The Effect of Aerobic Training Combined and Green tea (*Camellia sinensis* L.) Extract Consumption on Blood Glucose and Lipid Profile in Streptozotocin Induced Diabetic Rats

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Abstract

Background: Type 2 diabetes is a global health problem and a major cause of illness. Exercise, diet and medication are the three pillars in the treatment of type 2 diabetes.

Objective: The aim of the present study was to investigate the effect of aerobic training combined with green tea hydroalcoholic extract consumption on blood glucose and lipid profile on diabetic rats.

Methods: Diabetes was induced in 40 male Wistar rats by intraperitoneal injection of 50 ml/kg streptozocin. After two weeks the diabetic rats with fasting blood glucose of 150 to 300 mg/dl were divided into 4 groups of 10 rats each and named as: aerobic exercise, aerobic exercise with green tea, green tea, and control. Aerobic exercise was performed as running on Treadmill for 5 days a week daily for 30-90 minutes with Treadmill speed of 18 -24 meters per minute. Green tea extract (100 mg/kg) was gavages once a day for 5 weeks. At baseline and endpoint fasting blood glucose, cholesterol, HDL-c, LDL-c and triglyceride levels were determined in all groups.

Results: Fasting blood glucose level decreased significantly in all groups but triglyceride and cholesterol decreased in aerobic exercise and aerobic exercise + green tea groups at end point compared with baseline and also compared with control group. Fasting blood LDL-c level significantly decreased and HDL-c level significantly increase, in aerobic exercise and aerobic exercise with green tea at end point compared with baseline and also compared with control and green tea group.

Conclusion: Aerobic exercise favorably affects glucose and lipid profile and in combination with green tea it has synergistic effects.

Keywords: Aerobic exercise, Green tea, Lipid profile, Glucose, Rat

Introduction

Diabetes mellitus is a global health problem and a major cause of illness and is the sixth leading cause of death worldwide [1]. It has been reported that 6/6 % of people aged 20 to 79 years had diabetes in 2010 and it estimated that in the year of 2025 about 300 million people suffering from diabetes and in 2030 it will increase up to 366 million worldwide [2]. Hyperglycemia and hyperlipidemia are important cardiovascular risk factors [3]. Most people with diabetes consider using alternative treatments, including herbal medicine [4].

Green tea (*Camellia sinensis* L.) is one of the world's widely *consumed beverages* that used in traditional medicine for treatment of many chronic diseases including diabetes [5-7]. The beneficial effect of green tea has been reported not only on cardiovascular diseases but also on obesity and type 2 diabetes [8-9]. Green tea contains many chemical components such as epigallocatechin gallate, various amino acids, enzymes, minerals and vitamins [10]. The favorable effect green tea on insulin resistance, increase in fat metabolism and its consequent triglyceride and cholesterol lowering effects are key contributors for managements of hyperglycemia [11-12].

In addition exercise is universally acknowledged as vital component required for managing blood glucose levels and hyperlipidemia [13-14]. In type 2 diabetic patients aerobic exercise increases the action of insulin and indirectly increases glucose utilization by skeletal muscles [15].

Thus, according to the joint role of physical activity and consumption of green tea in the control of diabetes and presence of controversy reports, this study was undertaken to find out that whether aerobic training combined with green tea extract can effectively improve glucose and lipid profile in diabetic rats.

Materials and Methods

Plant material

Camellia sinensis leaves were collected at month of June from Tonekabon hills and identified by a botanist (M. Ahvazi). A voucher specimen of the plant was deposited in the herbarium (herbarium code no. 685) of the Institute of Medicinal Plants, ACECR. Samples were dried at 25°C and stored at 5°C.

Preparation of plant extract

About 500 g of dried leaf was extracted for 24 hour with 3 liter 70% aqueous ethanol using percolation method at room temperature. The procedure was repeated two times more for complete extraction. The extracts were mixed and filtered through Whatman no. 1 filter paper and evaporated to dryness under reduced pressure at a maximum of 40ºC using a rotary evaporator.

Determination of total phenols in extract

The concentration of total phenols in extract was measured by the method described by Kim et al (2003) with some modification [16]. Briefly, an aliquot (1 ml) of appropriately diluted extracts or standard solutions of gallic acid in water (50, 100, 150, 200 and 250 µg/ml) was added to a 25 ml volumetric flask containing 9 ml of ddH_2O. A reagent blank using ddH_2O was prepared. One milliliter of Folin & Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (25 ml) with ddH_2O and mixed thoroughly. After incubation for 90 min

at 23 ºC, the absorbance versus prepared blank was read at 750 nm. Total phenol contents of extracts were expressed as mg gallic acid equivalents (GAE)/g extract. All samples were analyzed in 3 replications.

Induction of diabetes in rats

Forty adult Wistar rats weighting 250-300 grams (75-90 days old) were obtained from our own breeding colony. Animals were maintained under standard environmental conditions and had access to standard rodent feed and water.

The rats were fasted overnight and diabetes was induced by single intraperitoneal injection of 50 mg/kg streptozotocin in citrate buffer. After 10 days time for the development of diabetes, the rats with blood glucose level of 150-300 mg/dl were considered as diabetic and selected for experiment.

Experimental design

Total 40 diabetic rats were divided into 4 groups: 1-control, 2-green tea, 3-aerobic training, and 4-aerobic training + green tea. After one week of adaptation the exercise protocol began with treadmill. Simultaneously green tea extract at the dose of 100 mg/kg was dissolved in distilled water and gavaged to the rats in both 2 and 4 groups for five weeks. Similarly distilled water was gavaged to the rats in both 1 and 3 groups. In exercise training groups at the first session of the first week, the rats run on treadmill at a speed of 18 meters per minute for 30 minutes and it was increased 15 minutes daily up to maximum 90 minutes at end of first week. From second to fourth week of study, exercise time was steady and treadmills speed increased to 24 m/min. Exercise time and treadmill speed was fixed at fifth week [17].

At baseline and end of the study, 48 hours after the last exercise session and green tea gavages and after eight hours of fasting, rats were anesthetized with chloroform and blood samples were taken from the rat's hart for determination of glucose, cholesterol, HDL-c, LDL-c and triglyceride levels. Fasting blood glucose level was determined by the glucose-oxidase method using a Beckman Glucose-2 Analyzer immediately after blood sampling. All other blood sample parameters were measured by auto analyzer Hitachi 902 using commercially available kits (Pars Azmon).

Statistical analyses

The results were expressed as means ± S.D. and analyzed with the Two-Way ANOVA followed by the tukey post hoc test. $P < 0.05$ was taken as significant.

Results

The green tea extracts possessed total phenol content expressed as gallic acid equivalent 553.04 ± 5.47 mg Gallic acid/g of the dried extract.

The fasting blood glucose level and lipid profile data are summarized in Figure 1 - 5. Result indicated that fasting blood glucose decreased significantly in aerobic exercise (P=0.04), aerobic exercise + green tea (P=0.00) and green tea (P=0.01) groups at endpoint compared with baseline and also compared with control group.

The fasting blood triglyceride decreased significantly in aerobic exercise and aerobic exercise + green tea groups at endpoint compared with baseline (P=0.02 and P=0.01 respectively) and also compared with control group (P=0.02 and P=0.01 respectively).

The fasting blood cholesterol level

significantly decreased in aerobic exercise and aerobic exercise + green tea groups at endpoint compared with baseline (P=0.03 and P=0.02 respectively) and also compared with control group (P=0.03 and P=0.01 respectively)

Blood HDL-c level increased significantly in aerobic exercise and aerobic exercise + green tea group at endpoint compared with control and green tea group (p=0.01 and p=0.00 respectively) and also compared with baseline (p=0.01 and p=0.00 respectively).

Blood LDL-c level was decreased significantly (p=0.00) in aerobic exercise and aerobic exercise + green tea group at endpoint compared with control (p=0.00) and green tea (p=0.00) groups and also compared with baseline (p=0.00).

Discussion

The results indicate that green tea and aerobic training alone or in combination produced a marked fall in blood glucose level but combination therapy did not showed any synergistic effect. Further, green tea did not show significant effect on lipid profile but aerobic training produced favorable effects on blood lipid profile however, combination of green tea and aerobic training produced more pronounced lowering effect on blood lipid profile. It means combination of green tea and aerobic training provides positive synergistic effect on lipid profile but not on blood glucose level.

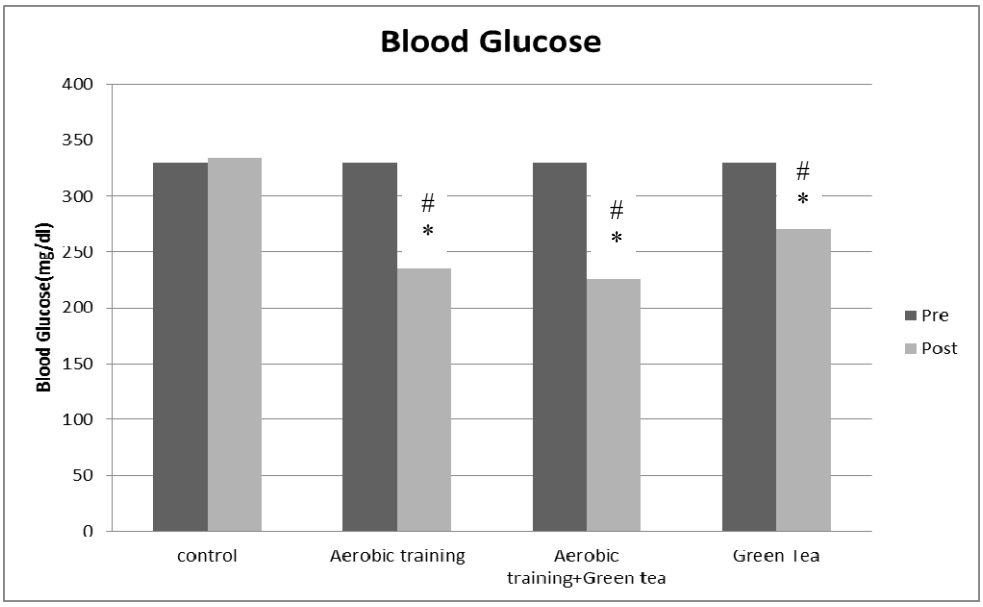

Figure 1- Changes in blood glucose level after five weeks of aerobic training and green tea (100 mg/kg) administration alone or in combination compared with each other and with control group. P < 0.05 was taken as significant (Two-Way ANOVA followed by the tukey post hoc test). *: Significant difference at endpoint compared with control group. #: Significant difference at endpoint compared with baseline.

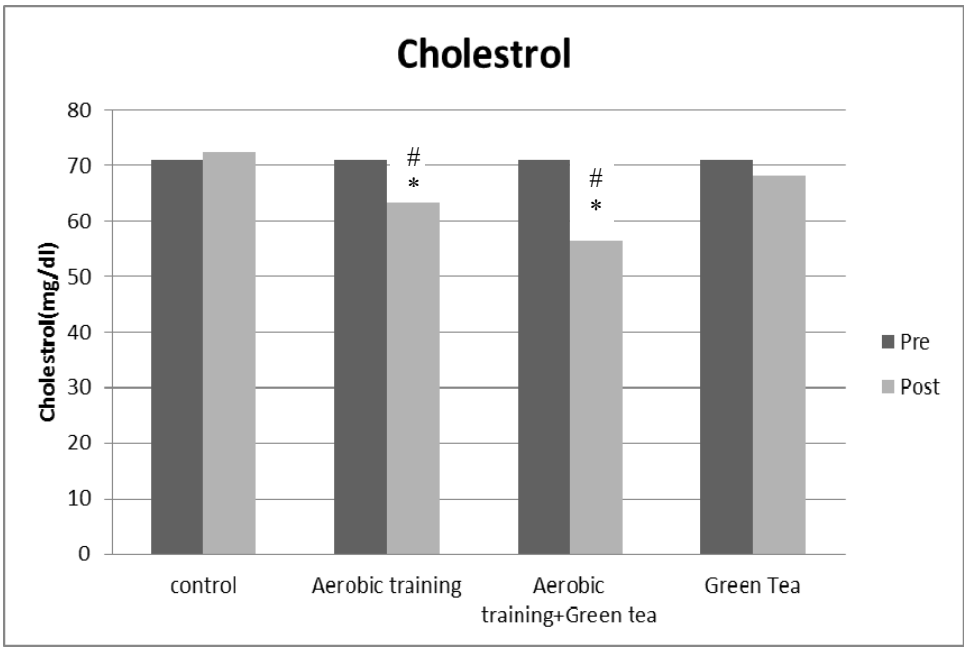

Figure 2- Changes in blood cholesterol level after five weeks of aerobic training and green tea (100 mg/kg) administration alone or in combination compared with each other and with control group.
P < 0.05 was taken as significant (Two-Way ANOVA followed by the tukey post hoc test).
***: Significant difference at endpoint compared with control group.**
#: Significant difference at endpoint compared with baseline.

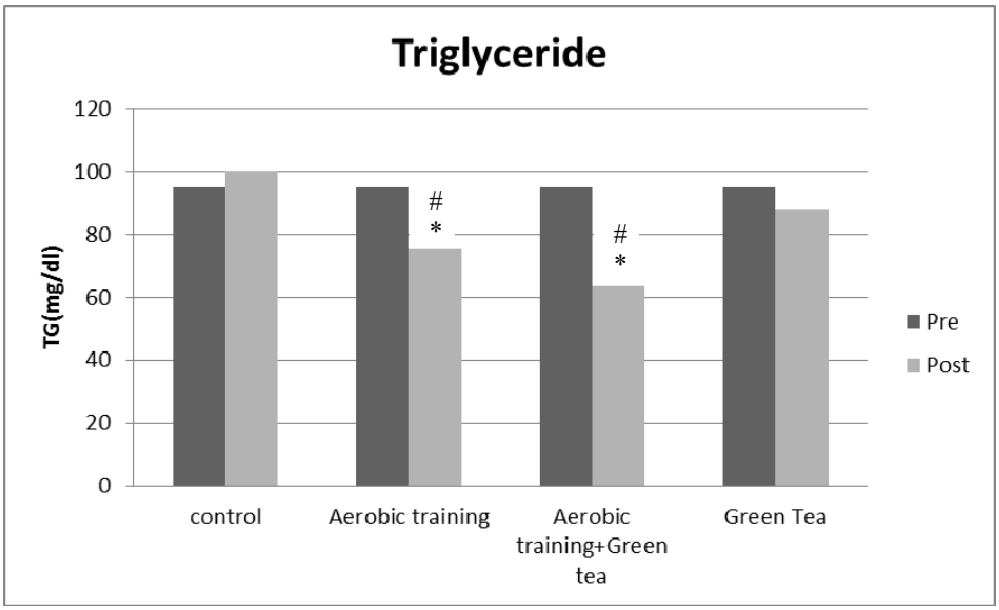

Figure 3- Changes in blood triglyceride level after five weeks of aerobic training and green tea (100 mg/kg) administration alone or in combination compared with each other and with control group. P < 0.05 was taken as significant (Two-Way ANOVA followed by the tukey post hoc test).
***: Significant difference at endpoint compared with control group.**
#: Significant difference at endpoint compared with baseline.

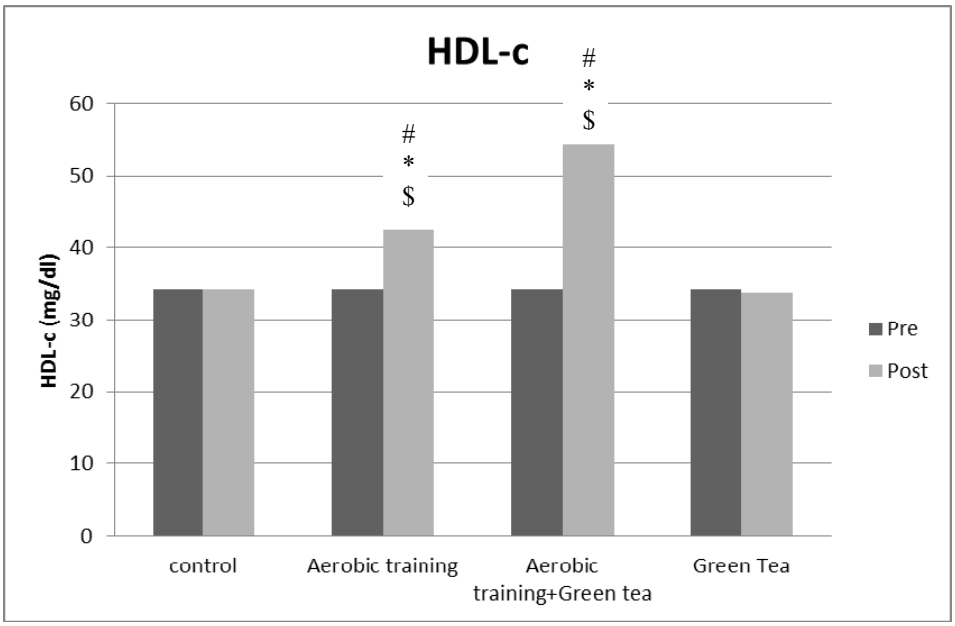

Figure 4- Changes in blood HDL-c level after five weeks of aerobic training and green tea (100 mg/kg) administration alone or in combination compared with each other and with control group. P < 0.05 was taken as significant (Two-Way ANOVA followed by the tukey post hoc test).
***: Significant difference at endpoint compared with control group. #: Significant difference at endpoint compared with baseline. $: Significant difference at endpoint compared with green tea group.**

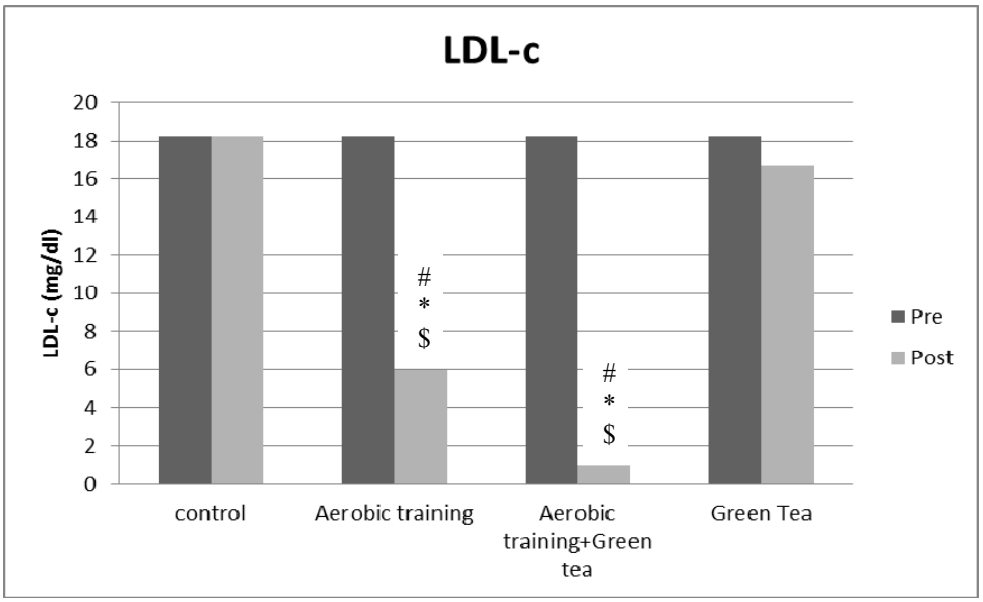

Figure 5- Changes in blood LDL-c level after five weeks of aerobic training and green tea (100 mg/kg) administration alone or in combination compared with each other and with control group. P < 0.05 was taken as significant (Two-Way ANOVA followed by the tukey post hoc test).
***: Significant difference at endpoint compared with control group.**
#: Significant difference at endpoint compared with baseline.
$: Significant difference at endpoint compared with green tea.

Although there are controversy reports regarding role of physical activity and consumption of green tea on blood glucose and lipid profile, several mechanisms are proposed for their favorable effects. It has been reported that, exercise can increase sugar consumption via muscle cells and there by decrease blood glucose concentrations [18]. During and after exercise, due to muscle activity, the tissue glucose metabolism rises [19]. The effects of green tea on of GLUT4 increase glucose transfer into the cells without increase in insulin level and its negative effect on amylase enzyme can reduce glucose uptake from foods [7-8]. Green tea caffeine and *catechin* components also increases fat metabolism and compound niacin in green tea reduces apo-lipoproteins A-I absorption and increases plasma pre βHDL-c, thereby increases plasma HDL-c level [20]. In addition green tea feeding to rats increases fecal excretion of cholesterol [11].

Physical activity also can lead to increase blood levels of HDL-c [21]. Exercise via effects on HDL-c, accelerate reverse transfer process and reduces LDL-c sticking to blood vessels [22], increased capillaries density, improves distribution of oxygen to the active muscles and improves efficiency of trained muscles to oxygen uptake [19].

Several reasons may propose for controversy effects of exercise and green tea on glucose and lipid profile compared with our study. The effect of green tea on blood glucose in our study is in contrary with Foniko, et al 2005 [23] finding. It seems that the cause of difference is due to lower dose of green tea used in that study.

The favorable effects of aerobic exercise on glucose metabolism in present study is in contrary with Yavari et al 2011[24] study that, may be due to difference in intensity and frequency of aerobic exercise between two studies. The effects of aerobic exercise combined with the consumption of green tea on triglyceride level in present is in contrary with results of Shimotoyodome research [25], that may be due to lower training time per session and lack of uniform training protocol in that study. Of note; use of single dose green tea and lack of glycosylated hemoglobin determination were the present study limitation.

Conclusion

Combination of aerobic exercises and green tea treatment to diabetic rat showed synergistic effect on improving lipid profile in diabetic rat but did not showed synergistic effect on reducing blood glucose level. Considering synergistic effect combination of aerobic exercises and consumption of green tea on lipid profile, conduction of human clinical trials regarding their safety and efficacy in hyperlipidemic patients are recommended.

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