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The Effect of yogurt enriched with green tea extract on specific health parameters in obese rats

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Abstract

This study sought to evaluate the effects of yogurt fortified with concentrated green tea extract and the drug orlistat on the health characteristics of obese experimental mice. The outcomes of administering a high-fat diet (T₂), a standard diet (T₁), and a high-fat diet supplemented daily with 2 cc of yogurt (T₃). The effects of a high-fat diet augmented with daily administrations of concentrated green tea extract (T₄) and orlistat (T₅) on several physiological parameters were evaluated, including body weight, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and liver enzymes in serum of male obese rats. The therapies were administered orally via gavage. The adult rats were weighed and randomly assigned to five groups. The primary findings indicate that obesity led to a marked decrease ($p \leq 0.05$) in HDL-C levels and a significant increase ($p \leq 0.05$) in animal body weight, as well as in cholesterol, triglycerides, and LDL-C concentrations, relative to the control group. The findings indicate that the groups consuming yogurt fortified with green tea extract had markedly elevated HDL levels and considerably reduced ($p \leq 0.05$) body weight, cholesterol, triglycerides, LDL-C, AST, ALT, and triglycerides.

Keywords: Green tea, extract, Yogurt, Blood lipids, Liver enzymes

Introduction

Food spoilage occurs due to contamination during the processing, preparation, and transportation of raw materials, as well as during manufacturing and packaging. This leads to the deterioration of food color, texture, and nutritional value, potentially promoting the growth of pathogenic microorganisms, thereby reducing food quality and making it unfit for consumption. Food preservation methods such as drying, freezing, heating, fermenting, and salting prolong the shelf life of food products; however, the potential for recontamination after processing remains. The contemporary antimicrobial packaging system is a sophisticated mechanism that incorporates antimicrobial chemicals to regulate the proliferation of specific microbes in food goods [1]. Growing awareness of nutritional health and longer life expectancies, which result in a larger percentage of senior people in society, may be the cause of the rising prevalence of functional foods in recent years. Additionally, rising costs of conventional medical treatments have driven consumers toward alternative therapies. Some nations have made functional foods easier to distribute and more readily available by issuing standards and specifications for them. Furthermore, a large body of research has been conducted that supports the idea that eating might help prevent or lessen the incidence of certain diseases. Additionally, from the early to mid-1990s, companies in the food sector have been able to develop and produce healthful items in large quantities because to advancements in genetic engineering and modern biotechnology, as well as speedier and greater manufacturing capacity [2]. A focus on antioxidants from natural sources has resulted from these discoveries as well as ongoing consumer interest in natural foods. Phenolic molecules are among the most significant natural antioxidants, according to research. Certain phenolic compounds, such rutin, which is mostly found in green tea, are superior to others in terms of efficacy and antioxidant capability [3]. Consumers are increasingly choosing to consume foods that are high in nutrients, even if they are not well known (e.g., food products including green tea). The secret behind the multiple benefits of green tea lies in its leaves' polyphenols, particularly flavonoids, specifically the catechin group, which constitute 80–90% of flavonoids and about 40% of water-soluble solids,

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making it beneficial as an anticancer agent, antimicrobial agent, treatment for circulatory system diseases, antioxidant, and anti-inflammatory agent ^[4].

Materials and Methods

- **Cow's milk:** In Salah Al-Din province, cow's milk was procured from a community in the Alam district and refrigerated during transportation. The samples were kept at $2\pm5^{\circ}\text{C}$ in the refrigerator.
- **Green tea:** The local marketplaces of Tikrit city supplied the green tea, as recognized by academic members from the Department of Field Crops at the College of Agriculture, University of Tikrit.
- **Orlistat drug:** Orlistat was procured from pharmacies, produced by the Italian firm Roche, in capsule format. The therapeutic dosage was 120 mg/kg of human body weight, deemed appropriate as per the manufacturer's guidelines and the Pharmacists Association's recommendations. The therapeutic dose for rats averaging 182 g was calculated as 0.3 mg per gram of body weight.
- **Yogurt production:** The University of Baghdad's College of Agriculture provided the dried form of a starter culture that included (*Lactobacillus bulgaricus*) and (*Streptococcus thermophiles*). The starter was turned on three times in a row before it was used. As a control sample, the first treatment was prepared using fresh cow's milk (M1). The second treatment substituted 15% green tea extract for fresh milk (M2), while the third treatment added 0.3 mg/L of orlistat. After 15 minutes of heating the milk to $85\text{--}90^{\circ}\text{C}$, it was cooled to 45°C , and 3% of the starter was added. After that, the mixture was put into plastic containers and let to sit at 42°C until yogurt started to form ^[5]. Until necessary analyses were conducted at various storage periods (1, 7, and 14 days), the resultant yogurt was kept in a refrigerator at $2\pm5^{\circ}\text{C}$.
- **Animals for experiments:** Adult male white rats (*Rattus norvegicus*) of the Sprague-Dawley strain, weighing 180–200 g and aged 2–3 months, were obtained from the College of Veterinary Medicine at the University of Tikrit. Animals were confined in metal cages with metal covers under controlled laboratory settings, with a light cycle of 12 hours of illumination and 12 hours of darkness. The temperature was sustained at $25\pm2^{\circ}\text{C}$. The animals were allotted three days to acclimate to their new environment and ensure they were clear of sickness. They were provided with sufficient quantities of food and water continuously (*ad libitum*) during the raising phase.^[6]
- **Balanced diet:** In accordance with the National Research Council/National Academy of Science norms, the balanced diet was created with the following ingredients: 536.5 g starch/kg, 5 g vitamin mixture/kg, 50 g mineral mix./kg, 50 g cellulose/kg, 100 g maize oil/kg, and 158.5 g casein/kg. After adding distilled water to create a cohesive dough, the material was formed into the proper shapes for the rats to eat, put in flat stainless steel pans, and dried entirely in an oven set at 50°C with a hot air stream. During the experiment, the dry pellets were kept in a refrigerator at $2\pm5^{\circ}\text{C}$ after being packaged in polyethylene bags.
- **High-fat diet preparation:** The high-fat diet was made in accordance with ^[8] and included the following

components: fat, protein, carbs, cholesterol, beef tallow, and a vitamin-mineral mixture at 58, 25, 17, 1, 13, and 0.6%, respectively.

The experiment's design

Six groups, each with six animals, were randomly selected from among the experimental animals. With the exception of the first group, which was given simply the balanced diet, they were fed the high fat diet for 4 weeks. The experimental animals were fed functional diets following the 28-day induction phase, and doses were given as follows, with one dose every 12 hours:

1. **Group 1:** (group under negative control): These animals remained healthy and were not given yogurt, but continued to receive water and balanced diet throughout the experiment (T_1).
2. **Group 2:** (group under positive control): fed a food heavy in fat (T_2) for the duration of the experiment.
3. **Group 3:** Throughout the trial, they were given a high-fat meal and 2 milliliters of yogurt orally every day (T_3).
4. **Group 4:** Fed a high fat diet and orally administered 2 ml of yogurt supplemented with green tea extract throughout the experiment (T_4).
5. **Group 5:** Fed a high fat diet and orally administered 2 ml of yogurt supplemented with orlistat throughout the experiment (T_5) ^[9].

Biochemical tests: Rats were fasted for around 12 hours at the conclusion of the experiment. Following the animals' anesthesia with chloroform, blood was extracted straight from the heart and put in tubes for tests (devoid of EDTA). After letting the samples sit in a water bath at 37°C for about 15 minutes, the serum was separated using a centrifuge set to 3000 rpm for 15 minutes. The serum was then kept at -18°C until biochemical analyses were completed. These included measuring the levels of triglycerides, cholesterol, HDL-C, LDL-C, Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and VLDL-C in animal serum using the methods described by ^[10].

Statistical analysis: The general linear model (General Linear Model) in the pre-made statistical software SAS ^[11] was used to analyze the experimental results in order to examine the impact of factors in accordance with the totally randomized design (CRD). At the 0.05 level, Duncan's test ^[12] was also used to assess the significance of variations in the means of the variables influencing the traits under study.

Results and discussion

Table 1 shows the impact of various therapies on body weight, it can be observed that feeding with a standard diet (T_1), a high fat diet (T_2), a high fat diet with daily oral administration of (2 ml) of yogurt (T_3), and a high fat diet with daily oral administration of (2 ml) of treatments (T_4 and T_5) had an impact. It is evident that different diets influenced the daily weight gain and final weight gain among experimental rat groups after 28 days of feeding and gavage. According to the results, rats given a typical diet gained 0.217 g of weight every day, reaching a final weight gain of 6.1 g. With a daily increase rate of 1.079 g/day and a final weight gain of 30.22 g, the table also demonstrates that rats fed a high-fat diet experienced the greatest daily gain. Rats fed a cholesterol-rich diet and given yogurt gained

0.852 g of weight per day, resulting in a final weight gain of 23.87 g. In contrast, rats fed a high in cholesterol diet and

given treatments (T₄ and T₅) gained 0.560, 0.941, and 15.69, 26.35 g of weight per day, respectively.

Table 1: Impact of various treatments on the weights of rats (g)

Daily rate of body weight gain (g)	Amount of increase or decrease in body weight after 28 days (g)	Body weight (g)		Treatments
		End of experiment	Beginning of experiment	
0,2170	6,1000	213,910 b0,270±	207,810 b1,450±	T ₁
0,0790	30,2200	268,460 a1,910±	238,240 a3,650±	T ₂
0,8520	23,8700	26,790 b2,05±0	236,920 a1,180±	T ₃
-0,5600	-15,6900	224,390 c2,11±0	240,080 a1,740±	T ₄
0,9410	26,3500	265,380 a3,280±	239,030 a2,970±	T ₅

The mean values ± standard deviation are represented by the numbers in the table.

Significant variations across the research groups ($p < 0.05$) are indicated by different letters in the same column.

T₁ = control (healthy), T₂ = Diseased control, T₃ = Diseased and handled with yogurt, T₄ = Diseased and handled with yogurt and green tea extract, T₅ = Diseased and handled with yogurt and orlistat.

Raised triglyceride and cholesterol levels, together with increased low-density lipoproteins, are the causes of the gaining weight seen in the mean weights of rats given cholesterol treatment. When animals are fed a high-calorie diet, this weight gain is regarded as natural [13]. This outcome is also in line with research done on rats fed a diet high in cholesterol [14]. The authors suggested that the cholesterol therapy may have interfered with the metabolism of fats and absorption through altering the mechanism of fat oxidation, which resulted in the accumulation of fat in particular body parts. These findings also concur with those of [15], who showed that fermented dairy products containing probiotic bacteria were efficient in helping experimental animals gain weight. Additionally, the outcomes align with the findings published by [16]. Consumption of green tea extract led to a significant reduction in rat body weight, which may be attributed to the high fiber content of this extract that improves digestion, promotes the movement of food mass with feces, helps eliminate toxins from the body, and induces a feeling of satiety. On the other hand, it might have an impact on hormones like insulin or leptin, which would effectively reduce weight [17].

Impact of various therapies on the lipid profile

Significant differences ($P \leq 0.05$) between the experimental groups were found in the biochemical tests, as indicated by

the experimental data in Table (2). In contrast to the group of healthy controls, which had a cholesterol level of 110.21 mg/100 ml, the diseased group's and group T₅'s levels rose to 217.55 mg/100 ml. On the other hand, as compared to the control group, therapies T₃ and T₄ significantly reduced cholesterol levels, reaching 150.18 and 147.29 mg/100 ml, respectively. Additionally, the table shows that, in comparison to the group of healthy controls, which had a triglyceride concentration of 88.39 mg/100 ml, the sick group's concentration increased significantly to 154.22 mg/100 ml and treatment T₅'s to 150.78 mg/100 ml. However, lipid levels in the serum of rats treated with T₃ and T₄ significantly decreased, reaching 131.17 and 125.30 mg/100 ml, respectively. The results of the biochemical study also disclosed that the sick group's high-density lipoprotein (HDL) levels had significantly decreased ($P \leq 0.05$) to 27.31 mg/100 ml from 41.48 mg/100 ml in the healthy control group. In comparison to the control, HDL levels in the treated diseased groups T₃ and T₄ rose noticeably to 38.81 and 39.22 mg/100 ml, respectively. Additionally, the table demonstrates that the diseased group's low-density lipoprotein (LDL) levels significantly increased to 159.40 mg/100 ml, compared to 51.06 mg/100 ml in the healthy control group. However, serum LDL levels in mice treated with T₃ and T₄ showed a considerable decrease, measuring 85.14 and 83.01 mg/100ml, respectively. Furthermore, the table indicates that the diseased group's very low-density lipoprotein (VLDL) levels increased to 30.84 mg/100 ml, whereas the values in the healthy control group were 17.67 mg/100 ml. On the other hand, VLDL levels significantly decreased with treatments T₃ and T₄, reaching 25.06 and 26.23 mg/100 ml, respectively.

Table 2: Impact of various therapies on the serum blood lipids profile (mg/100 ml) of male rats who are healthy and obese

LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	TG (mg/dl)	TC (mg/dl)	Treatments
53.320 d ±1.390	18.610 c ±0.670	44.650 a ±1.530	93.340 e ±1.660	115.450 d ±2.210	T ₁
162.110 a ±2.080	31.650 a ±1.240	31.430 d ±0.940	159.560 a ±2.480	222.320 a ±3.120	T ₂
88.380 b ±2.280	27.360 b ±0.850	39.730 b ±1.080	136.490 b ±1.640	155.270 b ±2.350	T ₃
85.290 b ±1.700	26.970 b ±0.880	41.400 b ±1.130	130.380 c ±2.220	152.540 bc ±3.600	T ₄
160.730 a ±2.770	31.540 a ±1.15	32.280 d ±0.840	155.640 a ±2.180	220.770 a ±3.260	T ₅

The mean values ± standard deviation are represented by the numbers in the table.

Significant variations across the research groups ($p < 0.05$) are indicated by different letters in the same column.

T₁ = control (healthy), T₂ = Diseased control, T₃ = Diseased and handled with yogurt, T₄ = Diseased and handled with yogurt and green tea extract, T₅ = Diseased and handled with yogurt and orlistat.

Recent research findings demonstrated that a high-fat diet in rats led to a significant increase in cholesterol, triglycerides, and low-density lipoprotein (LDL-C) levels, accompanied by a considerable decrease in high-density lipoprotein (HDL-C) concentration. These results correspond with those recorded by [18], who indicated that a fatty diet has a significant impact on health by promoting cholesterol formation, increasing LDL-C levels at the expense of HDL-C, elevating serum triglyceride levels, and forming arterial plaques, which obstruct blood flow and lead to various diseases. The reduction of HDL-C in rats subjected to a high-calorie diet may be attributed to elevated levels of LDL-C, triglycerides, and cholesterol, given its role in the reverse transport of cholesterol from tissues to the liver. The capacity of HDL-C to transport cholesterol is impeded by elevated triglyceride and cholesterol levels in tissues and blood vessels [19]. The results demonstrated that the oral feeding of yogurt to rats with increased cholesterol levels significantly reduced their blood lipid profile, supporting the findings of [20]. The study indicated that administering 1 cc of yogurt daily to hypercholesterolemic rats dramatically reduced blood lipid levels, ascribing this effect to the mechanism via which probiotic-rich fermented dairy products lower blood lipids in experimental animals with hyperlipidemia. Furthermore, providing yogurt fortified with green tea extract to rats reduces blood lipids owing to its fiber and pectin content, which promote the conversion of cholesterol into bile salts [4]. The lipid reduction process entails fatty acids and lipolytic activity through the activation of hormone-sensitive lipase or the suppression of

lipogenic enzymes, whereas dietary fiber positively affects cholesterol metabolism [21]. Green tea is low in calories and includes flavonoids that possess significant anti-oxidative characteristics, act as antioxidants, and neutralize free radicals, particularly the superoxide radical [22]. Green tea extract, containing water-soluble polysaccharides, unsaturated fatty acids, prostaglandins, and thromboxanes, mitigates fat formation in arteries, increases HDL-C levels, and diminishes the risk of myocardial infarction. [23].

Effect of different treatments on liver enzyme activity:

The consequences of consuming a typical diet, a diet high in cholesterol (2%), a diet high in cholesterol with everyday oral administration of 2 milliliters of yogurt, and a diet high in cholesterol with daily oral administration of yogurt supplemented with natural and synthetic antioxidants on the activity of liver enzymes in blood serum are displayed in Table (3). As can be seen from the table, the diseased control group's serum had the highest ALT level, measuring 43.75 units/liter, while the diseased groups treated with T₃, T₄, and T₅ had concentrations of 37.51, 29.17, and 39.41 units/liter, respectively, in comparison to the healthy control group's 25.89 units/liter. Regarding the AST enzyme, the diseased control group had the greatest concentration, measuring 85.26 units/liter, while the healthy control group had 61.08 units/liter. Significant differences were noted at P≤0.05. By contrast to the sick control group, the levels dropped to 78.18, 65.29, and 84.68 units/liter, respectively, after receiving T₃, T₄, and T₅ treatments. Furthermore, the diseased control group's serum had the highest ALP value, measuring 106.93 units/liter, whereas the sick groups treated with T₃, T₄, and T₅ had concentrations of 98.12, 92.36, and 103.06 units/liter, respectively.

Table 3: Effects of several treatments on serum liver enzyme activity (in international units per liter) in healthy and obese male rats

ALP (IU/L)	AST (IU/L)	ALT (IU/L)	Treatments
90.760 d3,11±	61.080 d1,25±	25.890 e0,09±	T ₁
106.930 a2,54±	85.260 a2,18±	43.750 a0,33±	T ₂
98.120 b2,15±	78.180 b0,91±	37.510 b0,89±	T ₃
92.360 c1,57±	65.290 c1,29±	29.170 d0,65±	T ₄
103.060 a2,34±	84.680 a2,20±	41.390 a0,78±	T ₅

The mean values ± standard deviation are represented by the numbers in the table.

Significant variations across the research groups ($p < 0.05$) are indicated by different letters in the same column.

T₁ = control (healthy), T₂ = Diseased control, T₃ = Diseased and handled with yogurt, T₄ = Diseased and handled with yogurt and green tea extract, T₅ = Diseased and handled with yogurt and orlistat.

The results regarding the elevation of liver enzymes in the hyperlipidemic animal group are consistent with [24], where the high fat content in the diet caused harm to the liver tissues due to health problems induced by fat, such as increased blood pressure and impaired blood flow within various organs, including liver tissue, leading to the release of liver enzymes into the bloodstream [25]. showed that giving rats a high-calorie diet raised the liver's levels of

saturated fatty acids, which could be a sign of liver damage because it raises the levels of liver enzymes. These results are also consistent with those reported by [20], who observed a significant decrease in liver enzyme levels (ALP, AST, ALT) in hyperlipidemic rats treated with yogurt, ascribes this decrease to the activity of lactic acid bacteria in boosting the liver's metabolic markers and, in turn, increasing its operations through improved metabolic processes. The study also showed that green tea extract-supplemented yogurt could help prevent liver damage. Consumption of green tea for a month in hyperlipidemic rats decreased liver enzyme levels (ALP, AST, ALT) attributable to its anti-inflammatory, antibacterial, and anticancer attributes. Furthermore, the diet's abundance of carotenoids bolsters immune response and mitigates liver disorders. [26]. Nicotinic acid and trigellion compounds,

which have been shown to be present in green tea extract, have been shown to decrease liver enzymes and have a protective effect against the damaging effects of free radicals. Because it doesn't raise cell membrane permeability—which is crucial because greater permeability allows enzymes to leak into the bloodstream—this keeps liver cells from changing structurally^[27].

Conclusion

Green tea has been shown to be instrumental in reducing weight and blood fat in obese rats.

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