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Hypocholesteremic and Antioxidant Effects of Garlic (*Allium sativum L.*) Extract in Rats Fed High Cholesterol Diet

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Abstract: The present study is designed to evaluate the effect of garlic extract on lipid profiles and oxidative stress in male albino rats fed a high cholesterol diet (HCD). A group of 24 male albino rats each weighing 125±5.0g, was divided into four groups. Group I was used as negative control and fed on standard diet and orally administered 1 ml distilled water. Group II was used as positive control and fed on high cholesterol diet and orally administered 1ml distilled water. Groups III and IV were fed on high cholesterol diet and orally administered garlic extract (0.2 and 0.4g/kg body weight/day, respectively). Garlic extract significantly increased ($p < 0.05$) plasma HDL-Cholesterol and decreased plasma TC, LDL-Cholesterol and TG as well as liver TC and TG as compared with positive control (group II). No significant difference was observed in plasma LDL-Cholesterol, HDL-Cholesterol as well as plasma and liver TG between the rats ingested with high or low dose of garlic extracts. However, there was a significant ($p < 0.05$) decrease in plasma and liver TC in rats ingested with a high dose of garlic extract (Group IV) as compared to low dose ingestion. Garlic extract significantly increased ($p < 0.05$) total antioxidant capacity, SOD and GSH-Px activities as compared to negative or positive control (group I and group II, respectively). No significant difference was observed in total antioxidant capacity, SOD and GSH-Px activities between the rats ingested with high or low dose of garlic extract. There was a significant decrease ($p < 0.05$) in plasma malondialdehyde in rats ingested with a high or low dose of garlic extract as compared to negative or positive control rats.

Key words: Garlic, lipid profile, total antioxidant capacity, malondialde

Introduction

Garlic (*Allium sativum L.*) is used as a spice and medicinal herb. Most recent research on garlic has used garlic in the form of tablets, flesh, raw, boiled, cooked and dried (Gorinstein *et al*., 2006). Commercially available garlic preparations 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 (Elkayam *et al*., 2003). Garlic exhibits a wide range of properties including immunomodulatory hepatoprotective, antimutagenic and anticarcinogenic effects (Horie *et al*., 1989; Agarwal, 1996). Garlic and garlic extracts are believed to possess beneficial effects for the prevention of cardiovascular diseases (Koscielny *et al*., 1999; Rahman, 2001; Steiner and Li, 2001). Garlic modulates lipid metabolism (Rassoul *et al*., 1992; Richter *et al*., 1992; Gebhardt, 1993; Walper *et al*.,1994; Yeh and Yeh, 1994; Steiner *et al*., 1996; Steiner and Lei, 2001; Slowing *et al*., 2001). Several studies have also shown that garlic contains active hypocholesterolemic and hypoglycemic components, known as diallyl disulfide and dipropyl disulfide (Bordia and Bansel, 1973; Jain and Vyas, 1975; Bordia *et al*., 1975; Jain, 1977). It has also been reported that garlic supplements in human subjects lead to the increased resistance of low density lipoprotein to oxidation and may be one of the powerful mechanisms accounting for the antioxidative and anti-atherosclerotic properties of garlic (Munday *et al*., 1999; Borek, 2001; Lau, 2001).

With regard to the antioxidative activity of garlic, Rahman *et al*. (2001) have demonstrated that components of aged garlic extract inhibit the in vivo oxidation of LDL by chelating Cu^{+2}, scavenging superoxide ions, thus inhibiting the oxidation of protein and lipid moiety of human LDL-Cholesterol (Dillon *et al*., 2003). As no exact molecular mechanisms on the protective function of garlic in the atheroselerosis have yet been clarified in detail, further studies need to be performed to establish the relation between garlic extract consumption, blood lipid profile and antioxidant status.

The purpose of the present study is to experimentally assess the hypocholesterolemic and antioxidative effects of garlic extract used in rats fed a high cholesterol diet.

Materials and Methods

Preparation of garlic extract: Fresh garlic bulbs (*Allium sativum L.*) were purchased from the local market in Riyadh, Saudi Arabia. Dried and ground bulbs (about 100g) were submitted to extraction with 300ml ethanol (80%) in a Soxhlet apparatus for 72 hours. After extraction, the solvent was filtered and made to evaporate by Rotavapor. The obtained garlic alcoholic

extract was stored at -20°C until use. Garlic extract was suspended in distilled water to give 0.4g garlic extract per one ml of the suspension and administered orally through orogastric tube. The volume of administrated extract was 1ml for each animal.

Animals and treatments: Male albino Wister rats weighing 115-125g were housed in clean cages at a 20-24°C temperature, 12-h light/12-h dark cycle and relative humidity (52% in the animal house at College of Medicine, King Saud University, Riyadh-Saudi Arabia). Rats were given free access to water and the standard diets that were prepared according to the American Institute of Nutrition (Reeves *et al*., 1993). Rats were allowed to acclimatize to the new environmental condition and received a standard diet for five days before the sixty-day experiment period. Twenty four rats were divided into 4 groups, each consisting of 6 animals. Group I: Negative control fed with a standard diet and orally administered 1ml distilled water. Group II: Positive control fed with a high cholesterol diet [standard diet+cholesterol (1% of diet weight)] and orally administered 1ml distilled water. Group III and IV: Fed with a high cholesterol diet [standard diet+cholesterol (1% of diet weight)] and orally administered garlic extract (0.2 and 0.4g/kg body weight, respectively) daily.

Analytical methods: On the 60th day, rats were fasted overnight and were anesthetized with light ether. A blood sample was collected in heparin-coated tubes, part of which was used for GSH-Px determination using Randox Assay Kits according to the methods of Poglia and Alenline (1970). Briefly, GSH-Px was catalyzed by the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The generation of nicotinamide adenine dinucleotide phosphate was then measured. The remaining blood sample was centrifuged at 2000g for fifteen minutes at 4°C. Erythrocytes were washed by saline and used for SOD determination, using Randox Assay Kits as described (Wolliams *et al*., 1983), which were based on the production of O_2^- anions by the xanthine/xanthine oxidase system. Plasma was analyzed for total antioxidant capacity, malondialdhehyde and lipid profile. Plasma concentrations of total antioxidant capacity were determined using the Total Antioxidant Status Assay Kits. According to the method of Miller *et al*. (1993), this assay relies on the ability of antioxidants in the sample to inhibit oxidation of 2, 2'-Azino-di- [3-ethylbenzthiazoline sulphonate] (ABTS) to ABT^+ by metmyoglobin. The amount of $ABTS^+$ produced can be monitored by reading absorbance at 600nm.

Plasma concentrations of malondialdehyde were determined by using the malondialdehyde Assay Kits according to the method of Ohkawa *et al*. (1979). Plasma concentrations of total cholesterol, HDL-Cholesterol and triglycerides determinations were performed by standard procedures with the Cobas Integra analyzer (Roche Diagnostic Systems, Switzerland) (Allain *et al*., 1974; Fossati and Prencipe, 1982; Hino *et al*., 1996). However, plasma concentration of LDL-Cholesterol was calculated with the Friedewald formula (Friedewald *et al*., 1972).

The liver was separated and cleaned from surrounding tissues, with blood removed by rinsing it in normal saline, then weighted and analyzed for liver concentration of total cholesterol and triglycerides. Liver lipids were extracted according to the method of Folch and Sloane (1975) while total cholesterol and triglycerides were determined as described previously by Heid *et al*. (1996).

Statistical analysis: Data analysis was performed using the *Statistical Package for the Social Science*s software (SPSS, version 11.0). Descriptive statistics were adopted to display data in means±SD. The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among the different groups. Differences were considered significant whenever the p-value is $P < 0.05$.

Results

Table 1 shows initial and final body weight, body weight gain and liver weight in rats fed a high cholesterol diet. Body weight gain was not significantly different between all the groups. However, weight gain was slightly higher in rats fed a high cholesterol diet (group II) as compared to other groups. Liver weight was significantly higher ($p < 0.05$) in rats fed a high cholesterol diet as compared to other groups.

Table 2 shows the effect of garlic extract on plasma and liver lipids profiles in rats fed a high cholesterol diet. There was a significant decrease ($P < 0.05$) in plasma HDL-Cholesterol in rats fed a high cholesterol diet (group II) as compared to group 1 (Normal). There was a significant rise ($p < 0.05$) in plasma total cholesterol, LDL-Cholesterol and triglyceride as well as liver total cholesterol and triglyceride in group II as compared to group 1 (Normal).

Garlic extracts significantly increased ($p < 0.05$) plasma HDL-Cholesterol and decreased plasma total cholesterol, LDL-Cholesterol and triglyceride as well as liver total cholesterol and triglyceride in group III and group IV as compared to rats fed a high cholesterol diet (group II).

No significant difference was observed in plasma LDL-Cholesterol, HDL-Cholesterol as well as plasma and liver triglyceride between the rats ingested a high or low dose of garlic extract (group III and group IV, respectively). However, there was a significant ($p < 0.05$) decrease in plasma and liver total cholesterol in the rats ingested a high dose of garlic extract (group IV) as compared to low dose ingestion (group III).

Table 1: Initial and final body weight, body weight gain and liver weight in rats fed high cholesterol diet*

Parameter	Group I (control)	Group II (HCD)††	Group III (HCD+0.2g garlic extract/ kg body wt.)	Group IV (HCD+0.4g garlic extract/ kg body wt.)
Initial body weight (g)	120.32±2.64	120.37±3.92	120.47±4.10	120.49±4.20
Final body weight (g)	373.52±4.84	384.37±5.63	372.47±6.10	372.49±6.10
Body weight gain (g/d)	4.22±2.20[a]	4.40±2.21[a]	4.20±2.02[a]	4.20±2.02[a]
Liver weight (g/100g)	3.40±0.24[a]	4.09±0.25[c]	3.44±0.24[a]	3.43±0.25[a]

*Values are expressed as mean±SD for six animals. †Body weight gain (g/d) = [Final body weight (g) - Initial body weight (g)] ÷ 60.
††High cholesterol diet (HCD). [abc]Different letters in a given row denote significant difference, $p < 0.05$.

Table 2: Effect of garlic extract on plasma and liver lipids profile in rats fed high cholesterol diet*

Parameter	Group I (control)	Group II (HCD)	Group III (HCD + 0.2 g garlic extract/ kg body wt.)	Group IV (HCD+0.4g garlic extract/ kg body wt.)
Plasma				
Total cholesterol (mg/dl)	70.21±3.7[a]	100.41±1.7[b]	65.50±2.33[c]	60.80±1.51[d]
LDL-Cholesterol(mg/dl)	53.22±1.62[a]	71.30±1.81[b]	45.61±3.21[c]	44.42±1.22[c]
HDL-Cholesterol (mg/dl)	16.81±1.10[a]	15.9±0.99[b]	17.1±1.01[a]	17.9±1.02[a]
Triglycerides (mg/dl)	45.60±1.10[a]	49.80±0.99[b]	33.41±1.13[c]	32.83±1.11[c]
Liver				
Total cholesterol (mg/g)	17.50±0.86[a]	32.01±0.99[b]	16.45±0.79[a]	15.50±0.91[c]
Triglycerides (mg/g)	33.94±2.11[a]	37.58±2.10[b]	27.81±2.34[c]	27.21±2.22[c]

*Values are expressed as mean±SD for six animals. †High cholesterol diet (HCD).
[abc]Different letters in a given row denote significant difference, $p < 0.05$.

Table 3 shows the effect of garlic extract on plasma antioxidant capacity and malondialdehyde, erythrocyte superoxide dismutase and blood glutathione peroxidase activities in rats fed a high cholesterol diet. There were significant decreases ($p < 0.05$) in total antioxidant capacity, SOD and GSH-Px in rats fed a high cholesterol diet (group II) as compared to normal rats (group I).

Garlic extracts significantly increased ($p < 0.05$) both total antioxidant capacity and SOD and GSH. Px in group III and group IV as compared to normal rats and rats fed a high cholesterol diet (group I and group II, respectively). No significant difference was observed in total antioxidant capacity, SOD and GSH-Px between the rats ingested a high or low dose of garlic extract (group III and group IV, respectively).

There was a significant increase ($p < 0.05$) in malondialdehyde among rats fed a high cholesterol diet (group II) as compared to normal rats (group I). Garlic extracts significantly decreased ($p < 0.05$) plasma malondialdehyde in group III and group IV as compared to group I and group II.

Discussion

Natural remedies have been investigated for centuries for a wide variety of ailments. Garlic has received special attention for its beneficial effects (Auer *et al.*, 1990; Santos and Grunwals, 1993), but until recently there has been little scientific support for its therapeutic and pharmacological properties. There is no satisfactory data from randomized controlled tests linking supplementation of garlic in diet with a reduction in cardiovascular morbidity and mortality (Silagy and Nail, 1994), though significant reductions in blood cholesterol and triglyceride levels were observed in some studies when garlic extract or powder were used. No satisfactory agreement has been reached on this kind of clinical and experimental data, since many of the tests have been limited by significant methodological shortcomings including inappropriate methods of randomization, poorly characterized subject groups, short duration and limited statistical methods (Silagy and Nail, 1994). Thus, the current study is conducted to assess the hypocholesterolamic and antioxidant effect of garlic extracts on rats fed a high cholesterol diet.

In this experiment, high cholesterol diets fed to rats (group II) have significantly increased their plasma TC, TG, LDL-Cholesterol as well as their liver TC and TG and have significantly decreased plasma HDL-Cholesterol. These results are consistent with Aouadi *et al.* (2000), who attributed these changes to endogenous synthesis.

Oral administration of 0.2 or 0.4 g/kg body weight/day of garlic extracts in rats fed high cholesterol diets (groups III and IV, respectively), has caused a significant reduction in plasma TC, TG, LDL-Cholesterol as well as liver TC and TG and significantly increased plasma HDL-Cholesterol. These effects have been more pronounced with the high dose of garlic extract. The results of this study confirm the earlier hypolipidemic effects reported for garlic (Bordia *et al.*, 1975; Augusti, 1977; Sodimu *et al.*, 1984; Banerjee *et al.*, 2002; Ou *et al.*, 2003; Thomson *et al.*, 2006); a similar

Table 3: Effect of garlic extract on plasma antioxidant capacity, malondialdehyde, erythrocyte superoxide dismutase and blood glutathione peroxidase activities in rats fed high cholesterol diet*

Parameter	Group I (control)	Group II (HCD)	Group III (HCD+0.2g garlic extract/ kg body wt.)	Group IV (HCD+0.4g garlic extract/ kg body wt.)
Antioxidant capacity (mM)	1.13±0.25[a]	0.99±0.17[b]	1.26 ±0.29[c]	1.27±0.31[c]
Malondialdhyde (nmol/mL)	1.44± 0.20[a]	2.65±0.27[b]	1.51± 0.23[c]	1.49± 0.28[c]
Superoxide dismutase (U/mL)	98.20±5.21[a]	81.30 ±4.10[b]	119.40±4.71[c]	120.31± 4.22[c]
Glutathione peroxidase (U/mL)	19.21±1.11[a]	17.9±0.89[b]	22.31± 2.10[c]	22.98±1.99[c]

*Values are expressed as mean±SD for six animals. †High cholesterol diet (HCD).
[abc]Different letters in a given row denote significant difference, $p < 0.05$.

hypocholesterolemic effect of garlic was observed previously in rats fed high-cholesterol diet in the presence of garlic oil (Gofman *et al*., 1996).

In the same respect, Aouadi *et al*. (2000) suggested that one reason for the preventive effect of garlic against the atherosclerotic process may be the changes in the portions of lipoprotein cholesterol fractions. Moreover, previous studies have shown that ingestion of garlic appears to inhibit hepatic fatty acid synthesis (Gebhardt, 1991) by lowering key enzymes activities in supplying substrates, thus reducing lipid accumulation in the liver and TG level in plasma.

With respect to the cholesterol lowering property of garlic, it has been suggested that some constituents of garlic may act as inhibitors for some enzymes such as hydroxyl methyl glutaryl CoA reductase, which participates in cholesterol synthesis (Gebhardt and Beck, 1996; Durak *et al*., 2004).

As seen in the present study, garlic extract can lower the blood cholesterol level and improve blood lipid profile to a significant extent. It also increases the plasma antioxidant capacity and oxidation resistance by increasing antioxidant enzymes activities (SOD and GSH-Px) and decreasing the plasma malondialdehyde level, which is an important indicator of lipid peroxidation.

The data in Table 3 shows that feeding rats high cholesterol diets significantly decreased plasma antioxidant capacity and antioxidant enzyme activities and significantly increased plasma malondialdehyde.

Oral administration of garlic extract in rats fed a high cholesterol diet (groups III and IV) caused significant increases of total antioxidant capacity and antioxidant enzyme activities (SOD and GSH-Px), but decreased plasma malondialdehyde concentration.

The significantly lower concentration of malondialdehyde in rats fed a high cholesterol diet and orally administrated garlic extract (groups III and IV), suggests that the addition of garlic extract increased the antioxidant potential and antioxidant enzyme activities (SOD and GSH-Px) as a protective mechanism against oxidative stress (Arivazhagan *et al*., 2000).

Oral administration of garlic extract reversed the changes induced by feeding high cholesterol diet, supporting the hypothesis that plant products are effective chemopreventive agents (Arivazhagan *et al*., 1999a).

Garlic has been reported to modulate lipid peroxidation levels and enhance the status of antioxidant (Arivazhagan *et al*., 1999a; Arivazhagan *et al*., 1999b; Balascnthil *et al*., 1999).

Allium components have been reported to elevate the levels of SOD, GSH-Px and Catalase (Pinto *et al*., 1997; Wei and Lau, 1998). Other beneficial effects of garlic can be attributed to the presence of non-enzymatic antioxidants such as selenium and copper metals, vitamin C and other phytochemicals such as organo-sulphur compounds (Prasad *et al*., 1995).

In conclusion the result of the present study demonstrates that garlic extracts may have a beneficial effect on the blood lipid profile and antioxidant status by improving lipid and antioxidant metabolic indices in rats' plasma.

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