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Original Research Article

BIOCHEMICAL EFFECTS OF GINGER AND/OR GREEN TEA EXTRACTS IN HIGH FAT DIET - INDUCED OBESE RATS

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Abstract: The current study was designed to clarify the sequel of administration of green tea and/ or extract of ginger on body weight, lipid profile, some hormones and some fertility variables in high fat diet (HFD) - induced obese rats. The results revealed that treatment of HFD obese rats with ginger extract decreased the elevated body weight (from 530±5.29 to 295±9.11), lee index (from 0.33±0.008 to 0.27±0.004), serum total cholesterol (from 25±2.57 to 187±2.79), triacylglycerol (TAG) (from 201±2.15 to154±1.82), low density lipoprotein cholesterol (LDL-C) (from 187.8±3.36 to119.6±2.31), very low density lipoprotein cholesterol (VLDL-C) (from 40.21±0.12 to 30.8±0.13), leptin (from 7.74±0.05 to 5.66±0.09), luetinzing hormone (LH) (from 0.30±0.006 to 0.19±0.005) and follicular stimulating hormone (FSH) (from 0.74±0.128 to 0.52±0.012) levels, but significantly elevated the decreased high density lipoprotein cholesterol (HDL-C) (from 21.40±0.50 to 36.40±0.81), serum testosterone (from 0.84±0.02 to 1.32±0.14), sperm count (from 44.20±2.08 to 65.20±0.54), motility (from 64±1.18 to 75.60±0.81) and normality (from 53.80±1.42 to 67±1.51). Similar results were observed with green tea treatment with minor variations. So, the inclusive outcomes may suggest that extracts of ginger and/ or green tea have a significant hypolipidemic effect with body weight reduction in rats fed high fat diet. In addition, the extracts may be qualified for ameliorative the ruined fertility parameters.

Key words: grean tea; ginger; obesity; lipids; sperm count; leptin

Introduction

Obesity is the excess accumulation of fat in the body. It is a condition medicinally termed to storage of superfluous body fat to amplitude that cause a negative health impact, resulting in reduction of life anticipatancy and excess health problems (1). Obesity is mainly caused by combination of excessive intake of high energy food, lack of physical activity and genetic susceptibility (2). Several effects are caused due to obesity including diabetes mellitus (3), hypertension (4), dyslipidemia (5), cardiac alterations (6), metabolic syndrome (7), lung diseases (8), cancer (9) and neurological disorders (10). In last few years, ginger and green tea have become a subject of interest because of their beneficial effect on human health.

Zingiber officinale (Ginger) has been consumed for over 2000 years as a spice. Its root contains polyphenol compounds including derivatives of 6-gingerol, which possess high antioxidant vigor (11). The piquancy of caller

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ginger is due principally to the gingerols that are symmetrical chains of phenols. The most plentiful is 6-gingerol (1), with presence of small quantities of another gingerols with varied chain lengths. The spiciness of dried ginger fundamentally results from shogaols (for example, 6-shogaol (2)), that are exsiccated forms of gingerols. Shogaols are created from the related gingerol during thermic processing. Dissolution averages of 6-gingerol to 6-shogaol were also appeared to be pH dependent and the paramount constancy at pH 4 (12). Many studies revealed several physiological responses of ginger, which may also be salutary for preventing and treat of some medical problems as hyperlipidemia (13), hyperglycemia (14), over weight (15), hepatic diseases (16), renal diseases (17) and gastrointestinal tract **(**GIT) illness (18).

Green tea is a pop drink from the plant of *Camellia sinensis* (Theacease family). Since many thousands years, it is supposed that green tea consumption possess medical effectiveness in the avoidance and treat a lot of diseases and so extend life is often connected to habit of green tea drinking (19). Green tea is manufactured from newly cropped young leaves by prompt steaming to avoid enzymatic fermentation. Steaming procedure inactivate enzymatic action of polyphenolic oxidase, which has the ability for conversion of tea catechins to polymeric derivatives and oligomeric. Though, young leaf handled for completely enzymatic fermentation, in which catechins is modified into arubigins and aflavins, which is a trait of the black tea, while semi-fermentation produces Oolong tea (20). In the recent past years, several studies revealed that green tea promotes health and has been informed for treatment of hyperlipidemia (21), overweight by increasing fat oxidation and energy expenditure (22) and hyperglycemia (23). The purpose of this study is to investigate the possible effects of green tea and ginger extracts on rats obesity induced by a high fat diet (HFD).

Material and methods

Experimental Animals

The present experiment was conducted on 50 apparently healthy male albino rats with average body weight (170-187 g). They were obtained from the Animal House, Zagazig Scientific and Medical Research Center (ZSMRC), Faculty of Medicine, Zagazig University. Rats were kept in clean hygienic house in metal cages and housed in controlled temperature room (19–23 °C), with dark light cycle of 12 hours during all experimental period. The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University.

Experimental Diets

Basal and fattening diets with their composition are shown in Table (1). The basal diet was used to meet the rat's nutrient requirements, while the high fat diet (HFD) was formulated to induce obesity in rats as previously mentioned (24). Water and feed were available all the time during the experimental period.

Herbal Plants

Ginger extracts (GE) (Multi-treat Arab Company for Pharmaceutical and Medicinal plants (MEPACO MEDIFOOD)), Egypt. It is present as tablets; one tablet contains 400 mg/kg BW ginger dry extract.

Green tea extracts (Multi –treat Arab Company for Pharmaceutical and Medicinal plants (MEPACO MEDIFOOD)), Egypt. It is present as tablets; one tablet contains 300 mg/kg BW green tea dry extract.

Experimental Design

A 50 mature male Albino rats were divided into 5 equal groups. Group (1) fed on basal diet (control group). Group (2) fed only HFD (60 % fat) for 45 days and served as obese control. Group (3) fed HFD and given one tablet ginger extracts (400 mg/kg BW) in 1 ml distilled water by gavages daily for 45 days. Group (4) fed HFD and given one tablet green tea extracts (300 mg/kg BW) in 1 ml distilled water by gavages daily for 45 days. Group (5) fed HFD and both ginger and green tea extracts in the same aforementioned doses and duration.

Table 1: Composition of the basal and experimental diet fed to healthy mature male albino rats

Ingredient	Basal diet (%)	Experimental diet (%)
Wheat Flour	77	16.07
Meat Meal	6.0	20
Animal Fat	3.5	57.93
Wheat Bran	10	3.0
Lysine	0.6	0.1
Methionine	0.4	0.4
Di-calcium phosphate	1.0	1.0
Sodium chloride	0.5	0.5
Vit. & Min. Mix	1.0	1.0
Calculated Nutrient Composition		
Crude protein	14.01	12.67
Energy (ME; kcal /kg)	3296.6	6239.23
Ether Extract	5.27	60.02
Crude fiber	3.83	3.75
Ash	3.06	5.77
Lysine	1.09	0.81
Methionine	0.61	0.58
Calcium	0.91	2.06
Phosphorus	0.64	1.12

ME: Metabolizable energy.

Determination of body weight, body length and Lee Index

The analyses were done in anaesthetized rats intraperitoneally injected by 0.1 ml of sodium barbiturate (1%) in order to observe the changes in both live body weights and lengths (25). The body weight and body length were measured according to the described methods (26,27). Lee Index: The final body weights and body lengths were used to calculate the Lee index = cube root of body weight (g) divided on nose to anus tall (cm) (26).

Collection of blood samples

By the end of the experiment, blood samples were collected after overnight fasting from retro-orbital venous plexus in a sterile test tube without anticoagulant for separation of serum for biochemical tests.

Biochemical studies

Lipid profile including serum total cholesterol, TAG, and HDL-C were evaluated using colorimetric methods (28-30), respectively; LDL-C and VLDL-C were calculated according to the equation of Friedewald (31) and serum leptin level (32). Serum levels of FSH, LH and testosterone were determined by Enzyme Linked Immunosorbent Assay (ELISA) as previously described **(**33-35), respectively.

Sperm analysis

Sperm motility and count, as well as normality were determined according to the described methods (36, 37), respectively.

Statistical analysis

The obtained data was statistically analyzed using one way ANOVA (SPSS) (38). The significance was set as $P \leq 0.05$. All values were represented as mean± standard error and the highest value was represented by the letter (a).

Results

Rats fed high fat diet (HFD) alone, HFD plus ginger and HFD plus green tea showed a significant rise in final body weight and lee index in comparison to the normal control as illustrated in Table (2). The highest values were recorded in G2. While groups 3 and 4 exposed a statistical falloff in the body weight and lee index, in comparison with G2 and the lowest values were reported in the G3. Moreover, HFD fed rats and treated with both ginger and green tea showed a significant diminution compared with groups (2-4), while in comparison with the

control group, lee index was returned to the normal values.

Lipid profile as illustrated in Table (3) disclosed a significant rise in the serum cholesterol, TAGs, LDL-C, VLDL-C and leptin (Table 4) levels in groups (2-5), in comparison with normal control group. The highest values were recorded in G2. On the other hand, groups (3-5) showed a significant decrease in the aforementioned parameters compared with obese rats (G2) and the lowest values were recorded in the G5. However HDL-C revealed a significant decrease in groups 2-5 compared with normal control. The lowest value was recorded in G2 and the highest value was reported in G5. Obese rats treated with either ginger, green tea or both showed a significant increase in HDL-C compared with obese group.

The serum reproductive hormones (FSH, LH and testosterone) results were shown in Table (4). Serum FSH and LH levels exposed a significant increase in G2 compared with the negative control and a significant decrease in groups 3-5 when compared with the positive control (G2) and return to normal levels in G5.On opposing, serum testosterone level showed a significant decrease in rats fed HFD alone (G2), HFD plus ginger and HFD plus green tea when compared with the normal rats as illustrated in Table (4). Groups (3 and 4) publicized a significant rise in serum testosterone level when compared with G2 and the highest values were reported in G3. Moreover, rats fed HFD diet and treated with both ginger and green tea showed insignificant statistical changes, in comparison with normal control and significant increase compared with groups 2-4.

Regarding semen examination in the present study, Table (5) revealed a significant decrease in semen motility and normality in groups 2-5, in comparison with the negative control group. On the other hand, treatment of obese rats (G3-5) showed a significant increase in the aforementioned parameters compared with obese rats. Sperm count showed similar results except for extracts combination which showed insignificant changes compared with the normal group and significant increase when compared with groups 2-4.

Table 2: The initial body weight, final body weight and Lee index in rats received high fat diet (HFD), ginger and or green tea

Parameters / Groups	Initial body weight (g)	Length (cm)	Final body weight (g)	Lee index
Negative control (G1)	181.00±0.96	24.00±0.03	221.00±6.37^{e}	0.24±0.003^{d}
Obesity (G2)	181.00±0.97	23.84±0.02	530.00±5.29^{a}	0.33+0.008^{a}
Obesity+Ginger (G3)	182.00±1.02	23.92±0.08	295.00±9.11^{c}	0.27±0.004^{c}
Obesity+Green tea (G4)	181.00±0.92	23.92±0.04	400.00±2.7^{b}	0.30±0.005^{b}
Obesity+Ginger+Green tea (G5)	181.00±1.50	24.00±0.06	238.00±4.42^{d}	0.25±0.002^{d}
P value	0.431	0.522	0.000	0.000

Means values ± S.E. within the same column having different letters were significantly different at $P \leq 0.05$.

Table 3: Lipid profile in rats received high fat diet (HFD), ginger and or green tea

Parameters / Groups	Cholesterol mg/dl	Triacylglycerols mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
Negative control (G1)	152.00±1.77^{d}	127.00±1.09^{d}	42.60±1.02^{a}	87.20±1.98^{c}	25.40±0.10^{c}
Obesity (G2)	251.00±2.57^{a}	201.00±2.15^{a}	21.40±0.50^{d}	187.8±3.86^{a}	40.21±0.12^{a}
Obesity+Ginger (G3)	187.00±2.79^{b}	154.00±1.82^{b}	36.40±0.81^{c}	119.6±2.31^{b}	30.80±0.13^{b}
Obesity+Green tea (G4)	191.00±1.06^{b}	154.00±1.15^{b}	35.20±0.58^{c}	124.4±1.16^{b}	30.80±0.10^{b}
Obesity+Ginger+Green tea (G5)	159.00±1.74^{c}	139.00±0.81^{c}	39.60±0.50^{b}	92.00±2.12^{c}	27.80±0.2^{c}
P value	0.000	0.000	0.000	0.000	0.000

Means values ± S.E. within the same column having different letters are significantly different at $P \leq 0.05$.
HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, VLDL-C: very low density lipoprotein cholesterol.

Table 4: Hormonal changes in rats received high fat diet (HFD), ginger and or green tea

Parameters / Groups	Leptin (ng/ml)	FSH (mIu/ml)	LH (mIu/ml)	Testosterone (ng/ml)
Negative control (G1)	4.48±0.10^{e}	0.36±0.005^{d}	0.15±0.003^{d}	1.65±0.02^{a}
Obesity (G2)	7.74±0.05^{a}	0.74±0.128^{a}	0.30±0.006^{a}	0.84±0.02^{d}
Obesity+Ginger (G3)	5.66±0.09^{c}	0.52±0.012^{c}	0.19±0.005^{c}	1.32±0.14^{b}
Obesity+Green tea (G4)	6.20±0.10^{b}	0.63±0.007^{b}	0.23±0.003^{b}	1.24+0.14^{c}
Obesity+Ginger+Green tea (G5)	4.92±0.21^{d}	0.37±0.008^{d}	0.16±0.005^{d}	1.62±0.18^{a}
P value	0.000	0.000	0.000	0.000

Means values ± S.E. within the same column having different letters are significantly different at $P \leq 0.05$.
FSH: follicular stimulating hormone, LH: luetinzing hormone.

Table 5: Sperm analysis in rats received high fat diet (HFD), ginger and or green tea

Parameters / Groups	Sperm count (million/epididymes)	Sperm motility %	Sperm normality %
Negative control (G1)	74.20±2.98^{a}	82.60±1.43^{a}	75.40±1.12^{a}
Obesity (G2)	44.20±2.08^{c}	64.00±1.18^{e}	53.80±1.42^{d}
Obesity+Ginger (G3)	65.20±0.54^{b}	75.60+0.81^{c}	67.00±1.51^{b}
Obesity+Green tea (G4)	60.40±3.65^{b}	70.40±0.50^{d}	61.60±1.07^{c}
Obesity+Ginger+Green tea (G5)	72.60±1.36^{a}	79.00±0.83^{b}	70.20±0.86^{b}
P value	000	000	000

Means values ± S.E. within the same column having different letters are significantly different at $P \leq 0.05$.

Discussion

Obesity is a complex disease gives rise to the interaction of dietary, a myriad of genetic, environmental factors and lifestyle which make a positive energy balance and lead to augmented mass of body fat (39). A highly significant increase was observed in the body weight and lee index of rats received HFD when compared with the normal control group. Our results nearly parallel to those previously obtained (40). This rise in body weight and Lee index may be attributed to the high caloric diet (contain 60% fat) resulting in fat accumulation in adipose tissue. Not only body mass high energy calorie from overeating; it also promote capacity of stomach which stimulate the gastric mechanoreceptors that adjust signals for eating greedily (41).

Obese rats treated with ginger and/ or green tea extracts showed a significant decline in body weight and lee index compared with obese rats as they stimulate thermogenesis. Also, ginger washes the body by minimize the acidity of stomach and cleaning the digestive tract from food logged in it (42). The obtained results agree with the findings of other researchers (39,40,43). The thermogenic possession of green tea was attributed to its high fulfilled of caffeine and catechin polyphenols (44).

Obese rats treated with ginger and green tea mixture disclosed insignificant changes in lee index compared with the normal control and an improvement in the body weight results compared with high calorie rats and other treated groups. In our opinion, this may be owing to the synergistic effect of both herbal plants.

Regarding to the lipid profile, obese rats revealed a highly significant rise of cholesterol, TAGs, LDL-C and VLDL-C with a highly significant reduction in HDL-C level compared with the negative control. These findings were similar to those of other authors (43,45,46). The obesity is the common source of dyslipidemia as lipid oversupply in a status of obesity results in excess availability of non esterified fatty acid and, in turn, elevated TAGs accumulates in non-adipose tissues, e.g. the liver, muscle and pancreas (5). Ginger and green tea administered to obese rats improved the previous results towards the normal control levels and agree with previous findings (47-49). It accomplished that the hypocholesterolemic effect of ginger or green tea could have probably outcome from the restrain of cellular cholesterol biosynthesis after extract consumption (50). Furthermore, it

was reported that the decrease of cellular cholesterol biosynthesis is closely correlated with increased activity of the LDL receptor, which enhancing the removal of LDL from blood, resulting in a reduction in the serum or plasma cholesterol concentration (51). Administration of combination of ginger and green tea extracts to obese rats improved lipid profile results than individual administration. There is also evidence that polyphenol contents in the tea enhance fecal cholesterol and bile acid excretion (52). Moreover, the hypocholestrolemic effect of ginger may be due to the presence of some chemical constituents in ginger, which inhibit the absorption of dietary fat by inhibiting its hydrolysis, it also stimulate the activity of hepatic enzyme cholesterol-7-alpha hydroxylase, which in turn stimulates the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body (53).

Studying serum leptin levels in obese animals revealed that there is a highly significant rise compared with the normal rats. Leptin is released as a response to obesity to correct the problem in which leptin is manufactured by white adipose tissue to signal fat accumulates reserves in the body and regulates energy expenditure and food intake (45). However, treatment of rats with obesity showed an improvement in the leptin level in comparison with the obese group. These results nearly analogous to those previously conveyed (47,54). In contrast another study showed no difference in leptin level (55). In our opinion, the cause of decrease in serum leptin was attributed to the beneficial effect of ginger and green tea which represented by decreased body weight and fat store. However, the improvement in rats given the combination of extracts is more than other groups which returned to normal levels. This normalization in serum leptin or lipid profile may be due to the effect that comes from the usage of the green tea and ginger extracts which leads to a lowering effect of free radicals on the cell membrane of cells (49).

Concerning the analysis of reproductive hormones in this study, rats fed with HFD revealed a highly significant diminution in serum testosterone. These results were in harmony with others (56,57). Moreover, obesity that associated with a significant reduction in the levels of testosterone and increased estrogen levels as excess fat indeed causes the male hormone, testosterone, to be transformed into estrogen which decreases testicle stimulation (58). While, ginger or green tea treated rats showed an improvement in testosterone levels compared with obese rats. These results were in groups (2-4) coordination with other findings (59,60). The improvements confirmed the effect of ginger and green tea was very clear in obese animals given the two extracts combination that showed insignificant changes in testosterone compared with the normal control group. However, it has been explained earlier that green tea extract polyphenols has inhibitory effect on leydig cell testosterone production probably through cell signaling pathway, P-450 side chain cleavage and the function of 17β HSD (61).

Rats fed HFD alone revealed a significant upsurge in the serum LH and FSH levels compared with the normal rats. Related findings were previously stated (62). While, ginger or green tea -treated obese rats showed a fall in serum levels of FSH and LH when compared with obese animals. Similar results were previously reported (63). The obtained results in were attributed to the compensatory mechanism as a response to the increased testosterone level by feedback.

A major observation of the current investigation is sperm analysis, feeding rats with HFD revealed a significant decrease in sperm count, motility and normality compared with the normal rats. These results were in line with others (62,64). It has been suggested that hypercholesterolemia causes testicular and reproductive damage by excessive generation of free radicals and oxidative stress, which are cytotoxic to spermatozoa (65-67). While obese rats treated with ginger or green tea showed an improvement in the results of sperm count, motility and normality compared with the obese rats. Ginger and green tea are rich in antioxidant components which display sperm antimutagenic activities due to their radical scavenging and their ability in suppression of the reactive

oxygen species (ROS) activity in a dose-dependent manner and to inhibit apoptosis and so improve semen quality in male rats (68). The improvement in sperm evaluation in obese rats treated with mixture of both plants was marked and consequently, these results confirmed the synergistic effect of both herbal plants.

Conclusion

It could be concluded that feeding high fat diet has deleterious effects on the body weight, lipid profile, leptin, reproductive hormones and fertility. Moreover, administration of ginger and green tea has favorable effects on the studied parameters in obesity with rats induced by high fat diet.

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Conflicts of interest

None of the authors have any conflict of interest to declare.

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