

Research Article/Özgün Araştırma

Effect of tarhana soup on serum lipid profile in BALB/c male mice fed a high-fatdiet

Tarhana çorbasının yüksek yağlı diyetle beslenen BALB/c erkek farelerde serum lipit profili üzerine etkisi

Efsane YAVUZ¹^[10], Mustafa ÖRKMEZ¹, Mehmet Akif BOZDAYI¹, Seren ORHAN¹, Davut Sinan KAPLAN², Ayhan BALKAN³

¹Gaziantep University, Faculty of Medicine, Department of Medical Biochemistry, 27310, Gaziantep-Turkey ²Gaziantep University, Faculty of Medicine, Department of Physiology, 27310, Gaziantep-Turkey

³ Gaziantep University Faculty of Medicine Department of Gastroenterology, 27310, Gaziantep-Turkey

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Abstract

Aim: With industrialization, there has been an increase in chronic diseases due to continuous change of nutrition. Tarhana is a traditional grain-based product fermented by lactic acid bacteria in Turkish cuisine. This study aims to evaluate possible effects of tarhana on the impaired lipid profile parameters and serum glucose values in male BALB/c mice fed a high-fat diet.

Materials and Methods: Male BALB/c mice were grouped into three different strain male mice groups: control group (n=10), high fat diet (HFD) (n=10) and high fat diet supplemented with tarhana (n=10). Standard pellet feed was given to the control group, high-fat feed with 60% fat content in the HFD group, high-fat feed and 0.3 mL (8-10mL/kg) of tarhana were given to the other group.

Results: A significant difference was detected in epididymal fat weights, lipid profiles and serum glucose values between the groups (p < 0.05).

Conclusion: It may have beneficial effects in many metabolic diseases, especially coronary heart disease and diabetes with its positive effects on lipid profile, epididymal adipose tissue and glucose level.

Keywords: Fermented food; High fat diet; Lipid profile; Hyperlipidemia; Tarhana.

Öz

Amaç: Sanayileşme ile beslenme alışkanlıklarının değişmesine bağlı olarak kronik hastalıklarda artış meydana gelmiştir. Tarhana, Türk yemeklerinde laktik asit bakterileri tarafından fermente edilen geleneksel tahıl bazlı bir üründür. Fermente gıdalar probiyotikler, prebiyotikler ve organik bileşikler açısından oldukça zengindir. Bu araştırma, tarhananın yüksek yağlı diyetle beslenen erkek BALB/c farelerde bozulmuş lipit profili parametreleri ve serum glukoz değeri üzerindeki olası etkilerini değerlendirmeyi amaclamaktadır.

Gereç ve Yöntem: BALB/c cinsi erkek fareler üç farklı gruba ayrılmıştır: kontrol grubu (n=10), yüksek yağlı diyet (YYD) (n=10) ve tarhana ilave edilmiş YYD (n=10). Kontrol grubuna standart pelet yem, YYD grubuna %60 yağ içeriğine sahip yüksek yağlı yem diğer gruba yüksek yağlı yem+0,3 mL (8-10mL/kg) tarhana verildi.

Bulgular: Gruplar arasında epididimal yağ ağırlıkları, lipid profilleri ve serum glukoz değerleri arasında anlamlı bir ilişki tespit edilmiştir (p<0.05).

Sonuç: Tarhananın lipid profili, epididimal yağ dokusu ve glukoz düzeyi üzerine olumlu etkisi ile başta koroner kalp hastalığı ve diyabet olmak üzere birçok metabolik hastalıkta faydalı etkileri olabileceği düşünülmektedir.

Anahtar Kelimeler: Fermente gıda; Yüksek yağlı diyet; Lipit profili; Hiperlipidemi; Tarhana.

Yazışma Adresi/Address for Correspondence: Efsane YAVUZ, Gaziantep University, Faculty of Medicine, Department of Medical
Biochemistry, 27310, Gaziantep-Turkey, E-mail: efsanee.yavuz@gmail.comGeliş Tarihi/Received:04.03.2024Kabul Tarihi/Accepted:15.07.2024Yayım Tarihi/Published online:30.08.2024



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Bu makale araştırma ve yayın etiğine uygun hazırlanmıştır. **Thenticate** intihal incelemesinden geçirilmiştir.

Introduction

The diets of people living before industrial expansion and agricultural production included a wide variety of unprocessed wild herbs, eggs, fish, meat and various raw seeds. At the beginning of the 20th century, the refining, storage and processing of foods accelerated with industrialization.¹ Economic growth and prosperity have led to major changes in the eating habits of societies, such as an overall increase in total saturated and trans fats and an increase in intake of foods containing animal products. Economic development has also led people to turn to ready-made high-fat refined foods and highsugar drinks. While these foods are low in vitamins, minerals, fiber, amino acids, alinolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and antioxidants, they are rich in saturated fat, simple sugar and salt. Diets in which such foods are commonly consumed are called the "Western diet".² A diet rich in fats, particularly those with high saturated fat content, is linked to a higher risk of several diseases, including obesity, hyperlipidemia, hypertension, increased adipose tissue, insulin resistance, type 2 diabetes mellitus (T2DM), and heart diseases.³ The 2017 Global Burden of Disease study reported that dietary risk factors were associated with 11 million deaths, primarily due to cardiovascular heart disease and T2DM. Globally, there is a suboptimal consumption of healthy foods/nutrients with overconsumption of unhealthy foods that, combined with insufficient physical activity, causes metabolic overload.⁴ Around 650 million people worldwide are currently classified as obese, according to data from the World Health Organization. Obesity can have many possibilities, including cardiometabolic rates of T2DM, non-alcoholic fatty liver disease, and cancer.⁴ The non-pharmacological and most effective method of preventing and treating metabolic syndrome includes changes in eating habits and lifestyle.⁵ Tarhana is a traditional grain-based fermented food unique to Turkey, fermented by lactic acid bacteria. Tarhana has both probiotic and prebiotic nutritional properties characterized by rich biological value and easy digestibility.⁶ The positive effects of probiotic, prebiotic and symbiotic foods or supplements on lipid metabolism disorder and related diseases have been proven by many studies⁷⁻⁹. It has been observed that probiotics and prebiotics improve the lipid profile of the host, decrease in adipose tissue, decrease in weight gain, increase in intestinal hormones such as Glucagon-like peptid 1 (GLP-1) and Peptide YY(PYY), decrease in proinflammatory markers and improve insulin resistance.¹⁰ Probiotics, prebiotics and symbiotics play an important role in reducing cholesterol levels.⁹

This investigation explores the influence of tarhana on altered lipid profiles cholesterol derivatives, (LDL, HDL and total cholesterol) triglycerides (TG) and serum glucose measurements in BALB/c male mice on a high-fat diet. This research enriches the literature by emphasizing the advantages of traditional natural foods in preventing and treating various diseases, including obesity, metabolic syndrome, coronary artery disease, and diabetes mellitus (DM), supported by scientifically validated data.

Material and Methods

The experimental study received authorization from the Gaziantep University Experimental Animal Local Ethics Committee with approval granted during the meeting on 18.02.2020 under decision number 2020/10. The study was conducted at in the Gaziantep University Medical Biochemistry Department. In addition, the study was supported by Gaziantep University Scientific Research Projects Management Unit no: TF.YLT.20.18.

Animals and treatments

Experimental animals and diets

In this study, 30 BALB/c male mice weighing 20 ± 0.5 grams were used. The experimental animals were acquired from the Gaziantep University Center for Experimental Animal Research. Male BALB/c mice (20 ± 5 g) were acclimated for 10 days in a 12 hours light/dark cycle at $23\pm2^{\circ}$ C ambient temperature, 50-60% relative moisture and ventilation system. They were fed ad libitum with a standard diet and water. At the start of the study, the animals were weighed and

grouped into units of 10 per cage, then housed in standard plastic cages.

High fat diet (HFD)

A high-fat diet consisting of 60-70%¹¹ of the energy of saturated fats was prepared by melting and adding $100g/40g^{12}$ of butter to the standard feed supplied in powder form from a private commercial feed producer by the Gaziantep University Experimental Animals Research Center. The carbohydrate, fat, protein, moisture, ash and caloric value analysis of the obtained high-fat feed was carried out at the Gaziantep University Food Engineering Department. Initial results showed the following values: carbohydrate 21.9 g/100, protein 9.3 g/100, fat 52.04 g/100, energy 589.37 kcal/100 g, moisture 11.2 g/100 and ash content 6.4 g/100. High-fat feeds given to experimental animals were prepared weekly and stored at -20°C.

Tarhana

Tarhana prepared for use in the study the stages of a traditional method¹³ including the following ingredients: 3 kg of yogurt, 4 kg of whole wheat flour, 2 kg of onions, 2 cups of cooked chickpeas, 3 kg of strained yogurt, 1 kg of red peppers, 1 kg of jalapeno peppers, 1 bunch of parsley, 1 bunch of dill, salt and flour using mint fame. After mixing the ingredients, it was kneaded into the dough and left to fermentation process by covering it at room temperature for 7 days. After the fermentation process was completed, the dough was spread thinly and allowed to dry. After drying, it was powdered and stored. Tarhana reviewed content includes 17% protein, 15% fat, 68% carbohydrates and 547.3 grams of fiber (Carbs 74.4 g/100g, Protein 18.1 g/100 g, Fat 7.4 g/100g, 12.6 g/100g fiber). The pH of tarhana was 4.92. The total bacteria, mold-yeasts and lactic acid bacteria content of the obtained tarhana were made at Gaziantep University, Department of Food Engineering. Total bacteria were evaluated using the TS 7703/ISO 4833 test method and $1x \ 10^2 \text{ cfu/g}$ result was found. The total mold-yeast was evaluated using the TS 6580 test method and was found to be 2.25×10^3 cfu/g. The total lactic acid was evaluated using the TS 7725/ ISO 4831 test method and the result was found to be 3.5×10^2

cfu/g. 10 grams of powdered tarhana were thoroughly mixed with 100 ml of boiled water, homogenized and passed through a fine wire strainer. Tarhana prepared daily by homogenizing with water was given to the experimental animals by oral gavage in an amount of 0.3 mL/kg.

Experimental groups

Thirty male mice of the BALB/c strain were allocated divided three groups of ten and followed specific dietary protocols over the course of 8 weeks (56 days)^{14,15}.

Control group (n=10): Mice in good health were provided with standard pellet feed and unrestricted water. They also received 0.3mL^{16} (8-10mL/kg) of water through oral gavage, identical to the volume administered to the tarhana group.

High-fat diet group (HFD) (n=10): Mice were provided with a prepared high-fat diet and water ad libitum. Additionally, they received 0.3 mL (8-10mL/kg) of water via oral gavage, the same volume given to the tarhana group.

Tarhana + High-fat diet (n=10): Mice fed with HFD ad libutum were given 8-10mL/kg of tarhana prepared daily by oral gavage.

Weight tracking

Experimental animals were weighed with precision scales in the same group order between 09.00 and 10.00 every Monday for 8 weeks from the beginning of the study.

Collecting blood samples and obtaining serum samples

At the conclusion of the 8-week experimental period, BALB/c male mice received an intraperitoneal injection of 60 mg/kg Ketamine and 10 mg/kg Xylazine for anesthesia. Subsequently, intracardiac blood samples were collected into gel serum separation tubes.

After a 30-minute waiting period, blood samples were centrifuged at 4000 rpm for 10 minutes and the serum samples obtained were transferred into numbered Eppendorf tubes. Serum samples were stored at -80°C until analysis of biochemical parameters.

Removal of epididymal fat tissue

Post-euthanasia, epididymal visceral fat tissues were removed from the anterior, lateral and dorsolateral regions of the prostate in male BALB/c mice and precisely measured on a sensitive scale.

Measurement of biochemical parameters in blood samples

On the day the study was conducted, serums stored at -80°C were removed and mixed with a vortex after they were allowed to thaw at room temperature. Measurements of cholesterol derivatives (total cholesterol, HDL and LDL cholesterol), TG and serum glucose values in serum samples were conducted on a Beckman Coulter A45800 biochemistry autoanalyzer using commercial kits.

Statistical analysis

Statistical evaluation of the data was conducted using IBM SPSS version 22.00. The Kolmogorov-Smirnov test was utilized to assess whether the gathered data conformed to a normal distribution. Given that the data followed a normal distribution and involved more than two groups, a one-way analysis of variance (ANOVA) test was conducted. Subsequently, a post-hoc test for multiple

Table 1. Average body weight values of groups by day

comparisons was employed to assess the differences among the groups. One-way ANOVA and post-hoc (Tukey) tests were used to statistically evaluate the weight changes between the groups. A value of p<0.05 was used to determine statistical significance.

Ethics committee approval

The study received authorization from the Gaziantep University Experimental Animal Local Ethics Committee, with approval granted during the meeting on 18.02.2020 under decision number 2020/10.

Results

Body weight increase findings

The average weight changes of the groups during the 8-week experiment are shown in Table 1. At the start of the experiment, the weight changes among the groups did not show statistically significant differences any Significant (p>0.05).differences were observed between the groups during the 6th and 7th weeks (p < 0.05). At these weeks, the weight gain of the control group was found to be significantly lower compared to the tarhana+HFD and HFD groups (p<0.05). There was no significant difference between the tarhana+HFD and HFD groups (p>0.05).

Days	Control weight(g) (n=10)	HFD weight(g) (n=10)	Tarhana+ HFD weight (g) (n=10)	p value
	Mean±SD	Mean±SD	Mean±SD	
Beginning (week 1)	22.7±2.8	24.2±1.4	23.8±1.9	0,29
7 th days (week 2)	24.1±3.2	23.7±1.7	24.4±2.2	0,82
14 th days(week 3)	23.7±2.8	24.5±1.8	24.6±1.5	0,60
28th days(week 4)	25 ± 3.2	25.4±1.5	24.9±1.2	0,86
35 th days(week 5)	25.3±2.6	27.1±1.6	27.2±2.5	0,14
42 th days(week 6)	24.5±2.3 °	26.8±1.8ª	25.9±0.7	0,02
49th days(week 7)	25.1±2.07 ^b	27±1.5	$28.4{\pm}2.9^{ab}$	0,01
56 th days(week 8)	25.8±2.3	26.7±1.5	28.3±3.09	0,08

ANOVA* p<0.05 (post-hoc multiple comparison test)

a: statistically significant compared to the control group (p < 0.05). b: it is statistically significant according to the tarhana+HFD group (p < 0.05). c: statistically significant compared to the HFD group (p < 0.05). HFD: high fat diet. SD: standart deviation

Epididymal fat weights

At the end of the 8-week period, the average epididymal fat weights obtained from the groups are shown in Table 2. There was a statistically notable difference in the average weights of epididymal fat among the experimental groups (p<0.05) (Figure 1).

Evaluation of biochemical parameters

A statistical analysis of the results obtained from the biochemical analysis of blood samples collected from experimental groups at the end of 8 weeks is shown in Table 3. Significant statistical differences were observed in the average serum glucose levels between the groups (p<0.05) as shown in Figure 2. Table 2. Average epididymal fat weights of the groups

Weight	Control	HFD	Tarhana+HFD	
(g)	(n=10)	(n=10)	(n=10)	
	Mean±SD	Mean±SD	Mean±SD	
epididymal fat	0.54±0.34 ^b °	1.28±0.08 ª b	$1.02{\pm}0.04$ ac	

ANOVA* p < 0.05 (post-hoc multiple comparison test)

a: statistically significant compared to the control group (p<0.05). b: it is statistically significant according to the tarhana+HFD group (p<0.05). c: statistically significant compared to the HFD group (p<0.05). HFD: high fat diet. SD: standart deviation

Table 3. Average serum	glucose and li	pid profile	values of the groups

Biochemical findings	Control	HFD	Tarhana+HFD
	Mean±SD	Mean±SD	Mean±SD
Glucose (mg/dL)	290.66±210.716°	465.66±51.73 ^{ab}	325.8±65,28 °
Total cholesterol (mg/dL)	97.33±19.65 bc	227.0±30.61 a b	188.2±25.78 ac
HDL cholesterol (mg/dL)	69.6±16.8 bc	166.3±16.1 ª	171.4±13.9ª
LDL cholesterol(mg/dL)	26.6±11.59 bc	74.3±15.9 ^{ab}	57.8±12.63 ac
Triglyceride (TG) (mg/dL)	131.3±20°	169.3±32.59 ^{ab}	131.6±26.28 °

ANOVA * p<0.05

post-hoc multiple comparison test

a: statistically significant compared to the control group (p<0.05). b: it is statistically significant according to the tarhana+HFD group (p<0.05). c: statistically significant compared to the HFD group (p<0.05).

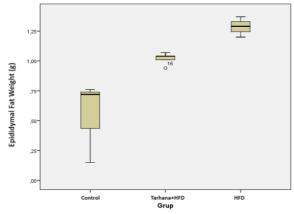


Figure 1. Comparison of epididymal fat weights across different groups

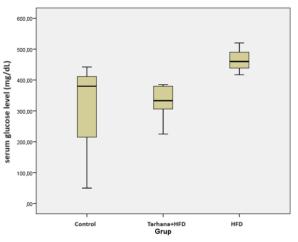


Figure 2. Comparision of the serum glucose (mg/dL)between groups

There was a statistically significant difference in the average total cholesterol levels among the groups (p<0.001) (Figure 3). There was a statistically significant difference in the average TG levels across the groups (p<0.001) (Figure 4).

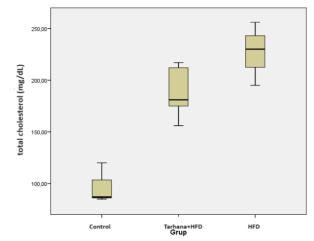


Figure 3. Comparison of the total cholesterol (mg/dL) between groups

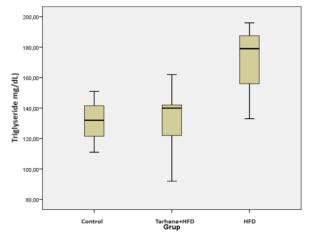


Figure 4. Comparision of the triglyseride (mg/dL) between groups

Discussion

The World Health Organization reports that cardiovascular diseases top the list of global causes of death, accounting for around 32% of all deaths.¹⁷ In Turkey, this rate is 38.4%

according to the 2018 data of TUIK (Turkish Statistical Institute).¹⁸ Among the most important causes of cardiovascular diseases are obesity. metabolic syndrome and its components, hyperlipidemia, an increase in white adipose tissue, high blood pressure and insulin resistance.⁵ According to the Turkey Nutrition and Health Research 2017, the incidence of obesity in Turkey is 30.3%. The regional data of the respective research indicate that, the obesity rate in the Southeastern Anatolia region is 22.9%. In Gaziantep province, which is located in the Southeastern Anatolia region where this study was conducted, the incidence of obesity was found to be 36.8% higher than the average in Turkey.¹⁹ Hyperlipidemia is a primary contributor to mortality in coronary heart diseases.²⁰ Modifiable risk factors include eating habits, physical activity, lifestyle, etc. The most practical and effective method to enhance lipid profiles and lower the risk of cardiovascular diseases involves making lifestyle adjustments, such as adopting a healthy diet and interested in regular physical exercise.²¹ Education is essential at all stages of intervention to achieve these behavioral changes in the management of $obesity^{22}$.

The study evaluated the effect of adding a strong symbiotic tarhana rich in probiotics and prebiotics, a traditional grain-based fermented Turkish food. We believe the results of this study point to the possibility that tarhana might be used as an alternative supplement to enhance lipid profiles, which would help to prevent metabolic disorders that are common throughout the world.

Fat-rich diets not only trigger obesity and associated disorders in humans, but they also trigger the same disorders in animals. Studies in both rats and mice have shown a positive relationship between the amount of fat in the diet and the increase in body weight and adipose tissue.²³

In this study, serum glucose levels in the tarhana- supplemented group were significantly decreased compared to those in the HFD only group. Recent research has concentrated on exploring the connection between gut microbiota and the regulation of glucose levels. In a review conducted by

Somayyeh Firouzi et al.¹⁷ the main emphasis was on evaluating how probiotics influence glucose homeostasis. Parameters like lipid profiles, body weight, and energy consumption were also examined, but with secondary importance. In total 17 animal based and 4 human based studies were examined. Of these studies, 16 involving animals and three humans involving demonstrated improvements in at least one parameter related to glucose homeostasis.²⁴ The antidiabetic effect of a fermented food supplement consisting of rice bran and soy fermented by Bacillus sp. for 10 weeks on rats with type 2 diabetes was investigated by Seong Lim and Boo-Yong.¹⁸ As a result of the study, diabetic rats receiving fermented food supplements showed decreased HbA1c, glucose and serum triglyceride levels.²⁵ In a Ray et al.¹⁹ study conducted including HFD-Fed male mice fermented by Bifidobacterium sp during 8 weeks rice-based fermented beverage of fermented foods by supplementation in the control of obesity, lipid metabolism, effects on glucose homeostasis were investigated. As a result of the study, similar to this study, lower epididymal adipose weights were observed in Bifidobacterium supplemented the and HFD+fermented food supplemented groups compared to the groups fed only HFD. The anti-obesity effect of Bifidobacterium sp. fermented foods is thought to be due to the content of bioactive organisms and many compounds such as phenolic compounds, prebiotics, dietary fibers, vitamins, minerals, peptides, antioxidants. As a result of this experimental study, it was found that the development of grain-based fermented foods could be an alternative method for the cure of obesity and associated disorders.²⁶ In this study, epididymal fat content was significantly higher in the HFD-fed group compared to the other two groups. Nevertheless, the amount of epididymal fat in the tarhana supplemented fed group was found to be significantly lower than in the HFD-Fed group. In a research project carried out by Shouman Lasker et al.²⁰ yogurt supplementation was given to rats fed an HFD for 8 weeks. The study found that rats fed a diet of yogurt plus a high-fat diet exhibited significantly lower weights of epididymal fat and serum glucose levels compared to those

fed only an HFD. As a result of this study, it was found that yogurt supplementation used in the diet may provide an alternative option in the treatment of metabolic syndrome.²⁷ In the study, the total cholesterol level of the tarhana supplemented group was remarkably lower than the cholesterol level of the group fed only an HFD. It is believed that probiotics exert their effect on cholesterol metabolism through various mechanisms.²⁸ A study conducted by Rehab F.M. Ali examined the effect of different amounts of Kishk administration on lipid parameters and biochemical pain, parameters in rats fed a hypercholesterolemic diet²⁹. Kishk is a traditional fermented grain food whose main ingredients are yogurt and whole wheat. The groups supplemented with Kishk experienced a notable reduction in body weight gain, which was attributed to the increased intake of fiber-rich foods, especially grains, as components of a nutritious diet. It was observed that the lipid profile of the Kishk-supplemented groups also improved significantly.³⁰ There is a significant inverse association between the level of HDL cholesterol in the plasma and the risk of cardiovascular diseases induced bv atherosclerosis.³¹ In animal based studies, it has been observed that the cholesterol and fat type of the diet can change the structure and function of HDL cholesterol. In particular, long-term HFD has been shown to impair the antioxidant and anti-inflammatory functions of HDL cholesterol in mice, and this effect was related to elevated HDL lipid hydroperoxide content.³² In some studies, low HDL cholesterol levels were observed in obese mouse models, while in other studies, an increase in HDL cholesterol was observed.³³ is а functional plant Inulin derived polysaccharide consisting of a fructan blend of oligosaccharides and polysaccharides (also known as oligofructose). Onion, wheat, barley, garlic, artichoke, banana and chicory are the most important sources of inulin.³⁴. It is known that inulin has an improving effect on lipid profile and glucose metabolism.³⁵ The tarhana used in the study was made at home by following traditional methods. Onion and whole wheat flour are important sources of inulin. Cooked onions contain 3g/100 inulin³⁶ and 2 kg of onions (60g inulin) are used in the

recipe. Whole wheat flour contains $2.4g/100^{36}$ inulin and 4 kg of whole wheat flour (96 g inulin) is used in the recipe. In this study, the results obtained showed an important decrease in the blood glucose and lipid levels of the tarhana + HFD group compared to the HFD. In a rapidly developing and changing world, people's eating habits have also changed greatly. The demand for more easily accessible and ready-made foods has increased instead of home-prepared foods. As these diets become more common, a range of disorders is becoming more prevalent, including Type 2 diabetes, liver diseases, obesity, metabolic and notably cardiovascular syndrome, diseases. These diseases are also a great burden on countries from an economic point of view. The importance of nutrition in the treatment and prevention of diseases is increasing day by day. Protective foods with rich nutritional content, which are not in the form of drugs, capsules or powders, but are a part of our daily diet are called functional foods. Fermented foods belong to the class of functional foods. Almost 60-70% of the products called functional foods are probiotics.³⁷ It is pointed out that tarhana is a symbiotic and functional food due to its high nutritional value, probiotic bacteria strains and prebiotics³⁸. Lactic acid bacteria are recognized as potential mediators of beneficial effects on hypercholesterolemia. Lactobacilli possess enzymes that participate in the deconjugation of bile salts to form bile acids, thus inhibiting micelle formation, intestinal cholesterol absorption and enterohepatic circulation of cholesterol³⁹. Prebiotics may reduce cardiovascular diseases risk by reducing inflammatory markers⁴⁰. The main mechanism of action of prebiotics on lipid metabolism is based on their ability to reduce lipid levels in the bloodstream by the presence of short-chain fatty acids (SCFA) produced upon selective fermentation of the prebiotic substrate by the gut microbiota⁴¹.

A healthy diet should consist of a balanced pattern based on nutritional diversity. We can benefit from the protective effects by adding fermented foods to our daily diet in a way that does not disrupt the pattern. The production of traditional fermented foods is mostly done at home in a way that is sufficient for households. By popularizing the consumption of these foods, it can be achieved to replace readymade foods, and they are also easily accessibility also. Tarhana, a functional food used in study, significantly reduced the risk of developing many diseases with its positive effects on lipid profile, epididymal fat weight and glucose level.

Conclusion

As a result of our study, epididymal adipose tissue, blood glucose levels, total cholesterol and triglyceride levels decreased significantly. These effects indicate that the protective effect of tarhana on maintaining a healthy weight and preventing chronic diseases important. Further similar studies is contributing to the literature are needed to further investigate and analyze the different parameters of the effects of tarhana on maintaining healthy diet and well-being.

Ethics Committee Approval

The study received authorization from the Gaziantep University Experimental Animal Local Ethics Committee, with approval granted during the meeting on 18.02.2020 under decision number 2020/10.

Author Contributions

All of the authors contributed at every stage of the study.

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Declaration of Interest

There is no disagreement between the authors.

Financial Disclosure

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Statements

This study was presented as a poster paper.

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