

The enhancement of the anti inflammatory effect of Caffeine on Green tea extract and EGCG on obese rats

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Abstract: Obesity is linked directly with the increased acute phase protein like c-reactive protein (CRP) and increased production of some cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) as a result of an inflammatory signaling pathway. The health benefits of green tea are related to its catechins particularly epigallocatechin-3-gallate (EGCG). Green tea contains caffeine which stimulate thermogenesis and fat oxidation. The aim of this study was to evaluate the effect of caffeine on EGCG –and green tea extract–administered obese rats induced by high-fat diet. The animals were divided into two groups. The first group served as healthy control group. The second group was fed high fat –diet for 8 week and was divided into 6 subgroups. IL-6, CRP and TNF- α showed a high significant increase ($p < 0.01$) at zero level and after 5 and 9 weeks of obesity induction as compared to control group. The results showed a decrease in proinflammatory cytokines effect of EGCG, green tea extract and caffeine on TNF- α after 5 weeks ($P < 0.051, P < 0.039, P < 0.002$), for IL-6 were ($P < 0.001, P < 0.001, P < 0.000$), and for CRP ($P < 0.066, P < 0.007, P < 0.002$) respectively. The 9 weeks treatment by EGCG, Green tea extract and caffeine on TNF- α ($P < 0.312, P < 0.122, P < 0.057$), on IL-6 ($P < 0.155, P < 0.029, P < 0.033$), on CRP ($P < 0.387, P < 0.168, P < 0.088$). The addition of caffeine to green tea showed enhancement of the effect with decrease of TNF- α , IL-6 and CRP after 5 weeks ($P < 0.03, P < 0.001, P < 0.034$) respectively where after the 9 weeks all the parameters reversed back to normal. The addition of caffeine to EGCG after 5 weeks showed enhancement of the effect on TNF- α , IL-6 and CRP ($P < 0.04, P < 0.011, P < 0.066$) respectively, at the end of the 9 weeks all the inflammatory parameters return to normal levels. As conclusion, the addition of caffeine to EGCG or green tea extract enhance their anti-inflammatory effects on the low grade inflammation state accompanied with obesity by decreasing the levels of TNF- α , IL-6 and CRP.

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1. Introduction

Obesity is the direct result of the imbalance between the energy intake and energy expenditure (Uljaszek 2008). Over the past several decades, there has been an alarming increase in the prevalence of obesity both in high-income as well as middle- and low-income countries (Lobstein et al. 2004). Several studies have shown that experimental animal and human obesity associated with the development atherosclerosis and increased prevalence of inflammatory diseases (Silver et al. 2007). The link between the obesity and the inflammation has been reported by the increase in plasma levels of several pro-inflammatory molecules including cytokines such as tumor necrosis factor-alpha (TNF α) and interleukin-6 (IL-6) which may have local effects on adipose tissue physiology and also systemic effects in other tissues (Bastard et al. 2006). Obesity is also closely associated with chronic inflammatory state resulted from increased acute phase proteins and other mediators and activation of a network of inflammatory signalling pathway. C-reactive protein (CRP) is a major human acute phase protein largely

synthesized in hepatocytes following inflammatory stimuli (Black et al. 2004). Cytokines particularly IL-6 has been found to induce hepatic CRP synthesis in primary hepatocytes and hepatoma cells at the transcriptional level (Reza et al. 2009). TNF- α is a multifunctional circulating proinflammatory cytokine found to play a major role in the cytokine cascade as it stimulate the synthesis of other cytokines like IL-6 which is a central mediator of the acute phase protein response and the primary determinant of CRP production there by it contribute to the maintenance of chronic low grade-inflammation involved in the progression of obesity and its associated of co morbidities (Harris et al. 2008). It has been reported that TNF α was overexpressed in adipose tissue of obese animal and human and its concentration is reduced after weight loss (Ziccardi et al. 2002).

The 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 evidences from in vitro and animals studies on the underlying mechanisms of green tea catechins and their antiobesity and ant-

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.2001).Oral administration of EGCG in aged rats fed a high –fat diet was also shown to significantly decrease CRP and TNF- α versus the unsupplemented group (Senthil et al.2009). 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(Diepvens and Westertrep 2007).

The increased and prolonged sympathetic stimulation of thermogenesis by the interaction between EGCG and caffeine could be of value in assisting the management of obesity by suppressing the production of proinflammatory cytokine TNF- α and IL-6 (Dulloo et al.2006). It was appeared that, Nature is, in fact ,the richest botanic pharmacy created for living beings and the scientific efforts must certainly be more oriented towards the discovery of nature in future. Thus, the present study aimed to investigate the effect of caffeine on EGCG –and green tea extract – administered in obese rats tacking some pro inflammatory cytokines in account.

2. Materials and Methods

Materials:

1. Green tea extract was obtained from the leaves of the Chinese green tea obtained from a local market.
2. Caffeine was obtained from Sigma chemical company U.S.A.
3. E.G.C.G was obtained from Sigma chemical company U.S.A.
4. Green tea extract, was obtained from boiling the chine's green tea leaves.
5. The Stock solution of Caffeine and EGCG were prepared by dissolving in sterile double distilled H₂O at a final concentration of 100Mm. Stock solutions were aliquots and kept at -20C for long term storage.

Animals and drug administration:

From four to six weeks old male albino rats (Sprague Dawely strains), about 150 Dokki, Giza, Egypt. The environmental conditions were properly standardized with a 12- hours light cycle and a constant temperature of 20°C and humidity of 48%.

Animals were fed on a standard laboratory pellets and tap water ad libitum. All experimental procedures conformed to the National Health and

Medical Research Council guidelines and were approved by an institutional animal ethics committee.

The 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 2-1: the first subgroup served as obese positive control group continue to feed high fat-diet and supplied distilled water through stomach lavage.

2-2: the second group was supplied with green tea extract (4.86mg/200gm.body weight) through stomach lavage.

2-3: the third group were supplied with epigallocatechin-3-gallate (EGCG)(1.44mg/200gm. Body weight) through stomach lavage.

2-4: the fourth group was supplied with caffeine (2.7 mg/200gm body weight) through stomach lavage.

2-5: the fifth group were supplied with a mixture of caffeine (2.7mg/200gm body weight)and green tea extract (4.86mg/200gm body weight)through stomach lavage.

2-6: the six group were supplied with a mixture of caffeine (2.7mg/200gm body weight) and EGCG (1.4mg/200gm body weight) through stomach lavage. The supplement dose of the treatment was given according to Paget and Barnas (1964), for rats 200gm = human dose *0.018 by intragastric lavage using stomach tube.

Sample collections:

The 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 parameter. Blood samples were collected after induction of obesity (zero level), 5weeks after prevalence of obesity and after 9 weeks of prevalence of obesity.

Biochemical analysis:

Serum TNF- α was determined by using high sensitivity ELISA, enzyme –linked immunosorbent assay for quantitative detection of rat tumor necrosis factor - α from Immunotech A Beckman culture company. Sensitivity 5pg/ml with intra-assay coefficient of variation ranged between 1.6 and 10 % and inter-assay variation coefficient ranged between 5.4 and 12.8%

Rat IL-6 was determined by using Platinum ELISA, enzyme –linked immunosorbent assay for quantitative detection of rat IL-6 from Bioscience Bender Medsystem , Vienna. Sensitivity 12pg/ml

with intra-assay coefficient of variation was <5% and inter-assay coefficient of variation <10%.

Rat CRP was determined by using a solid phase capture sandwich enzyme-linked immunosorbent assay ELISA kit, from Assay Pro Company .sensitivity less than 70ng /ml with intra-assay coefficient of variation 5.5% and inter-assay coefficient of variation 7.6%

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

TNF- α at zero level (obese rats) showed a high significant increase ($p < 0.001$) and highly significant increase ($p < 0.000$) after 5 weeks and after 9 weeks of induction of obesity as compared to control group (Table1).

IL-6 at zero level (obese rats) showed a high significant increase ($p < 0.001$) and high significant increase ($p < 0.002$) after 5 weeks and after 9 weeks of induction of obesity as compared to control group (Table1).

CRP levels showed high significant increase at zero level (obese rats) ($p < 0.014$) and after 5 weeks and after 9 weeks of induction of obesity ($p < 0.002$ ($p < 0.001$) respectively as compared to control group (Table1).

After 5 weeks treatment with EGCG, TNF- α showed high a significant decrease ($p < 0.05$) as compared to normal control .There is no statistical difference between the 9 weeks result and normal control ($p < 0.312$) (Table2).

IL-6 showed a high significant decrease ($p < 0.001$) after 5 weeks of treatment with EGCG and with no significant difference ($p < 0.155$) after 9 weeks of treatment as compared to control group (Table2). CRP levels showed no significant difference from control after 5 weeks of treatment ($p < 0.066$) and showed no significant difference after 9 weeks of treatment with EGCG ($p < 0.387$) as compared to control group (Table 2).

TNF- α showed high significant decrease after 5 weeks ($p < 0.020$) of caffeine treatment and showed no statistically significant difference ($p < 0.06$) after 9 weeks of treatment as compared to control group (Table3).IL-6 showed highly significant decrease after 5 weeks of treatment ($p < 0.000$) with caffeine and high significant decrease ($p < 0.033$) after 9 weeks of treatment as compared to control group Table (3). Treatment with caffeine showed high significant decrease of CRP after 5 weeks of treatment ($p < 0.002$) in comparison to

control. It decreased after 9 weeks of treatment ($p < 0.088$) put showed no significant difference when compared to control group (Table 3).

Treatment with green tea extract showed high significant decrease of TNF- α after 5 weeks ($p < 0.039$) when compared to control and showed no statistical significant difference ($p < 0.122$) after 9 weeks of treatment as compared to control group (Table4).

IL-6 showed high significant decrease after 5 weeks of treatment ($p < 0.001$) and high significant decrease ($p < 0.03$) after 9 weeks of treatment as compared to control group (Table4). CRP level showed high significant decrease after 5 weeks of treatment with green tea extract ($p < 0.007$) and showed no statistical significant difference after 9 weeks of treatment ($p < 0.168$) as compared to control group (Table 4).

The addition of Caffeine to green tea extract showed enhancement of the effect with a significant decrease of TNF- α after 5 weeks of treatment ($P < 0.03$), with no significance difference from control after 9 weeks of treatment ($P < 0.06$) (Table5).

Where IL-6 showed a high decrease after 5 weeks of treatment with the caffeine green tea mixture ($P < 0.001$) and no statistical difference from normal control after 9 weeks of treatment ($P < 0.11$) (Table5).CRP showed a significant decrease after treatment with mixture of caffeine and green tea extract ($P < 0.034$) with no statistical difference than the normal control ($P < 0.150$) (Table 5).

The administration of a mixture of EGCG and caffeine cause decrease in TNF- α after 5 weeks of treatment ($P < 0.04$) where after 9 weeks of treatment TNF- α showed no significance difference from normal control ($P < 0.606$) (Table (6)).The level of IL-6 after 5 weeks of administration of the mixture showed significant decrease ($P < 0.011$) with no significant difference from normal control after 9 weeks of treatment (Table6).The administration of the EGCG and caffeine mixture cause a high significant decrease of CRP ($P < 0.066$) where after 9 weeks of administration the level of CRP was with no statistical difference compare to control ($P < 0.497$) (Table 6).

The comparison of the effect of the obese, EGCG,, Caffeine, Green tea extract ,Caffeine plus Green tea extract and caffeine plus EGCG on TNF- α after 5 weeks of treatment showed an enhancement effect of caffeine on green tea and EGCG ,(121mg/ml), (66mg/ml), (80mg/ml), (98mg/ml), (75mg/ml)(63mg/ml) respectively (Fig.1)

The comparison of the effect on TNF- α after 9 weeks of treatment showed enhancement of caffeine on green tea and EGCG obese(138mg/ml), EGCG(37mg/ml),green tea extract(51mg/ml),

caffeine (43mg/ml), caffeine plus green tea extract (38mg/ml) and caffeine plus EGCG (33mg/ml) (Fig2).

The administration of caffeine in mixture with green tea or EGCG cause enhancement of their effect on IL-6 in comparison to other groups after 5 and 9 weeks (Fig3 and fig 4) respectively.

The effect of administration of obesity, EGCG, green tea extract, caffeine in comparison to

administration of mixture of caffeine plus green tea extract or caffeine mixed with EGCG on CRP after 5 weeks (4.8mg/ml, 2.8mg/ml, 3.4mg/ml, 3.06mg/ml, 2.85mg/ml and 2.7mg/ml) respectively and after 9 weeks (5.1mg/ml, 2.36mg/ml, 2.74mg/ml, 2.59mg/ml, 2.47mg/ml, 2.59mg/ml and 2.30mg/ml) respectively were represented in (fig 5, and fig 6).

Table (1) Comparison of pro-inflammatory cytokine between obese groups without treatment compared to control group.

	control	Obese at zero level	Obese after 5 weeks	Obese after 9 weeks	Obese at zero level VS control	Obese after 5 weeks VS control	Obese after 9 weeks VS control
TNF-α mg\ ml	32.5 \pm 2.04	105.63 \pm 12.48	120.88 \pm 11.61	137.75 \pm 3.4	P < 0.001	P < 0.000	P < 0.000
IL-6 mg\ ml	197.5 \pm 28.75	407.5 \pm 17.07	432.75 \pm 29.5	486.75 \pm 26.17	P < 0.001	P < 0.002	P < 0.000
C R P mg\ ml	2,037 \pm 0.39	4.3 \pm 0.49	4.8 \pm 0.21	5.1 \pm 0.23	P < 0.014	P < 0.002	P < 0.001

- Data are expressed as mean \pm standard deviation.

P < 0.05 Significant P < 0.001 High significant P < 0.000 highly significant - .

Table (2): The effect of EGCG alone on the pro-inflammatory cytokine at zero level and after 5 and 9 weeks of treatment in obese rats compared to the control group.

	control	EGCG at zero level	EGCG after 5 weeks	EGCG after 9 weeks	EGCG at zero level VS control	EGCG after 5 weeks VS control	EGCG after 9 weeks VS control
TNF-α mg\ ml	32.5 \pm 2.04	105.63 \pm 12.48	65.50 \pm 20.74	36.75 \pm 5.62	P < 0.001	P < 0.051	P < 0.312
IL-6 mg\ ml	197.5 \pm 28.75	407.5 \pm 17.07	346.5 \pm 18.5	220.00 \pm 6.9	P < 0.001	P < 0.001	P < 0.155
C R P mg\ ml	2,037 \pm 0.39	4.30 \pm 0.49	2.80 \pm 0.58	2.36 \pm 0.36	P < 0.014	P < 0.066	P < 0.387

- Data are expressed as mean \pm standard deviation.

- P < 0.05 Significant P < 0.001 High significant P < 0.000 Highly significant-

Table (3): The effect of caffeine alone on the pro-inflammatory cytokine levels after 5 and 9 weeks of treatment in obese rats compared to the control group.

	Control	caffeine at zero level	caffeine after 5 weeks	caffeine after 9 weeks	caffeine at zero level VS control	caffeine after 5 weeks VS control	caffeine after 9 weeks VS control
TNF-α mg\ ml	32.5 \pm 2.02	105.63 \pm 12.48	80.125 \pm 20.56	42.88 \pm 7.78	P < 0.001	P < 0.020	P < 0.057
IL-6 mg\ ml	197.5 \pm 28.75	407.5 \pm 17.07	374.5 \pm 28.8	260.05 \pm 24.71	P < 0.001	P < 0.000	P < 0.033
C R P mg\ ml	2,04 \pm 0.39	4.3 \pm .48	3.06 \pm 0.27	2.47 \pm 0.23	P < 0.014	P < 0.002	P < 0.088

- Data are expressed as mean \pm standard deviation.

P < 0.05 Significant P < 0.001 High significant P < 0.000 Highly significant.

Table (4): The effect of the extract of green tea alone on the pro-inflammatory cytokine levels after 5 and 9 weeks of treatment in obese rats compared to the control group.

	control	Extract at zero level	Extract after 5 weeks	Extract after 9 weeks	Extract at zero level VS control	Extract after 5 weeks VS control	Extract after 9 weeks VS control
TNF-α mg\ ml	32.5 ± 2.04	105.63 ± 12.48	97.63 ± 35.3	50.63 ± 15.35	P < 0.001	P < 0.039	P < 0.122
IL-6 mg\ ml	197.5 ± 28.75	407.5 ± 17.07	390.5 ± 19.25	263.5 ± 13.9	P < 0.001	P < 0.001	P < 0.029
C R P mg\ ml	2,037 ± 0.39	4.30 ± 0.49	3.41 ± 0.79	2.74 ± 0.39	P < 0.014	P < 0.007	P < 0.168

- Data are expressed as mean \pm standard deviation.

- P < 0.05 Significant P < 0.001 High significant P < 0.000 Highly significant

Table (5): The effect of the extract of green tea with caffeine in a combination on the pro-inflammatory cytokine levels after 5 and 9 weeks of treatment in obese rats compared to the control group.

	control	(extract-caffeine) at zero level	(extract-caffeine) after 5 weeks	(extract-caffeine) after 9 weeks	(extract-caffeine) at zero level VS control	(extract-caffeine) after 5 weeks VS control	(extract-caffeine) after 9 weeks VS control
TNF-α mg\ ml	32.5 ± 2.04	105.63 ± 12.48	74.63 ± 21.61	37.5 ± 2.88	P < 0.001	P < 0.031	P < 0.066
IL-6 mg\ ml	197.5 ± 28.75	407.5 ± 17.07	371.25 ± 15.01	229.00 ± 16.85	P < 0.001	P < 0.001	P < 0.110
C R P mg\ ml	2,037 ± 0.39	4.30 ± 0.49	2.85 ± 0.42	2.95 ± 0.40	P < 0.014	P < 0.034	P < 0.150

- Data are expressed as mean \pm standard deviation.

- P < 0.05 Significant P < 0.001 High significant P < 0.000 Highly significant

Table (6): The effect of EGCG with caffeine in a combination on the pro-inflammatory cytokine levels after 5 and 9 weeks of treatment in obese rats compared to the control group.

	control	(EGCG - caffeine) at zero level	(EGCG - caffeine) after 5 weeks	(EGCG - caffeine) after 9 weeks	(EGCG - caffeine) at zero level VS control	(EGCG - caffeine) after 5 weeks VS control	(EGCG - caffeine) after 9 weeks VS control
TNF-α mg\ ml	32.5 ± 2.04	105.63 ± 12.48	62.63 ± 16.6	33.13 ± 0.75	P < 0.001	P < 0.041	P < 0.606
IL-6 mg\ ml	197.5 ± 28.75	407.5 ± 17.07	334.5 ± 25.52	172.5 ± 9.7	P < 0.001	P < 0.011	P < 0.248
C R P mg\ ml	2,037 ± 0.39	4.30 ± 0.49	2.70 ± 0.19	2.3 ± 0.43	P < 0.014	P < 0.066	P < 0.497

- Data are expressed as mean \pm standard deviation.

- P < 0.05 Significant P < 0.001 High significant P < 0.000 Highly significant

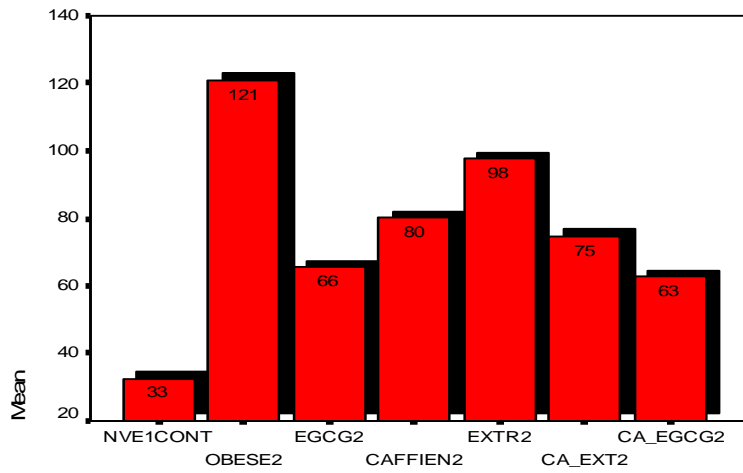


Fig (1): comparison of TNF- α between different groups and normal group 5weeks of treatment

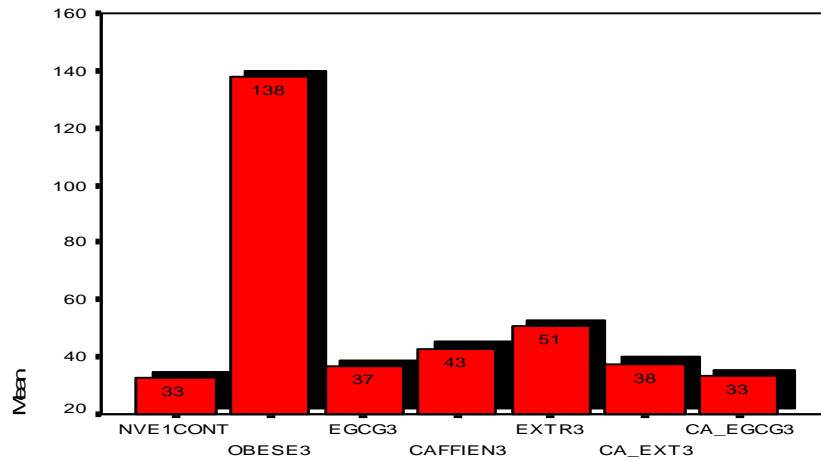


Fig (2): comparison of TNF- α level between different groups and normal group 9weeks of treatment

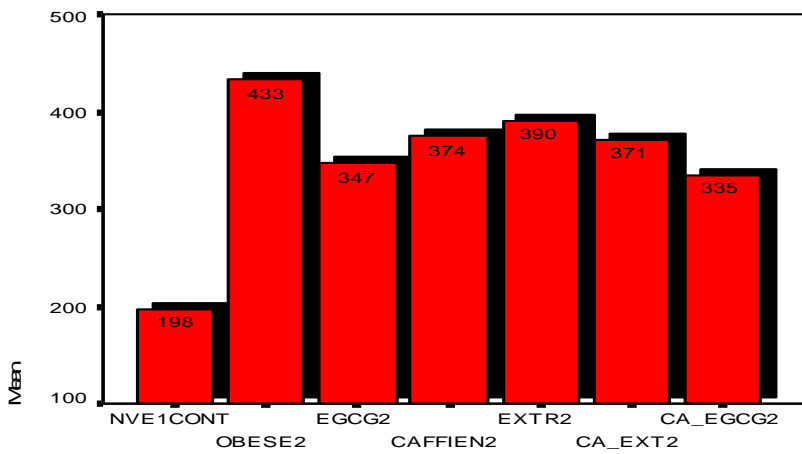


Fig (3): comparison of IL-6 level between different groups and normal group after 5 weeks of treatment

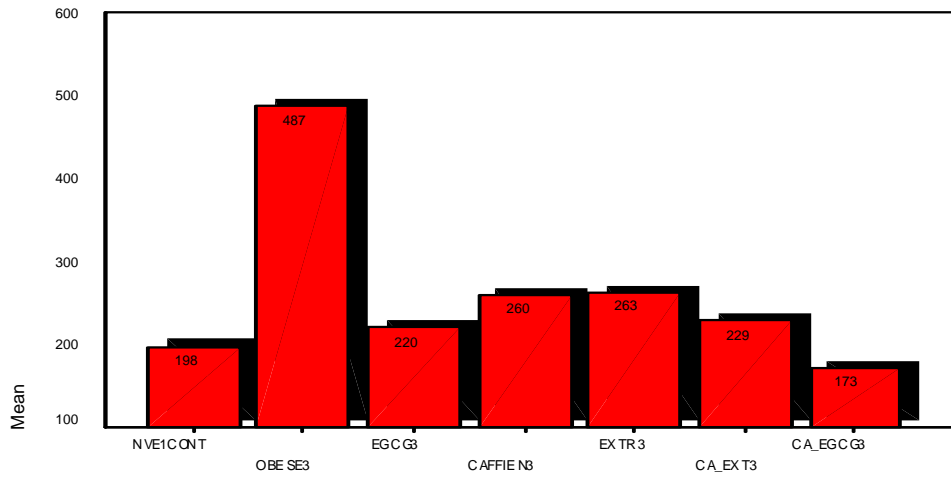


Fig (4): Comparison of IL-6 level between different groups and normal group after 9 weeks of treatment

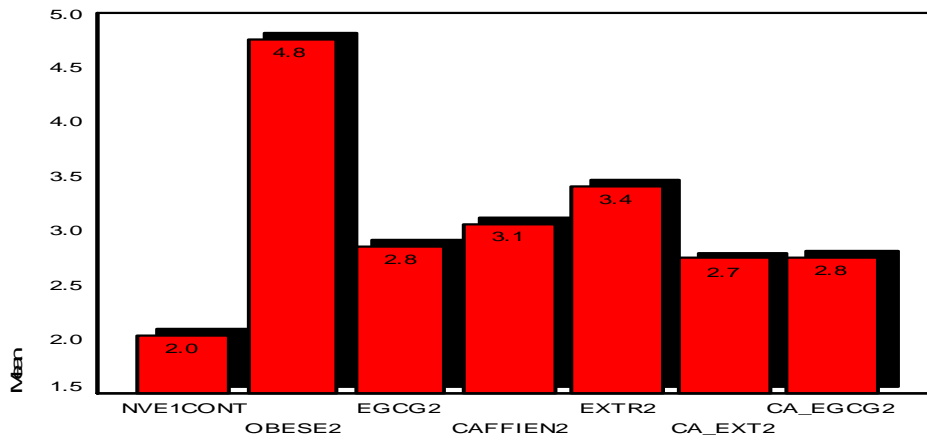


Fig (5): comparison of CRP levels in different groups and normal group 5weeks of treatment

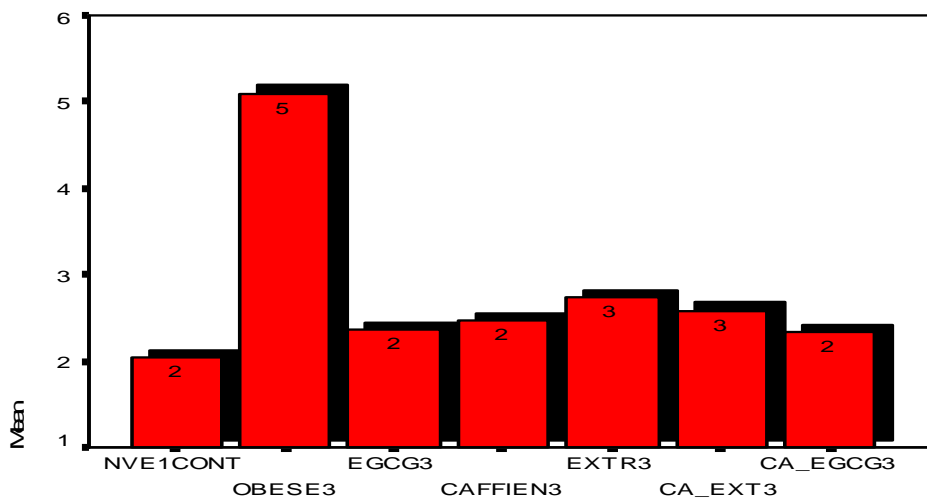


Fig (6): comparison of CRP levels in different groups and normal group 9weeks of treatment

4. Discussion:

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

Inflammatory activity is a characteristic of pathological process in obesity. Adipose tissue is an important source of cytokines and adiposity contributes to pro-inflammatory milieu, (Yu-Wen et al; 2009). It is well known that higher amount of adipocytes leads to misbalance in the immune system causing a state of low grade chronic inflammation leading to marked elevated concentration of proinflammatory cytokines, (Dixit; 2008, Fain; 2006).

Plasma TNF- α and IL-6 are produced by adipocytes and are elevated in obese subjects. TNF- α also induces IL-6 production which in turn regulates the production of CRP in the liver. Therefore, as TNF- α and IL-6 production is reduced by weight loss, one would expect CRP to be reduced as well. There was some evidence that caloric restriction alone dampens down the inflammatory response by reducing the DNA binding affinity of NF- κ B which modulates the production of cytokines IL-6 and TNF- α (Leonie et al., 2002). Adipocytes secrete IL-6, one of the chief inducers of CRP production by the liver. Approximately 30% of circulating IL-6 is estimated to be from adipose tissue. (Hye Soon Park et al., 2005). Proinflammatory cytokines were increased 3-fold for IL-6 and 7-fold for TNF- α in obese animals (Patrick et al., 2010). High level of IL-6 are responsible for the increase in acute-phase proteins seen in obese patients, particularly CRP, via its action on CRP-production cells in the liver (Michael et al., 2008). The elevated CRP concentration can be ascribed to increased expression of IL-6 in adipose tissue and its release into circulation. IL-6 is a pro-inflammatory cytokine that stimulates the production of CRP in the liver (Das, and FAMS., 2001).

Visser et al; (2001), demonstrated that higher BMI is associated with higher CRP concentration in young adults aged 17-39 years, thus confirming a state of low systemic inflammation on overweight and obese persons. (Das, and FAMS., 2001).

Our results showed elevated levels of TNF- α , IL-6 and CRP in obese rats fed high-fat diet when compared with the normal control rats fed normal diet. Which were coinciding with the previous studies. These results were reversed by

supplementation the rats with a combination of EGCG with caffeine, EGCG, caffeine, caffeine with green tea, and green tea extract alone. (Chen et al; 2004), found a decrease in lipid peroxidation and inflammation in carbon tetrachloride treated mice which were fed high-fat diet when supplemented with EGCG.

EGCG has been shown to increase NF- κ B related factor-2 (Nrf2) nuclear translocation and antioxidant response element (ARE) and up regulate Nrf2 protein expression in mouse model. The antioxidant activities are important because membrane peroxidation induces cellular damage and activates NF- κ B mediated responses that induces proinflammatory cytokines expression including TNF- α . EGCG has been shown to decrease TNF- α production in vitro by blocking NF- κ B activation via the inhibition of I κ B kinase, (Chen et al; 2002). EGCG inhibit transcription factor-mediated gene activation such as that via NF- κ B and AP-1, (Ahmed et al; 2000). Inhibition of NF- κ B and AP-1 mediated gene activation is the central phenomenon that explain the convergence in the antioxidant activity of the green tea catechins and the effect on specific mediator targets NF- κ B, which in response to ROS, activates transcription of many proinflammatory and anti-apoptotic/survival gene, (Schoon and Piette; 2000). The ROS-scavenging activity of green tea catechins inhibits NF- κ B activation leading to inhibition of expression of the proinflammatory and survival genes. In addition, EGCG has been shown to directly inhibit proteasome activity, (Nam et al; 2001), leading to accumulation of NF- κ B inhibitory protein I κ B, and other pro-apoptotic proteins such as Bax. Inhibition of NF- κ B mediated gene activation is also the likely mechanism of inhibition of inducible nitric oxide synthase observed with green tea and EGCG, which mediates its anti-inflammatory actions, (Singh et al; 2002; and Vadivel et al; 2009), showed that EGCG decreased lipopolysaccharide induced TNF- α production in mice and in a macrophage cell line.

Abe et al; (2005), stated that green tea cause down regulation of TNF- α gene expression. The molecular mechanism of anti-inflammatory effects of green tea may partially due to its ability to increase mRNA levels encoding anti-inflammatory factors such as TTP/TISII/ZF P36 and/or ELAVL1/HUA/HUR (Tris tetraprolin Family proteins, zinc finger protein 36, Human antigen R, Embryonic lethal abnormal vision-like 1) and decrease mRNA levels encoding pro-inflammatory factors such as TNF- α and or Cox-2/PTGs 2 (cyclooxygenase-2/prostaglandin endoperoxidase), (Heping et al; 2007). Green tea extract regulate TNF- α gene expression by modulating NF- κ B activation

though their inhibition effect on I κ B kinase activity and as scavenger of free radicals. Treatment of green tea reduced oxLDL-induced production of pro-inflammatory cytokines TNF- α and NF- κ B activation in a dose dependent manner (Wahyudi, and Sargowo; 2007). (Ramesh et al; 2009), have shown anti-inflammatory effects of intraperitoneal administration of EGCG in significantly reducing serum CRP levels and hematologic markers of inflammation on rats fed on atherogenic diet versus the untreated group. In same time (Ryua et al; 2005), examined the effect of green tea on inflammation, they stated that the inflammatory markers such as CRP and IL-6 were unchanged after green tea consumption.

Hirofumi; (2011), found that EGCG reduced Toll-like receptor-4 (TLR-4) expression (which induce inflammatory mediators) through 67LR (67LR receptor). EGCG induced a rapid up regulation of Tollip protein, a negative regulator of TLR – signaling, and this EGCG action was prevented by 67LR silencing or anti-67LR antibody treatment. (Hirofumi and Tatsuo; 2011),

Our results showed that caffeine had an anti-inflammatory effect that appeared through inhibiting TNF- α and subsequently IL-6 and CRP. It enhances the anti-inflammatory effect of EGCG and green tea extract when it was added to them.

Caffeine exerts most if not all of its effect via antagonism of adenosine receptors. Caffeine is also a well –known inhibitor of cyclic adenosine monophosphate-phosphodiesterase (cAMP-PDE). As adenosine mediates many anti-inflammatory effects, antagonism of adenosine receptors by caffeine would be expected to inhibit the anti-inflammatory effects of endogenous adenosine, and possibly elicit a pro-inflammatory effect. However, as the evidence clearly indicates that caffeine is generally anti-inflammatory, attention has been directed toward other possible mechanisms of action, (Horrigun et al; 2004).

Caffeine increased the concentration of intracellular cAMP and subsequent activation of protein kinase A (PKA). cAMP is very potent immunomodulatory, exerting generally suppressive effect on the functions of inflammatory and immune competent cells. Activation of cAMP/PKA pathway inhibits lipopolysaccharide (LPS) stimulated production of proinflammatory cytokines such as TNF- α , (L.H Horrigun et al; 2006). Furthermore the suppressive effect of caffeine on TNF- α production was blocked by the high specific cAMP antagonist Rp-8-Br-cAMPS. Similarly Rosental et al ;(1992) demonstrated that HA1000, an inhibitor of cAMP and cyclic guanosine monophosphate (cGMP) dependent protein kinases, blocked caffeine – mediated inhibition of sheep red blood cells specific

antibody responses by murine spleen cells in vivo. These data suggest that the immune-modulatory actions of caffeine are mediated by inhibition of cAMP-PDE and resultant increase in intracellular cAMP concentration. Furthermore approximately 80% of caffeine is converted to paraxanthine which is one of phosphodiesterase (PDE) inhibitors. Xanthines have extensive effects on immune function inducing suppression of pro-inflammatory cytokine release (including TNF- α , IL-2 and interferon- γ). Stimulating of anti-inflammatory cytokine (IL-10), and suppression of neutrophil and eosinophil chemotaxis and superoxide generation, (L.A.Horrigun et al; 2006).

Another pathway for immune modulatory effect of caffeine is through inhibition of poly (ADP-ribose) polymerase-1 (PARP-1) a nuclear enzyme present in eukaryotes, PARP-1 is reported to play a role in NF- κ B and AP-1 mediated production of pro-inflammatory cytokines, (Liesbeth et al; 2006).

We conclude that the addition of caffeine to EGCG or green tea extract enhance their anti-inflammatory effects on the low grade inflammation state a combined obesity by decreasing the levels of TNF- α , IL-6 and CRP. As inflammation known to be one of the major causes of CVD and Diabetes, this result could be beneficial in decreasing the side effects of obesity which cause low grade chronic inflammation.

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