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Hypocholesterolemic Effects of Purslane Extract on Serum Lipids in Rabbits Fed with High Cholesterol Levels

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Abstract: The purpose of this study was to investigate the effect of hydroalcoholic extract of Purslane leaves on serum lipids of rabbits fed with a hypercholesterolemic diet. Therefore different groups of animals were fed either the normal chow diet or a diet enriched in cholesterol (0.5%). Moreover, hypercholesterolemic animals were treated with or without different doses of Purslane extract (200, 400, 800 mg kg^{-1} body weight) orally for 12 weeks. Blood samples were obtained at 0, 6 and 12 weeks after treatment to analyze serum lipids and Atherogenic Index (AI) which is equal to total cholesterol- HDL cholesterol/HDL cholesterol. We show that the serum total cholesterol decreased in all groups treated with purslane extract. Its also found that the distribution of cholesterol between lipoproteins were Changed so, low density lipoprotein cholesterol (LDL-C) decreased significantly in all of the groups treated with purslane extract with respect to positive control group. All treated animals also showed a decrease in AI These findings indicate that this plant may be useful for the treatment of hypercholesterolemia.

Key words:*Portulaca oleracea* (Portulacaceae), purslane, antihypercholesterolemic, antihyperlipidemic, rabbit

INTRODUCTION

Hypercholesterolemia has been considered as a major risk factor for coronary heart disease and atherosclerosis. Hyperlipidemia, particularly elevated serum cholesterol and Low-Density Lipoproteins (LDL) levels, is a risk factor in the development of atherosclerotic heart disease (Romero-Corral *et al*., 2006). Although a range of synthetic drugs are available as antihyperlipidemic drugs, many of them do not fulfill all requirements and their numerous side effects and potential interference with drug metabolism are common (Moosmann and Behl, 2004). The search for compounds from nutraceutical sources for reduction of serum cholesterol and reduction of hypercholesterolemic atherosclerosis is on (Sajjadi *et al*., 1998; Movahedian *et al*., 2006; Prasad, 2005). Thus a survey among medicinal herbs is also still important and might provide a useful source for therapy or alternatively as simple dietary adjuncts to existing therapies. *Portulaca oleracea* (family Portulacaceae), commonly known as purslane in English and khorfeh in Persian is an herbaceous weed. It can be found growing wild or cultivated in the world (Nafisi, 1986; Minaiyan *et al*., 2005). Purslane leaves have been used in foods like soups, salads and pickles and Iranian folk medicine to treat several disorders such as hyperlipidemia (Nafisi, 1986), pain and inflammatory disorders and some other urinary and topical diseases (Minaiyan *et al*., 2005; Chan *et al*., 2000). *P. oleracea* contains many biologically active compounds and is a source of many nutrients. Some of the active compounds include omega-3 fatty acids, flavonoids, coumarins, vitamins A, C and E, β-carotene, melatonin, dopamine, noradrenalin, oxalates and minerals and traces of alkaloids, cardiac and anthraquinone glycosides (Chan *et al*., 2000; Simopoulos *et al*., 2005; Liu *et al*., 2000; Xu *et al*., 2006; Chen *et al*., 2003; Palaniswamy *et al*., 2004; Xiang *et al*., 2005; Lim and Quah, 2007). Compared to other vegetables, it has high contents of omega-3 fatty acids and protein (Liu *et al*., 2000). In pharmacological tests and clinical trials, different extracts of *P. oleracea* are reported to have analgesic, anti-inflammatory, smooth and skeletal muscle relaxant, anti-convulsant, anti-ulcerogenic, wound healing, bronchodilatory, antioxidant and antifungal effects (Karimi *et al*., 2004; Rashed *et al*., 2003; Malek *et al*., 2004; Oh *et al*., 2000). Because the traditional

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and popular uses of purslane leaves in Iran are supported by a long history of human experience, this plant may be a valuable source for the isolation of potential drugs. The purpose of the present study was to evaluate the antihyperlipidemic activities of the hydroalcoholic extract of the plant leaves in rabbits using the standard tests.

MATERIALS AND METHODS

Plant material and extraction: The leaves of *P. oleracea*, growing wild in Zarrinshahr, near to Isfahan province, were collected in June 2003. The herbarium department of Shiraz Faculty of Pharmacy, Shiraz, Iran, confirmed the plant identity as *P. oleracea*. A voucher specimen was deposited in the herbarium of our school (No. 1056). For preparation of hydroalcoholic extract, air-dried and powdered leaves of the plant (100 g) were percolated with 1000 mL of EtOH-H_2O (7:3) for 72 h. The extract was then shaked, filtered and evaporated in a rotating evaporator under reduced pressure until dryness (Hajhashemi *et al.*, 2003). Evaporation and solvent removal of hydroalcoholic extract gave semi-solid mass yielded 10.8% w/w.

Phytochemical analysis of purslane: The phytochemical profile of the purslane leaves was determined according to the Iranian Herbal Pharmacopoeia (2003) for detection of alkaloids, saponins, anthraquinones, tannins, flavonoids and cardiac glycosides. Phytochemical analysis was also carried out by TLC of purslane extract using conventional protocols (Wagner and Bladt, 1996; Harborne, 1998; Iranian Herbal Pharmacopoeia, 2003).

Diet and treatment: Thirty Male New Zealand white rabbits weighing 1.5 kg on arrival were obtained from the central animal house of the Tehran Pasteur Institute, Tehran, Iran. The animals were housed in temperature (21-23°C) and light controlled room with a 12 h light-dark cycle and ambient humidity (50-60%). All the rabbits were initially fed a normal diet (Pars Dam, Tehran, Iran) for 2 weeks and then randomly divided in to a hypercholesterolemic control group (n = 6, fed 0.5% high cholesterol diet) and three treatment groups (n = 6, fed 0.5% high cholesterol diet along with 200, 400, 800 mg kg^{-1}.b.w of purslane leaf extract orally by feeding tube). Rabbits fed a normal diet were used as normal control. Animals were fed for 12 weeks and each diet was set 100 g $rabbit^{-1}$ with water available ad libitum (Movahedian *et al.*, 2006).

Experimental procedure: The rabbits were weighed weekly. Blood samples from a marginal ear vein were taken at 0, 6 and 12 weeks after 12 h of fasting to monitor any changes in the total cholesterol, triglyceride (TG) and lipoprotein cholesterol. Serum was separated by centrifugation (2000 g, 20 min, 4°C) and used for biochemical analysis. Serum cholesterol and triglyceride levels were determined using commercially available kits (Pars Azmoon, Tehran, Iran). The lipoprotein cholesterol concentrations (HDL-C and LDL-C) were assessed colorimetrically with an enzyme assay kits (Pars Azmoon, Tehran, Iran). The atherogenic index (total cholesterol-HDL cholesterol/HDL cholesterol) was also calculated. All data are presented as mean±SD. Statistical analysis of the data was done using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. Statistical significance was accepted at $p<0.05$.

RESULTS

The phytochemical analyses performed in the present study showed that the plant and its extract contain alkaloids, flavonoids and cardiac glycosides while saponins, anthraquinones and tannins were absent.

All groups exhibited a body weight increase during the experimental period, whereas there were no significant differences in the body, liver and heart weights between the groups (results not showed). The serum concentrations of total cholesterol and lipoproteins cholesterol (LDL-C and HDL-C) were increased in hypercholesterolemic control group with respect to the normal control group in the period of 12 weeks (Table 1). The effects of purslane leaf extract on serum lipids in rabbits fed high cholesterol diet are presented in Fig. 1. The results showed that upon administration of hydroalcoholic extract of purslane for 12 weeks, serum triglyceride, total cholesterol, LDL-C and VLDL-C decreased significantly at doses of 200, 400 and 800 mg kg^{-1} bw in comparison with the

Table 1: Levels of serum lipoprotein lipids and atherogenic index (AI) in cholesterol fed rabbits compare with normal control *

Group	Time (weeks)	Total cholesterol	Triglyceride	LDL-C	HDL-CAI	AI
		(mg dL^{-1})				
NC	0	108.0±18	72.0±13	57.0±21	19±4	4.7
	6	98.0±17	77.0±17	46.0±19	18±6	4.4
	12	110.0±19	70.0±15	51.0±21	20±6	4.5
HC	0	101.0±16	65.0±14	49.0±20	18±4	4.6
	6	895.0±59**	275.0±22**	465.0±57**	41±7**	20.1**
	12	1628.0±79**	393.0±29**	881.0±74**	58±9**	27.1***

* Values are means±SD (n = 6), ** Data In columns were significantly different at $p<0.05$. NC: Normal Control; HC: Hypercholesteroleruic Control

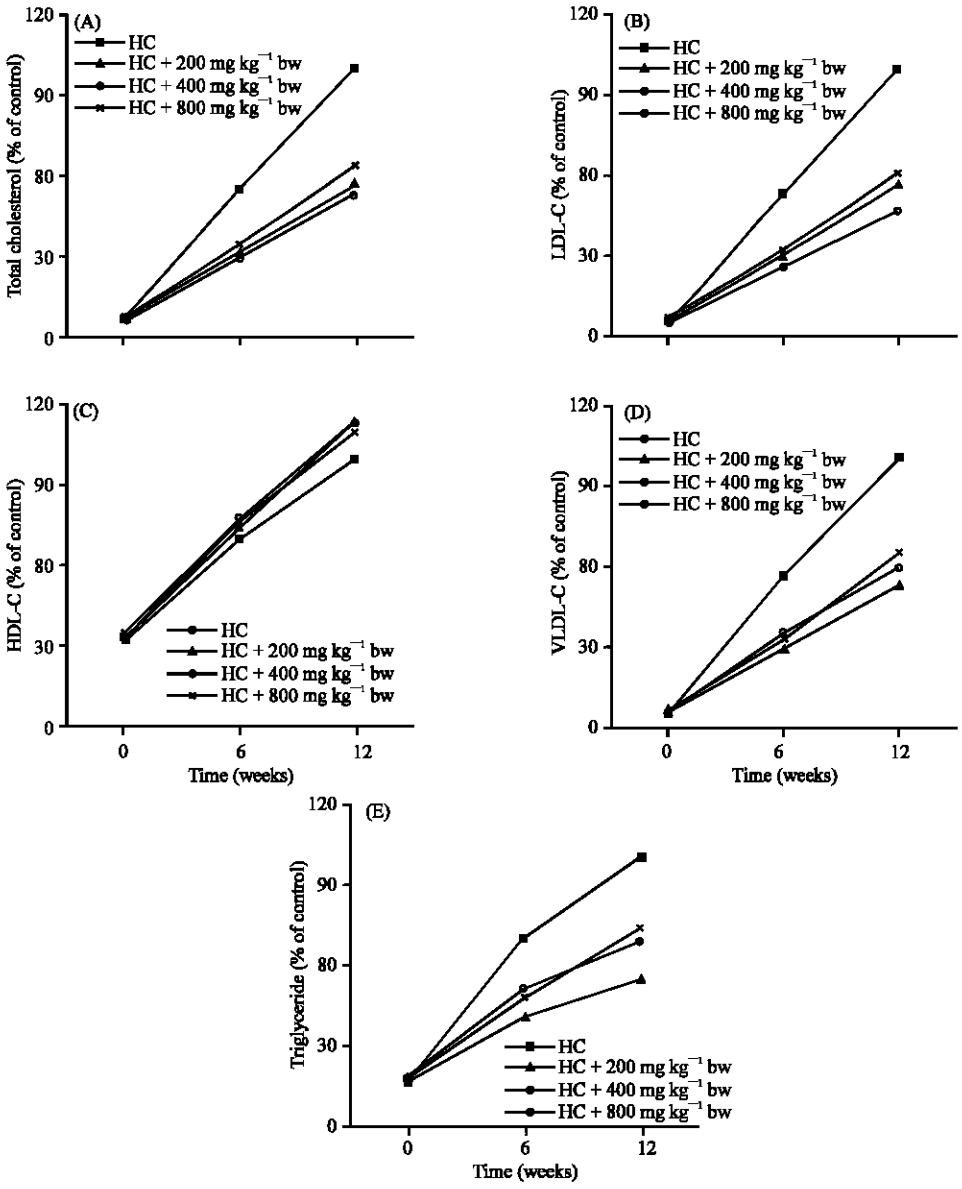

Fig. 1: Lipid profiles at 0, 6 and 12 week of treatment with 200, 400, 800 mg kg^{-1} of body weight (bw) of purslane leaf extract compare with Hypercholesterolemic Control (HC)

Table 2: Effect of purslane leaf extract on atherogenic index in cholesterol fed rabbits at 12 weeks of treatment

Group	Atherogenic index
Normal control	4.5±1.1
Hypercholesterolemic control	27.1±2.3
Purslane extract (mg kg^{-1} of body weight)	
HC + 200	15.2±1.6[a]
HC + 400	12.4±1.3[a]
HC + 800	15.4±1.2[a]

Data are mean±SD (n = 6); [a]Data are significantly different at $p<0.05$

hypercholesterolemic control group (Fig. 1). But serum HDL-C was elevated insignificantly in hypercholesterolemic rabbits treated with the above doses of purslane extract (Fig. 1). Oral administration of purslane leaf extract also showed a decrease in atheroginic index in all treatment groups with respect to the hypercholesterolemic control group (Table 2).

DISCUSSION

The elevation in serum total LDL and HDL cholesterol observed in our study upon cholesterol feeding is not surprising and is in agreement with several studies

(Prasad and Kalra, 1989; Keaney *et al*., 1993). In contrast, a significant decrease in plasma HDL-cholesterol in cholesterol-fed rabbits was also reported by Tsai and Chen (1979). However it was obvious in our study that the dramatic increase in serum total cholesterol mainly originated from the remarkable increase in LDL cholesterol rather than HDL-cholesterol. Hypercholesterolemia could produce oxidative stress in various ways and increases synthesis of arachidonic acid and prostaglandins. Reactive Oxygen Species (ROS) generation is increased during synthesis of prostaglandins and leukotrienes. ROS have been implicated in the development of hypercholesterolemic atherosclerosis (Prasad, 2005).

With regard to the effect of dietary supplements, our results showed that addition of purslane leaf extract to the cholesterol-enriched diet improved the hypercholesterolemia induced by a high-cholesterol diet in rabbits. The results showed that upon administration of hydroalcoholic extract of purslane for 12 weeks significantly decreased the serum total cholesterol, LDL-C and VLDL-C at doses of 200, 400 and 800 mg kg^{-1} bw. in comparison with the hypercholesterolemic group (Fig. 1). But serum HDL-C was elevated insignificantly in hypercholesterolemic rabbits treated with the above doses of purslane extract (Fig. 1). Most of the reduction in serum cholesterol occurred in the fraction of LDL. Because of apo B containing lipoprotein fractions are thought to be responsible for cholesterol deposition in atherosclerotic plaques (Schaefer and Asztalos, 2006), a reduction in LDL would be advantageous clinically and in fact it was shown clearly that the present crude extract had an improving effect on the hypercholesterolemia induced by a high fat diet.

Purslane treated animals also showed a decrease in the atherogenic index with respect of hypercholesterolemic groups (Table 2), which is generally believed to be beneficial since the HDL level inversely correlated with coronary heart disease and reduction in this ratio is considered as an anti atherosclerotic factor.

It has reported that some flavonoids, omega 3 fatty acids and melatonin have antihyperlipidemic properties. Melatonin recently identified in fresh purslane leaves. The melatonin concentration in purslane was found to exceed that reported in a number of other fruits and vegetables (Simopoulos *et al*., 2005). Melatonin has a variety of important functions including direct free radical scavenging and anti-inflammatory properties (Simopoulos *et al*., 2005; Rodriguez *et al*., 2004). Hoyos *et al*. shows that the increase in total cholesterol and LDL-C induced by the cholesterol enriched diet was reduced significantly by melatonin administration (Hoyos *et al*., 2000). The presence of these compounds in purslane leaf may play a role in the observed hypocholesterolemic effects.

The use of *P. oleracea* in Iranian folk medicine for hyperlipidemia has been justified by this work, as it showed antihypercholesterolemic activities in rabbits. Significant antihypercholesterolemic and antihyperlipidemic activities of the plant leaves have been found in this model, suggesting a rational basis for folk and traditional uses of this herb in Iran. Since the role of omega-3 fatty acids, antioxidant vitamins and other natural antioxidants like melatonin in hyperlipidemia are very clearly defined, antihypercholesterolemic and antihyperlipidemic potential of *P. oleracea* may be partly due to the antioxidant activity of the plant. Further experiments are needed to test the effect of these plant fractions in future.

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