Hypolipidemic effect of aqueous extract of *Carum carvi* (black Zeera) seeds in diet induced hyperlipidemic rats

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Abstract: Medicinal plants play a key role in preventing various diseases. Hyperlipidemia is a major contributor to the pathogenesis of cardiovascular diseases. The purpose of the present study was to assess the effect of aqueous extract of *Carum carvi* seeds in diet induced hyperlipidemia in rats. 2% cholesterol diet were given to rats for six weeks and rats showed high lipid levels were included in the study. Then all rats were divided into, normal control group (A), hyperlipidemia positive control group (B), and the remaining two groups (C and D) served as experimental groups. Group C hyperlipidemic experimental rats received aqueous dried extract of *Carum carvi* seeds at 60 mg/kg of body weight for eight weeks on daily basis. On the other hand group D rats received simvastatin at 1.0 mg/kg body weight for eight weeks. Blood samples were collected after eight weeks. The hyperlipidemic positive control group rats showed variable increase in serum triglycerides, LDL and total cholesterol levels. Serum HDL levels decreased in hyperlipidemic positive control groups. *Carum carvi* and simvastatin significantly decreased the levels of these parameters in rats. On comparison *Carum carvi* reduced lipid levels more, effectively than the simvastatin. *Carum carvi* constituents, especially flavonoids and carvone have strong anti-oxidant activity which might be involved in hypolipidemia. In conclusion, *Carum carvi* aqueous seeds extract decrease lipid levels in diet induced hyperlipidemic rats.

Keywords: *Carum carvi*, diabetic nephropathy (DN), oxidative stress, total cholesterol, serum triglycerides, LDL (low density lipoproteins), HDL (high density lipoproteins).

INTRODUCTION

Cardiovascular diseases are leading cause of death in the west. Dyslipidemia including hypercholesterolemia, high levels of LDL (low density lipoproteins) and low levels of HDL (high density lipoproteins) are major causes of increased atherogenic risks (Mahley and Bersot, 2006). Hyperlipidemia may be primary anomaly or it may be manifestation of some other conditions e.g., diabetes mellitus, hypothyroidism, cholestasis. It is also the major cause of conditions associated with atherosclerosis like coronary artery diseases, ischemic cerebrovascular disease and peripheral vascular disease (Gaziano, 2005). Dyslipidemia is characterized by elevated LDL (low density lipoproteins), high triglycerides and low HDL level. High levels of HDL are found consistently to be associated with long life in diverse population while LDL is the principal atherogenic component of cholesterol and major constituent of atherogenic plaques (Enas, 2000). Many *in vivo* and *in vitro* studies have indicated that oxidative stress is one of the major pathophysiological mechanisms involved in hyperlipidemia (Anjaneyulu and Chopra, 2004). Oxidatively modified LDL particles are thought to promote specifically the early development of atherosclerotic lesions (Hertog *et al.*, 1993).

Dietary modifications and drug therapy has shown promising results to regulate HDL and LDL cholesterol levels and to reduce subsequent risk of coronary artery disease associated pathological conditions. But due to high cost and adverse effects of lipid lowering drugs, peoples are now diverting to certain natural substances. The use of such substances are grown faster over the past few years which is undoubtedly driven by the belief that they are relatively safe, easily available and affordable (Yokozawa *et al*., 2006).

Caraway locally known as Black Zeera is a member of the group of aromatic, umbelliferous plants. Caraway is one of the oldest spices cultivated in Europe. It is naturally found in Northern and Central Europe, Siberia, Turkey, Iran, India and North Africa. The fruit of caraway is a schizocarp, which at harvest splits into two halves, called, seeds (DeCarvalho and DeFonseca, 2006). Caraway is known to have anti-hyperglycaemic effect. Caraway was commonly used in phytomedicine as antibacterial, anti-proliferative and laxative (Singh, 2002; Nakano, 1998). The major constituents of seeds are carvone, flavonoids and limonene. Myrcene, beta caryophyllene, thujone, anethole and pinene are present as minor components (DeCarvalho and DeFonseca, 2006). The flavonoid constituents of caraway have been separated by means of chromatography on cellulose columns and constituents such as quercetin-3-glucuronides, isoquercitrin, quercetin 3-0 caffeylglucoside, and kaempferol 3-glucoside were obtained (Kunzemann and Hermann, 1977).

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The purpose of the present experimental model was to observe the effect of *Carum carvi* in diet induced hyperlipidemic Wistar rats.

MATERIALS AND METHODS

Animals

Sixty adult male Wistar rats weighing 200-250g were procured for this study. They were kept in the experimental research laboratory of University of Health Sciences, Lahore under day and night conditions. Prior to the commencement of the experiments, all animals were kept for one week under the same laboratory conditions, at a temperature of 22 ± 2°C, relative humidity of 70 ± 4% and 12 hour light/day cycle. They received nutritionally standard diet and tap water. The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of experimental animals.

Chemicals/Instruments

2g Cholesterol, extra pure, Scharlau (Spain), 500mg Cholic acid, minimum 98%, Sigma-Aldrich (Germany), commercially available kits (Randox) for biochemical analysis of hyperlipidemia, pre-coated TLC (Thin Layer Chromatography) plate silica gel GF254, toluene, ethyl acetate, formic acid and methanol as solvent system. The standard compounds used are ellagic acid, gallic acid and protocatechuic acid. The instruments used were incubator and centrifuge (Germany), TLC scanner III (Camag, Switzerland) with win CATS software.

Plant materials and preparation of the extract

Seeds of *Carum carvi* were collected from local market of Lahore and were authenticated from a botanist (Dr. Ghazala, Professor of Botany, Punjab University, Lahore, Pakistan). *Carum carvi* seeds were coarsely powdered using a grinder. Dried powdered *Carum carvi* seeds (50g) were mixed with 100 ml of water and the mixture was blended. After blending, the mixture was stirred with the help of a magnetic stirrer for 3 days. The decant settled down, supernatant was separated and was centrifuged, incubated and dried to get powdered extract. The supernatant was collected in Petri dishes and labeled properly. This supernatant was then dried at 40°C in incubator. The dried material then scraped carefully and was kept in dark vials at -20°C. The percentage yield was 6.8 (w/v). The seeds (Voucher No.0786) and extract (Voucher No.0787) were deposited in Pharmacology laboratory, University of Health Sciences, Lahore. This supernatant got standardised from Pakistan Council for Scientific and Industrial Research (PCSIR) laboratories in Lahore.

Standardization of plant extract

TLC was used for standardization. TLC was performed on a pre-coated TLC plate silica gel GF_{254}. Sample was applied on the plate as 8 mm wide bands with an automatic TLC sampler. The development was carried out in trough chamber (20 cm × 10 cm), which was pre-saturated with mobile phase (solvent system, toluene-ethyl acetate-formic acid-methanol (30:30:8:2), for 20 min at room temperature (25 ± 2°C and 40% relative humidity). Subsequent to the development, TLC plates were dried under stream of hot air and then subjected to densitometric scanning using a TLC scanner III (Camag, Switzerland) with win CATS software (version 1.4.1) in the absorbance- reflectance scan mode. Quantitative evaluation of the plate was performed in absorption-reflection mode at 338 nm. The standard compounds used are ellagic acid, gallic acid and protocatechuic acid.

Experimental procedure

After acclimatisation, 10 rats were labelled as control. All other rats were fed on 2% cholesterol diet for six weeks to induce hyperlipidemia. After six weeks, blood lipid profile was done and rats having hyperlipidemia were included in the experiment. Now all rats are divided in four groups (10 rats in each group). The control rats (Group A) were fed on standard diet with tap water and received no drug. Group B hyperlipidemic rats fed only on standard diet with tap water for eight weeks and received no drug. Group C i.e. experimental hyperlipidemic group rats received standard diet with tap water and aqueous dried extract of *Carum carvi* seeds in a daily oral dose of 60 mg/kg for eight weeks. Group D i.e., experimental hyperlipidemic group rats received standard diet with tap water and simvastatin in a daily oral dose of 1.0mg/kg for eight weeks.

Sample collection

Blood sampling through tail vein was performed at 3 intervals (0, 6, 14 weeks) following same protocol every time. Twenty four hour after administration of the last dose of extract i.e., on 60^{th} day and after overnight fasting, the animals were weighed and anaesthetised under ether vapours. A sample of 2ml blood was drawn from tail vein from all animals. Blood was transferred to the sterile vacuotainers with gel and allowed to clot at room temperature for one hour. It was then centrifuged for ten minutes at a speed of 3000 rpm. Serum was separated and stored in sterile eppendorf tubes at -20°C for analysis of biochemical parameters (Shad *et al.*, 2003).

Biochemical analysis

Total cholesterol levels were estimated using commercially available kit (Randox, UK) based on enzymatic endpoint method (Thomas, 1998). Serum triglycerides was estimated by commercially available kits (Randox, UK), based on GPO-PAP method (Jacobs and Van Denmark, 1960) while serum HDL by precipitant method (Lopes-Virella, 1977). Serum LDL was estimated using commercially available kit (Randox, UK) based on an established method (Friedewald *et al.*, 1972).

STATISTICAL ANALYSIS

The data was entered and analysed using SPSS 17.0 (Statistical Package for Social Sciences). All data are shown as mean ± S.E.M. One way ANOVA was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. A p-value of <0.05 was considered as statistically significant.

RESULTS

The biochemical parameters showed that the 2% cholesterol diet given for six weeks caused a significantly ($p<0.01$) increased serum triglycerides, LDL and total cholesterol levels while significantly decreased serum HDL levels in the rats of group B, C and D as compared to control group.

The total cholesterol levels showed that 2% cholesterol diet given for six weeks caused a significantly ($p<0.01$) increased total cholesterol levels in the rats of group B, C and D as compared to control group. On the other hand, administration of aqueous dried extract of *Carum carvi* to group C and simvastatin to group D for eight weeks resulted in a significant ($p<0.01$) decrease in the total cholesterol levels of the rats in groups C and D when compared with that of group B.

Serum triglycerides levels showed that 2% cholesterol diet given for six weeks caused a significantly ($p<0.01$) increased triglycerides levels in the rats of group B, C and D as compared to control group. On the other hand, administration of aqueous extract of *Carum carvi* to group C and simvastatin to group D for eight weeks resulted in a significant ($p<0.01$) decrease in the triglycerides levels of the rats in groups C and D when compared with that of group B.

Serum LDL levels showed that 2% cholesterol diet given for six weeks caused a significantly ($p<0.01$) increased LDL levels in the rats of group B, C and D as compared to control group. On the other hand, administration of dried aqueous extract of *Carum carvi* to group C and simvastatin to group D for eight weeks resulted in a significant ($p<0.01$) decrease in the LDL levels of the rats in groups C and D when compared with that of group B.

Serum HDL levels showed that 2% cholesterol diet given for six weeks caused decrease HDL levels in the rats of group B, C and D as compared to control group. On the other hand, administration of aqueous extract of *Carum carvi* to group C and simvastatin to group D for eight weeks resulted in a significant ($p<0.01$) increase in the HDL levels of the rats in groups C and D when compared with that of group B.

DISCUSSION

Hyperlipidemia is a major contributor to the pathogenesis of cardiovascular diseases which is a leading health problem in the world. Many studies have shown that there is a direct correlation between incidence of coronary artery disease and total cholesterol levels (Bertolotti *et al*., 2005). According to the oxidation hypothesis, Steinberg and Witztum, 2010 proposed that LDL, in its oxidized form, is crucial to cellular uptake to form macrophages derived from cells in early development of atherosclerotic lesions. Oxidized LDL has many atherogenic properties in addition to the formation of foam cells. It behaves as a potent inflammatory agent and can induce the expression of adhesion molecules, chemokines and pro-inflammatory cytokines. Oxidized LDL may play a significant role in the initial endothelial damage that leads to atherogenesis. Oxygen radicals can oxidize LDL, which injures the endothelial wall and thereby promotes atherosclerotic change (Young and McEneny, 2001).

Good control of cholesterol through diet, exercise and drugs can reduce clinical complications in hyperlipidemic patients; however alternative treatment strategies are needed to prevent oxidative stress complications and to optimise recovery. It is well documented that modulations of oxidative stress through treatment with antioxidants can effectively reduce lipid levels (Evans *et al*., 2002).

The present study showed a significant elevation observed in the levels of serum triglycerides, LDL and total cholesterol of group B hyperlipidemic rats as compared to group A normal rats. Serum HDL levels was reduced in group B rats as compared to group A rats. Administration of *Carum carvi* seeds extract to group C and simvastatin to group D brought the levels of these diagnostic parameters in the serum of group C and D animals towards normal as compared to group B rats (table 1). When we compare mean values of group C with group D, although both decrease lipid levels, but *Carum carvi* extract reduced the levels more as compared to simvastatin. *Carum carvi* also increased the level of HDL more as compared to simvastatin (table 1), showing better effectiveness of *Carum carvi* extract over simvastatin. Our results are in accordance with the reports by others who used chemical antioxidants and diet of natural antioxidant plants (Sharma, 2003; Akpanabiatu, 2005).

The main constituents in *Carum carvi* are carvone, limonene and flavonoids. The proposed mechanism of *Carum carvi* in reducing the lipid levels could be due to the antioxidant mechanism. Robards and Antolovich in 1997 critically reviewed the analytical chemistry of bioflavonoid and it was found that flavonoids possess antioxidant activity, they are potent free radical

Table 1: Mean±SEM values of different biochemical parameters in all groups (A, B, C and D)

Parameter	Group A	Group B	Group C	Group D
Total Cholesterol (mg/dl)	65.19±1.36	152.81±1.83*	91.80±1.52**	92.01±1.78**
Serum Triglycerides (mg/dl)	94.66 ±1.71	166.53±1.71*	110.01±2.55**	111.50±2.67**
Serum LDL (mg/dl)	23.79±1.32	103.62±1.24*	23.71±1.19**	26.69±2.71**
Serum HDL(mg/dl)	21.53±0.72	17.53±0.69	46.77±1.36**	39.83±1.71**

*p<0.05 when compared with group A (control), **p<0.05 when compared with group B (diabetic)

scavengers and metal chelators and they also inhibit lipid oxidation which is a key step in the formation of atherosclerotic plaque. Stefania and Jerzy in 2005 also showed lipid lowering effect of quercetin which is a major flavonoid of plant kindom. Elmastas *et al.* (2006) also reported that carvone had a strong antioxidant activity. Therefore, in our study flavonoids and carvone in *Carum carvi* might have a role in decreasing lipid levels in rats.

In conclusion, the results of the present study indicate that the treatment with *Carum carvi* aqueous seeds extract decreased lipid levels in hyperlipidemic rats. The *Carum carvi* aqueous seeds extract, showed better results as compared to simvastatin. The overall hypolipidemic effect of *Carum carvi* is probably due to a counteraction of free radicals by its antioxidants i.e. Quercetin (flavonoids) and Carvone.

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