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Full Length Research Paper

Postprandial hypolipidemic and hypoglycemic effects of *Allium hertifolium* and *Sesamum indicum* on hypercholesterolemic rabbits

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Oxidative stress associated with postprandial hyperlipidemia contributes to endothelial dysfunction, which shifts hemostasis to a more thrombogenic state. The present study was undertaken to evaluate the effect of *Allium hertifolium* and *Sesamum indicum* on postprandial lipemic, glycemic profile and endothelial markers in hypercholesterolemic rabbits. A total of 32 male rabbits were randomized into 4 groups: Group 1: Control group (normal group); Group 2: Hypercholesterolemic diet (1% cholesterol); Group 3: 1% cholesterol administered with *A. hertifolium* extract (2% of diet); Group 4: 1% cholesterol administered with *S. indicum* powder (10% of diet). The serum was analyzed for lipid profile (total cholesterol (TC); triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), ApoB), glucose, nitrite, nitrate, serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels and plasma was analyzed for fibrinogen and factor VII. These factors were measured in blood samples following 15 h of fasting and 3 h after feeding. The results showed *A. hertifolium* and *S. indicum* to be effective in reducing SGPT, fibrinogen, total cholesterol (TC) and LDL-C values in comparison with hypercholesterolemic diet group. Consumption of *A. hertifolium* and *S. indicum* did not significantly change factor VII, ApoB and nitrite levels in comparison with hypercholesterolemic diet group. Intake of *S. indicum* significantly decreased serum SGOT, nitrate, glucose and TG compared to hypercholesterolemic diet group. *A. hertifolium* and *S. indicum* with a high content of phenolic compounds change the postprandial profile lipids, endothelial markers and thrombogenic factors and might be beneficial in patients with cardiovascular diseases.

Key words: *Allium hertifolium*, *Sesamum indicum*, postprandial, serum glutamic pyruvic transaminase (SGPT).

INTRODUCTION

In recent years, so many researches have confirmed and approved the correlation between the loads of postprandial elevated triglyceride and atherosclerosis.

There are so complicated and harmful reactions which happen in postprandial status. Postprandial triglyceride-rich lipoproteins are enhanced through smooth muscle cells and macrophages. Furthermore, they can be discovered as a part of the foam cells inside the vascular lesions and brain spots (Colin and Bruce, 2003; Jagla and Schrezenmeir, 2001). Postprandial oxidative stress (POS) which is a sub-form of nutritional oxidative stress

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is followed as a consequence of sustained elevation of postprandial hyperlipidemia (or hyperglycemia) and is correlated with a higher risks for excess overweight, hardening of arteries and diabetes mellitus etc. (Bowen and Borthakur, 2004). POS can be tapered when dietary antioxidant is supplied with meal. Many believe that dietary antioxidants are partly the reason for the benefits of diets which are rich in fruits and vegetables which have been suggested in global disease and control prevention (Hung et al., 2004).

Because of the fact that *Allium hirtifolium* (shallot) and *Sesamum indicum* (sesame) contain high levels of antioxidant polyphenols and perhaps reduce cardiovascular risk; the present study was designed to evaluate the impact of these two species on the postprandial status.

Sesame seeds are extremely rich in magnesium, copper, manganese, calcium and iron (90 mg/tablespoon for unshelled seeds, 10 mg for shelled seeds), and contain vitamin B1 and vitamin E (thiamine and alpha-tocopherol respectively).

These seeds contain lignans such as sesamin which are phytoestrogens along with antioxidant and have anticancer properties. Out of 6 plants having the edible oils, sesame oil had the highest volume of antioxidant. Furthermore, sesame seed contains phytosterols which; are related to reducing blood cholesterol level. Nutrient substances inside sesame seeds can be absorbed better provided that you grind or pulverize it before eating similar to *tahini* (sesame paste seed or Halva). Sesame seeds contain high levels of phytic acid which is an antinutrient (Cheung et al., 2007).

A. hirtifolium belongs to the Alliaceae species which originally is a Persian traditional herb which is used as condiment seasoning (Persian Shallot). It is recognized as a traditional medicine among Persian people and the bulbs also are widely used for treating rheumatic and inflammatory diseases (Jafarian et al., 2003). *A. hirtifolium* possesses certain beneficial secondary biological metabolites including S-allyl-cysteine (SAC), diallyl disulphide (DADS), diallyl trisulphide (DATS), alliin, alliinase, allicin, and methyl allyl trisulphide. Alliins will be changed to allicin when their bulbs are crushed (Block et al., 1992).

In this study, the effects of *A. hirtifolium*'s extract (2%) and *S. indicum* powder (10%) were studied on the atherosclerosis postprandial risk factors in rabbits fed with a high cholesterol diet.

METHODS AND MATERIALS

Preparation of *A. hirtifolium* and *S. indicum*

The plant identity such as *A. hirtifolium* and *S. indicum* was approved by the Herbarium Department of Shahrekord University of Medical Sciences. Some factors such as anthocyanins (Francis, 1982) and polyphenoles (Singleton et al., 1965) were measured in order to standardize the *A. hirtifolium* and *S. indicum*.

Experimental design

Thirty two male New Zealand rabbits (2100 ± 234 g) were purchased from Razi Vaccine and Serum Research Institute (Tehran, Iran). The rabbits were kept in a standard environment that had access to water and normal diet for a fortnight. Thereafter, to evaluate the effect of *A. hirtifolium* and *S. indicum* on atherosclerosis risk factors in hypercholesterolemic rabbits, the animals were divided into four groups (8 rabbits for each group). The first group was the control (normal), the second group fed a hypercholesterolemic diet (1% cholesterol in addition with a normal diet), the third group was fed a hypercholesterolemic diet in addition with 10% *S. indicum* powder and the fourth group received hypercholesterolemic diet and 2% *A. hirtifolium* extract.

The rabbits were fasted for 12 to 15 h and venous blood samples were taken to obtain underlying data. Three hours (this period included in the steady-state period of lipid absorption) after the dosage of the experimental diet, venous blood samples were again collected in order to investigate the acute effects of *A. hirtifolium* and *S. indicum* (Daher et al., 2005; Setorki et al., 2010a, b).

Total flavonoids determination

The total amount of flavonoids in the garlic extract was determined through colorimetric method which was described by Chang et al. (2002).

Hereby, 0.5 ml garlic extract (standard flavonoid compound or rutin) was mixed with aluminum chloride (0.1 ml of 10% solution), methanol (1.5 ml), potassium acetate (0.1 ml of 1 M), and distilled water (2.8 ml); this mixture was left at a room temperature for 30 min. The absorption of the mixture was measured at 415 nm using rutin solutions at 25 to 500 ppm concentrations in methanol. Total flavonoids were expressed in terms of rutin equivalents (in mg/g).

Total phenol determination

The colorimetry method with Folin-Ciocalteu reagent described by McDonald et al. (Singleton et al., 1965) was used to determine the amount of total phenolic compounds. Then, 0.5 ml of rutin solutions was mixed with the Folin-Ciocalteu reagent (1:10 dilution with distilled water) and aqueous Na₂CO₃ (0.4 ml of 7.5% solution) and the mixture were left a room temperature for 30 min; then, spectrophotometer (Unico UV-2100, USA) at 765 nm was used to determine the amounts of total phenols. Using 12.5, 25, 50, 62.5, 100 and 125 mg/L solutions of gallic acid in methanol and water (60:40, v/v), a standard curve was prepared. Total phenol values were expressed in terms of gallic acid equivalent (mg/g), as a common reference compound.

Blood sampling and analyses

In order for biochemical analyses, the blood serum and plasma were extracted by centrifuging blood samples for 20 min at 3000 rpm. Serum apolipoprotein B (ApoB), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), glucose, glutamic pyruvic transaminase (SGPT), and glutamic oxaloacetate transaminase (SGOT) were measured using standard enzymatic kits (Pars Azmoon Co., Iran) via auto-analyzer (Hitachi 902, Japan). Factor VII was measured using clotting time; it was derived from the sample being tested (Diagnostic Stago, French) and fibrinogen was measured using coagulation kit (Mahsayaran Co, Iran). Nitrite and nitrate were measured using a colorimetric assay kit (R&D Systems, USA) which involved the Griess Reagent.

RESULTS

The measured level of physicochemical factors in *S. indicum* and *A. hirtifolium*

The amounts of anthocyanin in *S. indicum* and *A. hirtifolium* respectively were 63.6 and 23.7 mg / 100 g and the amounts of flavonoid in *S. indicum* and *A. hirtifolium* respectively were 43 and 45 mg/1 g and also the amounts of their phenolic compounds were 54 and 89 mg/1 g, respectively.

The measured biochemical factors in rabbit

In hypercholesterolemic diet group, the amounts of glucose, triglycerides, fibrinogen, LDL-C, TC, SGPT, and SGOT significantly increased in comparison with the normal diet group. Consumption of shallot showed a significant reduction in glucose level compared to hypercholesterolemic diet group. No significant difference was found between the *S. indicum* and hypercholesterolemic diet groups in terms of glucose level (Table 1). In terms of lipid profile, consumption of *A. hirtifolium* and *S. indicum* showed a significant reduction in LDL-c level and cholesterol compared to hypercholesterolemic diet group. *S. indicum* consumption showed no significant change on TG; however, shallot showed a significant reduction on TG level compared to hypercholesterolemic diet group. None of the *A. hirtifolium* or *S. indicum* group showed a significant reduction on ApoB level compared to hypercholesterolemic diet group (Table 1).

Consumption of *A. hirtifolium* and *S. indicum* caused a significant reduction in SGPT level; however, only shallot caused a reduction in SGOT level compared hypercholesterolemic diet group (Table 1).

In terms of inflammatory factors, consumption of both *A. hirtifolium* and *S. indicum* caused a significant reduction in fibrinogen level. Consumption of these two species had no effect on the factor VII (Table 1).

In terms of endothelial markers (nitrite and nitrate), consumption of shallot caused a significant reduction in nitrate level. No significant difference was found between the *S. indicum* and hypercholesterolemic diet groups in terms of nitrate level. None of the *A. hirtifolium* and *S. indicum* groups had effect on the nitrite level (Table 1).

DISCUSSION

The present study was designed to evaluate the impact of *A. hirtifolium* and *S. indicum* on the postprandial status.

Postprandial (PP) lipidemia which is known by an increase in lipids or lipoproteins after a meal is a dynamic, non-steady state condition in which humans

spend the majority of their time. Many evidences indicate that PP lipemia enhance the atherogenesis risk (Hyson et al., 2003). There is a strong positive correlation between the progression and pathogenesis of atherosclerosis with importance and duration of postprandial hyperlipidemia (Karpe et al., 1994).

The results of this study showed that *A. hirtifolium* and *S. indicum* could lessen the cholesterol and LDL-C. In addition, *A. hirtifolium* could reduce blood triglyceride compared to hypercholesterolemic diet group.

The mechanism which caused the hypocholesterolemic effect could be due to viscosity, binding of bile salt and fermentability. The products of colonic fermentation that is short chain fatty acids, acetate and propionate inhibit HMG-CoA reductase by feedback mechanism that results in reduction of hepatic cholesterol synthesis (Carol et al., 1990).

The blood glucose level was also less in the forth group (*A. hirtifolium* group) than in hypercholesterolemic diet group. The effect of dietary *A. hirtifolium* on the PP glycemia was well established which may delay gastric emptying, slowing the carbohydrate uptake; besides, another mechanism that may contributed to the PP effect was the sequestration of carbohydrates ingested with the meal, retarding carbohydrates access to digestive enzymes (Hunninghake et al., 1994; Bell et al., 1989).

In this study, *A. hirtifolium* and *S. indicum* could reduce the blood fibrinogen in hypercholesterolemic rabbits.

Studies have indicated that endothelial dysfunction is one of the initial moves in development of atherosclerosis, and it is recognized by a thrombogenic status caused by an imbalance between procoagulant and profibrinolytic activities (Nossel, 1981). In some cases of procoagulant factors, plasminogen activator inhibitor-1 (PAI-1) and factor VII (FVI) concentrations have been associated with coronary heart disease (Smith et al., 2005), and both can be partly regulated by alimentary lipemia (Byrne et al., 1998). It has been more focusing on investigating whether or not different components of diet can regulate the acute postprandial changes in coagulation and fibrinolysis.

Plant compounds play a role in this process by preventing from the blood coagulation through reducing fibrinogen, increasing fibrinolysis, prothrombin time and inhibition of platelet aggregation (Schoene and Wguidry, 1999).

Consumption of antioxidants increase antioxidant capacity and reduce the risks of elevated fibrinogen and cardiovascular disease (Caen et al., 1993). In this study, the *A. hirtifolium* group reduced the nitrate in comparison with hypercholesterolemic diet group.

This is especially obvious on the effects of nitric oxide in atherosclerotic plaques which have already been reported (Rubbo et al., 1996; Naruse et al., 1994). It is getting more apparent that this controversy is due to diverse biological effects of reaction products formed through interactions of nitric oxide with diverse oxygen-

Table 1. Effects of hydroalcoholic extract of Sesame and *A. hirtifolium* on selective biochemical parameters in rabbits fed with high cholesterol diet.

Biochemical parameter	Cholesterolemic diet	Extract of <i>Allium</i>	Extract of <i>Sesame</i>	Normal diet
LDL-C (mg/dl)	39.31 ± 9.6	22 ± 6*	21.87 ± 6.5*	24.12 ± 3.5*
TG (mg/dl)	120.5 ± 16.3	94.7 ± 11.8	130.62 ± 15.2*	50.5 ± 4*
TC (mg/dl)	91 ± 10.1	62.57 ± 7.2*	72.78 ± 8*	56.62 ± 1.9*
Glucose	132 ± 10.7	115.57 ± 9.64	126.12 ± 6.95*	51.25 ± 8.8*
SGOT	40 ± 4	30.42 ± 2.93	35.1 ± 6.1*	2.62 ± 1.4*
SGPT	43.22 ± 7.8	30.42 ± 3.69*	37.25 ± 3.5*	29.75 ± 1.4*
ApoB	30.77 ± 3	30.42 ± 2.2	30.37 ± 2.66	27.78 ± 2.47
Factor VII (% activity)	298.1 ± 16.2	285 ± 10	295 ± 5.8	295.7 ± 9.2
Nitrite (µmol/l)	249.3 ± 25.5	233.4 ± 125.4	205.3 ± 41.4	250.4 ± 22.5
Nitrate (µmol/l)	430 ± 90	42.2 ± 53	192.9 ± 194.5*	305.6 ± 243.3
Fibrinogen (mg/dl)	251 ± 13.7	212 ± 8.9*	217.1 ± 8.8*	216.6 ± 9.6*

TC, Total cholesterol; ApoB100, apolipoprotein B100; nitrite, nitrate, factor VII, glucose; LDL, low density lipoprotein; fibrinogen, SGPT and SGOT, glucose and TG in each group (n = 8 for each experimental group) *, p < 0.05, comparison between cholesterolemic diet group and each of other 3 groups (*A. hirtifolium* and *S. indicum* and normal diet). Results are expressed as mean ± SEM.

based radicals. Furthermore, a basic component of our knowledge of nitric oxide role in vascular diseases is the identification of cell signaling pathways that are regulated by nitric oxide and effect which significantly is on the atherosclerotic process (Denninger and Marletta, 1999). The generated nitric oxide through inducible nitric oxide synthase (iNOS) causes cardiovascular diseases. Gene expression of iNOS is depending upon two NF-κB and signal transducers and activators of transcription protein (STAT) transcription factors (nuclear factor-κB). NF-κB is activated through lipopolysaccharides (LPS), IL-1, TNF (tumor necrosis factor) and STAT-1 (signal transducers and activators of transcription protein) also activated through INF (inflammation necrosis factor). Anthocyanins can reduce iNOS expression. Excessive iNOS expression by macrophages causes excessive nitric oxide in damaged tissues and onset of inflammatory process (Okopien et al., 2004).

Conclusion

People are regularly exposed to PP which is a non-fasted state and it has become clear that alongside basal or fasting metabolism, postprandial disturbances may also play a significant role in development and progression of cardiovascular diseases. This study indicated that dietary of *A. hirtifolium* and *S. indicum* are strong hypolipidemic, hypoglycemic and antithrombotic factors. This observation may be explained by an effect of *A. hirtifolium* and *S. indicum* antioxidant compounds on atherosclerosis postprandial agents. Further studies are required to determine the long-term effects of *A. hirtifolium* and *S. indicum* on atherosclerosis.

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