Effects of *Pinus eldarica* Medw. Nut Extract on Blood Glucose and Cholesterol Levels in Hypercholesterolemic Alloxan-induced Diabetic Rats

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Abstract

Background: Diabetes mellitus type 2 with dyslipidemia is a common disease. Previous studies suggest that chemical constituent present in *pinus eldarica* (*P. eldarica*) nut posesess antioxidant properties and positively affect glucose metabolism. However blood glucose and cholesterol lowering effects of *P. eldarica* nut have not been studied so far.

Objective: The present study was undertaken to explore the possiblility of anti-hyperglycemic and anti-hypercholesterolemic effects of the *P. eldarica* nut extract in hypercholesterolemic diabetic rats.

Methods: Sixty male wistar rats six months of age from central animal house of the institute of medicinal plants were selected. 10 rats were kept as normal group and diabetes was induced in the remaining rats by intraperitonial injection of 120 mg/kg aloxan monohydrate. After one week the diabetic rats with fasting blood glucose between 180 to 250 mg/kg were assigned to 5 groups of 10 rats each and were fed on hypercholesterolemic diet. One group was kept as control group (untreated diabetic rats) and *P. eldarica* nut extract in doses of 50, 100, 200 and 400 mg/kg was gavaged daily to the remaining rats. After one month, the fasting blood glucose, cholesterol and triglyceride levels were determined in all groups.

Results: The results indicate that fasting blood glucose in 200 and 400 mg/kg *P. eldarica* nut extract treated groups significantly decreased (P=0.000 and P=0.000) and fasting blood cholesterol and triglyceride levels did not change significantly compared with control group.

Conclusion: *P. eldarica* nut extract lowers blood glucose level withought affecting blood cholesterol and triglyceride levels in hypercholesterolemic diabetic rats.

Keywords: *Pinus eldarica* nut, Blood glucose, Rat, Diabetes, Hypercholesterolemia

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Introduction

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post-prandial blood glucose levels. Diabetes mellitus type 2 is one of the most prevalent and fastest growing diseases in almost all countries [1]. Apart from conventional anti-diabetic therapy, several studies have shown that medicinal plants have beneficial effects and improve glucose homeostasis in diabetic patients [2, 3]. A number of potential anti-diabetic and anti-hyperlipidemic plants were used by herbalists in the Iranian folk medicine [2]. It has been reported that medicinal plants with antioxidant properties or dietary antioxidant intake have beneficial effects on diabetes and hyperlipidemia [4-6]. *P. eldarica* nut is one of the dietary antioxidants which is used as food and medicine in several countries and contains several phenols and essential fatty acids with antioxidant properties [7]. The Iranian pine, *Pinus eldarica* Medw. (*P. eldarica*) belongs to the botanical family pinaceae and is native to Transcaucasian region between Europe and Asia, and has been widely planted in Iran, Afghanistan and Pakistan [8, 9]. Various parts of *P. eldarica* (e.g., needles, buds, resin and nuts) have been widely used in traditional medicine for the treatment of bronchial asthma, skin wounds, skin irritations, allergic rashes and dermatitis in Russia and the Central Asian countries [10, 11]. In a study, analysis of the *P. eldarica* nut oil indicated several components including: β-caryophyllene, α-pinene, longifolene, α-humulene, δ-3-carene and β-pinene with antioxidant properties [7]. In a previous unpublished study, we found high concentrations of total polyphenols (table 1) and fatty acids in *P. eldarica* nut indicating its antioxidant properties. Experimental studies in fact, strongly support the efficacy of polyphenols in chronic diseases including diabetes mellitus [12, 13]. In view of the traditional use of *P. eldarica* nuts as food and medicine and its established antioxidant properties, the present study was conducted to evaluate the possible anti-hyperglycemic and anti-hypercholesterolemic effects of *P. eldarica* nut in diabetic hypercholesterolemic rats.

Material and Methods

Plant material

P. eldarica cones were collected from Chitgar forest park (West of Tehran). The cones were collected between June and July of 2010. *P. eldarica* is preserved in the herbarium of Institute of Medicinal Plants (ACECR). Herbarium code of *P. eldarica* is 689 and it was identified by M. Ahvazi. Samples were dried in a dark place and at room temperature. The nuts were removed from cones and grouned to a powder by grinder.

Preparation of extract

The hydroalcholic extract of *P. eldarica* nut powder was prepared using 70% ethanol in water, using percolation method at room temperature. The powdered plant material was soaked initially in a solvent in a percolator and then sufficient amount of the solvent was added to cover material and kept for 24 hours with occasional stirring. The outlet of the percolator then was opened and the liquid contained therein was allowed to drip slowly. The procedure was repeated twice and the combined extractions was clarified by filtration and concentrated to dryness on rotatory evaporator at a maximum of 40ºC

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temperature and under reduced pressure. The extract in the dosages of 50, 100, 200 and 400 mg was suspended in vehicle of 3% Tween 80 in distilled water.

Polyphenols determination

The polyphenols content of *P. eldarica* nut was determined by the High-performance liquid chromatography (HPLC) method developed by Dogan *et al.* [14].

Hypercholesterolemic diet

The hypercholesterolemic diet was prepared according to modified metod of Groot AP et al. In brief 3% cholesterol powder, 1% cholic acid and 6% animal ghee were mixed with 90% rats chow powder [15]. The food mixture was shaped into small pellets by a hand operated device and then dried it in oven at 50ºC temperature.

Animals

60 adult male wistar rats aged six months with 200 ± 10 g weight were purchased from the central animal house of institute of medicinal plants. The animals were housed under standard conditions of light and dark cycle (12 hr light and 12 hour dark) with free access to food and water one week before starting the study.

Study protocol

10 rats were kept as normal group and alloxan monohydrate was injected intraperitonially in the doses of 120 mg/kg to the remaining 50 rats [16]. After one week the diabetic rats with fasting blood glucose between 180 to 250 mg/kg were selected and caged in five groups of 10 rats each and fed on hypercholesterolemic diet. One group was kept as control group (untreated diabetic rats) and *P. eldarica* nut extract in doses of 50, 100, 200 and 400 mg/kg was gavaged daily to the remaining rats for one month. In control group, extract vehicle was gavaged daily for one month. After one month the fasting blood glucose, cholesterol and triglyceride levels were determined. The experiment protocol was approved by the Institute of Medicinal Plants ethical committee. The above doses were selected according to a previous pilot study in which the anti-hyperglycemic effect of several *P. eldarica* nut extract doses was tested in diabetic rats for three days.

Blood samples were drawn after 6 hour fasting. Blood glucose levels were determined by the glucose-oxidase method using Beckman Glucose-2 Analyzer. Blood cholesterol and triglyceride levels were measured by the auto analyzer Hitachi 902 using commercially available kits (Pars Azmon).

Statistical analysis

All values are expressed as means ± SE. Data obtained were analysed using student's t-test to determine the statistical significance. $p < 0.05$ was considered as significant.

Results

The values for polyphenols contents of the *P. eldarica* nut are presented in Tables 1.

In alloxan induced diabetic rats fed on hypercholesterolemic diet the fasting blood glucose and cholesterol levels were significantly increased compared with normal (non-diabetes) group. In *P. eldarica* nut extract treated groups in the doses of 200 and 400 mg/kg, fasting blood glucose levels significantly ($p=0.000$ and $P=0.000$) decreased compared with control group (Table 2).

Administration of *P. eldarica* nut extract in different doses (50, 100, 200 and 400 mg/kg)

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did not producing any significant changes in blood cholesterol and triglyceride levels compared with control group (Table 2).

Table 1- The polyphenols content (mg/1000 g extract) of *P. eldarica* nut (HPLC method)

Polyphenols	mg/1000g extract
Total phenols	483
Catechin	10.1
Epicatechin	10.3
Gallic acid	1.6
Vanillic acid	—
Para-coumaric acid	1.4
Ferullic acid	1.7
Ortho-coumaric acid	0.12
Tyrosol	29.1
Dimers of catechin and epicatechin	7.5
Unknown	38.18

Table 2- The effects of different doses of *P. eldarica* nut extract on fasting blood parameters of diabetic rats (n=10)

	Fasting blood glucose (mg/dl)	P- value compared to control	Triglyceride (mg/dl)	P- value compared to control	Cholesterol (mg/dl)	P- value compared to control
Normal	94.2 ± 20.7		98.2 ± 11.5		84.1 ± 9.3	
Control	168.5 ± 25.3*	P=0.000	114.1± 33.5	P=0.068	98.8 ± 14.0	P=0.042*
***P. eldarica* nut extract 50 mg/kg**	172.2 ± 33.9	P=0.808	112.1 ± 21.9	P=0.196	103.2 ± 15.2	P=0.261
***P. eldarica* nut extract 100 mg/kg**	165.8 ± 22.3	P=0.842	109.5 ± 33.5	P=0.167	104.1 ± 15.3	P=0.496
***P. eldarica* nut extract 200 mg/kg**	115.7 ± 19.5**	P=0.000	106.0 ± 15.6	P=0.112	99.8 ± 14.6	P=0.889
***P. eldarica* nut extract 400 mg/kg**	114.8 ± 13.0**	P=0.000	107.9 ± 12.8	P=0.129	100.8 ± 16.0	P=0.811

Values are given as Mean ± SD.
*= comparison with normal (non-diabetic) group (student t-test).
**= comparison with control group (student t-test).

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Discussion

In the present study we found that *P. eldarica* nut extract reduced fasting blood glucose in diabetic rats fed on hypercholesterolemic diet without any significant effects on cholesterol and triglyceride. The mechanisms involved in the blood glucose lowering effect of the *P. eldarica* nut extract are not yet clearly established. However few theories try to explain the mechanisms involved in its effect on hyperglycemia. It has been reported that fatty acids an important component of *Pinus nut oil* by suppressing appetite may contribute to a successful caloric-restriction regimen and directly influence the blood glucose metabolism [17]. Another suspected anti-hyperglycemic mechanism may be due to effects of antioxidant components of *P. eldarica* nut extract. Hyperglycemia causes oxidative damage by generation of reactive oxygen species resulting in the acceleration of onset and progression of diabetes [18]. There are increasing evidences that plant polyphenols offer protection against oxidative stress and positively influence carbohydrate metabolism [19, 20]. *P. eldarica* nut contains appreciable amounts of phenolic compounds and other chemicals such as α-pinene, β-pinene and β-caryophyllene with antioxidant properties [7, 21, 22]. The anti-diabetic and antioxidant activities of α-pinene, β-pinene, β-caryophyllene present in Juglans regia L. leaf essential oil have been reported [23, 24]. As these chemicals are important components of *P. eldarica* nut [7], the anti-hyperglycemic effect observed in the present study may be due to effects of these components on body metabolism of diabetic rats.

Conclusion

In conclusion, since *P. eldarica* nut induced significant anti-diabetic effects in hypercholesterolemic diabetic rats, further studies for determination of its active constituents and possible mechanisms responsible for anti-hyperglycemic effects are recommended.

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