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## Effect of green tea on obesity and serum lipid profiles in both normo- and hyper-cholesterolemic rabbits

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### ABSTRACT

Obesity has been linked with several comorbidities like cardiovascular disorders, diabetes, and hypertension. Weight reduction is directly associated with the consumption of green tea. So, with this premise of background, we evaluated the potential of green tea consumption on obesity and serum lipid profiles in both normo- and hyper-cholesterolemic rabbits. Sixty New Zealand white rabbits (age: 6-8 months; weight 1.5-2 kg) were used for this experiment. Rabbits were randomized and equally divided into three different groups. Group I (control group) received 10 ml/kg of distilled water twice daily, Group II received high cholesterol diet twice daily, and Group III received high cholesterol diet along with green tea solution 10 ml/kg, twice daily for 90 days. Weight measurements and serum analysis were conducted on Day 0 and day 90. For the obtained data, SPSS analysis was performed (SPSS 21). A significant increment of body weight was observed in control ( $1.97 \pm 0.12$  vs  $2.87 \pm 0.21$ ,  $p < 0.0001$ ) and high cholesterol groups ( $1.91 \pm 0.23$  vs  $3.13 \pm 0.31$ ,  $p < 0.0001$ ) at Day 90 when compared with Day 0. Though the results were not significant, there was a reduction in the body weight in the green tea group ( $2.20 \pm 1.47$  vs  $1.72 \pm 0.22$ ) at Day 90 when compared with Day 0. Consumption of green tea for 90 days resulted in weight loss and inhibition of the increase in the levels of TC, LDL, and VLDL. So, green tea can act as a natural food supplement for the management of obesity and dyslipidemia.



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### INTRODUCTION

Tea has been considered as the most popular beverage worldwide especially in China and Japan. It is consumed worldwide (Chen and Lin, 2015). Tea is prepared from the leaves collected from the plant *Camellia sinensis* and has been widely used for medical purposes since ancient times. On the

basis of the method of processing of the leaves, tea can be classified as green tea (unfermented), black tea (fermented), and oolong tea (partially fermented). Green tea is more consumed in China and Japan while black tea is more popular worldwide (Yang *et al.*, 2015; Fujimura *et al.*, 2011). Black tea is prepared by drying and crushing the tea leaves after harvesting to facilitate the process of oxidation. In the process of oxidation, the indigenous tea polyphenols (primarily catechins and gallic acid) are converted into polyphenols (mainly theaflavins and thearubigins). Oolong tea is prepared by partial fermentation while green tea is unfermented tea leaves (Yuan, 2011).

Green tea is composed of various healthy components such as flavonols, flavonoids, polyphenols and other constituents such as organic acids, vitamins, lipids, polysaccharides, and thiamine. Polyphenols are considered to be the most important

active ingredient in green tea. Among the polyphenols present in green tea, the most predominant polyphenols are catechins accounting for about 25-35% of dry green tea weight (Rahmani *et al.*, 2015).

Over the past three decades, the severity of obesity has gradually enhanced in the world (Tsai *et al.*, 2011; Ligibel *et al.*, 2014). Obesity is considered as an important growing health problem. It has been associated with cardiovascular risk factors like hyperlipidemia and hypertension (Spiotta and Luma, 2008). Obesity also has been reported to cause hypercholesterolemia which is a metabolic disorder characterised by elevated level of total cholesterol in the blood (Csonka *et al.*, 2016). Hypercholesterolemia affects mainly the adult population especially in the developed countries (Kuklina *et al.*, 2009).

Green tea has been inspected broadly for its role in the management of several systemic diseases like metabolic disorders, obesity, diabetes, and growth (Yang *et al.*, 2018). Existing investigations according to the function of green tea in weight reduction have been conflicting in indicating an advantageous potential of green tea consumption on weight reduction (Quinhoneiro *et al.*, 2018; Rothenberg *et al.*, 2018).

So, in an attempt to find an appropriately safe and available natural product for the treatment of both obesity and hypercholesterolemia, we investigated the role of green tea on both obesity and lipid profile.

## MATERIALS AND METHODS

### Animals

Since rabbits are considered as the most suitable model for the studies of human hypercholesterolemia (Fan *et al.*, 2015), 60 New Zealand white rabbits were used in this study. Their age ranged from 6-8 months, and body weights ranged from 1.5-2 kg. The rabbits were examined physically and were adopted before the study for two weeks by allocating them in a single animal cage. They were maintained at the optimal room temperature (22-25°C) and were then introduced to the artificial light for about 12 hrs per day. The Scientific and Ethics Committee of the College of Medicine, University of Kirkuk have approved this study protocol.

### Green Tea

Green tea was obtained from Lipton in the form of tea backs. Each tea back contained 2 grams of green tea.

### Preparation of green tea solution

The green tea solution was heated to 80 °C for 30 minutes in 8:1 water to tea ratio (Vuong *et al.*, 2011).

## Cholesterol

Cholesterol powder was purchased from Hi-Media.

### Experimental design

Sixty rabbits were randomized and equally divided into 3 groups (with 20/group). Group I (control group) rabbits were administered with 10 ml/kg distilled water twice/day orally for 90 days by oral gavages. Group II consisted of rabbits that were fed with high cholesterol diet (2% w/w cholesterol mixed with conventional animal diet and 10 ml/kg of distilled water), twice daily orally for 90 days by oral gavages. Group III consisted of the rabbits that were fed with high cholesterol diet (2% w/w cholesterol mixed with conventional animal diet) and green tea solution 10 ml/kg, twice daily for 90 days.

### Blood Sampling and estimation of serum lipids

The blood samples were obtained from the rabbit's marginal ear vein and centrifuged at 3000 rpm for 10 minutes. The serum collected by micropipette (Cobas system Roche, Germany) was then analysed for triglycerides (TGs), Total cholesterol (TC) high-density lipoprotein cholesterol, (HDL-c), and low-density lipoprotein cholesterol (LDL-c) by kit based methods (Cobas co. Germany).

### Body Weight and food intake

The animals were weighed on day 0 and at the end of the 90th day to see the effect of green tea administration on animals' weight measured by a sensitive electronic balance. All animal groups were provided with similar food spills. The food pins were cleaned and refilled daily.

### Statistical Analysis

Data analysis was done by SPSS version 21. All the data were represented as mean±SD and n (%). The levels of all the parameters at Day 0 were compared with the levels at Day 90 in each group by paired t-test. The levels of all the parameters at Day 0 and Day 90 were compared between groups by One-way ANOVA. A p value<0.05 was considered significant.

## RESULTS

### Body weight

The average body weight at Day 0 was 1.97 ± 0.12, 1.89 ± 0.23, and 2.21 ± 1.39 Kg for the control group, high cholesterol group and high cholesterol with green tea group respectively. The weights at Day 0 did not vary significantly between the different groups (p=0.15) (Table 1).

There was a considerable increment in the body weight in control (1.97 ± 0.12 vs 2.87 ± 0.21 Kg, p<0.0001) and high cholesterol groups (1.89 ±

**Table 1: Effect of Green Tea on body weight**

Group	Body Weight (Kg) (Day 0)	Body Weight (Kg) (Day 90)	P value
Group I (n=20)	1.97±0.12	2.87 ± 0.21	<0.0001
Group II (n=20)	1.89±0.23	3.13 ± 0.30	<0.0001
Group III (n=20)	2.21±1.39	1.73 ±0.20	0.13
P value (overall, I Vs. II, II Vs. III, I Vs. III)	0.15, 0.15, 0.32, 0.45	0.002, 0.002, <0.0001, <0.0001	

Values represented as Mean ± SD. Weight at Day 0 was compared with weight at Day 90 in each group by paired t-test. Weight at Day 0 and weight at 90 days was compared between groups by One-way ANOVA. A p value<0.05 was considered significant.

**Table 2: Comparison of lipid profiles on Day 0 and Day 90 in different groups**

Parameters	Groups	Day 0	Day 90	P value
TC (mg/dL)	I	42.6 ± 8.33	42.1 ± 6.75	0.84
	II	41.75 ±9.36	1100.00 ± 287.00	<0.0001
	III	40.25 ± 13.72	451.25 ± 223.83	<0.0001
P value (overall, I vs. II, II vs. III, I vs. III)	-	0.78	<0.0001, <0.001, <0.001, <0.001	-
TG (mg/dL)	I	67.25 ± 9.66	67.50 ± 10.11	0.94
	II	70.50 ± 8.87	197.75 ± 32.55	<0.0001
	III	67.63 ± 7.14	196.75 ± 23.47	<0.0001
P value (overall, I vs. II, II vs. III, I vs. III)	-	0.44	<0.0001, <0.001, >0.05, <0.001	-
LDL (mg/dL)	I	10.85 ± 3.38	12.05 ± 3.80	0.31
	II	11.10 ± 4.09	784.50 ± 212.19	<0.0001
	III	10.85 ± 4.32	198.5 ± 16.71	<0.0001
P value (overall, I vs. II, II vs. III, I vs. III)	-	0.97	<0.0001, <0.001, <0.001, <0.001	-
VLDL (mg/dL)	I	7.25 ± 2.10	7.85 ± 2.72	0.43
	II	6.10 ± 1.86	531.50 ± 91.78	<0.0001
	III	7.4 ± 1.88	200.00 ± 12.57	<0.0001
P value (overall, I vs. II, II vs. III, I vs. III)	-	0.07	<0.0001, <0.001, <0.001, <0.001	-
HDL (mg/dL)	I	24.67 ± 1.59	24.95 ± 2.67	0.68
	II	23.60 ± 4.55	49.75 ± 9.24	<0.0001
	III	22.35 ± 4.92	50.75 ± 6.94	<0.0001
P value (overall, I vs II, II vs III, I vs III)	-	0.32	<0.0001, <0.0001, 0.46, <0.0001	-

Values represented as Mean ± SD. Parameters at Day 0 was compared with parameters at Day 90 in each group by paired t-test. Parameters at Day 0 and Parameters at 90 days were compared between groups by One-way ANOVA. A p-value <0.05 was considered significant. TC: Total cholesterol, TG: tri-glycerides, HDL-c: high-density lipoprotein cholesterol, and LDL-c: low-density lipoprotein cholesterol.

0.23 vs. 3.13 ± 0.30 Kg, p<0.0001). Although not significant (p=0.13), there was a fall in the body weight in the high cholesterol with the green tea group (2.21 ± 1.39 vs 1.73 ± 0.20 Kg) (Table 1).

Out of the 20 rabbits in the high cholesterol with green tea group, 15 rabbits (75%) showed weight reduction, 2 rabbits (10%) showed a gain in weight, and without any weight change in 3 rabbits (15%) at Day 90 as compared to Day 0. Of the 15 rabbits that showed weight reduction with green tea, 13 (86.67%) showed a reduction in weight by ≥15%, and the rest 2 rabbits (13.33%) showed more than 5% weight reduction (Table 1).

When the mean body weight at Day 90 was compared between the groups, the mean body weight varied significantly (p=0.002) between the groups at Day 90. The mean body weight in the high cholesterol with green tea group was the lowest (1.73 ± 0.20 Kg) I compared with the other two groups (high cholesterol group: 3.13 ± 0.30 Kg, p<0.0001; control group: 2.87 ± 0.21 Kg, p<0.0001). The mean body weight was the highest among the high cholesterol group when compared with the other two groups (Table 1).

**TC:** The mean cholesterol level at Day 0 was 42.6±8.33, 41.75±9.36, and 40.25±13.72 mg/dL

for the control group, high cholesterol group and high cholesterol with green tea group respectively. The cholesterol levels at Day 0 did not vary significantly between the different groups ( $p=0.78$ ) (Table 2). No considerable fluctuation was observed in the cholesterol levels in control ( $p=0.84$ ). However, there was highlighting an increase in the levels of cholesterol in the high cholesterol groups ( $p<0.0001$ ) and high cholesterol with the green tea group ( $p<0.0001$ ) (Table 2).

There was a considerable change in the TC levels at Day 90 among the groups ( $p<0.0001$ ). TC levels were the highest in the cholesterol group ( $1100.00 \pm 287.00$ ) in comparison with other two groups (high cholesterol with green tea group:  $451.25 \pm 223.83$ ,  $p<0.001$ ; control group:  $42.1 \pm 6.75$ ,  $p<0.001$ ). However, the mean TC levels in the high cholesterol with green tea group ( $451.25 \pm 223.83$ ) were higher than the control group ( $42.1 \pm 6.75$ ,  $p<0.001$ ) but lower than the cholesterol group ( $1100.00 \pm 287.00$ ,  $p<0.001$ ) (Table 2).

The mean increase in the cholesterol levels in the high cholesterol with green tea group ( $411.00 \pm 218.54$  mg/dL) was significantly low ( $p<0.0001$ ) when compared with the high cholesterol group ( $1058.25 \pm 289.62$  mg/dL).

**TG:** The mean TG level at Day 0 was  $67.25 \pm 9.66$ ,  $70.50 \pm 8.87$ , and  $67.63 \pm 7.14$  mg/dL for the control group, high cholesterol group and high cholesterol with green tea group respectively. The TG levels at Day 0 did not vary significantly between the different groups ( $p=0.44$ ). There wasn't enough difference in the TG levels in control ( $p=0.94$ ). However, there was a significant increase in the levels of TG in the high cholesterol groups ( $p<0.0001$ ) and high cholesterol with the green tea group ( $p<0.0001$ ) (Table 2).

However, there was a clear change in the TG levels at Day 90 among the groups ( $p<0.0001$ ). TG levels were found to be considerably higher among the high cholesterol with the green tea group when compared to control group ( $p<0.001$ ). Not much difference was observed in the TG levels between the high cholesterol with the green tea group and the high cholesterol group ( $p>0.05$ ). The TG levels were considerably higher among the high cholesterol group in comparison with the control group ( $p<0.001$ ) (Table 2).

Not many changes were seen ( $p=0.67$ ) in the mean increase in TG levels between the high cholesterol with green tea group ( $130.00 \pm 25.11$  mg/dL) and high cholesterol group ( $127.25 \pm 33.74$  mg/dL).

**LDL:** The mean LDL level at Day 0 was  $10.85 \pm 3.38$ ,  $11.10 \pm 4.09$ , and  $10.85 \pm 4.32$  mg/dL for the control group, high cholesterol group and high cholesterol with green tea group respectively. The LDL

levels at Day 0 did not vary significantly between the different groups ( $p=0.97$ ). Not enough changes in the LDL levels were observed among the control ( $p=0.31$ ). However, a notable increase in the levels of LDL among the high cholesterol groups ( $p<0.0001$ ) and high cholesterol with the green tea group ( $p<0.0001$ ) was observed (Table 2).

LDL levels were quite different at Day 90 among the groups ( $p<0.0001$ ). LDL levels were notably on the higher side among the high cholesterol with green tea group in comparison to the control group ( $p<0.001$ ) but there were significantly lower levels of LDL in the high cholesterol with green tea group as compared to the high cholesterol group ( $p<0.001$ ). The LDL levels were significantly higher in the high cholesterol group as compared to the control group ( $p<0.001$ ) (Table 2).

The mean increase in the LDL levels in the high cholesterol with green tea group ( $187.65 \pm 17.99$  mg/dL) was significantly low ( $p<0.0001$ ) as compared with the high cholesterol group ( $773.4 \pm 211.33$  mg/dL).

**VLDL:** The mean VLDL level at Day 0 was  $7.25 \pm 2.10$ ,  $6.10 \pm 1.86$ , and  $7.4 \pm 1.88$  mg/dL for the control group, high cholesterol group and high cholesterol with green tea group respectively. The VLDL levels at Day 0 did not vary significantly between the different groups ( $p=0.07$ ). There was no significant change in the VLDL levels in control ( $p=0.43$ ). However, there was a significant increase in the levels of VLDL in the high cholesterol groups ( $p<0.0001$ ) and high cholesterol with the green tea group ( $p<0.0001$ ) (Table 2).

A considerable change in the VLDL levels was observed at Day 90 among the groups ( $p<0.0001$ ). VLDL levels were significantly higher in the high cholesterol with green tea group as compared to the control group ( $p<0.001$ ) but there were significantly lower levels of VLDL in the high cholesterol with green tea group as compared to the high cholesterol group ( $p<0.001$ ). The VLDL levels were significantly higher in the high cholesterol group as compared to the control group ( $p<0.001$ ) (Table 2).

The mean increase in the VLDL levels in the high cholesterol with green tea group ( $192.60 \pm 12.75$  mg/dL) was significantly low ( $p<0.0001$ ) as compared with the high cholesterol group ( $525.40 \pm 91.85$  mg/dL).

**HDL:** The mean HDL level at Day 0 was  $24.67 \pm 1.59$ ,  $23.60 \pm 4.55$ , and  $22.35 \pm 4.92$  mg/dL for the control group, high cholesterol group and high cholesterol with green tea group respectively. The HDL levels at Day 0 did not vary significantly between the different groups ( $p=0.32$ ). There was no significant change in the HDL levels in control

( $p=0.68$ ). However, there was a significant increase in the levels of HDL in the high cholesterol groups ( $p<0.0001$ ) and high cholesterol with the green tea group ( $p<0.0001$ ) (Table 2).

There was a significant difference in the HDL levels at Day 90 between the groups ( $p<0.0001$ ). HDL levels were significantly higher in the high cholesterol with green tea group as compared to the control group ( $p<0.001$ ) but there was no significant difference in the levels of HDL in the high cholesterol with green tea group as compared to the high cholesterol group ( $p=0.46$ ). The HDL levels were significantly higher in the high cholesterol group as compared to the control group ( $p<0.001$ ) (Table 2).

There was no significant difference ( $p=0.34$ ) in the mean increase in HDL levels between the high cholesterol with green tea group ( $28.40 \pm 8.26$  mg/dL) and high cholesterol group ( $26.15 \pm 9.57$  mg/dL).

## DISCUSSION

Obesity is an excessive accumulation of body fat. Overeating, consumption of food rich in fats and sugars combined with poor and unhealthy lifestyle, and less physical activity results in weight gain and subsequently leads to obesity (Singh *et al.*, 2011). Obesity has turned into an outstanding overall medical issue. Worldwide the occurrence of obesity is increasing consistently, and in this manner, the related mortality, morbidity, and medical cost also are increasing. The common comorbid conditions associated with obesity are cardiovascular diseases, type 2 diabetes mellitus, hypertension, lung disorders, and dyslipidemia. Moreover, obesity affects the cardiovascular health by inducing high fasting plasma triglycerides, low HDL cholesterol, high LDL cholesterol, elevated insulin levels and blood glucose, and high blood pressure (Klop *et al.*, 2013). Approximately 60-70% of patients with obesity are dyslipidemic (Feingold and Grunfeld, 2018).

Green tea has been examined widely for its usefulness in the prevention of several systemic illnesses especially in metabolic diseases, obesity, diabetes, and cancer (Yang *et al.*, 2018). So, in this study, we have investigated the potential of green tea consumption on obesity and related dyslipidemia. In the present study, we observed the reduction of body weight by the consumption of green tea consumption in the group of rabbits consuming high cholesterol diet supplemented with green tea. This is suggestive of the fact that green tea is a natural supplement that can be used for weight reduction if taken regularly. Previous studies on the mechanism of green tea in weight reduction have been inconsistent in showing beneficial effects of green tea consumption on weight loss (Yang *et al.*, 2018;

Quinhoneiro *et al.*, 2018). There are several mechanisms to explain the mechanism of action of green tea in weight reduction, but the most accepted hypothesis is that green tea inhibits the expression and activity of enzymes involved in carbohydrate metabolism, and also it plays a significant role in the interaction of these undigested carbohydrates with the gut microbiome. These interactions among the polyphenols present in green tea, undigested carbohydrates, and gut microbiome generate short-chain fatty acids that improve lipid metabolism by activating AMP-activated protein kinase (AMPK) (Rothenberg *et al.*, 2018).

Several studies have examined the performance of green tea on obesity. Most of which used green tea extracts for studying its effect on obesity by testing it on animals by dividing them into three groups with - high-fat diet, normal diet, and high-fat with green tea extract added. Most studies have examined the efficacy of more than one type of tea on weight reduction. In the present study we divided the rabbits into three groups of normal diet ( $n=20$ ), high cholesterol diet ( $n=20$ ), and high cholesterol and green tea diet ( $n=20$ ). Recently a study reported that green tea consumption reduced the subcutaneous body fat accumulation in the group consuming high-fat diet along with green tea as compared to the groups of animals consuming normal diet and high-fat diet (Henning *et al.*, 2018). In another study, the anti-obesity effects of green tea and goishi tea was studied, and it reported that both green tea and goishi tea showed significant anti-obesity effects, but the mechanism of action was different. Green tea effectively reduced fat accumulation, and goishi tea increased the rates of lipid digestion or lipolysis (Jobu *et al.*, 2013). Consistent with the findings of these studies we also observed in the present study that consumption of green tea reduced the body weight in the rabbits consuming high cholesterol diet supplemented with green tea while rabbits in the other two groups that were on normal diet and high cholesterol diets showed an increase in body weight over a period of 90 days. Of the 13 rabbits (72.22%) that showed weight reduction with green tea, 11 showed a reduction in weight by  $\geq 15\%$ , and the rest 2 rabbits showed more than 5% weight reduction.

The present study observed that green tea reduced the rate of increase in the levels of cholesterol, LDL, and VLDL. There are studies to support our findings that report the fat accumulation-suppressing properties of green tea (Jobu *et al.*, 2013; Sae-Tan *et al.*, 2011; Lee M *et al.*, 2017).

There are several ant obesity drugs available in the market. However, the side effects, safety and their impact on normal body physiology is a matter of

concern. These drugs are reported to cause hypertension, headache, cardiovascular diseases, dry mouth, and insomnia. So, many of them have been withdrawn from the market (Kang and Park *et al.*, 2012). This warrants for the development of an alternative to these existing therapies or drugs. More studies should be done to evaluate the candidature of natural products as potential anti-obesity therapeutics. With an intention to prove the effect of green tea in treating and managing obesity and hypercholesterolemia, we analyzed the effect of green tea on obesity in both normal and hypercholesterol groups of rabbits. Very few research has been performed to evaluate the role of green tea on obesity and has documented mixed results (Hursel *et al.*, 2011; Mielgo-Ayuso *et al.*, 2013). The major drawback of these studies is the lack of statistical power. The findings of the present study successfully and consistently support the previous findings and suggest that green tea can be treated as a natural supplement to reduce obesity and control hypercholesterolemia. However, the findings of the present study should be tested extensively in human subjects of hypercholesterolemia and obesity to establish green tea as a therapeutic agent for obesity and hypercholesterolemia.

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