Biological Effect of Green Tea on Hypercholesterolemia Level in Rats

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ABSTRACT

The present study is conducted to evaluate the preventive effects of green tea (*Camellia sinensis*) on rat feed on high fat diet. Twenty-four male albino rats each weighing 100±30g, was divided into 4 groups. Group (1) negative control group feeding basal diet, Group(2)positive control group fed in high fat diet , Group (3) hypercholesterolemic rats fed on basal diet containing 10% green tea and Group (4) hypercholesterolemic rats fed on basal diet containing tablets atorvastatin. At the end of experiment, the four groups were compared in terms of the proportion of fat in the blood. The results indicated that, high fat diet caused deleterious metabolic effects, including hypertriglyceridemia, and liver dysfunction. Rats fed high fat di*et al*one showed increased activities of hepatocellular enzymes in plasma, significant decline in antioxidants, and elevated lipid peroxidation indices in liver. Green tea treatment significantly reduced elevated lipid peroxidation product, and brought back the liver antioxidants and the over accumulation lipids in serum towards normal. In addition, results showed that green tea significantly reduced total cholesterol (TC), plasma triglyceride (TG), LDL-C, VLDL-C and elevated plasma antioxidant in green tea treated rats groups (3) compared to group (2). Therefore, the results are clearly indicative of the beneficial effects of green tea in reducing lateral side effects of hyperlipidemia.

Keywords: High fat fed diet, Green tea (*Camellia sinensis*), Hyperlipidemia and Hepatic statuses .

INTRODUCTION

Hypercholesterolemia is a world-wide problem faced by many societies and is a cause critical concern for health professionals, since it constitutes one of the major risk factors for the development of cardiovascular diseases and liver injury such as atherosclerosis and its complication (Banerjee *et al*.,2003). Moreover, there is a close correlation between these diseases and lipid abnormalities, especially high level of plasma cholesterol, and blood pressure (Mohammadi and Oshaghi 2014). Cholesterol-enriched diet has been reported to adversely affect the health of humans and animal species. High level of blood cholesterol is a contributory factor of atherosclerosis and many lipid associated ailments like obesity and kidney failure (Sathivel, *et al*., 2008). Natural plant products have been used throughout human history for various purposes. Having co-evolved with animal life, many of the plants from which these natural products are derived are billions of years old. Tens of thousands of these products are produced as secondary metabolites by higher plants as a natural defense mechanism against disease and infection. Many of these natural products have pharmacological or biological activity that can be exploited in pharmaceutical drug discovery and drug design. Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern (Newman *et al*., 2003 ; Balunas and Kinghorn 2005 ; Gurib-Fakim , 2006 and Newman and Cragg , 2007).

Tea is one of the most popular drinks in the world, and its consumption is nearly as great as coffee. Tea has been used for centuries by ancient cultures for its medicinal properties and is popularly consumed in unfermented (green tea), semifermented (orang), and fermented (black tea) forms. Black tea is commonly consumed in the West whereas the consumption of green tea is especially popular in Asia, mainly for its health benefits. Several studies have reported that green tea extract has antioxidant, antibacterial, antiviral, anticarcinogenic, and antimutagenic functions (Higdon and Frei, 2003; Lin *et al*., 2008).

Tea's beneficial health effects are thought to stem from polyphenols with antioxidant properties. Green tea contains polyphenols which include flavanols, flavandiols, and phenolic acids (up to 30% of dry weight). The most important flavonoids are catechins, which are present at about 10% of the dry weight basis. Six major catechins known to display biological activity are (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-gallocatechin gallate (GCG), (-)-epigallocatechin gallate (EGCG) and (-) - epicatechin gallate (ECG). These native phenolic compounds are strong antioxidants with antimutagenic, anticarcinogenic, hypocholesterolemic, antibacterial, antiallergic and other clinically relevant activities (Yamamoto *et al*., 1997).

Green tea contains considerable amounts of catechins, which contribute most to its antioxidant properties. Besides, green tea is also reported to reduce serum cholesterol levels and inhibit hypertension in several experiments (Muramatsu *et al*., 1986 ; Hodgson *et al*., 1999).

Studies have shown that tea possesses diverse pharmacological properties which include anti-inflammatory (Mutoh *et al*., 2000), anti-mutagenic (Steele *et al*., 2000), antiangiogenic (Jung and Ellis, 2001), antiaging effects (Esposito *et al*., 2002), and preventive effects against cancers.

This study was designed to determine the total phenols, total flavonoids and antioxidative effect of green tea on hypercholesterolemic in rats.

MATERIALS AND METHODS

Materials

Green tea(*Camellia sinensis*), was obtained from a local market, El-Mansoura City , Egypt.

All chemicals and reagents used, in this study, were purchased from EL- Gomhouria Company, Dokki, Cairo, and Alamana Company, Cairo City, Egypt.

Male albino rats (Sprague Dawley strain) weighing (100g±10 g) were obtained from the Medical Experimental Research Central in the Faculty of Medicine , Mansoura University, Egypt.

Methods

Analytical Methods

Determination of total flavonoids:

Flavonoids compounds were determined according to the method of (Zhisen , 1999).

Fractionation of flavonoids compounds :

Flavonoid compounds were determined by HPLC according to the method of (Mattila , *et al* ., 2000).

Determination of total phenolic compounds : .

The total phenolic content of investigated sample were determined using Folin-Ciocalteau reagent according to (Velioglu , *et al*., 1998).

Fractionation of phenolic compounds:

Phenolic compounds were determined using HPLC according to the method of (Goupy, *et al* ., 1999).

Determination of Antioxidant activity (DPPH%):

Antioxidant activities were determined by HPLC according to the method of (Brand – Willims, *et al*., 1995).

Biological evaluation

Experimental Animals

Twenty four adult albino rats weight (100 ±10 g.) were kept under normal healthy conditions. All animals were housed in bottomed cages, fresh and clean drinking water was supplied through specific nipple. Rats were kept at a constant environmental and nutritional conditions throughout the period of the experiment (temp 24 ± 2 °C) and (12 hour light - dark cycle). Rats were fed on basal diet for acclimatization, for 10 days (adaptation period). The composition of basal diet (mg/ 100 g) as described by A.O.A.C (1990), salt and vitamins mixture was described by Abo-El naga (2002).

Experimental design

After adaptation period rats were divided to four groups (n=6), Group (1) as negative control group and the others feed on high cholesterol diets to induce hypercholesterolemia by the method used by (Osman , 2001) for 21 days. body weight was measured on every week of the experiment the rats were fed for eight weeks according to the following scheme:

Group (1): Rats fed on basal diet (negative control group).

Group (2): hypercholesterolemic rats fed on basal diet (positive control group).

Group (3): hypercholesterolemic rats fed on basal diet containing 10% green tea.

Group (4): hypercholesterolemia rats fed on basal diet containing tablets atorvastatin.

At the end of experiment (64 days), rats were fasted overnight and anesthetized using diethyl ether and blood samples were collected from the vein plexus eye by capillary tube into a clean dry centrifuge tubes. The serum was separated by centrifuge at 4000 rpm for 10 minutes and kept at- 18^{o} C until analysis.

Determination of body weight (BWG) and feed efficiency ratio (F.E.R):

Feed and growth parameters of hypercholesterolemic rats were determined. These parameters were food intake body weight gain (BWG) and food efficiency ratio (FER) according to Chapman *et al*., (1950).

$$\mathbf{B.W.G\ \%} = \frac{\textbf{Final body weight – initial body weight}}{\textbf{Initial body weight}} \textbf{x100}$$

$$(\boldsymbol{F.E.R}) = \frac{\boldsymbol{Daily\ body\ weight\ gain}}{\boldsymbol{Daily\ food\ intake}} \boldsymbol{x\ 100}$$

Biochemical analysis of serum:

Serum samples were analyzed at Medical Research Center Experimental lab, in the Faculty of Medicine, Mansoura University.

Blood picture

White blood cells (WBCs) count, red blood cells (RBCs) count, hemoglobin (HGB) concentration and blood platelets (PLT) count were determined according to methods of Cynthia *et al*., (1993).

Lipid profile

Serum total cholesterol (T.C.) was determined by enzymatic colorimetric method using kits according to Allain *et al*., (1974), Triglycerides (TG) were determined according to the method described by Fossati and Principe (1982),Serum HDL-cholesterol was determined according to (Lopez *et al*., 1977) and Serum Low Density Lipoprotein (LDL-cholesterol) was calculated by the difference between total cholesterol, HDL-c and very low density lipoprotein according to Friedewald *et al.,* (1972).

Determination of kidney functions:

Urea was determined by colorimetric enzymatic method according to Tabacco *et al*., (1979) and Creatinine was determined according to the method described by (Henry,1974).

Determination of liver functions .

Serum Alanine amino transferees (ALT) and Aspartate aminotransferees (AST) activites were calorimetric have been identified according to the method described by (Reitman and Frankel , 1957).While albumin in serum or plasma samples was done according to Doumas *et al.,* (1971).

Statistical analysis:

Data were statistically analyzed according to the technique of analysis variance (ANOVA), the least significant difference (L.S.D) and Duncan's methods was used to compare the deference between the means of treatment values to the methods described by Gomez, (1984). All statistical analyses were performed using analysis of variance technique by means of Co STATE Computer Software.

RESULTS AND DISCUSSION

Phenolic compounds, total phenolic compounds and antioxidant activity in aqueous and ethanol extract of green tea.

Phenolic compounds of green tea was tabulated in Table (1). From these results, it is evident that green tea had considerable amounts of phenolic compounds. Data in Table (1) showed that phenolic compounds appear in green tea water extract and recorded the highest value of Caffeine 7175.23 mg/100g, while results showed the lowest concentration Cinnamic 5.53 mg/100g . Also the highest content in phenolic compound in green tea ethanol extract was Caffeine 6069.07mg/100g , while Ferulic recorded the lowest concentration 14.84 mg/100g. The results at the same Table (1) showed that total phenolic compounds in ethanol extract of green tea higher than aqueous extract which recorded 176.326 and 94.87 mg/g ,respectively. While results showed the lower antioxidant activity in ethanol extract of green tea (86.166%) than aqueous extract (88.17%). These result agreed with those reported by Yao *et al., (*2006) and Friedman *et al.,* (2005) who reported The major component (cover 90% dry weight of phenolic) of total phenolic compounds in tea leaves is composed of flavonoids, in which flavan-3-ols (catechins) become the major constituent, which cover up to 30% of their dry weight. On the basis of the stereochemical configuration of the 3', 4'-dihydroxyphenyl and hydroxyl groups at the 2- and 3-positions of the C-ring, catechins can be categorized to

two isomers: trans-catechins and cisepicatechins. Both of them exists as two optical isomers: (+)-catechin and (-)-catechin and (-)-epicatechin and (+)- epicatechin, respectively. While, (-)-catechin can be converted by esterification with gallic acid to produce the esterified or galloyl catechins: (-)-catechin-3-gallate, (-)- epicatechin-3-gallate, (-)-epigallocatechin-3-gallate and (-) - gallocatechin-3-gallate.

Table 1. Phenolic compounds, total phenolic compounds and antioxidant activity (DPPH%) in both of aqueous and ethanol extracts of green tea.

Phenolic compound (mg/100g)	GREEN TEA	
	Water extract	Ethanol extract
Gallic	600.00	151.10
Pyrogallol	818.35	215.76
4-Amino-benzoic	159.94	181.76
3-OH-Tyrosol	74.38	43.57
Protocatechuic	335.37	256.24
Catechein	227.31	-
Chlorogenic	723.80	1206.40
Catechol	50.53	40.83
Epi-Cateechein	2289.51	2050.62
Caffeine	7175.23	6069.07
P.OH-.benzoic	506.58	4285.69
Caffeic	209.62	590.03
Vanillic	122.25	94.81
P-coumaric	421.45	251.57
Ferulic	30.19	14.84
Iso-Ferulic	10.83	37.56
Reversetrol	85.87	55.99
Ellagic	448.04	365.26
E-vanillic	2214.69	307.78
Alpha-coumaric	33.86	31.44
Benzoic	470.09	198.11
Salycilic	96.33	325.72
Coumarin	20.38	3.48
3,4,5.methoxy cinnamic	52.33	45.01
Cinnamic	5.53	18.54
Total Phenolic compounds(mg/g)	94.87	176.326
DPPH%	88.17	86.166

DPPH%=Antioxidant activity

Flavonoid compounds and total flavonoids in aqueous and ethanol extract of green tea.

Flavonoid compounds and total flavonoids in aqueous and ethanol extract of green tea are tabulated in Table (2).

Table 2. Flavonoid compounds and total flavonoids in aqueous and ethanol extract of green tea.

Flavonoid Compounds (mg/100g)	Samples	
	Green tea Water extract	Green tea Ethanol extract
Luteolin	110.76	45.17
Narengin	343.93	157.24
Rutin	198.79	-
Hisperidin	1399.97	1839.63
Rosmarinic	4142.01	2362.26
Quercetrin	146.39	119.64
Quercetin	70.58	85.99
Hispertin	74.79	40.37
Kampferol	7.17	19.69
Apegnin	3.69	7.29
7-OH-Flavone	0.07	0.26
Total flavonoids (mg/g)	38.1	98.198

These previous data point that total flavonoids in ethanol extract of green tea was 98.198 mg/g and decreased to 38.1 mg/g for aqueous extract. Also, date in Table (2) showed the content of flavonoid compounds ranged from 0.07 to 4142.01mg/100g in aqueous extract of green tea, while it ranged from 0.26 to 2362.26mg/100g in ethanol extract of green tea. In general, aqueous extract of green tea has higher amount of flavonoid compounds than ethanol extract of green tea such as rosmarinic, narengin, rutin, quercetrin, luteolin, and hispertin.

Effect of feeding on green tea on body bn weight gain, food intake and food efficiency ratio of rats :

Table (3) showed that the mean values initial of body weight gain (g), daily food intake (g) and food efficiency ratio, of rat fed on hypercholesterolemic containing 10%green tea and atorvastatin tablets.

Table 3. Effect of feeding on green tea on body weight gain , food intake and food efficiency ratio of rats

Parameters Groups	Weight gain (g)	Daily food intake (g/d)	Food efficiency Ratio (FER)
Group (1): -ve	113.33 ± 0.94^{b}	17.00 ± 0.01^{a}	10.41 ± 0.09^{ab}
Group (2): +ve	132.0 ± 0.01^{a}	16.85 ± 0.01^{ab}	11.80 ± 0.01^{a}
Group (3): Green tea	70.00 ± 0.01^{c}	13.99 ± 0.82^{cd}	7.82 ± 0.46^{cd}
Group (4): Atorvastatin tablets	48.66 ± 0.47^{d}	14.99 ± 0.28^{c}	8.86 ± 0.54^{c}

G1- = Basal died control (negative). G2+ = high - fat control (positive). G3 = Basal died +10% green tea. G4 =Basal died + atorvastatin tablets. a,b,c and d : means of lsd.

The obtained results illustrated that there are significant difference of body weight gain with all groups. Body weight gain showed significant difference between -ve control(G1) and +ve control(G2) 113.33g and 132.00g, respectively. There are significant difference in body weight gain between positive control group and all groups .On the other hand, the lowest value in body weight gain was 48.66 g in group (4) , While the body weight gain value was recorded 70.00 g in group (3). Food efficiency ratio , showed significant difference between the negative control and group 3 and 4 (10.41, 7.82 and 8.86, respectively).While , highest value was 11.80in group (2).

Effect of feeding on green tea on lipid profile in rats:

Results in Table (4) showed the effect of feeding on green tea on total cholesterol (TC), triglycerides (TG), LDL-c, HDL-c and vLDL-c of normal and hypercholesterolemic rats. From the obtained data, it could be observed that, levels of, LDL-cholesterol and vLDL-cholesterol at the end of experimental period, were decreased in groups which fed on diet contains of green tea (G3) and atorvastatin tablets (G4). While (+ve group) was recorded the higher blood LDL-cholesterol and vLDL-Cholesterol levels. It was the lowest decline in LDL-cholesterol and vLDL-Cholesterol levels appeared in the G1. On the contrary, the groups which fed on diet contains of green tea and atorvastatin tablets significantly increase HDL-cholesterol levels compared with(-ve group)) to reach its lowest level in G2(+ve group) reaching 35.00mg/dl. Concerning the results of triglycerides in blood serum recorded 90.0mg/dL blood serum for negative group, while, increased to 136.3mg/dL in positive

group. Also it was observed that no significant deference between rats treated with atorvastatin tablets and group 3(fed on diet contains of green tea) (110.0 and 110.0 mg/dl, respectively).While the total cholesterol of groups feed on diet contains of green tea (G3) and atorvastatin tablets (G4) were seen decreased significantly from 152.6 to 116.3mg/dl in +ve group and group (4), respectively. These results in parallel with those of other researchers (Jang *et al*., 2008).

The hypercholesterolemic effect may be ascribed to the increased dietary cholesterol intake , and Abdel-Maksod *et al.,* (2002) who reported that mice and rats received cholesterol-enriched diet showed hypercholesterolemia, elevated plasma serum LDLc and VLDLc compared with those fed a normal diet. Regarding garlic extract, our findings going with Mohammadi and Oshaghi (2014) .

Table 4. Effect of feeding on green tea on lipid profile in rats.

Parameters Groups	TC mg/dl	TG mg/dl	LDL-c mg/dl	HDL-c mg/dl	vLDL-c mg/dl
Group (1): -ve	103.6 ±2.4^{c}	90.0±2.2^{c}	37.00±3.2^{d}	48.66±1.70^{b}	18.00±0.4c
Group (2): +ve	152.6±7.8^{a}	136.3±9.0^{a}	90.40±7.29^{a}	35.00±1.63^{d}	27.26±1.80^{a}
Group (3): Green tea	116.0±1.6^{b}	110.0 ±1.6^{b}	51.00 ±1.45^{b}	43.00±0.82^{c}	22.00±0.33^{b}
Group (4): Atorvastatin tablets	116.3 ±1.2^{b}	110.6 ±1.7^{b}	39.20 ±1.72^{c}	55.00 ±2.83^{a}	22.13 ±0.34^{b}

G1- = Basal died control (negative). G2+ = high - fat control (positive).
G3 = Basal died +10% green tea. G4 =Basal died + atorvastatin tablets a,b,c and d : means of Sd.

Effect of feeding on green tea on liver functions.

The effect of green tea on liver functions in male rats, which, ALT and AST enzymes activity and Albumin were analyzed. Tabulated data in Table(5) show that ALT and AST sharply increased from 35.63 and 44.00 U/L to 66.63 and 81.00 U/L, respectively. Administration of atorvastatin tablets slightly reduced these values to 39.33 and 53.00 U/L. But feeding on green tea could significantly reduce these values to 37.66 and 58.00 U/L for ALT and AST, respectively. Albumin content ranged from 3.00 g /dl in +ve group to 3.90 g /dl in -ve group. The previous results reported that although treated with green tea helped to improve of liver functions.

Table 5. Effect of feeding on green tea on liver functions.

Parameters Groups	Albumin (g /dl)	ALT (IU/L)	AST (IU/L)
Group (1): -ve	3.90 ± 0.22^{a}	35.63 ± 1.70^{d}	44.00 ±2.94^{d}
Group (2): +ve	3.00 ± 0.08^{b}	66.63 ±2.05^{a}	81.00 ±3.27^{a}
Group (3): Green tea	3.60 ± 0.08^{b}	37.66 ±3.86^{c}	58.00 ±3.74^{b}
Group (4): Atorvastatin tablets	3.66 ± 0.05^{b}	39.33 ± 2.62^{b}	53.00 ±1.63^{c}

G1- = Basal died control (negative).
G2+ = high - fat control (positive).
G3 = Basal died +10% green tea.
G4 =Basal died + atorvastatin tablets
a,b,c and d : means of Sd.

These results agreed with those of Sudhahar *et al.* (2007) whose reported that High fat diet intake caused a highly significantly ellevated (ALT),(AST) of hypercholesterolemic control rats as compared to normal rats. Also, Thiemermann *et al.,* (1995) who reported that rats injected with LPS for 6 h was associated with a significant rise in the serum levels of a GOT or GPT and bilirubin, and hence, liver dysfunction. And our finding were in concomitant with Liu *et al.,* (2008) who observed that blood biochemistry indexes, including those of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL), had risen by 6 h post-LPS/D-GalN injection, reached a peak after 24 h and sustained high levels after 48 h. An abnormal liver appearance was found after 24 and 48 h post-injection.

Effect of feeding on green tea on kidney.

Data in Table (6) show that creatinine content was increased from 0.500to 0.008 mg/dl for -ve control group and +ve control group, respectively. These results agreed with those of (Hassan *et al.,* 2011) High fat diet intake caused a highly significantly elevated serum urea and creatinine concentrations of hypercholesterolemic control rats.as compared to normal rats. Green tea reduced this value to 0.567 mg/dl and 0.600 mg/dl for atorvastatin tablets. While, results show urea levels was significantly different ($P<0.05$) in all groups. Hypercholesterolemic control group has the highest urea level (61.00mg/dl) followed by group treated with green tea group (35.00mg/dl) followed by atorvastatin tablets group (33.66mg/dl) in compared with normal control group (31.00 mg/dl).These results agreed with those Waleed *et al.* (2017) who shows the effects of green tea on the urinary function of diabetic animals. After oral administration of green tea, urinary urea N excretion was significantly lower compared with that .Creatinine, glucose and protein excretion declined GT-treated animals compared with 12-week diabetic rats

Table 6. Effect of feeding on green tea on kidney.

Parameters Groups	Uric acid mg/dl	Creatinine mg/dl
Group (1): -ve	31.00±0.82^{d}	0.500±0.008^{d}
Group (2): +ve	61.00±1.63^{a}	0.811±0.008^{a}
Group (3): Green tea	35.00±1.63^{b}	0.567±0.040^{c}
Group (4): Atorvastatin tablets	33.66±0.62^{c}	0.600±0.008^{b}

G1- =Basal died control (negative). G2+ = high - fat control (positive).
G3 = Basal died +10% green tea.
G4 =Basal died + atorvastatin tablets a,b,c and d : means of Sd.

CONCLUSION

In conclusion, results of this study increase the current knowledge on the bioactive components of green tea. These results briefly could concluded that green tea had vital effects to prevent hypercholesterolemic level by its high content of polyphenols.

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التأثير البيولوجي للشاي الاخضر على فئران المصابة بارتفاع مستوي الكوليسترول

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أجريت هذه الدراسة لتقييم التأثير الواقي للشاي الأخضر على الفئران المغذاه على نظام غذائي عالي الدهون. تم تقسيم أربعة وعشرون فأرا وزن كل منها ١٠٠ ± ٣٠ جم إلى ٤ مجموعات. مجموعة (١) مجموعة كنترول سالبه تغذت على الوجبة القياسية، مجموعة (٢) مجموعة كنترول موجبه تغذيه على نظام غذائي غني بالدهون، مجموعة (٣) فئران مفرطة الكوليسترول تغذت على الوجبة القياسية تحتوي على ١٠٪ شاي أخضر ومجموعة (٤) فئران مفرطة الكوليسترول تغذت على الوجبة القياسية تحتوي على أقراص اتروفاستاتين. في نهاية التجربة، تمت مقارنة المجموعات الأربع من حيث نسبة الدهون في الدم. أشارت النتائج إلى أن النظام الغذائي مرتفع الدهون تسبب في حدوث خلل في الميتابولزم، بما في ذلك زيادة الجليسريدات الثلاثية في الدم، وخلل في وظائف الكبد.كما أظهرت الفئران التي تغذت على غذاء غني بالدهون فقط زيادة نشاط إنزيمات الكبد، وانخفاض مستوى مضادات الأكسدة، وارتفاع مؤشرات بيروكسيد الدهون في الكبد. خفض العلاج بالشاي الأخضر بشكل ملحوظ من منتج بيروكسيد الدهون ، وأعاد مضادات الأكسدة في الكبد وارتفاع مستوى الدهون الى المعدل الطبيعي. بالإضافة إلى ذلك، أظهرت النتائج أن الشاي الأخضر قلل بشكل كبير من مستوي الكوليستيرول الكلى والدهون الثلاثيه والكوليستيرول منخفض الكثافه والكوليستيرول منخفض جدا الكثافه في الدم كما رفع مستوى مضادات الاكسده في الكبد في مجموعه الفئران المعامله بالشاى الأخضر مجموعه رقم (٣) مقارنة بالمجموعة (٢). ولذلك، فإن النتائج تشير بوضوح إلى الآثار المفيدة للشاي الأخضر في الحد من الآثار الجانبية الجانبية لزيادة نسبه الدهون فى الدم.