

Original Article

THE EFFECT OF FRESH GARLIC ON THE LIPID PROFILE AND ATHEROSCLEROSIS DEVELOPMENT IN MALE RATS FED WITH A HIGH GHEE DIET

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ABSTRACT

Objective: Garlic has a protective effect against hyperlipidemia and ghee in diet is considered as a risk factor for hyperlipidemia. We examined the effects of fresh garlic administration on body weight, lipid profiles and plaque formation in the male rats fed with a high ghee diet.

Methods: Twenty-four male Wistar rats were randomly divided into 3 groups of 8. The control group consumed an ordinary diet, Group 2 received the 31% ghee pellets (high ghee diet) and group 3 received the 8% garlic+31% ghee pellets. After 9 mo, serum levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and atherosclerotic index (AI) were examined. Changes in body weight, daily food intake and plaque formation also were assayed in this survey.

Results: The high ghee diet induced the significant increase ($P<0.05$) in serum concentrations of TC, TG and HDL-C, and a decrease in LDL-C concentration (group 2). While fresh garlic increased TG ($P<0.05$) and HDL-C levels and declined TC and LDL-C in treated rats (group 3). Meanwhile, ghee and garlic showed a favorable effect in reducing AI ($P<0.05$). No histopathological changes were observed in the Aorta of the rats.

Conclusion: Evidence obtained from this study indicates that ghee and fresh garlic has potential effects in the prevention and control of hyperlipidemia complications and are beneficial when taken as a dietary supplement.

Keywords: Ghee, Fresh Garlic, Lipid profile, Rat.

INTRODUCTION

The prevalence of hyperlipidemia and its complications are on a rise worldwide. Hyperlipidemia is a heterogeneous group of disorders characterized by an excess of lipids in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides. Alterations in serum lipids and lipoprotein levels result in a variety of chronic diseases such as cardiovascular diseases (CVD) and atherosclerosis. CVD is now considered the most common cause of death worldwide [1]. Atherosclerosis is characterized by accumulation of lipids and fibrous elements in the large arteries. It is the narrowing or occlusion of the arteries by plaque, which consists of cholesterol, platelets and other substances [2].

Dietary factors mainly the type of fat consumption play a key role in the development of various human diseases like CVD and atherosclerosis. The amount of saturated fatty acid (SFA) in the diet can modify the lipid profile and lead to hyperlipidemia, which is directly related to the growth of atheroma plaque.

Hyperlipidemia can be reversible in many cases through healthy eating and reducing the consumption of SFA and cholesterol [3]. The strong focus on SFA as a risk factor for CVD originated in the 1960s and 70s from lines of evidence including ecologic studies across nations, short-term metabolic trials in generally healthy adults assessing total cholesterol (TC) and LDL-C, and animal experiments that together provided consistent support that SFA intake increased the risk of CVD [4]. Ghee, known as clarified butter or anhydrous milk fat, contains 47.8% saturated fat [5]. Ghee is usually called "yellow oil" or "Kermanshahi oil" in Iran, and used in the diet of Iranian people. It is prepared by heating butter or cream to just over 100 °C to remove water content by boiling and evaporation, then filtering out the precipitated milk solids. There has been concern about the possibility of ghee contributing to an increased risk of CVD and atherosclerosis, since it contains a high percentage of SFA and cholesterol [6].

Therefore, in the current survey ghee was used for preparing the hyper lipidemic rats. There is an increasing demand by patients for the use of natural products and other dietary modulators with hypo lipidemic activity. This is due to the fact that the wide array of synthetic drugs which is employed to control hyperlipidemia has undesirable side effects. Recent studies have directed their efforts toward the protective effects of plants such as garlic on hyperlipidemia [7].

The medicinal use of garlic (*Allium sativum*) in human health has been known for thousands of years and now, it has attracted particular attention of modern medicine because of its widespread health use around the world. The medicinal value of garlic is best known for its lipid lowering and antiatherogenic effects. During the last few decades, the hypolipidemic effect of garlic has been confirmed by many investigators although some of them did not confirm this effect.

The composition and quantity of the sulfur components in different garlic preparations, the subject recruitment, and duration of experiment, dietary control, lifestyle and methods of lipid analyses could account in part for the inconsistent findings [8-10]. The garlic antiatherogenic effects decreased lipid plaques in the arteries of hyperlipidemic animals and decreased accumulation of cholesterol in the walls of their blood vessels. These effects were attributed to the presence of sulfur compounds in the garlic [11]. Garlic has many organo sulfur compounds which may work synergistically whereas a single component of the garlic may not possess these properties [12], so fresh garlic was used for this study. There are not many studies about the effect of dietary fresh garlic on lipid profile in the serum of the male rats fed with a high ghee diet.

The present study was undertaken to survey the effect of fresh garlic on serum lipids as well as progression of aortic lesions and body weight in ghee-fed rats as a hyperlipidemic model.

MATERIALS AND METHODS

Preparation of ghee supplementation

Ghee (anhydrous milk fat) was purchased from Kermanshah (Iran), where it was prepared by heating butter at high temperatures. The 31% Ghee pellets were made by mixing 31 g of Ghee with 69 g of powdered standard rat pellet diet.

Preparation of fresh garlic supplementation

Cloves of fresh garlic were purchased from the local supermarket in Hamadan. The fresh garlic was identified by Botany department of Bouali University (Hamadan-Iran) as a local variety. The peeled garlic cloves were crushed mechanically in a mixer for 15 min. Then, the 8% garlic pellets were made by mixing 8 g of fresh crushed garlic with 31 g of Ghee and 61 g of powdered standard rat pellet diet and were stored at -20 °C until use.

Experimental animals and design

This experimental study was performed on 24 male Wistar rats, weighed in the beginning of the experiment 198±15 g (three months old). All experiments on animals were performed following the standards of European Community Council Directive of November 24, 1986 (86/609/EEC). The study protocol was revised and approved (3/2012) by the Ethical Committee of the Department of Physiology, Medical Faculty, Hamadan University of Medical Sciences, Iran.

The animals were housed individually in conventional wire mesh cages in a room temperature regulated at 21±1 °C, humidity 45-50 %, and light/dark cycles (12h). Environmental conditions such as humidity, heat, light, and ventilation were kept constantly for 24 h daily during the period of the study. The animals were kept on rodent chow for a week after this washout period and were randomly divided into three groups of 8 as follow;

Group 1 (control) received a normal diet (66% carbohydrates, 25% protein, and 9% fat).

Group 2 (high ghee diet) received the 31% Ghee pellets.

Group 3 (high ghee diet+fresh garlic) received the 8% fresh garlic+31% Ghee pellets.

In group 2 and 3, ghee and garlic were mixed with normal diet and produce the pellets.

All rats were allowed free access to the test diets and deionized water throughout the test period. After 9 mo of feeding, fasting rats were sacrificed under anesthesia by 40 mg/kg i. p. sodium pentobarbital. The blood was collected by cardiac puncture and allowed to clot, and the clotted blood was then centrifuged at 2200 g for 15 min. The serum was separated and stored at -80 °C until analysis.

Body weight and food intake

The body weight was recorded weekly and food intake daily. The weight gain of the rats was recorded on a weekly basis, the initial and final weight of rats was also recorded.

Measurement of biochemical factors

Serum total cholesterol (TC) and triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) concentrations were determined using commercial kits (Pars Azmun kit, Iran) according to the instructions

of the manufacturer by using an auto-analyzer (Hitachi 902 set, Japan). This measurement was done by someone who was blind to groups.

The Atherogenic Index (AI) was calculated as LDL-C/HDL-C.

$$\text{Atherogenic Index (AI)} = \frac{\text{LDL-C}}{\text{HDL-C}}$$

Tissue collection and histological analysis of aortic atherosclerotic lesions

The aorta was also removed at the time of sacrifice for evaluating the formation of plaque. After dissecting the bodies, specimen from aorta were quickly removed, weighed, and prepared for accurate detection and estimation of lipid deposits in the intima. The specimens were fixed with 100 ml of 10% formaldehyde solution made in 0.1M phosphate buffer saline (PBS), pH 7.33 at 4 °C, embedded in paraffin and stained with H and E (hematoxylin and eosin) according to Bancroft [10]. All sections of 5 µm were cut with a microtome and were coded and analyzed blindly by the pathologist without knowledge of related characteristics or diet. The prepared slides were observed using a light microscope and scoring was done based on a scale from zero to four. Zero shows no histopathological results, 1 shows slight severity, 2 mild, 3 moderate and 4 severe degrees of histopathological results.

Statistical analysis

Each experiment was performed in triplicate and repeated at least twice. To verify the statistical significance of the studied parameters, means of three time's analyzed samples were defined. When appropriate, differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) and followed by Tukey as post hoc test. The data were expressed as means±standard error of the means (S. E. M.). Differences were considered significant when P-values were <0.05. The data were analyzed using SPSS software version 14.

RESULTS

Changes in body weight and food intake

The baseline body weight (mean±SEM) increased from 195±2.23 g to 380±3.01 g (Group 1), from 196.8±0.44 g to 405.8±2.42 g (Group 2), and from 199.6±1.67 g to 361.6±3.98 g (Group 3). These elevations were not statistically significant. However, 31% dietary ghee yielded more elevation in final body weight compared with the two other groups (table 1).

The diets were well tolerated and all animals completed the study. During the study period, the relative mean±SEM of food intake was 14.5±0.8 g/d, 13±1.1 g/d and 16.5±1.2 g/d, in the Groups 1, 2 and 3, respectively. There was no significant difference in the mean food intake among the three study groups. The food intake in rats receiving ghee diets was significantly lower compared with the control group (table 1).

Biochemical analysis of the serum

Changes in total cholesterol (TC)

The effect of dietary treatments on serum TC is shown in fig. 1 for all diet groups. Animals on 31% ghee diet showed a significantly greater elevation in the level of serum TC than other study groups (P<.05). Moreover, addition of 8% dietary garlic to the high ghee diet (Group 3) produced a mild reduction in the serum TC compared with the control group.

Table 1: Initial and final body weight of the rats, and food intake (9 mo treatment)

Groups (n=8)	Initial Body weight (g) (mean±SEM)	Final Body weight (g) (mean±SEM)	Food intake (g/d) (mean±SEM)
Group1 (Control)	195±2.23	380±3.01 *	14.5±0.8
Group2 (31%Ghee)	196.8±0.44	405.8±2.42 ©	13±1.1
Group3 (31%Ghee+8% fresh Garlic)	199.6±1.67	361.6±3.98 #	16.5±1.2

There was not significant differences in initial and final body weight in each groups.* p=0.06, © p=0.07, # p=0.09

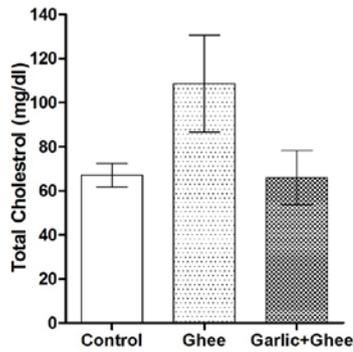


Fig. 1: The effect of garlic on serum TC in ghee-fed rats and control group (9 mo treatment). Values are presented as mean±SD for eight rats per group

Changes in LDL cholesterol

Current findings indicated that ghee individually, and ghee in combination with garlic reduced LDL-C levels in the Group 2 and 3, respectively in comparison to control (fig. 2). Apparently, ghee diet combined with fresh garlic, resulted in more reduction in LDL-C level.

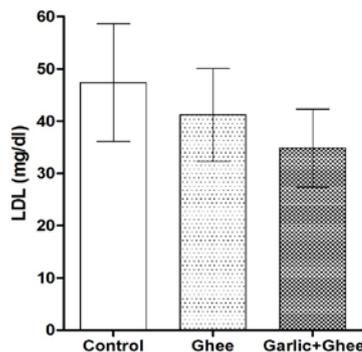


Fig. 2: The effect of garlic on serum level of LDL-C in ghee-fed rats and control group (9 mo treatment). Values are presented as mean±SEM for eight rats per group

Changes in HDL cholesterol

As shown in fig. 3, ghee diets individually and ghee in combination with garlic increased HDL-C level in Group 2 and 3, respectively. This rise in group 2 was significant in comparison with other groups ($p < 0.05$).

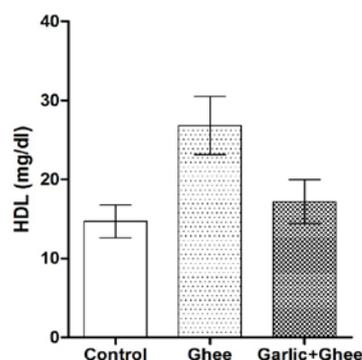


Fig. 3: The effect of garlic on serum level of HDL-C in ghee-fed rats and the control group (9 mo treatment). Values are presented as mean±SD for eight rats per group

Changes in triglycerides (TG)

The serum level of TG in the ghee-fed rats (Group 2 and Group 3) for a period of 9 mo significantly increased, as compared to the control

group ($p < 0.05$) (fig. 4). Similarly, the highest elevation in the TG level was observed in group 2, the differences were significant when compared with Group 3 ($P < 0.05$).

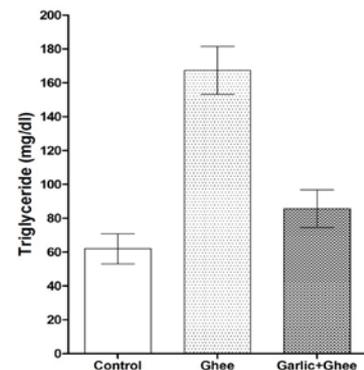


Fig. 4: The effect of garlic on serum TG in ghee-fed rats and the control group (9 mo treatment). Values are presented as mean±SEM for eight rats per groups

Changes in LDL-C/HDL-C ratios (Atherogenic index)

A significant reduction was observed in LDL/HDL ratio (AI) in the Group 2 and 3 compared with Group 1 ($P < 0.05$). However, there was no statistically significant difference between the Group 2 and 3 (fig. 5).

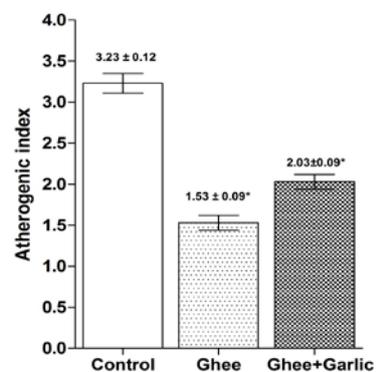


Fig. 5: The effect of garlic on LDL-C/HDL-C ratios (Atherogenic index) in ghee-fed rats and control group (9 mo treatment). Values are presented as mean±SEM for eight rats per groups. Control-received a normal diet, High ghee diet-received the 31% Ghee pellets, and High ghee diet+garlic-received the 31% Ghee+8% garlic pellets. * Significant differences versus Group 1 ($P < 0.05$)

Histological results

No histopathological change was detected in the aorta of the study animals (0 Grade). On the other hand, the Groups 2 and 3 did not demonstrate substantially developed lesions, enlarged intimal and total areas than those from the control group (fig. 6).

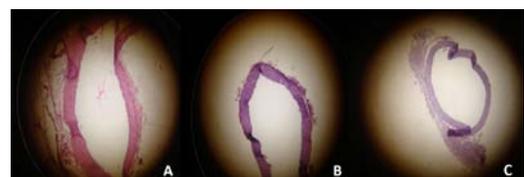


Fig. 6: Micrographs of cross-sections of the aorta showing no histopathological changes (H and E X 200) (0 Grade). (A) Group 1 (control) received normal diet, (B) Group 2 (high ghee diet) received the 31% Ghee pellets, and (C) Group 3 (high ghee diet+garlic) received the 31% Ghee+8% garlic pellets

DISCUSSION

The approaches to the prevention and treatment of atherosclerotic diseases are primarily based on the reduction of risk factors or rather modifiable factor such as hyperlipidemia. In the present survey ghee was used for inducing hyperlipidemia in male rats, because there is concern about the possibility of ghee contributing to an increased risk of hyperlipidemia as it contains a high percentage of SFA and cholesterol. The idea of harmfulness of ghee originated in the 1960s and 70s from lines of some evidences [4].

Current study does not support a conclusion of harmful effects of the moderate consumption of ghee in the rats. Our present study on male rats indicates that consumption of 31% ghee may increase triglyceride levels, but does not increase LDL-C that is linked to a higher risk of cardiovascular disease. Ghee also increased significantly the HDL-C level that negatively correlated with CVD. In consistent with our result, Spitteller [13] pointed out that atherosclerosis was not induced by the consumption of fats containing saturated fatty acids. Similar findings in a human study by Shankar, Yadav [14] demonstrated significant increase of TC, HDL-C with ghee consumption. In a clinical trial by Mohammadifard, Nazem [15] ghee did not significantly change TC, TG, LDL-C, and HDL-C levels.

Given these conflicting directions of effects, a more global risk marker such as the LDL-C: HDL-C ratio (AI) may provide the best overall indication of potential effects on CVD risk. The AI, defined as the ratio of LDL-C and HDL-C, is believed to be an important risk factor of CVD and atherosclerosis. It has been shown that abnormally high serum levels of LDL-C and low serum levels of HDL-C are associated with an increased risk for atherosclerosis [16]. Increase in the HDL-C concentrations and decrease in the LDL-C concentrations in ghee-fed rats indicates the anti atherogenic property of ghee. So, Ghee intake has the significant reducing effect on the AI in the present study. However, the evidence from randomized controlled trials (RCTs) of lipid and non-lipid risk factors, demonstrated that AI is non-significantly affected by consumption of SFA [4]. These studies provide evidence that dietary ghee in combination with fresh garlic does not have adverse effect on serum lipids profile in rats. Ravnskov (1998) found a positive ecological correlation between national intakes of SFA and cardiovascular mortality. Kumar and colleagues [17] have shown that the consumption of up to 10% ghee in the diet had a positive effect on serum lipid profiles in Wistar rats (an outbred strain).

The slightly hypo lipidemic effect of ghee may be mediated through the inhibition of cholesterol biosynthesis which enhances the fecal excretion of sterols and bile acids. Dietary ghee significantly increased the excretion of bile constituents and lowered serum cholesterol levels, how much ever ghee contains SFA and cholesterol. Bile is an important mode of transport for the excretion of cholesterol and its metabolites [18]. So ghee exhibited positive effects on HLD-C and LDL-C by enhancing the secretion of biliary constituents. Also, ghee contains conjugated linoleic acid which has been shown to decrease serum LDL-C and atherogenesis. Ghee contains antioxidants, including vitamin E, vitamin A, and carotenoids which may be helpful in preventing hyperlipidemia [18].

It is widely believed that the antioxidant micronutrients obtained from fruits and vegetables afford significant protection against diseases. Many research studies have been published, which report beneficial properties of herbal mixtures containing ghee [17, 19].

In the present study, the combination fresh garlic and ghee significantly reduced the serum triglycerides level and AI, increased significantly HDL-C, and also mildly lowered cholesterol and LDL-C levels. Since the concentration of LDL-C was not significantly changed, the cholesterol-lowering effect was in fact exclusively attributed to the elevation of HDL-C, making the most characteristic changes involved in cholesterol modulation by fresh garlic. Results here demonstrated that the daily administration of garlic to ghee-fed rats had a beneficial effect on the lipid profile.

Several studies have suggested that garlic may have beneficial effects on plasma cholesterol levels, while other studies did not find the same. The composition and quantity of sulfur components of

different protocol designs of garlic preparations and the different rat models and inappropriate methods of randomization, lack of dietary run in period, short duration, and inadequate statistical power could account in part for the inconsistent findings [8-10, 20]. The mechanism by which garlic or garlic preparations reduce plasma lipids has not been fully investigated. The proposed mechanisms for lipid-lowering include inhibiting enzymes involved in cholesterol synthesis and deactivation of HMG-CoA reductase and also reducing the hepatic activities of lipogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase [21]. It is, therefore, reasonable that the hypolipidemic effect of garlic may stem in part from impaired cholesterol synthesis. Garlic can also contain high levels of tellurium and selenium compounds, which contribute to the overall block in cholesterol synthesis by inhibiting squalene monooxygenase. Squalene monooxygenase plays an important role in the overall regulation of cholesterol biosynthesis [22].

Large population-based observational studies found that garlic fibers are associated with a reduced CVD risk. Soluble fibers in garlic are thought to bind bile acids during the intra luminal formation of micelles. This leads to increased bile acid synthesis, reduction in hepatic cholesterol content, up-regulation of LDL-C receptors, and increased LDL-C clearance. Other potential mechanisms include increased intra luminal viscosity, decreased cholesterol absorption and entrapment of cholesterol in the small intestine [23]. Most studies found that high fat diet results in increased body weight and food intake. Garlic could have interfered with satiety and influenced food choices, resulting in a reduction in dietary food intake and final body weight [24]. In contrary, we found that consumption of high ghee diet/garlic showed no significant changes in the total body weight and food intake during the study (table 1).

Aorta results indicate that ghee showed no histopathological changes. These results indicate that garlic diets have no favorable effect on aorta histology. The same results were obtained at low levels of garlic supplementation [25]. But previous study [26] investigated the effect of allicin as an active component of garlic on the formation of fatty streaks in an aorta in mice and illustrated the powerful effect of fresh garlic on arteries which is in contrast with our findings.

Therefore further research should be carried out to identify specific compounds from garlic or garlic products that are responsible for most of its biological effects and how dietary garlic might interact with ghee to influence lipid profile in serum.

CONCLUSION

In the last several decades, ghee has been implicated in the increasing prevalence of CVD but this study demonstrated that ghee supplementation have beneficial effects on some parameters of lipid profile in rats fed with high ghee diet. In addition, fresh garlic with ghee is more effective in controlling hyperlipidemia and could potentially play an important role in preventing atherosclerosis.

However, more extensive research is needed before recommending ghee and garlic supplementation to humans. Also, further studies are needed to elucidate the underlying mechanisms.

Compliance with Ethical Standards

Funding: This study was supported by Hamadan University of Medical Sciences, Grant number H2/458/6.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All experiments on animals were performed following the standards of European Community Council Directive of November 24, 1986 (86/609/EEC). The study protocol was revised and approved (3/2012) by the Ethical Committee of the Department of Physiology, Medical Faculty, Hamadan University of Medical Sciences, Iran.

CONFLICT OF INTERESTS

The authors of this manuscript have no invested interests in products described or used in this article. The authors have no conflicts of interest.

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