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Comparing the Effect of Garlic and Lemon Mixture with Mucilage of Okra Pods to Prevent the Increase of Plasma Lipids in Ratus Norvegicus Alluvia

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Abstract

Background: *Cardiovascular disease is one of the main causes of mortality in the world and is primarily caused by high blood lipids. This study aimed to compare the effect of garlic and lemon mixture with mucilage of the okra pods in preventing the increase of plasma lipids in male Ratus.*
Methods: *This study was performed on 34 Ratus norvegicus alluvia. The Ratus were randomly divided into two classes, which were further subdivided in three groups each. A class of Ratus received food mixed with market oils for 60 days. Moreover, the Ratus in the treatment group received 10% okra mucilage and the mixture of garlic and lemon as gavage for 3 weeks. Data were analyzed by SPSS-21 software. The Mann–Whitney nonparametric test was used to compare each pair of groups. P-value less than 0.05 was considered as statistically significant.*
Results: *Comparing the control group and the group receiving oil, we observed that that lipid intake was effective and significantly increased the blood cholesterol in the oil group (P<0.01). In Ratus receiving fatty foods, it was observed that treatment regimens reduced blood lipids. Moreover, a significant difference was observed between the two treatment groups, indicating a 10% mucilage advantage in reducing blood lipids compared with other treatment groups.*
Conclusions: *The results indicate that both treatments reduce blood lipids, but 10% mucilage in Ratus with high blood lipids has better efficacy than garlic and lemon mixture in reducing blood lipids.*

Keywords: Okra mucilage, Garlic, Lemon, Plasma lipids, Ratus.
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Introduction

Cardiovascular disease is one of the leading causes of mortality worldwide and according to ATP III guidelines the first outcome is a metabolic syndrome.[1] Metabolic syndrome is a branch of metabolic disorders, including disorders of glucose and insulin, lipid abnormality, obesity (especially abdominal obesity), and hypertension, all of which have been proven to be risk factors for cardiovascular disease.[2,3] One of the most important interventions for the treating dyslipidemia is the use of lipid-lowering drugs.[4] One issue of modern medicine is the increasing use of chemical drugs, which has numerous consequences, including the following:

- The gradual occurrence of an autoimmune phenomenon, which requires increased use of strong drugs with dangerous side effects.
- Undesirable side effects, which are sometimes more dangerous than the disease.[5]

Therefore, plant treatments without medication were provided. Herbal medicine is also one of the oldest treatments that have been considered in the last two decades.[6] The use of medicinal herbs and the history of traditional medicine or experimental medicine can be correlated with human life and civilization, since diseases are born with the advent of mankind. Therefore, today, all academic and industrial centers and WHO present extensive plans for the use of medicinal plants.[7] The use of food for treating diseases and promoting good health (prevention) is one of the most important strategies of traditional medicine in Iran.[8] Mixture of garlic, lemon, and okra mucilage are among the foods that can affect our desired index.

Garlic is of great nutritional importance. It has been cultivated since ancient times as one of the medicinal herbs and condiments, and nowadays, it is used worldwide in famous medicinal herbs.[9] The effects of garlic on the treatment of meningitis; parasitic diseases such as hymenolepiasis, trypanosomiasis, and leishmaniasis; platelet aggregation; thrombosis; lipid profiles; and blood pressure have been confirmed.[10]

The lime tree (lemon; scientific name: citrus aurantifolia) belongs to citrus family.[11] Lemon is clinically considered to have antitumor, anti-inflammatory, antitetracyclic, antiosteoporotic, antithrombotic, and antiviral properties. The mechanism of action of flavonoids is through their effect on nitrite; the direct removal of oxidative radicals; and their effect on the accumulation of leukocytes, oxides and reactions with other enzymatic systems. Flavonoids in Citrus also have a positive effect on the immune system.[12]

Okra (Hibiscus esculentus) belongs to the family of Malvaceae and is one of the tropical and semitropical plants distributed in the Middle East (Iran). This plant is rich in carbohydrates; phytosterols, tannins, and flavonoids.[13] Flavonoids have many pharmacological effects, such as LDL protection from oxidation, anticancer, anti-inflammatory and liver protection, antidiabetes, antiallergic, antitumor effects.[10] The slimy property of okra is due to the thick and slimy matter found in fruit pods, called mucilage. Mucilage has hypocholesterolemic and hypolipidemic properties and includes polysaccharides; proteins; minerals; natural sugars comprising rhamnose, galactose, galacturonic acid, and glucose; and palmitic acid, oleic acid, and linoleic acid.[14,15]

Fewer side effects and availability of the natural substances facilitate their use, and considering the studies on the effects of garlic and lemon, and that a high number of studies have been conducted on the relationship between separate use of garlic and lemon and their effects on the reduction of inflammatory bioindicators, lipid profiles and insulin resistance in cardiovascular patients, it is noteworthy to say that no study has compared the effects of the mixture of garlic, lemon and mucilage in okra pod. As a result, the present study aimed to investigate and compare the effect of garlic and lemon mixture with mucilage in okra pods in order to prevent the increase of lipids in Ratus Norvegicus alluvia.

Materials and Methods

This study was performed on 34 R. Norvegicus alluvia's weighing 180–200 g. Blood was directly extracted from the Ratus heart. This action increases blood volume and mortality; hence, it was conducted only at the end of the blood sampling. Basic information is needed for analysis so that the effect of the treatment can be measured. Therefore, Ratus were randomly divided into two classes, and then each class was further divided into three subgroups; First class (control group):

1. Control: Ratus receiving routine foods (N=8)

2. Control + garlic and lemon: Ratus receiving routine foods + garlic and lemon (N=4)

3. Control + okra mucilage: Ratus receiving routine foods + okra mucilage (N=4)

The second class (case group): Ratus that received food with market oil in order to increase their blood lipids and then received;

1. Oil: Ratus receiving the same diet containing oil with no other additives (N=4)

2. Oil + garlic and lemon: Ratus receiving food containing oil + garlic and lemon (N=7)

3. Oil + okra mucilage: Ratus receiving food containing oil + okra mucilage (N=7)

The diet was administered by initially crushing the food plates with the mill, followed by addition of the oil and mixing it with the crushed plate in order to make a paste. The Ratus in the case group received this diet for 60 days, and then, the Ratus in both case and control groups were subjected to the above treatment regimen for 3 weeks.

10% okra mucilage was prepared as follows: 1 kg of okra skin was mixed with 1 kg of water and boiled after 5–6 h for 30 min; after 5–6 hours, when mucilage was removed, it was filtered to obtain the aqueous extract of 10% okra mucilage and kept in the refrigerator (all of the aforementioned steps were performed under sterile conditions).[16]

To prepare garlic and lemon mixture, initially, 30 garlic were peeled and poured into the mixer with 5 (Shirazi) lemons with skin that had already been separated from their nuclei, and when all the contents were crushed in the mixer, they were mixed and boiled with 1 L of water (only once boiled). After cooling down, the mixture was filtered and the contents were kept in a glass and preserved in the refrigerator.[17]

Ratus were placed in different cages based on their treatment class and subgroups. For example, the Ratus in the first class that received routine food and lemon garlic extract were kept in one cage and the Ratus that received routine food and mucilage were kept in another cage. Food packages were prepared as previously described. There were two types of packaging: (with and without oil) and nine cages. To prevent information bias, a bar code was used on each envelope. The second digit on the right of this barcode represents the type of treatment and the second digit on the left of the barcode represents the cage number of the Ratus. Three types of vials were also coded (1: distilled water; 2: okra mucilage; and 3: garlic and lemon), as aforementioned. Ratus received the prepared solutions for 3 weeks as gavages (10% of their weight per day). The person analyzing the information was blinded to the study. Data were analyzed using SPSS-21 software. Considering the low volume of the sample and non-normality of data, the Mann–Whitney nonparametric test was used to compare each pair of groups. A value of 5% was considered as statistically significant.

Results

The studied Ratus were divided into six groups, and their descriptive information is presented in table 1 (considering the small sample size and nonnormality of data, the mean and first and third quartiles were displayed).

Comparison of the control group with the group receiving oil using the Mann–Whitney test revealed that lipid was effective and significantly increased the blood cholesterol ($P<0.01$).

Comparison of the first class revealed that both treatments led to a reduction in blood lipids; however, no significant difference was found between the two groups using the Mann–Whitney test (table 2).

Comparison of the second class revealed that the two treatments led to a decrease in blood lipids and a significant difference was observed while comparing the two groups using the Mann–Whitney test (table 3).

Table 1. Descriptive statistics (Mean (1st-3rd quartiles)) of studied variables

	Cholesterol (mg/dl)	Tri glyceride (mg/dl)	Glucose (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	123.5 (121.50-126.0)	103.5 (85.25-171.0)	92.5 (77.0-108.0)	59.5 (46.75-64.50)	52.5 (43.50-56.75)
Oil	146 (131.75-164.75)	102 (95.75-117.25)	113.5 (103.50-118.25)	56 (44.75-62.0)	64.5 (58.0-69.5)
Control + garlic and lemon	111 (105.75-117.0)	92.5 (68.0-129.75)	94.5 (77.50-108.50)	72.5 (65.0-77.0)	28.5 (24.0-30.75)
Control + okra mucilage	103.5 (78.75-109.50)	105.5 (71.75-117.50)	75.0 (70.50-80.25)	53.0 (46.75-63.75)	33.5 (28.50-47.50)
Oil + garlic and lemon	102.5 (100.25-120.0)	82.0 (70.50-115.25)	91.0 (70.75-104.0)	65.0 (59.0-75.50)	34.0 (27.0-38.50)
Oil + okra mucilage	124 (115.0-128.0)	77 (57.0-112.0)	88 (85.0-91.0)	61 (56.0-65.0)	45 (38.0-50.0)

Table 2. Comparison of under treatment groups in the first class

	Cholesterol P.V	Tri glyceride P.V	Glucose P.V	HDL P.V	LDL P.V
Control + garlic and lemon	0.01	0.44	1.00	0.02	0.01
Control + okra mucilage	0.23	0.73	0.73	0.23	0.03
P-value Comparison of two groups	1.00	0.69	1.00	0.11	0.06

Table 3. Comparison of under treatment groups in the second class

	Cholesterol P.V	Tri glyceride P.V	Glucose P.V	HDL P.V	LDL P.V
Oil + garlic and lemon	0.02	0.13	0.01	0.30	0.01
Oil + okra mucilage	0.01	0.20	0.03	0.09	0.01
P-value Comparison of two groups	0.04	0.53	0.80	0.16	0.01

Discussion

The results of this study revealed that blood lipids of the Ratus that received routine food with market oil for 60 days were significantly higher than the group that did not receive the lipid. After 3 weeks of treatment, the Ratus group with high blood lipids revealed a significant decrease in cholesterol and LDL as the result of the given treatment; however, in the group with normal blood lipids, it was observed that okra mucilage reduced cholesterol and LDL, but the mixture of lemon and garlic only significantly decreased the level of LDL in the blood of the Ratus.

Essence in sour lemon peel significantly reduces cholesterol and triglyceride levels. Garlic is also effective in reducing cholesterol and triglycerides, and in lowering blood pressure and preventing atherosclerosis,[18] which is consistent with the results of our study. Bok et al. reported lower levels of liver and plasma cholesterol in the blood of Ratus receiving lemon peel and concluded that this reduction was related to the flavonoids.[19] Hertog proved that the flavonoids in the sour lemon reacted with active oxygen due to its antioxidant properties and prevented the oxidizing of LDL.[20]

A study that investigated the effect of allicin in garlic powder and its effects on blood lipids, triglycerides, and blood pressure, reported that allicin in garlic reduces the content of blood lipid in Ratus.[21] Animal studies have reported that garlic decreases the blood lipid parameters in rabbits. Another study on rabbits revealed that garlic reduced blood lipids in rabbits with high cholesterol,[22] which is consistent with the results of the present study.

Parastooei et al. conducted a study on the effect of garlic pork on blood glucose and lipid of the diabetic people and concluded that garlic consumption reduced cholesterol and LDL in diabetic individuals. Garlic and thyme mixture decreased the lipid indices in Guinea pigs.[23] In animal models; the consumption of garlic powder reduces the accumulation of lipids in the liver and increases bile acid secretion.[24]

The fiber-rich diet reduces triglyceride levels by inhibiting lipogenesis in the liver. Fiber reduces lipid parameters and LDL-cholesterol levels by preventing the absorption of bile acids and cholesterol and increasing LDL receptor activity.[25]

Mucilage inhibits the absorption of harmful cholesterol and decreases serum and tissue lipids; in cells isolated from the rat liver, it was observed that mucilage reduces the synthesis of VLDL and ApoB. Moreover, the polysaccharides in the fruit of the okra plant are bound to bile acid and prevent its continuous flow.[26,27] The secretion of pectin in this fruit increases the bile acid removal, and thereby, increases the synthesis of bile acids from cholesterol, reducing the cholesterol and the risk of cardiovascular disease.[28]

Gum and mucilage in plants increase lipoprotein lipase (LPL) activity in the heart and fat tissues. As a result, the adsorption of triglyceride-rich lipoproteins increases in the non-liver tissue, which leads to a decrease in glyceride, and considering that the highest amount of cholesterol is present in LDL, reducing the cholesterol levels leads to reduction in LDL.[29]

Comparing the two groups revealed that only in groups with high blood lipids, a difference between the two groups was observed, indicating that 10% mucilage had a better effect on the Ratus with high blood lipid. The results of the study indicate that both treatments reduce blood lipids; however, 10% mucilage in Ratus with high blood lipids has better efficacy than the mixture of garlic and lemon in reducing blood lipids.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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