

The enhancement effect of administration of caffeine in combination with Green tea and its component on lipid profile elements in obese rats.

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Abstract: Obesity-associated dyslipidemia is linked directly with increased risks of atherosclerosis and cardiovascular diseases. Dyslipidemia is recognized by the increase in total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL); and the decrease in high density lipoprotein (HDL) concentrations. Beneficial effects of green tea are related to its main constituent; Catechins; particularly epigallocatechin-3-gallate (EGCG). Green tea contains caffeine which known to stimulate thermogenesis and fat oxidation. The aim of this study was to evaluate the effect of caffeine on EGCG and green tea extract-administered to obese rats fed with high-fat diet. Animals were divided into two groups. The first group served as healthy control group. The second group was divided into 6 subgroups according to the treatment supplementation after 8 weeks of feeding high fat-diet. The first subgroup served as obese positive control group continue to feed on high fat diet, the second one was supplied with green tea extract, the third was supplied with EGCG, the fourth was supplied with caffeine, the fifth was supplied with a mixture and the sixth was supplied with a mixture of caffeine and EGCG then TC, LDL, TG and HDL were determined. A very high significant changes ($p < 0.000$) were observed in TC, TG, LDL and HDL concentrations in the obese group and after 5 and 9 weeks of the treatment as compared to the control group. Results showed a high significant decrease ($p < 0.001$) in TC, TG and LDL levels while a high significant increase (0.001) was observed in HDL levels in all studied groups (EGCG, caffeine and green tea extract groups) after 5 and 9 weeks of the treatment as compared to the obese group. Caffeine in combination with EGCG or green tea extract showed enhancement effect with the decrease in TC, TG and LDL after 5 weeks ($p < 0.002$, $p < 0.027$, $p < 0.002$ with EGCG and $p < 0.001$, $p < 0.010$, $p < 0.000$ with green tea extract) and the increase in HDL ($p < 0.009$ with EGCG and $p < 0.021$ with green tea extract). The enhancement effect was clearly observed after 9 weeks of treatment by the decrease in TC, TG and LDL ($P < 0.079$, $P < 0.471$ and 0.058 with EGCG and $p < 0.030$, $p < 0.048$ and $p < 0.016$ with green tea extract) and the increase in HDL ($P < 0.145$ with EGCG and $p < 0.087$ with green tea extract) indicating that at the end of the experiment, all the lipid profile elements tend to return to the normal levels in response to the lipolytic effects of green tea and its constituents. The synergistic effect of caffeine and green tea component should be recommended in the management of obesity.

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Key words: Obesity, green tea, EGCG, caffeine, TC, TG, LDL, HDL.

1. Introduction:

Obesity is defined as, an excessive fat accumulation in the body. According to the world health organization (WHO), obesity is one of the greatest public health challenges of the 21st century

(Aurore and Marie 2010). It is generally associated with an increased risk of excessive fat related metabolic and chronic diseases including type 2 diabetes mellitus, hypertension and dyslipidemia (Bogaert and Linas 2009). Obesity-associated pattern of dyslipidemia (high Triglyceride levels, low HDL concentrations and high LDL particles) plays a crucial role in the development of atherosclerosis and cardiovascular diseases in obese subjects, because all the elements of dyslipidemia normally associated with these diseases have been shown to be atherogenic (Jernas *et al.*, 2006). Health benefits of green tea were

reported. Many of these benefits of green tea are related to its catechins particularly epigallocatechin-3-gallate (EGCG). There were evidences from *in vitro* and *in vivo* studies on underlying mechanisms of green tea catechins and their anti-obesity and anti-inflammatory effects (Sabu *et al.*, 2010). These mechanisms may be related to certain pathways such as through the modulation of energy balance, endocrine system, food intake, lipid and carbohydrate metabolism and redox status (Yang *et al.*, 2002). Green tea was reduced significantly serum cholesterol and triglyceride, improved plasma lipid profile, reduced LDL and VLDL oxidation and concurrently increase HDL in hamster fed normal or high cholesterol diet (Hea *et al.*, 2010). The purified EGCG was found to reduce or prevent the increase in body weight in obese rats, the effect that in turn inhibit lipid oxidation and

modulate glucose level (Tsuneki *et al.*, 2004). Green tea contains caffeine which is a member of methylxanthine family of drugs that may stimulate thermogenesis and fat oxidation through inhibition of phosphodiesterase (Belza *et al.*, 2007). Caffeine has been previously utilized in therapies for weight loss, due to claimed action of amplifying the lipolytic effect of ephedrine (Diepven *et al.*, 2007). Thus, the present study aimed to investigate the effect of caffeine in combination with EGCG and green tea extract administered in obese rats taking elements of lipid profile in consideration.

2. Material and Methods

Materials:

Leaves of the Chinese green tea obtained from a local market, Caffeine was obtained from Sigma chemical company (U.S.A.), EGCG was obtained from Sigma chemical company (U.S.A.), Green tea extract was obtained from boiling the Chinese green tea leaves. Stock solutions of Caffeine and EGCG were prepared by dissolving in sterile double distilled water at a final concentration of 100Mm and they kept at -20 degree for long term storage.

Animals and drug administration:

Four to six weeks old male albino rats (Sprague Dawley strains), about 150 -200 g were purchased from animal house of national research centre, Dokki, Giza, Egypt. Environmental conditions were properly standardized with 12- hours light cycle and a constant temperature of 20 degree and humidity of 48%. Animals were fed on a standard laboratory pellets and tap water ad libitum. Experimental procedures conformed to the National Health and Medical Research Council guidelines and were approved by an institutional animal ethics committee. Animals were divided into two groups. The first group served as healthy control group and composed of 20 rats. Obesity was induced in the second group by feeding high fat diet for 8 weeks. The second group (obese rats) was divided into 6 subgroups each composed of 20 rats. The first subgroup served as obese positive control group continue to feed high fat diet and supplied with distilled water through the stomach lavage, the second subgroup was supplied with green tea extract (4.86mg/200g body weight) through the stomach lavage, the third subgroup was supplied with epigallocatechin-3-gallate (EGCG) (1.44mg/200g body weight) through the stomach lavage; the fourth subgroup was supplied with caffeine (2.7 mg/200g body weight) through the stomach lavage; the fifth subgroup was supplied with a mixture of caffeine (2.7mg/200g body weight) and green tea extract (4.86mg/200g body weight) through the stomach lavage, and the sixth subgroup was

supplied with a mixture of caffeine (2.7mg/200g body weight) and EGCG (1.4mg/200g body weight) through the stomach lavage. Supplemented doses of the treatment were given according to Paget and Barnas (1964).

Sample collection:

Fasting blood samples were collected from the retro orbital plexus of rats (Schemer, 1967), under diethyl anesthesia by clean heparinized capillary tubes and were left to clots, then centrifuged at 5000 rpm for 10 minutes to separate sera which in turn were used for the determination of different biochemical parameters. Blood samples were collected after the induction of obesity, after five weeks of the prevalence of obesity and after nine weeks of prevalence of obesity.

Biochemical assays:

Cholesterol was determined using colorimetric method (Richmond, 1973), using Biodiagnostic kit, Egypt. High Density lipoprotein cholesterol (HDL-C) is determined according to the CHOP- PAP method by photometric systems (Rifai *et al.*, 1999), using Diasys Diagnostic systems GmbH Kit, Germany. Low density lipoprotein cholesterol (LDL-C) was determined colorimetrically using CHOD- PAP- method (Rifai *et al.*, 1999), using Diasys Diagnostic systems GmbH Kit, German. Triglycerides were determined econometrically according to Fassati and Principle, (1982) method using Biodiagnostic kit, Egypt.

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Prenciple., (1982) method using Biodiagnostic kit, Egypt.

Statistical analysis:

The results were expressed as mean \pm standard deviation .statistical analysis of differences between means were performed using student “t” test (Sendecor and Cochran, 1967).

3. Results

Lipid profile results in normal ,obese and treated groups:

Results of lipid profile were represented by mean levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) in normal, obese and treated groups. The significant changes between obese group compared to control group were recorded in Table (1) and the significant changes between treated groups and obese group were recorded in Tables (2 and 3).These results showed a very high significant increase in TC,TG and LDL and high significant decrease in HDL in obese group and after 5 and 9 weeks of treatment as compared to control animals.

Total Cholesterol normal ,obese and levels in treated groups:

Differences between total Cholesterol levels in EGCG group compared to the control group were recorded in Tables (2 and 3).These results showed a very high significant increase ($p < 0.000$) in the obese group, a high significant increase after 5 weeks of the treatment ($p < 0.001$) (Table 2) and after 9 weeks of the treatment ($p < 0.024$) as compared to the control group (Table 3). Total cholesterol levels were decreased significantly after 5 weeks ($p < 0.002$) and after 9 weeks ($p < 0.001$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between total cholesterol levels in the Caffeine group compared to the control group were represented in Tables (2 and 3). These results showed a very high significant increase ($p < 0.000$) in the obese group, a high significant increase after 5 weeks of the treatment ($p < 0.001$) (Table 2) and after 9 weeks of the treatment ($p < 0.011$) as compared to the control group (Table 3).Total cholesterol levels were decreased significantly after 5 weeks ($p < 0.001$) and after 9 weeks ($p < 0.002$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between total cholesterol levels in the green tea extract group compared to the control group were recorded in Tables (2 and 3). These results showed a very high significant increase ($p < 0.000$) in the obese group , a very high significant increase ($p < 0.000$) after 5 weeks of the treatment (Table 2) and a

high significant increase ($p < 0.003$) and after 9 weeks of the treatment as compared to the control group (Table 3). Total cholesterol levels were decreased significantly after 5 weeks ($p < 0.003$) and after 9 weeks ($p < 0.007$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between total cholesterol levels in the green tea extract plus Caffeine group compared to the control group were represented in Tables (2and 3). These results recorded a very high significant increase ($p < 0.000$) in the obese group , a high significant increase after 5 weeks of the treatment ($p < 0.001$) (Table 2) and after 9 weeks of the treatment ($p < 0.030$) as compared to the control group (Table 3).Total cholesterol levels were decreased significantly after 5 and 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between total cholesterol levels in EGCG plus Caffeine group compared to the control group were recorded in Tables (2and 3). These results showed a very high significant increase ($p < 0.000$) in the obese group, high significant increase ($p < 0.002$) after 5 weeks of the treatment (Table 2) and an insignificant increase ($p < 0.079$) after 9 weeks of the treatment as compared to control group (Table 3). Total cholesterol levels were decreased significantly after 5 weeks ($p < 0.000$) and after 9 weeks ($p < 0.001$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Triglyceride levels in normal ,obese and treated groups:

Differences between triglyceride levels in the EGCG group compared to the control group were recorded in Tables (2and3). These results showed a high significant increase ($p < 0.005$) in the obese group , an insignificant increase after 5 weeks of the treatment ($p < 0.056$) (Table 2) and after 9 weeks of the treatment ($p < 0.545$) as compared to the control group (Table3).Triglycerides levels were decreased significantly after 5 weeks ($p < 0.003$) and after 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between triglyceride levels in the caffeine group compared to the control group were recorded in Tables (2 and 3). These results showed a high significant increase ($p < 0.005$) in the obese group , a high significant increase after 5 weeks of the treatment ($p < 0.008$) (Table 2), and after 9 weeks of the treatment ($p < 0.023$) as compared to the control group (Table 3). Triglyceride levels were decreased significantly after 5 and 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between triglyceride levels in the green tea extract group compared to the control group were recorded in Tables (2 and 3). These results showed a high significant increase ($p < 0.005$) in the obese group, a high significant increase after 5 weeks of the treatment ($p < 0.006$) (Table 2) and after 9 weeks of the treatment ($p < 0.013$) as compared to the control group (Table 3). Triglyceride levels were decreased significantly after 5 and 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between triglyceride levels in green tea extract plus caffeine group compared to the control group were recorded in Tables (2 and 3). These results showed a high significant increase ($p < 0.005$) in obese group, a high significant increase after 5 weeks of the treatment ($p < 0.010$) (Table 2) and after 9 weeks of the treatment ($p < 0.048$) as compared to the control group (Table 3). Triglycerides levels were decreased significantly after 5 and 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between triglyceride levels in the EGCG plus caffeine group compared to the control group were recorded in Tables (2 and 3). These results showed a high significant increase ($p < 0.005$) in the obese group high significant increase ($p < 0.027$) after 5 weeks of the treatment (Table 2) and a insignificant decrease ($p < 0.471$) after 9 weeks of the treatment level as compared to the control group (Table 3). Triglyceride levels were decreased significantly after 5 and 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Low density lipoprotein(LDL) levels in normal , obese and treated groups:

Differences between LDL levels in the EGCG group compared to the control group were recorded in Tables (2 and 3). These results showed a very high significant increase in the obese group ($p < 0.000$), a very high significant increase after 5 weeks of the treatment ($p < 0.000$) (Table 2) and a high significant increase after 9 weeks of the treatment ($p < 0.011$) as compared to the control group (Table 3). The LDL levels were decreased significantly after 5 weeks ($p < 0.000$) and after 9 weeks ($p < 0.001$) of the treatment when compared to obese group as shown in Tables (2 and 3).

Differences between LDL levels in the caffeine group compared to the control group were recorded in Table (2 and 3). These results showed a very high significant increase in the obese group ($p < 0.000$), a high significant increase after 5 weeks ($p < 0.002$) and 9 weeks ($p < 0.004$) of the treatment as compared to the control group. LDL levels were decreased

significantly after 5 weeks ($p < 0.003$) and after 9 weeks ($p < 0.001$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between LDL levels in the green tea extract group compared to the control group were recorded in Table (2 and 3). These results showed a very high significant increase in the obese group ($p < 0.000$) and very high significant increase after 5 and 9 weeks of the treatment ($p < 0.000$) (Tables 2 and 3) as compared to the control group. LDL levels were decreased significantly after 5 weeks ($p < 0.006$) and after 9 weeks ($p < 0.001$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between LDL levels in green tea extract plus Caffeine group compared to the control group were recorded in Tables (2 and 3). These results showed a very high significant increase in obese group ($p < 0.000$), a very high significant increase after 5 weeks of the treatment ($p < 0.000$) (Table 2) and a high significant increase after 9 weeks of the treatment ($p < 0.016$) as compared to the control group (Table 3). LDL levels were decreased significantly after 5 and 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between LDL levels in the EGCG plus caffeine group compared to the control group were recorded in Table (2 and 3). These results showed a very high significant increase in obese group ($p < 0.000$), a high significant increase after 5 weeks of the treatment ($p < 0.002$) (Table 2) and insignificant increase after 9 weeks of the treatment ($p < 0.058$) (Table 3) as compared to the control group. LDL levels were decreased significantly after 5 and 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

High density lipoprotein(HDL) levels in normal , obese and treated group:

Differences between HDL levels in the EGCG group compared to the control group were recorded in Tables (2 and 3). These results showed a high significant decrease in the obese group, a high significant decrease after 5 weeks of the treatment ($p < 0.021$) (Table 2) and an insignificant decrease after 9 weeks of the treatment ($p < 0.065$) (Table 3) as compared to the control group. HDL levels were increased significantly after 5 weeks ($p < 0.008$) and after 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between HDL levels in caffeine group compared to the control group were recorded in Tables (2 and 3). These results showed a high significant decrease in the obese group ($p < 0.005$), a high significant decrease after 5 weeks ($p < 0.014$)

(Table 2) and after 9 weeks ($p < 0.038$) (Table 3) of the treatment as compared to the control group. HDL levels were increased significantly after 5 weeks ($p < 0.001$) and after 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between HDL levels in green tea extract group compared to the control group were recorded in Table (2 and 3). These results showed a high significant decrease in the obese group ($p < 0.005$), a high significant decrease after 5 weeks ($p < 0.018$) (Table 2) and 9 weeks ($p < 0.025$) (Table 3) of the treatment as compared to the control group. HDL levels were increased significantly after 5 weeks ($p < 0.011$) and after 9 weeks ($p < 0.003$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between HDL levels in green tea extract plus caffeine group compared to the control group were recorded in Tables (2 and 3). These results showed a high significant decrease in the obese group ($p < 0.005$), a high significant decrease after 5 weeks of the treatment ($p < 0.018$) (Table 2) and an insignificant decrease after 9 weeks of treatment ($p < 0.087$) as compared to the control group (Table 3). HDL levels were increased significantly after 5 weeks ($p < 0.002$) and after 9 weeks ($p < 0.001$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Differences between HDL levels in the EGCG plus caffeine group compared to the control group were recorded in Tables (2 and 3). These results

showed a high significant decrease in the obese group ($p < 0.005$), a high significant decrease after 5 weeks of the treatment ($p < 0.009$) (Table 2) and an insignificant decrease after 9 weeks of the treatment ($p < 0.145$) (Table 3) as compared to the control group. The HDL levels were increased significantly after 5 weeks ($p < 0.003$) and after 9 weeks ($p < 0.000$) of the treatment when compared to the obese group as shown in Tables (2 and 3).

Table (1):- Results of lipid profile elements (TC, TG, LDL, and HDL) in obese group compared to normal control group.

Parameter group	Normal control	Obese group
TC(mg\dl)	99.4± 7.52	188.00± 9.8 *
TG(mg/dl)	90.00 ± 5.3	125.5 ± 10.5 *
LDL (mg\dl)	40.9± 6.05	132.75± 9.03 *
HDL (mg/dl)	57.4± 2.9	29.75±.50 *

Data are expressed as mean standard deviation Total Cholesterol(TC),Triglyceride(TG),Low density lipoprotein(LDL),High density lipoprotein(HDL).
P< 0.05 Significant, P< 0.001 High significant< 0.000 highly significant # Significant versus positive control *Significant versus normal control

Table (2):- Results of lipid profile elements(TC, TG, LDL, and HDL) in each group of experiment after 5 weeks of treatment.

Parameter group	Normal control	positive control group	EGCG group	Caffeine group	Green tea extract group	caffeine plus green tea extract group	caffeine plus EGCG group	
TC(mg/dl)	Value	99.4± 7.52	214.00± 5.20	171.25± 3.80	175.75± 0.96	182.25± 2.63	158.75± 6.60	135.25± 5.40
	sig		*	* #	* #	* #	* #	# *
TG(mg/dl)	Value	90.00± 5.3	138.05 ± 11.3	110.25± 11.50	119.12± 9.90	122.36± 9.03	116.09± 9.6	106.70± 8.70
	sig		*	#	* #	* #	* #	# *
LDL (mg/dl)	Value	40.9± 6.05	160.75± 8.05	100.25± 2.90	111.75± 8.42	125.75± 5.21	95.75± 4.57	83.00± 4.24
	sig		*	* #	* #	* #	* #	# *
HDL(mg/dl)	Value	57.4± 2.9	27.75± 0.95	35.00± 3.91	34.00± 0.82	34.00± 0.82	33.08± 1.40	36.18± 1.60
	sig		*	* #	* #	* #	* #	* #

Table (3):-Results of lipid profile elements (TC, TG, LDL, and HDL) in each group of experiment after 9 weeks of treatment.

Parameter group		Normal control	positive control group	EGCG group	Caffeine group	Green tea extract group	caffeine plus green tea extract group	caffeine plus EGCG group
TC(mg/dl)	Value	99.4± 7.52	288.5± 8.7	129.5± 10.21	147.75± 10.01	173.25± 8.9	119.50± 2.9	116.25± 11.18
	sig		*	* #	* #	* #	* #	#
TG(mg/dl)	Value	90.00± 5.3	144.36± 11.8	94.00± 9.8	107.25± 8.5	112.75± 9.03	105.2± 9.6	93.00± 7.6
	sig		*	#	* #	* #	* #	#
L D L (mg/dl)	Value	40.9± 6.05	175.5± 6.61	78.25± 9.53	91.75± 7.39	110.75± 5.43	75.75± 8.57	53.25± 5.21
	sig		*	* #	* #	* #	* #	#
HDL(mg/dl)	Value	57.4± 2.9	23.65± .75	36.19± 0.92	35.5± .58	34.5± 1.73	36.58± 1.4	37.25± .96
	sig		*	* #	* #	* #	* #	* #

Discussion:

Tea is one of the most widely consumed beverages in the world and is following water in popularity as beverage. Consumption of tea has been associated with many health benefits, including prevention of cancer and heart diseases. These effects are attributed to the polyphenol compounds in tea. Different mechanisms of action have been proposed for the observed beneficial effects of tea polyphenols (Shengmin *et al.*, 2011).

In the present study, effects of green tea and its components on the lipid profile of obesity with the concomitant effect on the inflammatory state that associated with obesity were observed. The lowering effect of green tea on different lipid parameters may be attributed to its lipogenic activity of adipose tissue, regulatory role of liver in lipid metabolism, or reduction of intestinal lipid absorption.

Green tea extract concomitantly suppresses adipose lipogenic and lipolytic activities. Thus in turn, would decrease the flux of non-esterified fatty acid from adipose to the liver where it would be otherwise esterified to triglycerides (Hea *et al.*, 2010). Green tea was reduced significantly serum cholesterol and triglycerides and improved plasma lipid profiles and reduced LDL and VLDL oxidation in hamster fed normal or high cholesterol diet (Yang and Koo, 2000). Moreover, the green tea ingestion decreased LDL-cholesterol, concurrently HDL-cholesterol increases. Green tea supplementation caused a decrease in total cholesterol and LDL-cholesterol in comparison to non-supplemented group (Arpita *et al.*, 2010).

Kuhn *et al.*, (2004) reported that, ester-bond containing green tea polyphenols inhibit ubiquitin/proteasome degradation of the active sterol

regulatory element binding protein-2 (SREBP-2) resulting in up regulation of LDL-receptor.

Susana *et al.*, (2006) stated that, green tea has been associated with lower serum levels of cholesterol, triglycerides, and LDL-cholesterol but higher serum levels of HDL-cholesterol. Green tea up regulate LDL-cholesterol receptor through the sterol-regulated element binding protein and inhibit ubiquitin-proteasome pathway with increased expression of sterol-regulating elements-binding protein - 2 and LDL receptor.

Furthermore, Mohamed *et al.*, (2007) found that, green tea extract reduced triglyceride beside total cholesterol. Green tea extract decreased the expression of hepatic lipogenic genes including sterol regulatory element binding protein-1c (SRBEP-1c) and down stream target genes, fatty acid synthase (FAS) and sterol- CoA desaturase.

All these previous investigations appears in the agreement with our results, current study which showed that, green tea extract reduced the levels of cholesterol, triglycerides, LDL-cholesterol with concomitant increase in HDL-cholesterol levels in obese rats, and suggested that, different mechanisms are involved in beneficial effects of green tea which may be strongly related to specific ingredients.

Inhibition of cholesterol absorption has been proposed as a mechanism to explain the cholesterol lowering effects of green tea. This is because the fecal excretion of total lipids and cholesterol were found to be higher in animals consuming green tea extract (Yang and Koo, 2000). It's evident that green tea effectively lower the intestinal absorption of lipids, in dose dependent manner in rats, investigators demonstrated that, tea catechins are less effective in inhibiting cholesterol absorption than EGCG. Ikeda *et*

al., (2003) stated that , green tea also inhibit luminal lipid hydrolysis by inhibiting pancreatic phospholipase A2 (PLA2), showed that , EGCG was the most effective in inhibiting PLA2 activity.

EGCG plus caffeine supplementation to the rats fed high-fat diet reversed these alterations with less effect with EGCG alone, caffeine ,green tea extract plus caffeine and the least effect was obtained by using green tea extract alone. These results are consistent with previous studies those showed that orally administered EGCG, caffeine and green tea caused a reduction in total cholesterol (Kao *et al.*,2000 and Tsuneki *et al.*,2004).

One of the possible mechanisms for the hypocholesterolemic activities of EGCG appears to be due to , the inhibition of intestinal cholesterol absorption by reducing micelles solubilization of cholesterol and subsequently fat excretion increases, causing a reduction in liver cholesterol concentration(Ikeda *et al.*, 1992). They proposed that , EGCG may form emulsion, hindering access to the substrate by PLA2 or directly with the enzyme protein altering its conformation and catalytic activity (Readerstorff *et al.*, 2003).

Christina *et al.*, (2007) stated that , EGCG was found to inhibit cholesterol synthesis and up regulate LDL receptor binding activity and LDL receptor protein, EGCG has been shown to increase both the expression and phosphorylation of adenosine mono-phosphate kinase and the phosphorylation of acetyl-CoA carboxylase which leads to suppression of etherification of fatty acids to triglycerides (Mohsen and Sayeda, 2010).

On the other hand, caffeine administration to obese rats in the present study showed Total cholesterol and triglycerides lowering effect. It has been reported that , caffeine elevates the activity of hepatic acyl-CoA oxidase in rats fed high-fat diet. Also, the decrease in hepatic TG is possibly due to the increased hepatic B-oxidation activity by caffeine. Caffeine increases the lipolysis through catecholamine. Unlike EGCG, caffeine is rapidly absorbed from the stomach and small intestine and may have little impact on the luminal hydrolysis and micelles solubilization of lipids, but influence the intracellular events of lipid processing and transport involving chylomicrons (Kabayashi *et al.*, 2005).

Caffeine may not interfere with the luminal processes leading to the cell uptake of lipid , but delay the intracellular movement of the absorbed lipid from the enterocyte into the lymphocytes. Caffeine induced decline in lymph flow was associated with the lowering of lipid absorption. Methylexanthine, including caffeine are known to elevate intracellular levels of cAMP by inhibiting phosphodiesterase, which responsible for the hydrolytic inactivation of

cAMP. The rise in cAMP was accompanied by the decrease in the secretion of triacylglycerol, cholesterol and apolipoprotein B via VLDL (Leonie *et al.*, 2002).

The previous results were coinciding with our work. Our results showed that , supplementation of caffeine in combination with EGCG or green tea extract enhance their effect on serum levels of lipid profile by decreasing total cholesterol, triglycerides, and decrease LDL-cholesterol with concurrent increase in HDL-cholesterol level in rats fed high-fat diet (obese rats) in comparison to normal control (fed normal diet).

Finally, it could be suggested that , dyslipidemia associated with obesity in the present study participates in abnormal changes in adiposities and adipose tissue macrophages. Moreover, these results showed that , green tea and its components enhance lipid profile of obese rats. These effects appear strongest in animals treated with caffeine in combination with EGCG and green tea. Therefore, the study indicates to the possibility to treat obesity and its complications by combinations of caffeine and green tea components , particularly EGCG.

In conclusion: It could be concluded that , green tea extract, EGCG and caffeine induced different beneficial effects on lipid disorders in obese rats. Caffeine increased the effect of EGCG and green tea extract on lipid profile. The synergistic action of caffeine and green tea components are recommended to be applied in the management of obesity and other related complications.

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