

Lipid Lowering Effect of Ethanolic Extract of Aerial Parts of *Peucedanum pastinacifolium* Boiss. and Hausskn. in Hypercholesterolemic Rats

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Abstract

Increased plasma cholesterol is known to be a major risk related to the development of coronary artery disease. The main purpose of this study was to determine possible effects of the ethanolic extract of aerial parts of *Peucedanum pastinacifolium* on the serum lipids in hypercholesterolemic rats. Experimental hypercholesterolemia was induced by feeding rats a cholesterol-enriched diet for 56 days. Hypercholesterolemic rats were classified into five groups. One group did not receive treatments and served as a control hypercholesterolemic group. The other four groups were fed the cholesterol-enriched diet in conjunction with 125, 250, 500 mg extract per kg of the body weight along with 20 mg atorvastatin per kg body weight, in a daily oral dose. A normal group of rats fed with a plain chow diet was also included in the study. After oral administration for 8 weeks, *P. pastinacifolium* extract produced significant decrease on serum total cholesterol, low-density lipoprotein cholesterol, triglyceride levels and atherogenic indices in hypercholesterolemic groups ($P < 0.05$). *P. pastinacifolium* extract had no effects on serum high-density lipoprotein cholesterol levels in these groups. It is concluded that the extract of aerial parts of *P. pastinacifolium* exhibits lipid lowering activity in hypercholesterolemic rats. However, further investigation would be necessary to evaluate the mechanism of action of the extract.

Keywords: *Peucedanum pastinacifolium*; Lipoproteins; Cholesterol; Hypercholesterolemia.

Introduction

Cardiovascular diseases are one of the leading causes of death in the world. Atherosclerosis plays a major role in the development of myocardial infarction and stroke (1). Elevated plasma cholesterol, especially in LDL, is known to be a major risk related to the development of coronary artery disease or arteriosclerosis (2).

Approximately 15% of middle-aged Americans have primary hypercholesterolemia, that is, plasma cholesterol levels in excess of 250 mg/dL. The risk for coronary heart disease in these patients is at least twice that of patients with a baseline level of 200 mg/dL (3).

Several publications coming from different laboratories included recommendations for a reduction in cholesterol consumption as a means of preventing heart disease. At present, dyslipidemia is most commonly treated with lipid-altering pharmacological therapies. However,

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safety concerns regarding the use of these agents have prompted the need for safe and efficacious nonpharmacological lipid-altering interventions (4). As such, the effects of dietary components on plasma lipid metabolism have recently received considerable attention, thereby highlighting the importance of naturally occurring compounds as cholesterol metabolism regulators.

Many herbal medicinal products have potential hypocholesterolemic activity and encouraging safety profiles, such as *Dracocephalum kotschyi* (5), *Allium porrum* (6), *Purslane* (7), *Eclipta prostrata* (8), *Scoparia dulcis* (9), *Trigonella foenum-graecum*, and red yeast rice (10). However, only a limited amount of clinical research exists to support their efficacy.

Peucedanum pastinacifolium Boiss. and Hausskn. is a plant from Apiaceae family (11). The plant have been used by local inhabitants in west of Iran, as an antihyperlipidemic vegetable. Chemical components of *P. pastinacifolium* are unknown, and there is no evidence for its pharmacological activity but there are some studies on other *Peucedanum* spices which demonstrates antibacterial (12, 13), antiplatelet (14) and antimutagenic (15) activities. Phytochemical studies on *Peucedanum* species led to the isolation of coumarins (16, 17) and essential oil (18, 19). In this study we report the effect of *P. pastinacifolium* aerial parts extract on serum lipids levels to evaluate hypolipidemic activity of the plant.

Experimental

Plant material and extraction

Aerial parts of *P. pastinacifolium* at full flowering stage were collected from Isfahan province in May 2005 at an altitude of 1530 m. The plant identity as *P. pastinacifolium* was confirmed by the Botany Department of Isfahan University. A voucher specimen of the plant was deposited in the Herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran (No. 1124). The air-dried plant material were roughly cut and ground to the powder. The plant material was macerated with

EtOH-H₂O (7:3) for 24 h. The hydroalcoholic extract was evaporated in a rotating evaporator under reduced pressure until dryness.

Diet and treatment

Six groups (n=36) of male wistar rats (obtained from the central animal house of the Tehran Pasteur Institute, Tehran, Iran) weighting 200±20 g at the beginning of the study were used. Rats were housed identically in an air-conditioned room under a 12 h light-dark cycle. The control group was fed a standard diet (normal control). Group 1 was fed a standard diet enriched with 1% cholesterol and 0.5% cholic acid (hypercholesterolemic control). Groups 2-5 receiving cholesterol-enriched diet plus 125, 250, 500 mg extract per kg of the body weight along with 20 mg atorvastatin per kg body weight, respectively (treated groups), in a daily oral dose. The experiment lasted eight weeks. All of the rats were initially fed a standard laboratory diet for at least 7 days after delivery to our laboratory for adaptation. Tap water was freely available. Food intake was monitored daily for all control and treated groups. The different doses of plant extract and atorvastatin were suspended in water and given orally each day.

Measurement of body weight and biochemical parameters

The body weight (BW) of each rat was determined before starting the treatment and each week afterward. Blood samples were collected from the jugular vein; serum was separated by centrifugation (2000 g, 20 min, 4°C) and used for biochemical analysis. Serum concentrations of cholesterol, triglyceride, LDL-C and HDL-C were determined with commercially available enzyme kits (Pars Azmoon, Tehran, Iran). The atherogenic index (total cholesterol -HDL-C/HDL-C) was also calculated. At the end of the treatment, the heart and liver were weighed and the organ/ BW ratio was calculated and expressed as a percentage.

Statistical analysis

All values used in analysis are presented as mean ± SD for 6 rats in each group of experiments. Comparisons among the different

Table 1. Effect of *P. pastinacifolium* extract on the organs / BW ratios at the end of the treatment.

Group	Body weight (g)	Heart / BW ratio (%)	Liver / BW ratio (%)
C	350 ± 24	0.41 ± 0.05	3.5 ± 0.3
HC	375 ± 28	0.43 ± 0.06	4.8 ± 0.4*
HC + 125 mg/kg BW extract	348 ± 25	0.45 ± 0.06	4.1 ± 0.3**
HC + 250 mg/kg BW extract	345 ± 28	0.42 ± 0.05	3.9 ± 0.4**
HC + 500 mg/kg BW extract	340 ± 29	0.41 ± 0.05	3.8 ± 0.3**
HC + 20 mg/kg BW atorvastatin	345 ± 22	0.44 ± 0.06	3.6 ± 0.4**

Values are means ± SD (n=6).

* P < 0.05 with respect to the normal control group (C).

** P < 0.05 with respect to the hypercholesterolemic control group (HC).

groups were performed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test and differences were considered significant when P < 0.05.

Results

All groups exhibited an increase in body weight through the treatment without significant differences among them (Table 1). There were no differences in the heart/BW ratio. However the liver/ BW ratio decreased in all of the treated groups compared with the hypercholesterolemic group (Table 1).

The serum concentrations of total cholesterol, triglyceride and lipoproteins (HDL-C and LDL-C) were increased in hypercholesterolemic group with respect to the normal control group at the end of the treatment (Table 2).

Serum total cholesterol and triglyceride decreased significantly in all groups treated with *P. pastinacifolium* extract but no significant differences were observed among them (Table 3). Changes in distribution of cholesterol in HDL or LDL were found, i.e., LDL-C decreased significantly with respect to the hypercholesterolemic group in all groups treated with *P. pastinacifolium* extract and atorvastatin. However there were no significant differences

among the groups (Table 3). Although a decrease in HDL-C was found in all treated groups in comparison with hypercholesterolemic group, this reduction was not significant (Table 3). Finally atherogenic index in all groups treated with *P. pastinacifolium* extract was decreased significantly compared with hypercholesterolemic group since no significant differences were found between the treated groups and control group (Table 4).

Discussion

The importance of plasma levels of cholesterol and lipoproteins in the pathogenesis of atherosclerosis has been noted by a number of studies. The relationship between dietary cholesterol and coronary heart disease (CHD) has been a topic of intense research, and considerable debate of the 20th century (20). The plasma cholesterol response to dietary cholesterol is highly variable across and within animal species, since rats exhibit little change in plasma total cholesterol even with high doses of dietary cholesterol (20).

Our results showed that serum total cholesterol and lipoprotein cholesterol levels in rats were increased in the hypercholesterolemic group at the end of the 56-day study (Table 2).

Table 2. Serum lipids in normal and hypercholesterolemic groups.

Group	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Normal control	82 ± 6	56 ± 13	20 ± 5	32 ± 6
Hypercholesterolemic control	202 ± 30*	114 ± 19*	109 ± 25*	53 ± 14*

Values are means ± SD (n=6).

* Represents significant difference from normal control group at P < 0.05.

Table 3. Effect of *P. pastinacifolium* extract and atorvastatin on serum total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C) levels in hypercholesterolemic rats.

Groups	TC (% of control)	TG (% of control)	LDL-C (% of control)	HDL-C (% of control)
Hypercholesterolemic control	100	100	100	100
125 mg/kg BW extract	48 ± 11*	42 ± 18*	25 ± 7*	78 ± 15
250 mg/kg BW extract	51 ± 8*	48 ± 11*	22 ± 3*	89 ± 10
500 mg/kg BW extract	50 ± 7*	44 ± 15*	22 ± 5*	87 ± 13
20 mg/kg BW atorvastatin	51 ± 11*	53 ± 6*	16 ± 4*	93 ± 20

Values are means ± SD (n=6).

* Represents significant difference from hypercholesterolemic control group at P < 0.05.

It was obvious in our study that the elevation in serum total cholesterol mainly originated from the remarkable increase in LDL-C rather than HDL-C. Various studies indicated that rats fed with a cholesterol-rich diet do not develop cholesterolemia, instead, cholesterol must be fed in combination with cholate. Diets supplemented with cholic acid increased cholesterol absorption (21, 22).

Because of folkloric use of *P. pastinacifolium* as a lipid lowering vegetable in west of Iran, we investigated the possible beneficial effects of the extract on the serum lipids levels in diet induced hypercholesterolemic rats. With regard to the effect of dietary supplements, our results showed that oral administration of *P. pastinacifolium* extract improved the hyperlipidemia induced by cholesterol-enriched diet in treated rats. As shown in the present work, oral administration of *P. pastinacifolium* extract for 8 weeks resulted in a significant reduction of serum total cholesterol, LDL-C and TG levels without any

significant changes in HDL-C at doses of 125, 250, and 500 mg/kg BW in comparison with the hypercholesterolemic control group (Table 3). However, there is no significant differences in serum lipid parameters in the all groups treated with different doses of the extract. Therefore, it can be concluded from our results that the effect of *P. pastinacifolium* extract was not dose dependent at selected doses (Table 3).

To the best of our knowledge, there is no phytochemical study on *P. pastinacifolium* in the literature, but presence of coumarinic compounds in other *Peucedanum* species (16, 17) led us to the probable biological activity of this group of secondary metabolites. It is proved that some furanocoumarines such as khellactons can inhibit lipolysis induced by adrenalin which can finally affect the lipid metabolism and reduction of lipoprotein levels (23). Laserpitin is another beneficial coumarin compound found in *Angelica keiskei* that can change lipoproteins metabolism and has beneficial effect on hyperlipidemia. This compound produces increase in serum HDL level and decreases in the hepatic triglyceride content (24). Another interesting study indicate that scoparone (6,7-dimethoxycoumarin), extracted from Chinese herb (*Artemisia scoparia*), has an antiatherogenic action in hyperlipidemic diabetic rabbits (25). Potential hypocholesterolemic effects of coumarins and presence of coumarins in other *Peucedanum* species indicate that coumarins are probably the active constituents of the plant. However, further investigation are necessary to prove this postulation and to evaluate the mechanism of action of the extract. Another possible

Table 4. Effect of *P. pastinacifolium* extract and atorvastatin on atherogenic index in hypercholesterolemic rats.

Groups	Atherogenic index
Normal control	0.60 ± 0.14
Hypercholesterolemic control	1.59 ± 0.54
125 mg/kg BW extract	0.55 ± 0.11*
250 mg/kg BW extract	0.47 ± 0.06*
500 mg/kg BW extract	0.47 ± 0.09*
20 mg/kg BW atorvastatin	0.39 ± 0.04*

Values are means ± SD (n=6).

* Represents significant difference from hypercholesterolemic control group at P < 0.05.

mechanism by which *P. pastinacifolium* exerted its cholesterol lowering effect is a decrease in cholesterol absorption, by binding with bile acids within the intestine and increasing fecal bile acids excretion. Another mechanism related to hypolipidemic effect of Apiaceae family is hypoglycemic effect and reduction in blood glucose. Continues supplementation of *Cuminum cyminum* for six weeks prevents elevation of plasma and tissue lipids secondary to the diabetic rats. This hypolipidemic effect of *C. cyminum* can be explained as result of direct reduction in the blood glucose concentration (26).

Rats treated with *P. pastinacifolium* extract also showed a decrease in the atherogenic index compared with hypercholesterolemic control group, which is generally believed to be beneficial since the HDL level inversely correlated with CHD and reduction in this ratio is considered as an anti atherosclerotic factor. This is an interesting result, as elevated concentration of serum cholesterol (especially LDL-C) is one of the risk factors in development of atherosclerosis.

In conclusion, the current results indicate that *P. pastinacifolium* extract exerts a hypocholesterolemic effect in cholesterol-fed rats and suggest a rational basis for folk and traditional uses of this herb in Iran.

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