

EVALUATION THE PROTECTIVE EFFECT OF GARLIC AND GREEN TEA ON LIPID PROFILE AND SOME HORMONES IN OBESE RATS

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ABSTRACT: Obesity represents a global challenge because its effect on life style of many peoples suffering from it. The present study aimed to evaluate the role of some adipose tissue hormones and enzymes level in obese rats associated with cardiovascular diseases, and to evaluate therapeutic role of some medicinal plants (garlic and green tea). A number of 80 male albino rats divided to 8 groups: G1 (normal group), G2 Non treated (fat lard feeding) group, G3 and G4 (fat lard feeding) for one month followed by treatment with green tea for a another month with 1 ml and 2ml dose of extract respectively, G5 and G6 (fat lard feeding) group for one month followed by treatment garlic for another month with 1ml and 2ml of extract respectively. G7 and G8 (fat lard feeding) group for one month , and treatment with mixture of green tea and garlic extract for another month with 1ml mixture and 2ml mixture of each respectively . At the end of experiment , the serum collected for analyses of the foldaway parameters (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, nitric oxide NO, lactate dehydrogenase LDH, aspartate transaminase AST and thyroid stimulating hormones (TSH ,T3, T4).

The obtained data estimate that the fat lard feeding leads to increase the levels of TSH, AST, triglyceride, total cholesterol, LDL-cholesterol and LDH. The treatment with green tea and garlic leads to decreases the levels of these parameters towards the normal levels, at the same time the fat lard feeding leads to decreases the level of NO, T3, T4 and the treatment with green tea and garlic improve the levels towards the normal levels. The garlic was more effective than green tea, while the blood indices don't affected in case of obesity and treatment.

Key words: Garlic, Green tea, Adipose tissue, Lactate dehydrogenase, Obesity.

INTRODUCTION

Obesity develops primarily as a result of an imbalance between energy intake and energy expenditure. Thyroid hormones influence energy expenditure by controlling cellular respiration and thermogenesis as well as identifying resting metabolic rate (Walczak & Sieminska, 2021).

Obesity is linked with elevated levels of triglycerides, cholesterol, and LDL (Low-Density Lipoprotein) cholesterol in the blood, as well as low levels of HDL (High-Density Lipoprotein) cholesterol (Jiménez *et al.*, 2020). Obesity also is a significant predictor of high serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (Ruhl and Everhart, 2003).

Many studies have indicated the vital role that plants and their effective components play in improving public health in general, especially improving blood lipid profile indicators (Abozid, and Farid, 2013; Abozid and Ahmed, 2013; Abozid *et al.*, 2014; Farid *et al.*, 2015; Ashoush *et al.*, 2017; El-Shennawy, and Abozid, 2017).

Garlic (*Allium sativum L.*) was used as a pharmacological food for a long time. Garlic and its components were shown to have powerful regulatory activities in body processes such as blood clotting, lipid metabolism, immunity, and xenobiotic metabolism. In a rat model of high fat diet-induced obesity, garlic oil inhibited body weight gain and white adipose tissue mass (Kagawa *et al.*, 2020).

Green tea contains catechins (such as epigallocatechin3-gallate) (Di Lorenzo *et al.*, 2015) as well as quercetin, thearubigins, theaflavins, theanine, caffeine, chlorogenic acid, and gallic acid, which are derived from the leaves of *Camellia sinensis* (Cercato *et al.*, 2015).

For the weight loss caused by green tea, several models have been suggested. These mechanisms include decreasing food intake, interfering with lipid emulsification and absorption, suppressing adipogenesis and lipid synthesis, and increasing energy expenditure through thermogenesis, fat oxidation, and fecal lipid excretion (Huang *et al.*, 2014).

MATERIALS AND METHODS

Materials

Experimental animals

A number of (80) male Albino rats (one month age) were obtained from the animal house of nuclear Research Centre .Atomic Energy authority of Egypt. The rats were kept for adaptation under normal laboratory conditions for 7 days before the beginning of the experiment.

Medicinal plants

Two medicinal plants were employed in this study. They were green tea and garlic. Green tea was purchased from Arab Company for Pharmaceuticals and Medicinal plants (MEPACO-MEDI FOOD), Enchas El Raml – Sharkia, Egypt. Garlic was purchased from SEKEM (ATOS Pharma) – Cairo. Egypt.

Methods

Experimental design

All rats were fed on balanced basal diet and allowed for access water (Saluja *et al.*, 2010). After 7 days, 7 groups of rats were fed on a mixture balanced basal diet and fat lard (40% and 60% balanced basal diet) from (Alabrar Feed Factory- AlShrakia- Egypt) for one month, the experiment extended for another month of treatment, treatment with extracts of green tea and garlic, during this month all rats were fed

on balanced basal diet only. All groups of rats were weighted two times weekly during all times of the experiment, the groups of rats were as the following:

1. **First group:** 10 normal rats (normal control) (basal diet only)
2. **Second group:** 10 rats fed with fat lard for one month
3. **Third group:** 10 rats fed with fat lard for one month and treated with 1 ml green tea daily for another month
4. **Fourth group:** 10 rats fed with fat lard for one month and treated with 2 ml green tea daily for another month
5. **Fifth group:** 10 rats fed with fat lard for one month and treated with 1 ml garlic daily for another month
6. **Sixth group:** 10 rats fed with fat lard for one month and treated with 2 ml garlic daily for another month
7. **Seventh group:** 10 rats fed with fat lard for one month and treated with 1 ml green tea and 1 ml garlic daily for another month
8. **Eighth group:** 10 rats fed with fat lard for one month and treated with 2 ml green tea and 2 ml garlic daily for another month.

Rats treated by using stomach tube. The dose was 15 mg of extract /kg rats. At the end of the experiment, rats were sacrificed and the serum collected for analysis.

Biochemical analysis

Lipid profile parameters (Triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol, serum lactate dehydrogenase (LDH) activity, serum aspartate amino transaminase (AST) activity and serum total nitric acid (TNO) level were determined according to commercial kits (Biodignostic Company, Egypt).

Results

Data in Table (1) showed that, the body weights levels rats all groups were more increased in comparison with normal group G1, and these levels of weights begin to return towards the normal growth in the treated groups

Table (1): The body weights of rats in all treated groups.

Groups	G1	G2	G3	G4	G5	G6	G7	G8
Weight (g)	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
1 st week	111± 11.2 a	117± 11.4 a	115± 12.1 a	118± 12.8 a	112± 11.9 a	111± 11.7 a	114± 11.8 a	111± 11.4a
2 nd week	152± 15.1 a	168± 16.3 a	167± 16.8 a	169± 16.8 a	164± 16.9 a	162± 16.7 a	165± 16.4 a	166± 16.9 a
3 rd week	186± 17.9 a	260± 27.1 b	270± 27.3 b	270± 27.1 b	269± 26.7 b	268± 27.1 b	269± 26.6 b	278± 26.9 b
4 th week	222± 23.1 a	328± 33.1 c	321± 32.9 c	320± 32.9 c	318± 33.1 c	317± 32.2 c	319± 31.7 c	320± 32.7 c
5 th week	261± 27.1 a	360± 35.9 c	350± 35.7 c	349± 35.1 c	347± 34.9 c	347± 34.8 c	349± 34.9 c	342± 34.8 c
6 th week	399± 40.1 a	399± 40.2 d	380± 39.1 c	375± 38.1 c	361± 36.1 c	360± 37.1 c	360± 37.2 c	362± 37.1 c
7 th week	332± 34.1 a	440± 44.9 d	416 ± 41.1 c	400± 41.2 c	382± 38.2 b	385± 38.9 b	381± 38.7 b	380± 38.2 b
8 th week	361± 40.1 a	479± 48.2 f	455± 46.7 c	420 ± 43.1 b	411± 41.2 b	411± 41.8 b	401± 41.1 b	399± 41.2 b

a,b,c,d,f means with a common within a row are significantly different at (P<0.05).

G1: Normal control

G2: Fed fat diet in treatment

G3: Fed fat diet treatment 1 ml green tea extract

G4: Fed fat diet treatment 2 ml green tea extract

G5: Fed fat diet treatment 1 ml garlic extract

G6: Fed fat diet treatment 2 ml garlic extract

G7: Fed fat diet treatment mix 1 ml green tea extract + 1 ml garlic

G8: Fed fat diet treatment mix 2 ml green tea extract + 2 ml garlic

Data in Table (2) represent the mean values of AST, it was found that, increased in case of obesity, and treatments with the tow extracts improve its level. On the contrary at the levels of NO, their levels decreased in obesity and begin to increased and improved after treatment with the extracts. The levels of (TC) total cholesterol, where its level was increased as the result of obesity and begin to decrease after treatment with the two extracts. Where the levels triglycerides increased in obesity and the treatment with the tow extracts leads to decrease and improve these levels.

Data in Table (3) gives the mean levels of HDL, where this level decreased as the result of obesity, and begins to increase after treatment with tow extracts. Also LDL-cholesterol levels increased as the result of obesity, and after treatment with the tow extracts, begin to decline.

Levels of T3 were decreased in obesity and it begins to be improved after treatment with tow extract. The same in T4 levels which were decreased as the result of obesity and were improved after treatment of two extracts. TSH levels, increased in case of obesity and begin to decline after treatment with tow extracts.

Table (2): Effect of green tea and garlic extract on LDH, AST activities and NO, TC, TG levels of all groups.

Groups	G1	G2	G3	G4	G5	G6	G7	G8
Parameters	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
LDH (U/L)	150± 11 a	275± 14 c	265± 12 c	240± 11 b	242± 10 b	238± 9 b	236± 9 b	235± 10 b
AST (U/dl)	55± 3.1 a	72± 5.8 d	69.8± 5.7 d	50± 4.8 c	49.2± 4.2 c	47.8± 4.1 c	48.1± 4.3 c	42± 3.7 b
NO (mg/dl)	24.5± 3.8 d	11± 1.7 a	13.9± 2.1 a	15± 2.2 b	15.4± 2.2 b	16.9± 2.7 b	17.1± 2.6 b	18.9± 2.8 c
TC (mg/dl)	120± 10 a	453± 20 a	390± 18 b	370± 15 b	315± 14 c	302± 12 c	290± 11 c	285± 12 c
TG (mg/dl)	105± 5 d	410± 17 a	340± 11 b	320± 9 b	260± 7 c	250± 6 c	245± 6 c	235± 5 c

a,b,c,d,f means with a common within a row are significantly different at (P<0.05).

G1: Normal control

G2: Fed fat diet in treatment

G3: Fed fat diet treatment 1 ml green tea extract

G4: Fed fat diet treatment 2 ml green tea extract

G5: Fed fat diet treatment 1 ml garlic extract

G6: Fed fat diet treatment 2 ml garlic extract

G7: Fed fat diet treatment mix 1 ml green tea extract + 1 ml garlic

G8: Fed fat diet treatment mix 2 ml green tea extract + 2 ml garlic

Table (3): Effect of green tea and garlic extract on HDL- cholesterol, LDL- cholesterol, T3, T4, TSH levels of all groups.

Groups	G1	G2	G3	G4	G5	G6	G7	G8
Parameters	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
HDL-cholesterol (mg/dl)	55± 3 a	15± 2 a	23± 3 b	25± 3 b	35± 4 c	37± 5 c	41± 6 c	43± 5 c
LDL- cholesterol (mg/dl)	58± 4 d	250± 15 a	210± 12 b	203± 10 b	195± 10 b	145± 9 c	140± 9 c	138± 7 c
T3 (pg/dl)	4.1± 0.3 d	1.8± 0.2 a	1.9± 0.1 a	2.8± 0.2 b	2.9± 0.3 b	3.6± 0.3 c	3.7± 0.2 c	3.7± 0.3 c
T4 (pg/dl)	1.7± 0.03 c	0.4± 0.01 a	0.5± 0.01 a	0.6± 0.01 a	0.6± 0.01 a	0.9± 0.02 b	1.0± 0.02 b	1.1± 0.02 b
TSH (mIu/L)	0.56± 0.04 a	7.9± 0.2 d	6.8± 0.2 d	5.1± 0.07 c	5.2± 0.06 c	4.9± 0.06 c	4.6± 0.04 b	4.5± 0.05 b

a,b,c,d,f means with a common within a row are significantly different at (P<0.05).

G1: Normal control

G2: Fed fat diet in treatment

G3: Fed fat diet treatment 1 ml green tea extract

G4: Fed fat diet treatment 2 ml green tea extract

G5: Fed fat diet treatment 1 ml garlic extract

G6: Fed fat diet treatment 2 ml garlic extract

G7: Fed fat diet treatment mix 1 ml green tea extract + 1 ml garlic

G8: Fed fat diet treatment mix 2 ml green tea extract + 2 ml garlic

Discussion

According to the World Health Organization (WHO), Egypt has the world's 18th highest obesity prevalence. Obesity causes approximately 4.7 million premature deaths each year (Aboulghate *et al.*, 2021).

Mak-Soon *et al.*, (2011) stated that dietary supplementation with garlic leads to reduction of body weight and resulted in a decreased mass of adipose tissue beside ameliorate plasma lipid profiles in mice with high – fat –diet induced obesity.

The obesity is associated with increased the level of all serum lipid profile except HDL - cholesterol. Therefore, patients presenting with these biochemical abnormalities are recommended to be investigated for obesity and vice versa (Kanwar & Kabra, 2016).

In this study which classifies the experiment to two stages, during the first stage (fat feeding) which extend for one month (4 weeks), there was a significant increase in body weight of all groups (7 groups) in comparison with control group, non fat feeding group. Figure (1)

The second stage which extends for another month of treatment using green tea and garlic extracts for (G3, G4, G5, G6, G7 and G8) beside G2 non treated group.

Scoring the levels of different parameters associated with obesity in G2, it was found that there was a significant increase of total cholesterol (TC), triglycerides (TG), low density lipoprotein levels (LDL) levels in comparison with normal control group G1, while the level of high density lipoprotein levels (HDL) was significantly decreased in G2, and these results are in coincidence with all previous studies

A number of randomized, controlled intervention trials have found that Green tea catechin (GTC) is thought to influence body weight and composition through a variety of mechanisms. GTC, increasing energy expenditure and promoting fat oxidation. Caffeine, which is naturally present in green tea, influences SNS activity and may work in tandem with GTC to increase energy expenditure and fat

oxidation. Modifications in appetite, up-regulation of enzymes involved in hepatic fat oxidation, and decreased nutrient absorption are all possible mechanisms (Rains *et al.*, 2011).

The present study are in coincidence with the previous studies where the growth data during the first month of fat feeding of G2,G3,G4,G5,G6,G7 are highly increased than the first group (G1). And after treatment with green tea (G3, G4) during the second month, it was found that the rate of growth was reduced in comparison with (G2) non treated group and the rate of reduction extended through the second month, and there is no significant difference between the two doses of green tea treatment

Salehi *et al.*, (2015) investigated the effects of fresh garlic administration on body weight, lipid profiles, and plaque formation. The high ghee diet resulted in a significant increase in serum concentrations of TC, TG, and HDL-C, as well as a decrease in LDL-C concentration. Fresh garlic increased TG and HDL-C levels while decreasing TC and LDL-C levels in treated rats.

The present study found also that garlic supplement has a protective effect on the elevated parameters of obesity, where elevated levels of TC, TG, and LDL were declined under effect of garlic extract, at the same time the level of HDL was significantly increased under garlic treatment .These results are in coincidence with most of many studies.

Several physiological functions of the cardiovascular system are controlled by nitric oxide (NO). The NO metabolites nitrite and nitrate were measured in the plasma of mice to determine the effect of aged garlic extract (AGE) on NO production. From 15 to 60 minutes after administration, AGE (2.86 g/kg, p.o.) temporarily increased NO production by 30-40% (Moriyama *et al.*, 2002).

The results of this study proved that the level of nitric oxide was significantly decreased in untreated fat feeding group (G2), and its level was significantly increased in the other treated groups (G3, G4, G5, G6, G7 and G8) with green tea, garlic and combined dose of them. These results proved the beneficial effect of garlic and green

tea on cardiovascular disease resulting from obesity and other causes and the study is in agreement with many studies.

Juvenile obesity is linked to increased risk of liver steatosis, which is thought to be involved in transaminases (AST and ALT), and lipid metabolism but not glucose metabolism. Even in the prepubertal stage, these changes are visible (Guzzaloni *et al.*, 2000).

Garlic supplementation significantly reduced AST levels but had no effect on ALT levels, according to Panjeshahin *et al.*, (2020) findings.

Pezeshki *et al.*, (2016) study looked into the effects of drinking green tea on non alcohol fatty liver disease (NAFLD) patients. respectively, and (AST) levels greater than 31 mg/dl and 47 g/dl in women and men, respectively, and no other hepatic disease. After 12 weeks, the green tea group had significantly lower AST levels. Green tea extract (GTE) supplement supplementation, according to the findings of this study, reduces liver enzymes in patients with NAFLD.

The present study chose AST enzyme to represent liver and heart function, and follow up the levels of this enzyme estimated that obesity leads to significant increase of the level of AST in comparison with normal control, and after treatment with different doses of garlic and green tea singly or combined resulted in significant decrease of AST levels toward the normal level, hence this result is in agreement with many studies regards liver and heart function in obesity and treatment with garlic and green tea.

Johari *et al.*, (2018) study purpose was to assess the levels of thyroid hormones and lactate dehydrogenase (LDH) in obese and/or diabetic patients. TSH levels increased significantly in the obese and obese diabetic groups. LDH levels were also significantly lower in obese and obese diabetic groups compared to diabetic patients. The percentage of LDH was significantly lower in both the diabetic and obese groups. LDH have the potential to be useful diagnostic markers for metabolic syndrome. This could aid in investigating the metabolic changes associated with obesity and diabetes complications.

Hamlaoui-Gasmi *et al.*, (2012) investigated the ability of high dose garlic administered orally (p.o.) or intraperitoneally (i.p.) to affect heart antioxidant status in rats. P.o. garlic acts as an antioxidant in this organ by decreasing H₂O₂ and lactate dehydrogenase (LDH) levels while increasing free iron levels. It had no effect on (MDA), catalase (CAT), or superoxide dismutase (SOD), but it did reduce peroxidase (POD) activity. When taken orally, a high garlic dosage is safer.

Regarding estimation of the levels of LDH in case of obesity and treated groups, the present study found that, the level of LDH increased in obesity group G2, and the level of the enzyme begin to decrease toward the normal level after treatment with garlic and high dose of green tea. These results are in coincidence with different studies and are in contrary with others.

Helal *et al.*, (2018) Changes in hormone levels, including thyroid hormones, cause physiological/clinical abnormalities. The purpose of study was to demonstrate the protective effect of garlic, or their combination against L-thyroxine-induced hyperthyroidism in male albino rats. Garlic, protects against hyperthyroidism.

Regarding to the thyroid gland function and scoring of its hormones (T₃, T₄) and TSH of the pituitary gland; the results of the present study proved that there were association between obesity and thyroid hormone levels, it was found that T₃ and T₄ were decreased in obese group G2, green tea and garlic increase the level of T₃, while green tea did not give any change of T₄ level, only garlic and combination of green tea and garlic increase the level of T₄. In contrary, in case of TSH, the obesity resulted in highly significant increase of TSH level and treatment with green tea and garlic decrease the level of TSH towards the normal level. The results of this study are in agreement with different studies especially in case of obesity.

Jeong *et al.*, (2022) investigated the links between hematological parameters and childhood and adolescent obesity Hematological parameters (such as white blood cells [WBCs], red blood

cells [RBCs], hemoglobin [Hb], hematocrit [Hct], and platelets) were measured in 7997 participants. In children and adolescents, a higher BMI was associated with higher WBC, RBC, Hb, Hct, and platelet counts. Hematological parameters should be evaluated in obese children and adolescents because higher levels of hematological parameters are potential risk factors for obesity-related diseases.

Rani *et al.*, (2018) The goal of study was to see how green tea affects hemoglobin and hematocrit levels in wistar albino. Twenty-four rats were divided into four groups and given a different dose of 5.6g in a 1.8ml/200gr solvent, 8.4g in a 1.8ml/200gr solvent, and 11.2g in a 1.8ml/200gr solvent for a month. Based on the findings of this study, it was concluded that green tea has an effect on the reduction of hemoglobin and hematocrit in wistar albino.

Shoshin *et al.*, (2020) study looks at the effects of different concentrations of continuous green tea consumption in rats. In the analysis of hematological parameters, there was no significant variation in RBC, MCHC, and platelet among all treated groups.

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تقييم التأثير الوقائي للثوم والشاي الأخضر علي دهون الدم وبعض الهرمونات في الجرذان المصابة بالسمنة

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(2) قسم الكيمياء الحيوية الزراعية - كلية الزراعة - جامعة المنوفية

الملخص العربي

السمنة تمثل تحدى عالمي بسبب تأثيراتها علي اسلوب الحياة لكثير من الناس الذين يعانون من السمنة. الدراسة الحالية : تهدف الي تقييم مستوي بعض الهرمونات والانزيمات في الفئران المصابة بالسمنة المرتبطة بأمراض القلب والاعوية الدموية وتقييم الدور العلاجي لكلا من الثوم والشاي الاخضر. اشتملت الدراسة علي عدد ٨٠ فأر من ذكور ألبينو وتم تقسيمهم الي ٨ مجموعات كل مجموعة ١٠ فئران علي النحو التالي: المجموعة الاولى وهي مجموعة طبيعية، أما المجموعة الثانية (مجموعة السمنة) تم تغذيتها على دهن الخنزير مضافا الي العلف، المجموعة الثالثة والرابعة فقد تم تغذيتهم على دهن الخنزير لمدة شهر ثم تم علاجها لمدة شهر آخر بمستخلص الشاي الاخضر بالحقن المعدي بمعدل ١ مل للمجموعة الثالثة و ٢ مل للمجموعة الرابعة. المجموعة الخامسة والسادسة بعد تغذيتهم لمدة شهر على دهن الخنزير تم معالجتهم عن طريق الحقن المعدي ايضا باستخدام انبوب معدي بمستخلص الثوم بمعدل حقن ١ مل للمجموعة الخامسة و ٢ مل للمجموعة السادسة. أما المجموعة السابعة والثامنة فتم تغذيتهم على دهن الخنزير لمدة شهر ثم تمت المعالجة لمدة شهر آخر بخليط من مستخلص الشاي الاخضر والثوم بمعدل حقن ١ مل شاي اخضر مع ١ مل ثوم للمجموعة السابعة ومعدل ٢ مل مستخلص شاي اخضر مع ٢ مل مستخلص ثوم للمجموعة الثامنة.

في نهاية التجربة تم تجميع السيرم لعمل التحاليل والقياسات التالية: (الكوليسترول الكلي - الجلوسريدات الثلاثية --LDL cholesterol- HDL-cholesterol - اوكسيد النيتريك NO - نشاط إنزيمي AST - LDH - صورة الدم الكاملة CBC - هرمونات الغدة الدرقية T, T3, T4 وهرمون TSH).

وأوضحت النتائج: ان السمنة تؤدي الى زيادة مستويات TSH ونشاط إنزيمي LDH, AST, الجلوسريدات الثلاثية والكوليسترول الكلي وكذلك LDL-cholesterol. والعلاج بمستخلص الشاي الاخضر والثوم أدى الي انخفاض مستويات هذه القياسات باتجاه المستويات الطبيعية.

وفى نفس الوقت ادت السمنة الي انخفاض مستويات كل من (NO - T3 - T4 - HDL-cholesterol) بينما عند العلاج بمستخلص الشاي الاخضر والثوم تحسنت مستويات هذه القياسات نحو المستويات الطبيعية.

وكان مستخلص الثوم أكثر فاعلية من مستخلص الشاي الاخضر وان قياسات الدم لم تتأثر بشكل ملحوظ سواء فى حالة السمنة او بعد العلاج.

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