Full Length Research Paper

Alterations in serum lipid, lipoprotein and visceral abdominal fat pad parameters of hypercholestrolemic guinea pigs in response to short term garlic consumption

Malihe Pourkabir*, Tahora Shomali and Farzad Asadi

Department of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran Iran.

Commercially available garlic preparation in the form of garlic oil, garlic powder and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile. The aim of the present study was to determine short term effects of dietary consumption of garlic on the serum levels of triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and abdominal fat pad status in hyperlipidemic guinea pigs. 15 animals categorized into three groups of 8 animals each as control, 2.5 and 5% garlic incorporation diet. 2.5 and 5% garlic made significant decrease in serum TG (15 and 29%), TC (73 and 78%), VLDL-C (65 and 70%) and LDL-C (69 and 70%) when compared to the control. However, we have not shown significant changes in the level of serum HDL-C. Moreover, TC concentrations per gram of abdominal fat pad showed 15 and 24% decrease in response to 2.5 and 5% garlic consumption. Garlic consumption decreased mean adipocyte abdominal fat pad (AFP) so that higher percentage of cells was in 0 - 5 µm range. Reduction in AFP (50%) and body weight decrease (14%) was shown for garlic groups when compared to the control. The beneficial note of short term dietary consumption of garlic will be done through both serum lipid status and cellular changes of the abdominal fat pad.

Key words: Guinea pig, garlic, lipid.

INTRODUCTION

Documents dating from the beginning of recorded history show that garlic was found in Egyptian pyramids and ancient Greek temple. In addition, medical texts from China, India, Egypt and Rome mentioned medical applications of garlic (Rivlin et al, 2006). Surpri-singly, the ancient Chinese consumed the garlic to achieve longevity (Ali et al., 2000).

The beneficial effects of garlic on the cardiovascular risk factors, mainly hyperlipidemia and thrombogenesis, have been reported in both animals and humans by Elkayam et al. (2003). Moreover, its hypolipidemic has been confirmed by many investigators. There have also been reports on the beneficial effects of garlic extract and oil in controlling hyperlipidemia in animals. In this regard, Chang and Johnson (1980) and Adamu et al. (1982) showed that adding garlic to sucrose and cholesterol in the diet of rat prevents an increase in serum and liver cholesterol, triglycerides and free fatty acid levels. High dose application of garlic (1 g/kg) in patients has beneficial effects in reducing hyperlipidemia. On the other hand, obesity is a common and serious disorder which requires appropriate management (Yanovski and Yanoviaki, 2002).

The aim of the present study was to extend literature

---

*Corresponding author. E-mail: pourkabr@ut.ac.ir. Tel: +982161117147. Fax: +982166933222.

Table 1. Serum lipid, lipoprotein, body weight and abdominal fat pad (AFP) parameters of guinea pigs in the control (n = 5) and treatment groups of feeding 2.5% (n=5) and 5% dietary garlic (n = 5) powder. Values were expressed as mean ± SD.

Biochemical parameters	Control (A)	2.5% Garlic powder (B)	5% Garlic powder (C)	P-values (significant differences among groups)
TG (mmol/l)	1.13 ± 0.19	0.95 ± 0.15	0.80 ± 0.15	A,C (0.02)
TC (mmol/l)	13.44 ± 1.07	3.57 ± 0.22	3.14 ± 0.40	A,C (< 0.001); A,B (< 0.001)
VLDL-C (mmol/l)	0.52 ± 0.09	0.19 ± 0.03	0.16 ± 0.03	A,C (< 0.001); A,B (< 0.001)
LDL-C (mmol/l)	8.26 ± 1.61	2.6 ± 0.01	1.76 ± 0.37	A,C (< 0.001); A,B (< 0.001)
HDL-C (mmol/l)	0.64 ± 0.1	0.55 ± 0.13	0.66 ± 0.12	P = 0.322
Body weight (g)	349.66 ± 16.92	364.67 ±17.79	298.33 ± 22.05	A,C (0.003); B,C (< 0.001)
Adipocytes number (per gram of AFP)	64166.67 ± 1814.54	52500 ± 1215	53750 ± 1767.77	P = 0.959
AFP triglyceride (mmol/g of AFP)	3.71 ± 0.36	3.15 ± 0.16	2.82 ± 0.32	A,C (0.01); A,B (0.027)
Fat pad (g)	3.84 ± 0.77	4.21 ± 0.68	1.945 ± 0.35	A,C (0.001); B,C (< 0.001)

on the pharmacological and nutritional effects of short term consumption of garlic on the serum states of lipid and lipoprotein in the dietary induced hyperlipidemic guinea pigs as a LDL animal model. Moreover, we followed alteration in the status of the abdominal fat pad in response to consumption of dietary garlic.

MATERIALS AND METHODS

Twenty one albino male guinea pigs (mean weight 230 ± 10 g) were purchased from Institute Pasteur Iran (Tehran-Iran). After 10 days acclimatization on a normal diet, three guinea pigs were randomly selected and were weighed after overnight fasting. Animals had free access to water. Blood was collected by cardiac puncture, allowed to clot, and centrifuged at 3500 g for 3 min; sera were separated and stored at - 80°C until analysis for cholesterol and triglyceride concentrations. The reminder of guinea pigs were fed by hyperlipidemic diet (16% cholesterol and 15% corn oil) (Chapman and Mills, 1977). After 16 days, serum total cholesterol (TC) and total triglyceride (TG) were measured in 3 guinea pigs using enzymatic method. The reminder of guinea pigs were divided into 4 groups of four animals each and fed as fallows for 21 days: hyperlipidemic diet, hyperlipidemic diet supplemented with 2.5% garlic powder, and hyperlipidemic diet supplemented with 5% garlic powder.

Garlic powder was prepared by freeze-drying method. At the end of experiment all guinea pigs were weighed, bled and their serum TG, TC, very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured. TG levels were measured using Gottfried and Rosenberg (Gottfried and Rosenberg, 1973) and TC were measured by Loeffler and Mc Dougald (Loeffler and McDougald, 1963). HDL- C levels were measured by modified method of Burstein (Burstein al., 1970). VLDL-C levels were estimated as one fifth of TG levels and LDL-C levels were calculated by Friedewald

equation as: LDL-C = TC-(HDL-C+VLDL-C). Guinea pigs were euthanized by deep anesthesia and placed in the refrigerator (4°C) over night. Abdominal fat pad were collected, homogenized and analyzed for size, count and triglyceride concentration of adipocytes per gram of fat pad according to the method of Ashwel and Priest (1977). Briefly, 1 g of separated adipose tissue was put into the plastic tube contain 3 ml bicarbonate buffer, bovine serum 3% albumin, 10 mg collagen and 3 μmol glucose per ml and incu-bated for 1 h at 37°C in the metabolic shaker (Memmert, D91126). Contents of tubes were mixed by a plastic rod and centrifuged at 400 g for 1 min. After three times washing, adipocytes were counted by a hemacitometer. The mean diameter of the adipocytes was determined by Rodbell (1964) method. Furthermore, the lipid content of the abdominal fat pad was extracted by Dole's method (1956) and its value was determined by Neri and Firing's method (1973). Descriptive analysis was done by Sigma stat 2.01 Software. Values of different parameters were compared among groups by 1-way ANOVA.

RESULTS

Table 1 shows the effect of garlic powder on the serum TG, TC, VLDL-C, LDL-C HDL-C and body weight in the control and treatment groups.

There was a decrease in the serum TG (p = 0.02) compared to the control group. The levels of serum TC showed a significant decrease in response to 2.5 and 5% garlic powder when compared the control group (p < 0.001). Serum VLDL-C levels showed a significant decrease in response to 2.5 and 5% garlic powder compared to the control group (p < 0.001). Meanwhile such decrease has been shown for LDL -C levels.

However, serum HDL-C levels did not show changes in

response to 2.5 and 5% dietary garlic powder when com-pared to the control group (p > 0.05).

Body weight showed a significant decrease in response to 5% dietary garlic powder compared to the control (p = 0.003). Meanwhile, the mean weight of abdominal fat mass showed a significant decrease in response to 5% dietary garlic powder (p = 0.014).

We have not shown any significant changes in the adipocyte counts per gram of abdominal fat pad in treatment groups compared to the control (p > 0.05). Furthermore, 2.5% garlic powder did not make any changes in the abdominal fat pad TG levels compared to the control (p > 0.05). However, a significant decrease has been shown in response to dietary 5% garlic powder (p = 0.012).

Compare to the base line, treatment with garlic powder made different distribution for adipocyte size. In this regard, while medium size adipocyte (50-100μm) had the highest distribution in the control group, small size adipocyte (0-50 μm) had the highest distribution in the treatment groups.

DISCUSSION

In the present study, the effect of 2.5 and 5% dietary garlic powder on the serum TG, TC, VLDL-C, LDL-C, HDL-C levels and abdominal fat pad has been studied in the hyperlipidemic guinea pigs.

Effect of garlic powder on serum TG

Consumption of 2.5 and 5% garlic powder decreased serum TG levels (about 16 and 29%) compared to the control. Qureshi et al. (1983) reported 10 - 26% decrease in serum TG levels when pooled Leghorn breed was fed by 3.8% etheric, methanolic and hydro extract of garlic for 4 weeks. At the present study, 5 and 2.5% dietary garlic powder made 16 and 29% decrease (respectively) in TG concentrations when compared to the control. In addition, Bordia et al. (1998) reported a decrease in plasma TG levels of atherosclerotic patients when compared to the garlic powder.

In the present study, serum TC levels in response to 2.5 and 5% garlic powder were 73 and 78% lower than the control group, respectively. In this respect, 5% garlic powder was more potent (about 19%) than 2.5%. Kamanna et al. (1982) and Marta et al. (2006) reported a decrease in the serum TC in response to consumption of 2 and 3% garlic powder. They showed that garlic powder made 52 and 33% decrease in serum TC when rats were fed by an atherogenic diet for 8 - 9 weeks. They discussed that 2% garlic powder was effective and desirable. Qureshi et al. (1983) reported 4 weeks feeding of 3.8% hydro, etheric and methanolic extract of garlic powder in the pooled Leghorn diet resulted in 20 - 25%

reduction in the serum TC levels. Bordia et al. (1998) reported a reduced plasma TC levels in affected peoples with cardiovascular lesions.

At present study, the effect of garlic powder on serum VLDL-C levels was also assessed. We found a reduction (about 65 and 70%) in the serum VLDL-C levels in response to 2.5 and 5% garlic powder, respectively. How-ever, decease of HDL-C levels in response to 2.5 and 5% dietary garlic powder was not significant. In this respect, Kamanna et al. (1982) and Goley and Bobbioni (1997) showed that supplementation of the rat chow with 2% garlic powder for 8 - 9 weeks had not any effect on the HDL-C levels in the atherosclerotic rats. Similarly, Qureshi et al. (1983) showed that 4 weeks feeding of the pooled Leghorn breed with hydro, etheric and methanolic extracts of the garlic did not make any significant changes in the HDL-C concentrations.

It has been documented that the ratio of LDL-C/HDL-C in guinea pigs was high such that HDL-C levels were very low and LDL-C levels were high. At the present study, consumption of 2.5 and 5% garlic powder led to a decrease (69 and 79%) in serum LDL-C concentrations. On the other hand, reduction of LDL-C concentrations in response to 5% garlic powder was 31% higher than those responses to 2.5% garlic powder. In line with our findings, Kamanna et al. (1982) showed that adding of 2% garlic powder to the atherogenic diet of rats for 8 - 9 weeks decreased their LDL-C concentrations by 42%. In a similar experiment, Qureshi et al. (1983) found that adding of 3.8% hydro, methanolic or etheric extract of garlic to the diet resulted in a 28 - 41% decrease in the serum LDL-C concentrations. Also, clinical trial showed that using garlic powder and its extracts significantly decreased plasma LDL-C concentrations in human. In this regard, Chi et al. (1982) and Yeh and Liu, (2001) discussed that dietary garlic led to a decrease in the activity of lipogenic and cholesterogenic enzyme of liver such as malic enzyme, fatty acid synthetase, glucose-6-phosphate dehydrogenase and 3- hydroxyl-3-methyl glutaryl coenzyme-A (HMG-CoA).

Effect of dietary garlic on mean diameter of adipocytes

Studies on laboratory rodents, domestic animals and human have shown that the volume of fat cells are affected by diet, consumption of meals, exercise, age, endocrine and genetic factors (Pond and Mattasks,1983). The mean adipocytes size has effect on its metabolism and sensitiveness with insulin. We have shown that the medium size (50 - 100 μm) and large size (100 - 150 μm) adipocytes had the highest (60%) and the lowest (8%) frequency in the control group, respectively. The frequency of small size adipocytes (0 - 50 μm) was about 32%. Consumption of 2.5% garlic powder resulted in about 20% decrease in the population of the medium size

adipocytes when compared to the control group. In this case, the population of smaller size adipocytes (0 - 50 μm) showed increase (about 1.6 times) when compared to the control group.

While feeding of 5% garlic powder decreased the population of the larger size adipocytes (100 - 150 μm), it increased the percentages of the smaller adipocytes (0 - 50 μm). Percentage frequency of the small size adipocytes in response to 5% garlic powder was 32% higher than the 2.5% garlic powder and 52% higher than the control.

Effect of dietary garlic on adipocytes number per gram of abdominal fat pad

The number of adipocytes in the abdominal fat pad of guinea pigs received 2.5 and 5% garlic powder and showed 18 and 16% decrease compared to the control, respectively. Moreover, there was no significant difference between two groups. We showed that short term use of the dietary garlic powder led to reduction in diameter and number of adipocytes.

Effect of dietary garlic powder on TG levels per gram of abdominal fat pad

The administration of 2.5 and 5% dietary garlic powder led to 15 and 24% decrease in TG concentrations per gram of abdominal fat pad, respectively. There was a dose dependent change in TG levels of fat pad. This reduction in response to 5% dietary garlic powder was 11% higher than that response to 2.5% garlic powder.

Effect of dietary garlic on the body weight

Body weight in 2.5% garlic powder group was 3.4 times higher than the control. However, such increase was not significant compared with control group. In this regard, body weight in response to 5% garlic powder was 14% lower than the control group.

It has been shown that allicin given to rats inhibited an increase in the body weight (Elkayam et al., 2003).

Effect of dietary garlic on the abdominal fat pad

Administration of 2.5 and 5% dietary garlic powder was also led to decrease (10 and 50%) in the abdominal fat mass when compared to the control. It seems that among the active constituent of garlic, the principal component is allicin (thio-2 propen-1- sulfinic acid S-allyl ester) which renders the garlic the above mentioned characteristics (Elkayam et al., 2003).

REFERENCES

Adamu I, Joseph PK, Augusts KT9 (1982). Hypo lipidemic action of onion and garlic unsaturated oils in sucrose fed rats over a two month period. Experientia, 38: 899-901.

Ali M, Al-Qattan KK, Al-Enezi F (2000). Khanafer RMA, Mustafa T Effect of allicin from garlic powder on serum lipids and blood presser in rats fed and with high cholesterol diet. Prostaglandin, Leukotrienes and Essential Fatty Acids. 62 (4): 253-259.

Ashwel M, Priest P (1997). Human fat cell sizing - a quick, simple method. J. Lipid Res .17: 190-192.

Bordia A, Verma SK, Strivasta KC (1998). Effect of garlic (*Allium stivum*) on blood lipids, blood sugar fibrinogen and fibrinolitic activity in patients with coronary artery disease. Prostaglandins Leukot. Essent. Fatty acids. 58: 257-263.

Chang MI, Jonson MA (1980). Effect of garlic on carbohydrate metabolism and lipid synthesis in rats, Nutrition, 110: 931-936.

Burstein M, Scholnic HR, Morfin R (1970). Rapid method for the isolation of lipoprotein from human serum by precipitation with poly anion. J. Lipid Res. 11: 583-595.

Chapman M, Mills G (1977). Characterization of the serum lipoprotein & their apo proteins in hype cholestrolemic guinea pigs. Biochem. J. 167: p. 921.

Chi MS, Koh ET, Stewart TJ (1982). Effect of garlic on lipid metabolism in rats fed cholesterol or lard. J. Nutr. 112(2): 241-248.

Dole VP (1956). A relation between non eterified fatty acid in plasma and metabolism of glucose. J. Clin. Investigations, 35: 150-154.

Elkayam A, Mirelman D, Peleg E, Wilchek M, Miron T, Rabinkov A, Oron - Herman M, Rosenthal T (2003). The effect of Allicine on weight in Fructose- Induced Hyperinsulinemic, Hyprlipidemic, Hypertensive Rats. AJH; 16: 1053-1056.

Golay A, Bobbioni E (1997). The role of dietary fat in obesity. Int. J. Obes. Relat. Metab. Disord. Jun; 21 Suppl 3: S2-11.

Gottfried R, Rosenberg B (1973). Improved manual spectophotometric procedure for determination of serum triglyceride. Clinical chemistry, principles and procedures. Annino JS, Giese RW 1967. Little Brown and Company. Boston Press.

Kamanna VS, Chandrasekhara N (1982). Effect of garlic (*Alliums sativ* Linn) on serum lipoproteins and lipoprotein cholesterol levels in albino rats rendered hyper cholesterolemic by feeding cholesterol. Lipids, 17: 483- 488.

Marta T, Khaled K, Al-Qattan TB, Muslim A (2006). Induction garlic in the diet may help lower blood glucose cholesterol and triglycerides. J. Nutr. 136: 800S-802S.

Neri Bruce P, Fringes Christopher S (1973). Improved method for determination of triglyceride in serum. Clin. Chem. 19: 1201-1202.

Pond MC, Mattasks AC (1983). The effect of food restriction and exercise on site -specific difference in adyposytes volume adipose tissue cellularity in the guinea pig. Br. J. Nutr. 51: 416-424.

Qureshi AA, Din ZZ, Abuirmeileh N, Ahmad WCY, Elson CE (1983). Suppression of avian hepatic lipid metabolism by solvent extract of garlic: Impact on serum lipid. Journal of nutrition .113(9): 1746- 1755.

Rivlin RS, Budoff M, Amagase H (2006). Significance of garlic and its constituents in cancer and cardiovascular disease. J. Nutr. 136: 713S-715S.

Rodbell M (1964). Metabolism of isolated fat cells , effect of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 239: 375-380.

Smith U, Sjostrom L (1972). Comparison of two methods for determining human adipose cell size. J. Lipid Res. 13: 822-824.

Yanovski SZ, Yaovski JA (2002). Obesity. N. Engl. J. Med. 346: 591-602.

Yeh Y, Liu L (2001). Cholesterol lowering effect of garlic extacts & organosulfur compounds: Human and animal studies. J. Nutr. 131: 989-993.