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

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Received: 1 Dec. 2012

Accepted: 17 Mar. 2013

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 possess antioxidant properties and positively affect glucose metabolism. However blood glucose and cholesterol lowering effects of *P. eldarica* nut have not been studied so far.

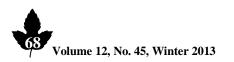
Objective: The present study was undertaken to explore the possiblility of anti-hyperglycemic and anti-hypercholesterolemic effects of the *P. eldarica* nut extract in hypercholesterolemic diabetic rats.

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



Introduction

Diabetes is а chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and postprandial 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 antidiabetic therapy, several studies have shown that medicinal plants have beneficial effects and improve glucose homeostasis in diabetic patients [2, 3]. A number of potential antidiabetic and anti-hyperlipidemic plants were used by herbalists in the Iranian folk medicine [2]. It has been reported that medicinal plants antioxidant properties with 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 40°C



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 chromatography (HPLC) liquid method developed by Dogan et al. [14].

Hypercholesterolemic diet

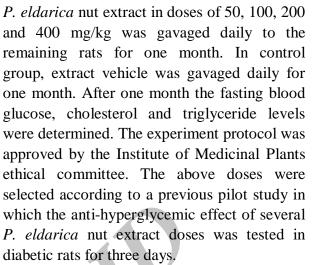
The hypercholesterolemic diet was prepared according to modified metod of Groot AP et al. In brief 3% cholesterol powder, 1% cholic acid and 6% animal ghee were mixed with 90% rats chow powder [15]. The food mixture was shaped into small pellets by a hand operated device and then dried it in oven at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



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

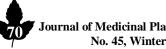
All values are expressed as means \pm SE. Data obtained were analysed using student's ttest to determine the statistical significance. p < 0.05 was considered as significant.

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 blood mg/kg, fasting 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)



Journal of Medicinal Plants, Volume 12, No. 45. Winter 2013

did not producing any significant changes in blood cholesterol and triglyceride levels

compared with control group (Table 2).

Table 1- The polyphenols content (n	mg/1000 g extract) of P.	eldarica nut (HPLC method)
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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*			
<i>P. eldarica</i> 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			
<i>P. eldarica</i> 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			
<i>P. eldarica</i> 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			
<i>P. eldarica</i> 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 \pm 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 fed glucose in diabetic rats on hypercholesterolemic without diet any significant effects on cholesterol and triglyceride. The mechanisms involved in the glucose lowering effect of the blood 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 influence directly the blood glucose metabolism [17]. Another suspected antihyperglycemic 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 increasing evidences that are 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.

Acknowledgements

This research was supported by a grant from the Institute of Medicinal Plants (ACECR), Karaj, Iran and Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences Tehran, Iran.

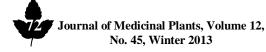
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