BASIC RESEARCH

ANTIHYPERLIPIDEMIC EFFECT OF *PEUCEDANUM PASTINACIFOLIUM* EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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INTRODUCTION: Dyslipidemia is one of the most common complications of diabetes mellitus, significantly contributing to cardiovascular morbidity and mortality in diabetic patients. *Peucedanum pastinacifolium* Boiss. & Hausskn. is commonly used as an antihyperlipidemic vegetable in Iranian folk medicine. **MATERIAL AND METHODS:** In this study, we examined a hydroalcoholic extract of the aerial parts of *Peucedanum pastinacifolium* to determine its lipid-lowering activity in normal and streptozotocin (STZ)-induced diabetic rats. Experimental diabetes mellitus was induced by a single intraperitoneal administration of streptozotocin. Normal and streptozotocin-induced diabetic rats were separated into four groups. The groups were fed with 0, 125, 250 or 500 mg/kg body weight of *Peucedanum Pastinacifolium* hydroalcoholic Extract (PPE) in aqueous solution for 30 days. **RESULTS:** The results show that there were significant ($P < 0.05$) increases in total serum cholesterol, triglyceride and low-density lipoprotein cholesterol (LDL-C) and a decrease in high-density lipoprotein cholesterol (HDL-C) in streptozotocin-induced diabetic rats. Treatment of diabetic rats with PPE over a period of a month returned these levels close to control levels. **CONCLUSION:** These results suggest that PPE has hypolipidemic effects in streptozotocin-induced diabetic rats.

KEYWORDS: *P. pastinacifolium*, Lipoproteins, Cholesterol, Hypercholesterolemia.

INTRODUCTION

The number of people with diabetes mellitus (DM) worldwide is rapidly increasing. There are currently more than 170 million people with diagnosed DM, and that number has been predicted to rise to more than 360 million by 2030.[1] In Iran, 7.7% of adults aged 25 to 64 (approximately 2 million people) have been diagnosed with DM, and 16.8% of them (about 4.4 million) have impaired fasting glucose.[2] DM is an endocrine disorder, and its complications are a major cause of disability and hospitalization, posing a significant financial burden.

Dyslipidemia [elevated plasma levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) and a low concentration of high-density lipoprotein-cholesterol (HDL-C)] is one of the most common complications of DM. It plays a significant role in the development of premature atherosclerosis, coronary insufficiency and myocardial infarction.[3] Lipid profile abnormalities in diabetes are mediated through derangements in a variety of regulatory processes, especially insulin deficiency, thereby rendering diabetic patients more prone to hypercholesterolemia and hypertriglyceridemia.[4]

Treatment of hyperlipidemia in diabetes involves improving glycemic control, getting exercise and using lipid-lowering diet and drugs.[5] At present, dyslipidemia is most commonly treated with lipid-lowering drugs, some of which are associated with serious adverse side effects. Thus, the effects of dietary components on plasma lipid metabolism have recently received considerable attention, highlighting the importance of natural products as lipid metabolism regulators.[6]

Many medicinal plants have been found to be useful to successfully manage hyperlipidemia; these include

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Dracocephalum kotschyi,[7] *Allium porrum*,[8] purslane,[9] *Eclipta prostrata*,[10] *Scoparia dulcis*,[11] *Trigonella foenum-graecum* and red yeast rice.[12] However, only a limited amount of clinical research exists to support their efficacy.

Peucedanum pastinacifolium Boiss & Hausskn. is a plant belonging to the Apiaceae family, commonly known as "Alafe-Tofangchi". The plant is eaten by people in the center and western regions of Iran, and it is believed to be useful as an antihyperlipedimic plant. A survey of the literature revealed no pharmacological studies of this plant. Some species of *Peucedanum* have been used as anti-inflammatory, analgesic and diuretic compounds in Europe,[13] and some members of the family have antispasmodic,[14] antihypertension,[15] antihypeglycemic,[16] antitumor, antibacterial[17] and antiplatelet aggregation activities.[18] The most important chemical constituents of the genus *Peucedanum* are furanocoumarines, which have been isolated from several species.[14,18-20]

We previously demonstrated the hypolipidemic activity of the *P. pastinacifolium* plant in cholesterol-fed rats.[21] There are several reports that hyperglycemia is accompanied by increases in serum TC and TG levels in streptozotocin (STZ)-induced DM.[22, 23] In the present study, we investigated the hypolipidemic effect of a *P. pastinacifolium* extract in STZ-induced diabetic rats.

MATERIALS AND METHODS

Plant material and extraction: Wild samples of *P. pastinacifolium* were collected from the Soffeh Mountains (1500 m) near Isfahan, Iran, in June 2007 and identified 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. 1146). The aerial parts of plants were air dried and extracted at room temperature with a 70:30 ratio of ethanol:water. The hydroalcoholic extract was concentrated *in vacuo*, resulting in a crude extract.

Animals and experimental induction of diabetes: Adult male Wistar rats weighing approximately 200-250 g (obtained from the central animal house of the Tehran Pasteur Institute, Tehran, Iran) were housed in an air-conditioned room under a 12-h light-dark cycle. The animals were allowed free access to tap water and standard laboratory rat food. All experimental procedures involving animals were approved by the Animal Research Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran.

Diabetes mellitus was artificially induced by a single intraperitoneal (i.p.) injection of a freshly prepared STZ solution (Sigma, St. Louis, MO, USA) dissolved in 0.1 M citrate buffer, pH 4.5, at a dose of 60 mg/kg Body Weight (BW) to overnight-fasted rats.[24] Control rats received an i.p. injection of citrate buffer alone. At three days post-administration, rats with stabilized diabetes, as indicated by a fasting blood glucose level of more than 250 mg/dl, were selected for the study. Treatment was started on the fourth day after STZ administration and continued for 30 days.

Treatment protocol: The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by our institutional committee on animal care. Normal and STZ-diabetic rats were randomly assigned to seven groups of six as follows:

Group I. Control rats receiving citrate buffer

Group II. Diabetic control rats

Groups III – V. Control rats orally treated with different doses of plant extract (125, 250, 500 mg/kg BW) in aqueous solution for 30 days.

Group VI. Diabetic rats fed with glibenclamide (5 mg/kg BW) in aqueous solution for 30 days.

Groups VII – IX. Diabetic rats orally treated with different doses of plant extract (125, 250, 500 mg/kg BW) in aqueous solution for 30 days.

At the end of the experimental period, the rats were anesthetized and killed by cervical dislocation. Blood samples were collected and the serum was separated by centrifugation (2000 g, 20 min, and 4°C) and submitted to biochemical analysis. The serum concentrations of glucose, TC, TG, LDL-C and HDL-C were determined with the use of commercially available enzyme kits (Pars Azmoon, Tehran, Iran). The atherogenic index [total cholesterol – HDL-C]/ HDL-C] was calculated.

Statistical analysis: The results are presented as mean ± SD. Statistical analysis of the data was performed using an unpaired t-test and analysis of variance (ANOVA) with SPSS/11.5 software. Significant differences are indicated by p-values of less than 0.05.

RESULTS

The serum lipid profiles of the control (group I) and STZ-induced diabetic rats (group II) are shown in Table 1. A significant ($P < 0.05$) increase in the levels of serum total cholesterol, triglyceride and LDL-C were observed in the diabetic rats, whereas the HDL-C level was markedly ($P < 0.05$) decreased compared to normal control rats (Table 1).

Table 2 shows the serum lipid profiles of the untreated control group (group I) and control groups treated with different doses of PPE (groups III-V). The treatment of control rats with PPE did not significantly affect any of the evaluated parameters (Table 2).

Table 1 - Comparison of serum lipid profiles in normal and STZ-induced diabetic rats.

Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Normal	97 ± 10	53 ± 13	48 ± 9	38 ± 4
Diabetic	128* ± 15	87* ± 12	89* ± 19	21* ± 5

Values are the mean ± SD (n=6). * Represents a significant difference from the normal control group at P<0.05. LDL-C: Low-Density Lipoprotein-Cholesterol, HDL-C: High-Density Lipoprotein-Cholesterol, STZ: Streptozotocin

Table 2 - Comparison of serum lipid profiles in normal and PPE-treated normal rats.

Groups	TC (% of control)	TG (% of control)	LDL-C (% of control)	HDL-C (% of control)
Normal control	100	100	100	100
Treated groups				
125 mg/kg B.W. PPE	89 ± 7	93 ± 8	102 ± 10	97 ± 8
250 mg/kg B.W. PPE	99 ± 6	101 ± 9	98 ± 9	90 ± 9
500 mg/kg B.W. PPE	98 ± 7	94 ± 7	102 ± 9	92 ± 8
5 mg/kg B.W. Glybenclamide	82 ± 9	80 ± 10	85 ± 10	84 ± 11

Values are the mean ± SD (n=6). * Represents significant difference from normal control group at P<0.05. LDL-C: Low-Density Lipoprotein-Cholesterol, HDL-C: High-Density Lipoprotein-Cholesterol, TC: Total Cholesterol, TG: Triglycerides, PPE: *Peucedanum pastinacifolium* hydroalcoholic Extract

Table 3 - Effect of PPE on serum lipid profiles of STZ-induced diabetic rats.

Groups	TC (% of control)	TG (% of control)	LDL-C (% of control)	HDL-C (% of control)
Diabetic control	100	100	100	100
Treated groups				
125 mg/kg B.W. PPE	89 ± 7	83 ± 8	91 ± 7	90 ± 7
250 mg/kg B.W. PPE	83 ± 8	86 ± 9	79 ± 9	98 ± 9
500 mg/kg B.W. PPE	70* ± 6	79* ± 7	40* ± 6	190* ± 10
5 mg/kg B.W. Glybenclamide	63* ± 6	52* ± 6	38* ± 6	186* ± 9

Values are the mean ± SD (n=6). * Represents significant difference from diabetic control group at P<0.05. LDL-C: Low-Density Lipoprotein-Cholesterol, HDL-C: High-Density Lipoprotein-Cholesterol, TC: Total Cholesterol, TG: Triglycerides, PPE: Peucedanum pastinacifolium hydroalcoholic Extract, STZ: Streptozotocin

Diabetic rats treated with PPE (group IX) or glibenclamide (group VI) for 30 days had significantly reduced serum total cholesterol, triglyceride and LDL-C levels, whereas the HDL-C level was significantly increased compared to control diabetic rats (group II). Drug treatment restored these values to near control group levels (Table 3). Finally, the atherogenic index in groups treated with PPE (groups IX) or glibenclamide (group VI) were significantly decreased compared with the diabetic control group (group II). The treated animals had values similar to the normal levels seen in the control group (Table 4). The body weights and blood glucose levels at the initiation and end of the study period in normal and diabetic control groups are shown in Table 5.

DISCUSSION

DM is associated with profound alterations in the serum lipid and lipoprotein profiles and with an increased risk of

Table 4 - Effect of PPE on the atherogenic index of STZ-induced diabetic rats.

Groups	Atherogenic Index
Normal control	1.56 ± 0.26
Diabetic control	5.36 ± 1.96
Treated groups	
125 mg/kg B.W. PPE	5.29 ± 1.09
250 mg/kg B.W. PPE	4.49 ± 2.10
500 mg/kg B.W. PPE	1.26* ± 0.31
20 mg/kg B.W. Glybenclamide	1.09* ± 0.24

Values are the mean ± SD (n=6). * Represents significant difference from the diabetic control group at P<0.05. PPE: *Peucedanum pastin*acifolium hydroalcoholic Extract, STZ: Streptozotocin

coronary heart disease.[5] Lowering serum lipid levels through dietary changes or drug therapy is associated with a decrease in the risk of vascular disease and related complications.[25]

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Table 5 - Changes in body weight and blood glucose in normal and diabetic control animals.

Groups	B.W (gr)		Glucose (mg/dl)	
	1st day	30th day	1st day	30th day
Normal control	208 ± 20	285 ± 30	97 ± 25	118 ± 20
Diabetic control	220 ± 18	160 ± 25	398 ± 89	333 ± 52

Values are the mean ± SD (n=6). B.W: Body Weight

Many herbs and plant products have been shown to have hypolipidemic properties[7-12], and the hypolipidemic activity of PPE in hypercholesterolemic rats has been previously reported by the authors.[21]

In the present study, the ability of PPE to partially reverse the hyperlipideimia of STZ-induced diabetic rats is confirmed. Induction of diabetes in rats by administration of STZ led to the development of dyslipidemia. In our study, a marked increase in the lipid content of serum was found in STZ-induced diabetic rats (Table 1). The increase in the total serum cholesterol, triglyceride and LDL-C levels in the diabetic rats is mainly due to increased mobilization of free fatty acids from peripheral deposits, as insulin inhibits the hormone-sensitive lipase. The increase in the serum LDL-C level may also result from glycosylation of the lysyl residues of apoprotein B, which leads to a decrease in LDL metabolism due to a decrease in the affinity of LDL for its receptors. Our results show that the serum HDL-C level decreased in STZ-induced diabetic rats (Table 1). A number of observations indicate that the plasma HDL-C level is low in untreated diabetics .[26] The hypolipidemic potency of PPE following daily administration indicated that it effectively reduces serum TC, TG and LDL-C levels and increases the serum HDL-C level in diabetic rats. Importantly, it had no effect on serum lipid profiles in normal rats (Tables 2 and 3).

The mechanism(s) of the hypolipidemic actions of PPE are not known; however, they could be mediated by control of tissue metabolism and improved insulin secretion and action because insulin lowers lipid levels and normalizes lipids in STZ-induced diabetic rats.[27]

The increase in the HDL-C level achieved by PPE significantly decreased the treated rats' atherogenic index (Table 4). The HDL level inversely correlates with the risk of atherosclerotic cardiovascular disease. HDLs protect against or reverse atherosclerosis by their ability to serve as acceptor particles for macrophage cholesterol efflux, prevention of endothelial dysfunction and maintenance of endothelial integrity.[28, 29] Thus, PPE has the potential to prevent the formation of atherosclerosis and coronary heart disease, which are secondary diabetic complications of severe DM.

In conclusion, alterations in lipid profiles were restored to near normal levels by PPE treatment of rats with experimentally induced diabetes. Several authors reported that secondary metabolites, such as furanocoumarines, saponins, flavonoids, phenolic compounds, and triterpenoids, have hypolipidemic activity.[30-32] Hence, the hypolipidemic and anti-atherogenic properties of PPE may be due to different types of active secondary metabolites, each with a single or diverse range of biological activities. Further biochemical and pharmacological investigations are in progress to isolate and identify the active compounds in *P. pastinacifolium*.

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