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## Research Article

# Effects of Green Tea on Adipose Gene Expression, Hepatic Antioxidants and Lipid Profile in Obese Male Rats

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## Abstract

**Background and Objective:** Role of green tea constituents in enhancing adipose Mesoderm Specific Transcript (MEST) expression of obese rats has not investigated yet. Evaluation of their effects on lipid profile, MEST expression and oxidative biomarkers were main objective of the current study. **Materials and Methods:** About 60 rats used in the current experiment (32 weeks) and divided into two unequal groups. Rats of the first group (n = 10) was negative control and fed basal diet (C). Rats of the second group (n = 50) was positive control, fed high fat diet and divided into five equal treatments (T1, T2, T3, T4 and T5). Rats of T1 were positive control and fed high fat diet. Rats of T2–T5 fed high fat diet mixed with green tea, Epigallocatechin gallate (EGCG), polyphenol 60 and EGCG+caffeine, respectively. **Results:** High fat diet elevated total cholesterol (TC), triacylglycerol (TAG), low-density lipoprotein cholesterol (LDL-C), malondialdehyde (MDA) and MEST gene expression and reduced high-density lipoprotein cholesterol (HDL-C), glutathione (GSH), catalase (CAT) and glutathione-s-transferase (GST). Green tea or its constituents particularly EGCG restored the changed values towards the negative control. **Conclusion:** These results concluded that EGCG might be potential therapy against obesity over whole herb or other studied constituents.

**Key words:** Green tea, catechins, polyphenols, adipose genes, malondialdehyde, obesity

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Obesity is a major syndrome connected with bad life style and eating habits characterized by high increase in body weight. The most significant cause of obesity is the high intake of fat-rich diet or high calorie-dense food consumption with decrease in physical exercise<sup>1</sup>. During the last two decades, the disease control and prevention centers showed a worldwide dramatic increase in the obesity rate<sup>2</sup>. The American Medical Association declared obesity as a disease<sup>3</sup> since 2013. Understanding the cause, costs and treatment opportunities of obesity are a major concern of many studies in metabolic, genetic, hormonal, behavioral, cultural and social aspects<sup>4</sup>. Herbal medicines broadly applied for prevention and treatment of many chronic diseases, involving obesity due to they have no side effects in comparison with chemical drugs<sup>5</sup>. Many of extracts of these herbs reduce body weight by regulating body energy balance<sup>6</sup>. Green tea (Theaceae or *Camellia sinensis*) is one of the most broadly expanded beverages. Moreover, many studies suggested that, it uses as anti-inflammatory, anti-cholesterol, anti-diabetic, anti-mutation, anti-microbial, anti-cancer, anti-stroke and anti-oxidant herbal<sup>7</sup>. The anti-obesity impact of green tea attributed to its ability in raising thermo genesis, fat oxidation, reduced lipid peroxidation and restraining appetite<sup>8</sup>. The most effective compounds found in green tea are polyphenols mainly catechins which representing almost 78% of green tea constituents<sup>9</sup>. Catechins including (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and (-)-epicatechin-3-gallate (ECG) and (-)-gallocatechin-gallate (GCG)<sup>10</sup>. The antioxidant activity of green tea influenced by the quantity and quality of polyphenols catechins. Polyphenols catechins eliminated the free radicals that involved in the pathogenesis of many diseases<sup>11,12</sup>. The EGCG one of the most important catechins of green tea recorded as anti-obesity agent<sup>13</sup>. Mesoderm Specific Transcript (MEST) gene expression was considered as an obesity biomarker<sup>14</sup>. Its expression in white adipose tissue had a relation with white adipose tissue expansion and body weight gain<sup>15</sup>. The effect of green tea and/or its constituents in expression of MEST gene have not investigated so far. Data regarding the comparison between the anti-obesity effects of whole green tea herb and its constituents are not fully covered. In addition, reports investigated the effect of green tea and/or its constituents in MEST gene expression of adipose tissues are scarce. Therefore, the current study aimed to evaluate the effects of green tea and/or its bioactive compounds

in lipid profile, selected serum biochemical parameters, MEST gene expression of adipose tissues and hepatic antioxidants of obese male rats.

## MATERIALS AND METHODS

**Animals and experimental design:** A total number of 60 Sprague Dawley adult male rats obtained from laboratory animal house, College of Veterinary Medicine, King Faisal University, Saudi Arabia. The experiments performed during August, 2017. The body weights of used rats were  $180 \pm 20$  g. The rats were located in a well-ventilated wire mesh cages under comfort weather conditions for 2 weeks as a sort of acclimatization. Afterwards, rats divided into two main groups. The first group (n = 10) fed on normal fat diet Teklad 7001, Envigo, USA, 4.4% fat (Table 1) for 16 weeks whereas rats in the second group (n = 50) fed high fat diet (TD 064114; Teklad rodent diets; Envigo®Teklad diet, USA (Table 1)) for the same period. After this period, rats of the first group (n = 10) continue as a negative control rats (C) who kept on low fat diet without any treatment for further 16 weeks experimental period. Rats of the second group (n = 50) were divided into five equal treatments (T1, T2, T3, T4 and T5; 10 rats for each). Rats of T1 (n = 10) continue in the experiment as a positive control rats, who kept on high fat diet without any treatment for further 16 weeks. Rats of T2 (n = 10) fed high fat diet mixed with green tea extract (Natural products lines™, China; 500 mg 100 mL<sup>-1</sup> for 16 weeks)<sup>16</sup>. Rats of T3 (n = 10) fed high fat diet mixed with EGCG (Sigma Aldrich, USA; 232 mg 100 mL<sup>-1</sup> for 16 weeks)<sup>17</sup>. Rats of T4 (n = 10) fed high fat diet mixed with polyphenol 60 (Sigma Aldrich, USA; 232 mg 100 mL<sup>-1</sup> for 16 weeks)<sup>17</sup>, whereas rats of T5 (n = 10) fed high fat diet mixed with EGCG (232 mg 100 mL<sup>-1</sup> for 16 weeks)+caffeine (92.8 mg 100 mL<sup>-1</sup>) incorporated in capsule (Applied Nutrition™, Los Anglos, USA). Daily food

Table 1: Estimated chemical analysis of experimental diets (unless stated %)

Macronutrients	Low fat diet <sup>†</sup>	High fat diet <sup>‡</sup>
Moisture	7.5	7
Dry matter	92.5	93
Crude fiber	3.5	3.1
Crude protein	24	24
Crude fat	4.4	34.3
Ash	9.7	10.2
Organic matter	82.8	82.8
Calcium	2	2
Phosphorus	1	1
Nitrogen free extract	50.9	21.4
Energy density (k cal g <sup>-1</sup> )	3	5.1

Feed chemical composition is reported on as fed basis, <sup>†</sup>Low fat diet: 7001 Teklad 4% rat diet (Envigo®Teklad diet, USA), <sup>‡</sup>High fat diet induced obesity: TD 064114 (Teklad rodent diets (60/fat) (Envigo®Teklad diet, USA)

intake recorded. Tap water was available *ad libitum*. All experimental procedures maintained and performed by national guidelines and protocols, approved by the University Scientific Research Ethics Committee, King Faisal University, Saudi Arabia (Approval #186026).

**Sampling:** By the end of experimental protocol, the rats in all groups anesthetized with intraperitoneal injection of thiopental sodium (50 mg kg<sup>-1</sup>). About 10 mL blood sample collected by cardiac puncture in heparinized and plain vacutainers for separation of plasma and serum, respectively. Plasma used for estimation of G6PD activity and MDA concentration. Sera kept frozen at -20°C until used for measuring levels of TC, TAG, HDL-C, LDL-C, blood glucose, total protein, TAC and activities of ALT and AST. After euthanization of rats, liver and adipose tissues quickly dissected and washed with ice-cold normal saline. Slices from liver and adipose tissues used to prepare tissue homogenates (10% weight/volume) in phosphate buffered saline (pH 7.4). Hepatic homogenate used for determination of GSH concentration and activities of GST and CAT. All biochemical variables were measured using commercially available kits according to the manufacturer's instructions supplied by Cayman Chemical, Ann Arbor, Michigan, USA. Adipose tissue homogenate centrifuged at 3000 × g for 10 min at 4°C after removal of the cell debris, the supernatant was stored at -80°C until further RNA extraction and estimation of relative mRNA expression of MEST.

**RNA extraction, cDNA synthesis and reverse transcription-PCR (RT-PCR):** RNA extracted from adipose tissue by using Trizol reagent (Invitrogen, Life Technologies, NY, USA) according to the manual instruction. About 2 µg of total RNA for each intact sample was reversing transcribed into cDNA. The amplification of cDNA was done using Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific, USA).

The analysis of MEST gene analyzed in comparison with rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH):

- The primer sequences of MEST gene were (F): '5AGAATCGTTCTGGCCGTCTC3 and (R): '5CCCGTCATTGTTGCGAATCC'3.
- The primer sequences for rat GAPDH were (F): 5TCCTCCTGAGCGCAAGTACTCT 3 and R: '5GCTCAGTAACAGTCCGCCTAGAA'3.

**Statistical analyses:** The SPSS software V.19 used for statistical analysis and The Kolmogorov-Smirnov test (KS test) applied to analyze the normality of the data before analysis<sup>18</sup>. All the measured biochemical parameters analyzed using one-way ANOVA followed by Duncan's multiple range tests at a significant<sup>19</sup> level of p ≤ 0.05. The data represented by the mean ± standard error.

## RESULTS

**Growth performance parameters:** Data presented in Table 2 indicated that, high fat diet in rats (positive control; T1) induced significant (p < 0.001) elevation in final body weight, total weight gain, total and daily feed intake and feed conversion ratio with significant reduction to feed and protein efficiency ratios compare to that of rats fed a basal diet (negative control; C). Inclusion of green tea or its ingredients in high fat diet of rats (T3) induced significant reduction to final body weight, total weight gain, total and daily feed intake and feed conversion ratio with significant elevation to feed and protein efficiency ratios compare to that of rats fed high fat diet alone (positive control; T1). These values were close to that of negative control rats fed basal diet (C). The superiority of this action arranged to be EGCG (T3) > EGCG + caffeine (T5) > polyphenol 60 (T4) > green tea powder (T2) in regards to all parameters except for total and daily feed intake and feed

Table 2: Effect of green tea or its ingredients on growth performance of rats fed high fat diet (Means ± SE)

Items	C	T1	T2	T3	T4	T5	p. value
Initial body weight (g)	169.91 ± 0.83	70.24 ± 1.63	172.48 ± 1.64	169.37 ± 1.62	171.82 ± 1.02	168.90 ± 1.01	0.37
Final body weight (g)	271.00 ± 1.21 <sup>e</sup>	397.90 ± 1.27 <sup>a</sup>	329.70 ± 6.41 <sup>b</sup>	263.90 ± 1.03 <sup>f</sup>	303.50 ± 0.84 <sup>c</sup>	284.60 ± 0.79 <sup>d</sup>	<0.001
Total weight gain (g)	101.09 ± 1.07 <sup>e</sup>	227.66 ± 2.21 <sup>a</sup>	157.22 ± 7.12 <sup>b</sup>	94.53 ± 1.55 <sup>f</sup>	131.68 ± 1.58 <sup>c</sup>	115.70 ± 1.17 <sup>d</sup>	<0.001
Total feed intake (g)	716.40 ± 10.24 <sup>b</sup>	861.84 ± 4.06 <sup>a</sup>	687.60 ± 7.44 <sup>c</sup>	665.40 ± 5.24 <sup>d</sup>	694.20 ± 4.01 <sup>c</sup>	692.20 ± 5.51 <sup>c</sup>	<0.001
Daily feed intake (g)	11.94 ± 0.17 <sup>b</sup>	14.36 ± 0.07 <sup>a</sup>	11.46 ± 0.12 <sup>c</sup>	11.09 ± 0.08 <sup>d</sup>	11.57 ± 0.07 <sup>c</sup>	11.57 ± 0.09 <sup>c</sup>	<0.001
Feed conversion ratio	7.09 ± 0.12 <sup>b</sup>	7.39 ± 0.04 <sup>a</sup>	5.98 ± 0.15 <sup>b</sup>	5.07 ± 0.05 <sup>c</sup>	5.43 ± 0.04 <sup>c</sup>	5.28 ± 0.05 <sup>d</sup>	<0.001
Feed efficiency ratio	0.14 ± 0.02 <sup>c</sup>	0.10 ± 0.02 <sup>d</sup>	0.19 ± 0.01 <sup>b</sup>	0.24 ± 0.02 <sup>a</sup>	0.19 ± 0.01 <sup>b</sup>	0.17 ± 0.02 <sup>b</sup>	<0.001
Protein efficiency ratio	0.58 ± 0.01 <sup>c</sup>	0.11 ± 0.01 <sup>d</sup>	0.69 ± 0.19 <sup>b</sup>	0.95 ± 0.01 <sup>a</sup>	0.79 ± 0.01 <sup>b</sup>	0.69 ± 0.09 <sup>b</sup>	<0.001

a-f: Means in the same rows with different superscripts differ significantly (p < 0.001). (n = 10), C: Control group; T1 (Treatment 1): High fat diet; T2 (Treatment 2): High fat supplemented with Chinese green tea; T3 (Treatment 3): High fat diet supplemented with Epigallocatechin gallate (EGCG); T4 (Treatment 4): High fat diet supplemented with polyphenol 60; T5 (Treatment 5): High fat diet supplemented with green tea capsules (EGCG + Caffeine)

and protein efficiency ratios in which the order started by EGCG (T3) followed by other treatments (T2, T4 and T5) of comparable effects.

**Lipid profile and liver function:** Supplementation of high fat diet in rats (positive control; T1) induced significant ( $p < 0.001$ ) elevation of TC, TAG, LDL-C, glucose, ALT and AST with significant ( $p < 0.001$ ) reduction in HDL-C and total proteins compare to that of rats fed a basal diet (negative control; C) (Table 3). Inclusion of green tea or its ingredients in high fat diet of rats induced significant decrease ( $p < 0.001$ ) to TC, TAG, LDL-C, glucose, ALT and AST with significant increase in HDL-C and total proteins compare to that of rats fed high fat diet alone (positive control; T1) and nears to that of negative control rats fed basal diet (C) (Table 3). In this context, EGCG (T3) and polyphenol 60 (T4) induced the same pronounced effect on reduction of TC concentration followed by EGCG+caffeine (T5) and green tea powder (T2) which remained comparable (Table 3). Moreover, the effects of EGCG (T3) and polyphenol 60 (T4) were recorded to be the best on reduction of TAG concentration followed by green tea powder (T2) and ended finally by EGCG+caffeine (T5) (Table 3). The most favorable effect in regards to HDL-c, LDL-c, ALT and total proteins recorded to EGCG (T3) followed by polyphenol 60 (T4) and ended by green tea (T2) and EGCG+caffeine (T5) best for reduction of glucose level followed by EGCG+caffeine (T5), green tea (T2) and ended by polyphenol 60 (T4) while the improvement of AST activity was mostly enhanced by green tea powder (T2) followed by polyphenol 60 (T4), EGCG (T3) and finally EGCG+caffeine (T5).

**Oxidative stress biomarkers and relative mRNA expression of MEST gene in adipose tissues:** High fat diet in rats (positive control; T1) induced significant ( $p < 0.001$ ) elevation in MDA concentration (Table 4) and MEST gene expression (Fig. 1) which remained comparable (Table 3). The EGCG was recorded the comparison to that of rats fed a basal diet

(negative control; C) (Table 4). In addition, significant ( $p < 0.001$ ) reduction to TAC and GSH concentrations and activities of G6PD, CAT and GST observed in rats fed high fat diet (positive control; T1) compare to that of negative control (Table 4). Green tea or its ingredients (T2-T5) induced similar significant reduction to MDA concentration and similar significant elevation to TAC and GSH concentrations and hepatic GST activity in rats fed high fat diet compare to that of rats fed high diet alone (T1) and were close to negative control values (C). Addition of EGCG (T3) or polyphenol 60 (T4) to high fat diet induced similar pronounced significant increase to the activities of G6PD in rats followed by green tea (T2) compare to positive control (T1) and near to negative control (C). However, EGCG+caffeine (T5) had no effect in this regard and remained comparable to that of positive control (T1). Addition of green tea (T2) or EGCG (T3) to high fat diet induced similar increase in the activity of hepatic

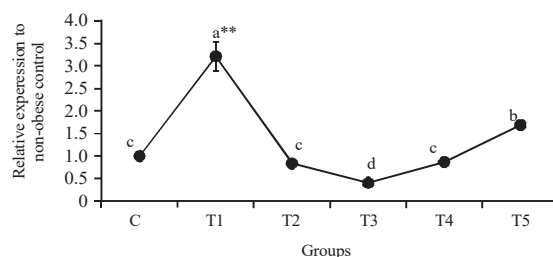


Fig. 1: RT-PCR analysis of MEST gene in adipose tissue of rats fed high fat diet and treated with green tea and/or its ingredients compare to negative control rats. \*\*a-d means with different letters differ significantly ( $p < 0.001$ ), C: Control group; T1: Treatment 1, high fat diet; T2: Treatment 2, high fat supplemented with Chinese green tea; T3: Treatment 3, high fat diet supplemented with Epigallocatechin gallate (EGCG); T4: Treatment 4, high fat diet supplemented with polyphenol 60; T5: Treatment 5, high fat diet supplemented with green tea capsules (EGCG+Caffeine)

Table 3: Effect of green tea or its ingredients on lipid profile, blood glucose and liver functions of rats fed high fat diet (Means  $\pm$  SE)

Groups	C	T1	T2	T3	T4	T5	p-value
TC (mg dL <sup>-1</sup> )	140.33 $\pm$ 1.31 <sup>d</sup>	225.36 $\pm$ 1.33 <sup>a</sup>	188.39 $\pm$ 11.1 <sup>b</sup>	165.84 $\pm$ 2.22 <sup>c</sup>	164.07 $\pm$ 4.15 <sup>c</sup>	187.30 $\pm$ 2.99 <sup>b</sup>	<0.001
TAG (mg dL <sup>-1</sup> )	77.88 $\pm$ 1.30 <sup>d</sup>	125.54 $\pm$ 0.41 <sup>a</sup>	97.36 $\pm$ 1.37 <sup>c</sup>	79.87 $\pm$ 1.13 <sup>d</sup>	79.02 $\pm$ 0.95 <sup>d</sup>	107.30 $\pm$ 2.13 <sup>b</sup>	<0.001
HDL-C (mg dL <sup>-1</sup> )	94.90 $\pm$ 1.02 <sup>a</sup>	30.80 $\pm$ 0.47 <sup>e</sup>	43.03 $\pm$ 1.24 <sup>cd</sup>	88.96 $\pm$ 2.71 <sup>b</sup>	46.05 $\pm$ 1.38 <sup>c</sup>	40.52 $\pm$ 1.001 <sup>d</sup>	<0.001
LDL-C (mg dL <sup>-1</sup> )	29.85 $\pm$ 2.02 <sup>e</sup>	169.45 $\pm$ 1.28 <sup>a</sup>	125.89 $\pm$ 10.13 <sup>b</sup>	50.90 $\pm$ 1.33 <sup>d</sup>	102.22 $\pm$ 4.42 <sup>c</sup>	125.31 $\pm$ 2.34 <sup>b</sup>	<0.001
Glucose (mg dL <sup>-1</sup> )	93.14 $\pm$ 0.14 <sup>e</sup>	168.02 $\pm$ 3.22 <sup>a</sup>	132.54 $\pm$ 5.84 <sup>c</sup>	115.38 $\pm$ 1.19 <sup>d</sup>	152.50 $\pm$ 2.45 <sup>b</sup>	123.20 $\pm$ 0.57 <sup>d</sup>	<0.001
ALT (U L <sup>-1</sup> )	43.17 $\pm$ 0.88 <sup>c</sup>	171.88 $\pm$ 2.14 <sup>a</sup>	84.39 $\pm$ 0.36 <sup>b</sup>	45.89 $\pm$ 1.08 <sup>e</sup>	50.79 $\pm$ 2.31 <sup>d</sup>	90.52 $\pm$ 6.67 <sup>b</sup>	<0.001
AST (U L <sup>-1</sup> )	90.65 $\pm$ 0.17 <sup>f</sup>	212.68 $\pm$ 1.34 <sup>a</sup>	117.04 $\pm$ 3.88 <sup>c</sup>	137.94 $\pm$ 0.85 <sup>c</sup>	129.80 $\pm$ 0.59 <sup>d</sup>	199.58 $\pm$ 0.66 <sup>b</sup>	<0.001
Total protein (mg dL <sup>-1</sup> )	7.19 $\pm$ 0.05 <sup>c</sup>	6.59 $\pm$ 0.13 <sup>f</sup>	7.08 $\pm$ 0.19 <sup>d</sup>	7.38 $\pm$ 0.05 <sup>a</sup>	7.28 $\pm$ 0.19 <sup>b</sup>	6.92 $\pm$ 0.07 <sup>d</sup>	<0.001

a-f: Means in the same rows with different superscripts differ significantly ( $p < 0.001$ ). (n = 10), C: Control group; T1 (Treatment 1): High fat diet; T2 (Treatment 2): High fat supplemented with Chinese green tea; T3 (Treatment 3): High fat diet supplemented with Epigallocatechin gallate (EGCG); T4 (Treatment 4): High fat diet supplemented with polyphenol 60; T5 (Treatment 5): High fat diet supplemented with green tea capsules (EGCG+Caffeine), TC: Total cholesterol, TAG: Triacylglycerol, LDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, ALT: Alanine aminotransferase and ST: Aspartate aminotransferase

Table 4: Effect of green tea or its ingredients on blood and liver tissue antioxidant and oxidant stress markers in rats fed high fat diet (Means  $\pm$  SE)

Groups	C	T1	T2	T3	T4	T5	p-value
<b>Blood:</b>							
G6PD (IU g <sup>-1</sup> Hb)	75.70 $\pm$ 0.81 <sup>a</sup>	38.82 $\pm$ 1.42 <sup>c</sup>	55.78 $\pm$ 0.43 <sup>d</sup>	60.59 $\pm$ 5.81 <sup>b</sup>	65.73 $\pm$ 0.62 <sup>b</sup>	39.63 $\pm$ 0.73 <sup>c</sup>	<0.001
TAC (mm L <sup>-1</sup> )	1.66 $\pm$ 0.09 <sup>a</sup>	0.86 $\pm$ 0.04 <sup>c</sup>	1.54 $\pm$ 0.06 <sup>ab</sup>	1.41 $\pm$ 0.04 <sup>b</sup>	1.47 $\pm$ 0.05 <sup>b</sup>	1.40 $\pm$ 0.05 <sup>b</sup>	<0.001
MDA (nmol mL <sup>-1</sup> )	41.89 $\pm$ 0.35 <sup>c</sup>	97.13 $\pm$ 2.01 <sup>a</sup>	55.42 $\pm$ 1.22 <sup>b</sup>	50.92 $\pm$ 1.29 <sup>b</sup>	55.36 $\pm$ 5.76 <sup>b</sup>	59.62 $\pm$ 6.31 <sup>b</sup>	<0.001
<b>Liver tissue:</b>							
GST (U g <sup>-1</sup> )	10.90 $\pm$ 0.13 <sup>a</sup>	3.01 $\pm$ 0.54 <sup>c</sup>	5.37 $\pm$ 0.12 <sup>b</sup>	4.99 $\pm$ 0.27 <sup>b</sup>	5.80 $\pm$ 0.15 <sup>b</sup>	5.46 $\pm$ 0.41 <sup>b</sup>	<0.001
CAT (U g <sup>-1</sup> )	2.63 $\pm$ 0.09 <sup>a</sup>	0.18 $\pm$ 0.02 <sup>e</sup>	1.92 $\pm$ 0.09 <sup>b</sup>	1.93 $\pm$ 0.24 <sup>b</sup>	1.14 $\pm$ 0.06 <sup>c</sup>	0.51 $\pm$ 0.03 <sup>d</sup>	<0.001
GSH (U g <sup>-1</sup> ) level	24.76 $\pm$ 0.62 <sup>a</sup>	4.96 $\pm$ 0.07 <sup>c</sup>	18.01 $\pm$ 1.26 <sup>b</sup>	23.48 $\pm$ 1.10 <sup>a</sup>	21.44 $\pm$ 2.27 <sup>ab</sup>	21.96 $\pm$ 0.97 <sup>ab</sup>	<0.001

a-e: Means in the same rows with different superscripts differ significantly ( $p < 0.001$ ). (n = 10), C: Control group; T1 (Treatment 1): High fat diet; T2 (Treatment 2): High fat diet supplemented with Chinese green tea; T3 (Treatment 3): High fat diet supplemented with Epigallocatechin gallate (EGCG); T4 (Treatment 4): High fat diet supplemented with polyphenol 60; T5 (Treatment 5): High fat diet supplemented with green tea capsules (EGCG+Caffeine), G6PDH: Glucose 6-phosphate dehydrogenase activity; TAC: Total antioxidant capacity, MDA: Malondialdehyde, GSH: glutathione s-transferase activity, CAT: catalase activity and GSH reduced glutathione

CAT followed by polyphenol 60 (T4) and ended by EGCG+caffeine (T5) compare to positive control (T1). Supplementation of green tea or its ingredients (T2-T5) induced significant down regulation to the expression of MEST gene compare to that of rats fed high fat diet alone, positive control (T1). The down-regulation was lower than negative control values in rats fed high fat diet and treated with EGCG and was higher than negative control value in rats fed high fat diet and treated with EGCG+caffeine (T5). Moreover, this down-regulation was comparable to the negative control values in rats fed high fat diet and treated either with green tea (T2) or polyphenol 60 (T4).

## DISCUSSION

The observed reduction of growth performance parameters nears to the negative control values in rats fed green tea or their active principals are consistent with the results of Suzuki *et al.*<sup>20</sup> indicated a significant decreased in body weight gain of rats fed either green tea or their active principals. The observed pronounced effect of EGCG on growth performance parameters compared to green tea and/or other constituents and even its mixture with caffeine required further investigation in regards of lipid-associated genes and its interaction with caffeine. The significant increases in TC, TAG and LDL-C in rats fed high fat diet and the restoring of this elevation in such parameters by inclusion of green tea or its active ingredients were parallel with earlier work in rats<sup>21</sup>. This hypolipidemic effect of green tea or its constituents may exhibited by decreasing the absorption of TAG and cholesterol and increasing the fat excretion in bile<sup>22</sup>. The pronounced effect of EGCG in correction of lipid profile parameters towards the control values as indicated in the current study may attributed to its lowering effect on food digestibility by inhibiting pancreatic lipase or due

to its role in inhibition of lipogenesis and increasing the post-prandial fat oxidation and diminishing merging of alimentary lipids into tissues as well<sup>23</sup>. The significant increase in ALT and AST in rats fed high diet alone indicated liver injuries<sup>24,25</sup>, that may attributed to deposition of fats on the liver. The significant reduction of elevated ALT and AST activities in rats fed high diet and treated with green tea or its constituents come in accordance with previous report in rats fed high diet and treated with aqueous extract of green tea<sup>26</sup>. The present study could argued that green tea or its constituents may protect the liver against fat deposition and subsequent injuries. The observed significant decrease of the total protein in serum of rats fed high fat diet only indicated liver affection and was parallel to the earlier reports in obese rats<sup>27</sup>. The observed improvement of serum total protein in rats fed high diet and treated with either green tea or its constituents confirmed the earlier results<sup>27</sup>. which indicated a sort of liver function improvement due to dietary supplementation of green tea in obese rats. Reactive oxygen species levels increased in obesity and the decline of these compounds noticed following weight loss<sup>28</sup>. In earlier reported<sup>29</sup>, antioxidant power of investigated doses of green tea or its constituents confirmed in the current study as reflected on observed significant increase of G6PD, GST, CAT, TAC and GSH concentration along with significant reduction of MDA concentration. The significant reduction of MDA as a result of incorporation of green tea or its constituents in high fat diets of rats may attributed to the positive effect of this herbs or its constituent on lipid profiles<sup>30</sup>. The significant increase of the activities of investigated antioxidant enzymes because of inclusion of green tea or its constituents in high fat diet of rats come in accordance with clinical data supported previously<sup>31-33</sup>. In the current work, high dietary fat induced significant up regulation of MEST mRNA expression in adipose tissue of untreated obese rats which down regulated

as a result of inclusion of green tea or its constituents. These findings confirmed the relation between obesity and MEST gene expression as mentioned earlier<sup>15,34</sup>. Lu *et al.*<sup>17</sup> concluded that the anti-obesity effect of green tea polyphenols attributed to restored expression of many genes of hepatic origin. These authors did not examine the expression of genes of adipose tissue origin as that done in the current study (MEST gene). Authors recommended green tea total polyphenol as anti-obesity therapy however; the current findings concluded that, EGCG is the preferable therapy of obesity over total polyphenols, mixture with caffeine and original green tea<sup>17</sup>. The distinguished effect of EGCG on performance parameters, lipid profile, antioxidant status and expression of MEST gene in rats fed high fat diet over whole herb and other constituents supported the previous work which indicated that, EGCG supplementation induced a significant regulation of lipids metabolism associated genes<sup>35</sup>. Therefore, future studies are recommended to examine the effects of the combination of EGCG with polysaccharides<sup>36</sup> or other ingredients at molecular level.

### CONCLUSION

The current study can concluded that green tea and/or its studied constituents improved growth performance, regulated the lipid profile, counteracted the oxidative stress, stimulated the activities of antioxidant enzymes and restored the expression of MEST gene of adipose tissues in rats fed high fat diet. EGCG might be a potential therapy against obesity over the whole herb or other studied green tea constituents.

### SIGNIFICANCE STATEMENT

This study suggested new evidence about the mechanism of action of anti-obesity effect of green tea and/or its ingredients representing in down regulation to Mesoderm Specific Transcript (MEST) gene in adipose tissues. The current study confirmed the anti-obesity effect of green tea and/or its ingredients by regulation of lipid profiles, reduction of lipid peroxidation and enhancement of antioxidant enzymes activities. This study will help the researchers to explore important aspect of the potential molecular mechanisms of anti-obesity effect of green tea and/or its ingredients particularly EGCG thereby, aiding in further researches into the treatment of obesity. Thus, a new theory on green tea and/or ingredients particularly EGCG and obesity perhaps arrived at.

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