

The Effects of *Portulaca Oleracea* Alcoholic Extract on Induced Hypercholesterolemia in Rats

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Article information	Abstract
<p>Article history: Received: 12 Nov 2011 Accepted: 6 May 2012 Available online: 29 Oct 2012 ZJRMS 2013; 15(6): 34-39</p> <p>Keywords: Atorvastatin Cholesterol Hypercholesterolemia <i>Portulaca oleracea</i> Triglyceride Rat</p> <p>*Corresponding author at: Department of Physiology, Arak University of Medical Sciences, Arak, Iran. E-mail: dr.ashtiyani@arakmu.ac.ir</p>	<p>Background: Hypercholesterolemia is a major risk factor for cardiovascular diseases. <i>Portulaca oleracea</i> is a vegetable, known in traditional medicine and has many medicinal properties. In the present study the effects of alcoholic extract of <i>Portulaca oleracea</i> on blood fat profiles are evaluated.</p> <p>Materials and Methods: In this experimental study, 60 Wistar rats in the weight range of 170±5 g in 6 groups (n=10) were selected as follows: Control group receiving normal diet, Sham group receiving high fat diet, experimental groups receiving the <i>Portulaca oleracea</i> extract with maximum dose(800 mg/kg), moderate dose (400 mg/kg) and minimum dose (200 mg/kg) as intraperitoneally and injection of 10 mg/kg atorvastatin and treated with high fat diet for 21 days. After the end of this period, blood sampling and measuring obtained samples, data was analyzed using SPSS-11.5 software</p> <p>Results: Based on the results obtained from all groups receiving the extract of <i>Portulaca oleracea</i> herb, it was found that the level of cholesterol concentration in these groups and the level of cholesterol and triglyceride concentrations in the group receiving atorvastatin significantly decreased ($p \leq 0.05$), but the plasma concentration of high and low-density lipoproteins did not show any significant changes.</p> <p>Conclusion: The results of this study show that oral intake of alcoholic extract of <i>portulaca oleracea</i> can play an important role in reducing cholesterol levels, similar to the use of atorvastatin. This performance is probably more related to the high density of antioxidants and omega-3 found in this herb and mechanisms of the cholesterol synthesis inhibition.</p>

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Introduction

Coronary diseases are of the most important and common diseases of recent years that have been spread in a wide range of advanced and poor societies, in all ages especially in middle ages [1]. The main cause of coronary artery disease is atherosclerosis which is now the most frequent cause of death in developed countries.

Spread of this disease is specifically attributed to the cholesterol and primarily to the lipid metabolism [2]. According to the various reports, more than 40% of deaths in Iran are due to the cardiovascular disease [3]. Studies show that high levels of cholesterol in the blood cause atherosclerosis and arterial disease and often lead to the heart attack. Low-density lipoprotein (LDL) is the main cholesterol-carrying lipoprotein in the blood which causes delivery of cholesterol from liver to the peripheral tissues.

LDL enters into the peripheral cells through endocytosis and delivers its contents to the peripheral cells. High-density lipoprotein (HDL) is involved in the transition of cholesterol to the center and in this process; HDL takes additional cholesterol away from the tissue [4].

Diet rich in monounsaturated fatty acids such as olive oil increases serum levels of HDL and decreases serum levels of LDL; while the multi-saturated oils decrease HDL and LDL levels [5]. Accordingly, discovery of cholesterol-lowering drugs is one of the priorities of pharmacology.

So, today, statins are used as the most common lipid-lowering drugs. In 2007, statins experienced a worldwide sale of \$ 35 million. Atorvastatin is one of the widely consumed types of statins [3].

All kinds of drugs belonged to the statin family inhibit cholesterol synthesis through inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). But side effects including increased liver enzymes in the blood have been reported as the side effects of taking these drugs. Therefore many studies have been conducted on the existence of an uncertainty in making decision about the use of statins by people suffered from coronary artery disease [6].

Hence, researchers are always looking for an optimal treatment that can overcome hyperlipidemia with minimal side effects. Therefore, drugs with natural origin, especially medicinal plants, have been considered for

many years and *Portulaca oleracea* is one of those plants that could be used in this regard [7, 8].

Khorfeh (In Persian) with the scientific name of *Portulaca oleracea* from the family of Oleracea (Fig. 1) is a herbaceous and yearling plant with a fleshy stem and thick, green and succulent leaves which facing each other and red stems and yellow or white small flowers and small black seeds that have medicinal properties. This plant grows in the most parts of the planet and today it is found in most of the countries as wild or cultivated herb. From the perspective of traditional medicine, the nature of *Portulaca oleracea* is cold and wet, astringent and diuretic, bile anodyne that relieves temperature of blood, liver and stomach. *Portulaca oleracea* is also useful in the elimination of headaches, thirst relief, stoppage of any bleeding, crushing of bladder stones, and reduction of coughing and irritation of the urethra, bladder, intestines and hemorrhoids.

Portulaca oleracea seed has anti-tapeworm properties and its stem and leaf extract is useful for the liver disease and kidney pain. Water, glaze material, pectin, protein, carbohydrates, unsaturated fatty acids, various antioxidants and minerals, including iron (F), copper, manganese, potassium, calcium, phosphorus and selenium are found in different parts of this herb. The amount of protein in this herb has been reported as 44.25 grams in 100 grams of dried leaves. It has also plenty of antioxidant compounds such as alpha-tocopherol, ascorbic acid and glutathione. *Portulaca oleracea* is also a good source of coenzyme Q₁₀ [1]. Photochemical experiments conducted on *Portulaca oleracea* extract showed that this herb contains vitamins B₁ and A, noradrenaline, dopamine, organic acids such as cinamic, caffoic, malic, oxalic, citric acids and also coumarins, flavonoids, cardiac anthraquinone glycosides and quercetin alkaloids [9]. It is noteworthy that no sign of significant toxicity associated with this plant has been reported yet [10].

Portulaca oleracea is the richest herbal source of omega 3 fatty acids [11]. Studies have shown that increased consumption of omega-3 fatty acids are often associated with the reduced risk of cardiovascular diseases and omega-3 fatty acids are effective in reducing the progression of cardiovascular diseases and reduction in the mortality resulted from this disease. Meanwhile, substantial reduction in the consumption of blood triglycerides containing Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) has been recently reported [11-13].

Therefore in this study the possible effects of oral consumption of alcoholic extract of *Portulaca oleracea* (stems and leaves) on hypercholesterolemia occurred in the rats and its comparison with the conventional oral drug (i.e. atorvastatin) used to treat hypercholesterolemia are evaluated. Since the cholesterol-lowering effects of some plants containing antioxidants and alkaloids have been proven [11, 14, 15], and on the other hand, few clinical studies have examined these features, so the purpose of this study is to examine the possible effect of oral

consumption of alcoholic extract of *Portulaca oleracea* (stems and leaves) on hypercholesterolemia created in the rat and to compare its effects with the oral drug (i.e., atorvastatin) used to treat hyperlipidemia

Materials and Methods

This study is an experimental research and all animals used in this study have been provided from Propagation and Breeding Center of Razi Institute, Fars Province and were kept in standard conditions of temperature and light. Also it was carried out based upon compliance with all ethical codes for working with laboratory animals developed by the Ministry of Health and Medical Education. Prior to the commencement of the studies, all animals were weighted in order to be sure they are in a particular range of weight. Average weights of male rats used in this study were 170±5 g. Test duration was 21 days. 60 rats were used in this study and they randomly classified into six groups of ten rats as follows:

A- Control group: During the test period, animals in this group did not receive any medication and they treated with normal diet. B- Case group of injection: 0.2 ml solvent (normal saline) was daily injected to the rats with hypercholesterolemia. C- Experimental group 1: rats with hypercholesterolemia that were daily receiving 200 mg/kg (minimum dose) alcoholic extract of the portulaca oleracea herb as intraperitoneally (IP).

D- Experimental group 2: rats with hypercholesterolemia that were daily receiving 400 mg/kg (average dose) alcoholic extract of the *Portulaca oleracea* herb as IP.

E- Experimental groups 3: rats with hypercholesterolemia that were daily receiving 800 mg/kg (maximum dose) alcoholic extract of the *Portulaca oleracea* herb as IP.

F-Atorvastatin group: Rats with hypercholesterolemia that were daily receiving 10mg/kg atorvastatin (Shafa pharmaceutical company), as oral (edible) emulsion and gavages. In order to prepare a meal with 2% cholesterol, 20 grams of pure Merck cholesterol powder (Fluka Chemika) should be solved in 5 ml of heated olive oil and mixed with 1kg of rat food (rat chow). To avoid deterioration of animals' food, it was tried to keep their food in the refrigerator for two days [14] in order to prepare alcoholic extracts of *Portulaca oleracea* (Fig. 1) standard methods of extraction were used.

After preparation of aerial parts of *Portulaca oleracea* and cleaning and drying the herb, it was powdered and 600 grams of dry powder were poured in a sealed glass container and 96% medical alcohol was added to it. Then the mixture was set aside for about 72 hours in order to absorb the moisture well. After this period of time, the mixture was centrifuged following filtering and it was placed in a bath of warm water to enable its alcohol to be completely evaporated.

After evaporation of the alcohol, extract was still fluid due to the existence of water and for the complete evaporation of water, the extract were primarily placed in 40°C fore and then was placed in the vicinity of calcium

chloride. The mentioned extract cannot be completely dried or powdered due to having oily carotenoid compounds and always has a jelly status and the weight of obtained extract was measured as 13% compared to the dry fruit. All experimental groups were treated with the fatty diet during the experiment. The test period was 21 days and during this period injections and gavages were conducting at 9 am every day. Injection was intraperitoneally done using an insulin syringe; and 10mg/kg atorvastatin was prescribed as oral (edible) emulsion. After termination of this period and in order to evaluate concentrations of biochemical factors in plasma, a blood sample from heart was prepared through a mild anesthesia with ether. After blood centrifugation of 3000rpm, the serum was separated and transferred to the laboratory for measuring considered factors. Serum cholesterol and triglyceride levels were determined using kits produced by Iran Darman Kav Co. and calorimetric method. Lipoproteins were measured based on a combination of precipitation and ultracentrifugation methods, using the kits produced by Iran Kav Co. HDL cholesterol was measured using precipitation method.

At the first stage, the precipitation reagent was added to the serum in order non-lipoprotein compounds of HDL to be gathered in one place. Then these compounds were precipitated by centrifugation for 10 min. Thereafter HDL cholesterol was measured using an enzymatic method. LDL cholesterol was calculated (measured) based on the Friedwald formula [16]. Average results obtained from the serum concentrations of biochemical factors and activity of the liver enzymes in various groups were reported as the mean and standard error (Mean±SD) and SPSS version 11.5 statistical software, one-way ANOVA followed by Tukey and Duncan tests were used to do inter-group comparison, while considering $p \leq 0.05$ as the significance level.

Results



Figure 1. Portulaca Oleracea

Table 1. Evaluation of the effect of alcoholic extract of portulaca oleracea and atorvastatin on lipid profiles in rats, comparison between the effects of portulaca oleracea and atorvastatin and comparison between different doses of portulaca oleracea

Groups	Control	Sham	P. oleracea (200 mg/Kg)	P. oleracea (400 mg/Kg)	P. oleracea (800 mg/Kg)	Atorvastatin (10 mg/Kg)
Parameters						
Cholesterol	85±1	97.25±3.1*	74.87±3.6 #	74.37±4.7#	71.25±3.1#	79.62±2.4 #
TG	86±3.6	113±8.7*	114.62±6.6 μF	117.50±4.8αF	158.50±10.6F#	71.37±6.6#
LDL	31.25±2.6	41.62±3.7*	36.50±2.1μ	38.75±2.1α	54.504 F#	34.75±1.6
HDL	23.62±1	28±1.4	29.87±2.9	26.50±1α	31.65±0.7	28.28±1

* = indicates significant changes compared to the control group,

= indicates significant changes compared to the case group,

F = indicates significant changes compared to the atorvastatin,

μ = indicates significant changes between the minimum and maximum doses of *Portulaca oleracea*

α = indicates the significant changes between the average and maximum doses of *Portulaca oleracea*.

Comparison of the results of statistical tests about the effect of *Portulaca oleracea* extract and Atorvastatin on blood fat shows that the cholesterol levels in the case group were significantly increased compared to the control group ($p=0.01$) and all experimental groups receiving the extract of *Portulaca oleracea* (minimum, average and maximum dose) indicated a significant decrease compared to the case group ($p=0.01$). Results obtained about triglyceride (TG), indicate that TG levels increased significantly in the case group compared to the control group ($p=0.01$) and in the experimental group received a maximum dose of extract of portulaca oleracea (800 mg/kg) a significant increase was observed compared to the case group ($p=0.01$), but experimental groups received an average and minimum doses of *Portulaca oleracea* extract (400 and 200 mg/kg) didn't show a significant increase compared to the case group. About LDL, the case group indicated a significant increase compared to the control group ($p=0.04$).

And also group receiving the maximum dose of *Portulaca oleracea* extract (800 mg/kg) indicated a significant increase compared to the case group ($p=0.01$), but in the experimental group received an average and minimum doses of *Portulaca oleracea* extract (400 and 200 mg/kg) no significant changes were observed compared to the case group. About HDL, case group didn't show any significant changes compared to the control group and also the experimental groups receiving maximum, average and minimum doses of portulaca oleracea extract (400 and 200 mg/kg) showed a significant increase compared to the case group. About the experimental group receiving atorvastatin, cholesterol and TG levels were significantly decreased compared to the case group ($p=0.01$) However, LDL and HDL levels showed no significant changes compared to the case group.

Also results of the statistical tests about the evaluation of the comparison between the effect of *Portulaca oleracea* extract and atorvastatin on blood fat showed that as far as cholesterol is concerned, none of the experimental groups receiving a minimum, average and maximum dose of *Portulaca oleracea* showed significant changes compared to the group receiving atorvastatin.

About TG, all experimental groups receiving the extract of *Portulaca oleracea* show significant increase compared to the experimental group receiving atorvastatin ($p=0.01$). Therefore, *Portulaca oleracea* extract is more effective on TG than atorvastatin. In the case of LDL, only the experimental group receiving a maximum dose of *Portulaca oleracea* extract showed a significant increase compared to the experimental group receiving atorvastatin ($p=0.01$). But the experimental groups receiving minimum and average doses of *Portulaca oleracea* extract had no significant changes compared to the

The results of this study showed that the *Portulaca oleracea* extract, at all doses, reduces the level of cholesterol and increases TG, LDL and HDL levels at a maximum dose and atorvastatin also reduces cholesterol and triglycerides levels, but it was ineffective on LDL and HDL. The results also showed that the effect of the maximum dose of *Portulaca oleracea* on the reduction of cholesterol level and the increase in HDL and LDL levels and the effects of all doses of *Portulaca oleracea* on the increase in the level of TG are significant compared to the atorvastatin (Table 1). HDL has major role in protection against cardiovascular disease through reducing the conversion of LDL to the oxidized form of the initial state and the reduction of the effect of oxidized LDL. The main properties of HDL are due to the proteins associated with it. The main reason for the antioxidant property of HDL is due the paraoxonase -1 enzyme which is attached to the HDL and moves in the blood together with it.

In a study conducted by Samani et al. it was found that *Portulaca oleracea* can be involved in reducing risk factors of cardiovascular diseases through increasing the level of HDL, activity of the Paraoxonase-1 (PON1) and Apolipoprotein A-1 (APOA1) enzymes and also reducing the level of total cholesterol, cholesterol contained in LDL (LDL-C), oxidized low-density lipoprotein (oxLDL), and especially triglycerides. Diet rich in monounsaturated fatty acids such as olive oil increases HDL levels [17]. Therefore, since this herb contains unsaturated fatty acids with high nutritional value including oleic acid, linoleic acid and linolenic acid that have only one double bond in their structure and are powerful antioxidant substances; they are essential even for the completion of the feeding of many animal species, including human and are called essential fatty acids in terms of their nutritional value. They also regulate the inhibition of the occurrence of glycolysis and Lypogenesis enzymes; therefore reduction in the level of serum lipids, including cholesterol and the increase in the HDL level are justifiable [18, 18].

The results of this study also indicate significant decrease in the level of cholesterol in different

experimental group receiving atorvastatin. About HDL, none of the experimental groups receiving the minimum, average and maximum doses of *Portulaca oleracea* extract showed any significant changes compared to the experimental group receiving atorvastatin.

Comparison of different doses of *Portulaca oleracea* extract also showed that in the case of cholesterol, the changes are not significant between different doses of the extracts. About TG and LDL, the changes between minimum and average doses of the extract are significant compared to the maximum dose ($p=0.01$), but changes between minimum and average doses are not significant and in the case of HDL, only the comparison between average and maximum doses shows a significant change ($p=0.05$) (Table 1).

Discussion

concentrations of *Portulaca oleracea* extract which shows The effective role of the extract of *Portulaca oleracea* fruit in controlling serum cholesterol and the increase in the level of triglycerides and HDL (Table 1). Previous studies have shown that polysaccharides, flavonoids, oligo-proteins, poly peptides, steroids and alkaloids present in the medicinal plants can justify the properties of some herbs in decreasing the blood sugar and fat which are effective in diabetes treatment, as far as preventing the biochemical changes of blood is concerned [17, 18].

One of the most important enzymes in the process of cholesterol synthesis is the coenzyme A reductase. Among the main inhibitors of this enzyme, cholesterol (negative feedback), glucagon, cortisol, and anti blood fat drugs such as lovastatin and atorvastatin can be mentioned.

These medications mainly reduce plasma triacylglycerol through reducing the secretion of very-low-density lipoprotein (VLDL) containing triacylglycerol and LDL by the liver. Generally speaking, methods of reducing cholesterol are rejection, inhibition of the synthesis and its absorption [18]. Studies conducted on this herb also show that *Portulaca oleracea* contains phenolic alkaloids [20]. Alkaloids are substances that can inhibit cholesterol synthesis [21]. Many of the plants containing plenty of antioxidants, omega 3 and omega-6 fats can inhibit lipid peroxidation.

And this property is applied through breaking the existing oxidative structure by cytochrome P450 and neutralizing free radicals. *Portulaca oleracea* also inhibits oxidative stress induced by streptozocin in laboratory rats [22]. Mahmoodi et al. [23], studied the effects of supplementation with omega-3 unsaturated fatty acids along with vitamins C and E and zinc, on lipid and lipoprotein parameters in two groups of postmenopausal women with diabetes who had a normal diet and was compared to the control group. The findings of this study suggest the highest influence of omega 3 fatty acids plus vitamin E on plasma triglyceride levels on the reduction of the level of triglycerides and no significant difference

between other indices of lipid and lipoproteins of the plasma was observed among the 3 groups. In some other studies, supplementation with omega-3 fatty acids resulted in significant increase in the HDL cholesterol [23].

In a study, Mokhtarian-Dalouei showed that daily consumption of 2 eggs enriched with omega-3 fatty acids for 4 weeks significantly reduced the level of triglycerides and blood pressure and is also associated with the significant increase in the level of HDL [24]. Intake of fiber has also cholesterol-lowering effect, whereas it is ineffective on the TG level. These cholesterol lowering properties may be due to the fibers found in this herb. Fiber changes the rate of the fat and carbohydrates absorption in the intestine [25]. Certainly following the reduction in the level of serum cholesterol, the level of liver Lipidosis and the activity of liver enzyme are significantly reduced [26, 27]. In some studies, the blood sugar lowering properties of polysaccharides derived from *Portulaca oleracea* in laboratory animals (rats) have also been reported [11].

Sun et al. showed that the fiber composition may improve glucose metabolism. The mechanism of this effect is associated with the increase in tissue sensitivity to insulin [28]. Fibers may affect releasing gastrointestinal hormones and modify the pancreas secretes and digestive processes and alter speed and manner of the absorption and metabolism of carbohydrates, fats, protein and the balance of elements [29]. When insulin is present, it delivers glucose to the cells and cells use this glucose. But when the insulin is not present, cells cannot use the glucose. Thus, cells begin to use fats and release them into the blood and eventually hyperlipidemia is occurred [30]. About the *Portulaca oleracea*, given the fiber compounds found in this herb and its anti-diabetic properties, reduction in the levels of cholesterol seems quite reasonable. Fiber found in *Portulaca oleracea* is likely prevent cholesterol absorption from the gastrointestinal tract through binding to the cholesterol in the diet and thereby lowers the level of cholesterol [11].

Active radicals such as hydroxyl superoxide and hydroxyl anions are able to remove hydrogen atoms from the side-chain saturated fatty acids in biological membranes and cause lipid peroxidation damage. Mammalian cells have the enzymatic and non-enzymatic ability against free radicals attached to them. The non-enzymatic ability includes vitamin E, beta carotene and vitamin C and involved enzymes also include superoxide dismutase, catalase and glutathione peroxidase and if the antioxidant defense system of the body is destroyed, increased formation of free radicals can cause cellular oxidative stress [31, 15]. *Portulaca oleracea* is rich in vitamins A, E and C [11]. Laboratory studies have shown that antioxidant vitamins can prevent lipid peroxidation; and E and C vitamins have intensifying effects [23].

Recently, researchers have also shown that alpha-tocopherol can reduce oxidative stress and inflammation

by reducing pre-inflammatory cytokine release, decreasing monocyte adhesion to the endothelium and reducing the amount of C-reactive protein (CRP) in the plasma. Reduction in the amount of the Mellon dialdehyde in the plasma plays an important role in preventing atherosclerosis [23]. *Portulaca oleracea* herb has anti-diabetic properties and alters the activity of glutathione reductase enzyme. It also causes a significant decrease in the peroxidation of lipids dependent on the increase in the activity of superoxide dismutases (SOD) and catalase (CAT) [32].

This herb also causes a significant decrease in the concentration of tumor necrosis factor alpha (TNF- α) and a significant increase in the level of mRNA in lipoprotein lipase (LPL mRNA) in the liver [33]. In this study, probably powerful antioxidant substances present in this extract reduces plasma cholesterol level. Previous studies have shown that the *Portulaca oleracea* is a good source of coenzyme Q₁₀ [41]. Shojaei et al., showed that carnitine and coenzyme Q₁₀ supplements can reduce levels of serum lipoproteins [34]. Coenzyme Q₁₀ is widely found in organs having high oxygen consumption, such as heart, liver and brain and acts as a potent antioxidant in the removal of free radicals. Also it has a key role in the production of other antioxidants like vitamin C and E. This helps to prevent the start and continuation of lipid peroxidation. Therefore, it prevents LDL oxidation and has an important role in reducing atherosclerosis. Several studies have shown that this coenzyme can protect patients with heart failure and myocardial ischemia against oxidative stress [35].

Polysaccharides, flavonoids, glycoprotein, and polypeptides, steroids and alkaloids present in the medicinal plants can reduce fat and sugar. Findings of this study also indicate that alcoholic extract of *Portulaca oleracea* can play an important role in the treatment of hypercholesterolemia. This role is probably because of the antioxidant substances, alkaloids, fiber, coenzyme Q₁₀ and abundant quantities of omega-3 found in *Portulaca oleracea* and mechanisms of inhibition of cholesterol synthesis. However, further studies are needed to be conducted to discover the involved mechanisms.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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