comparable to the untreated group.

Table 1 summarizes the overall outcome of the study.

DISCUSSION

Reverse cholesterol transport (RCT) is the biochemical process by which cholesterol is transported from the peripheral tissues and cells to the liver. The main function of RCT is to inhibit atherogenesis by decreasing the plasma cholesterol level [12]. The process of RCT can be divided into three stages: (1) the efflux of cellular cholesterol from peripheral cells as HDL; (2) conversion of cholesterol in HDL to cholesteryl esters (CE) by lecithin-cholesterol acyl transferase and the transport of cholesterol as CE within the core of HDL particles to the liver; (3) delivery of CE from HDL to hepatocytes [12, 13].

In the present study, we found that DMPA significantly increased the level of LDL-c and cholesterol/HDL-c ratio compared to the untreated animals. In addition, the HDL-c levels were also significantly lower in the DMPA group than those of the untreated control group. This finding indicates that DMPA may alter the reverse cholesterol transport. As mentioned above, previous studies reported inconsistent results regarding the lipid metabolism alteration by DMPA treatment [3, 4]. In the present study, low and medium
doses of the green tea extract significantly prevented DMPA-induced increase in LDL-c levels, but can’t reach the level of the untreated control group. However, administration of 10.6 mg/kg extract (low dose) to the DMPA-treated rats elevated HDL-c levels to nearly untreated control values. Moreover, the lowest and highest doses of green tea extract decreased the cholesterol/HDL-c ratio, comparable to the untreated group. These results confirmed the previous reports that green tea intake induces significant reductions in total cholesterol and LDL-c level [6]. After drinking green tea, a significant beneficial improvement in the lipid profile, including a decrease in total cholesterol, LDL-c, ratio of cholesterol/HDL-c, but an increase in HDL-c level was reported [7]. Decrease in the level of cholesterol/HDL-c ratio and HDL/LDL-c ratio is linked to a reduction in the risk of morbidity and mortality in cardiovascular diseases [13] due to DMPA treatment.

In conclusion, green tea extract inhibits the increase in LDL-c and cholesterol/HDL-c ratio induced by DMPA. This result may provide a natural therapeutic alternative for normalizing the lipid profile found in subjects exposed to DMPA.

### REFERENCES