Journal of Ethnopharmacology 126 (2009) 525–532

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

The effect of total extract of *Securigera securidaca* L. seeds on serum lipid profiles, antioxidant status, and vascular function in hypercholesterolemic rats

Alireza Garjani[a,*], Fatemeh Fathiazad[b], Arezoo Zakheri[b], Negar Allaf Akbari[b], Yadollah Azarmie[c], Ashraf Fakhrjoo[d], Sina Andalib[b], Nasrin Maleki-Dizaji[b]

[a] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
[b] School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

A R T I C L E I N F O

Article history:
Received 16 February 2009
Received in revised form 31 August 2009
Accepted 2 September 2009
Available online 12 September 2009

Keywords:
Hypercholesterolemia
Securigera securidaca
Lipid profile
Vascular function

A B S T R A C T

Aim of the study: Seeds of *Securigera securidaca* are used for the treatment of disorders such as hyperlipidemia, diabetes, and epilepsy in Iranian folk medicine. The possible hypolipidemic and antioxidative effects of hydroalcoholic extract of *S. securidaca* seeds as well as the effect of the extract on vascular reactivity were investigated in hypercholesterolemic rats.
Materials and methods: High-fat fed wistar rats received orally different doses of the extract for 20 days. At the end of the experiment vein blood and liver were collected to measure the lipid profile, lipid peroxidation, and antioxidative enzyme activities. The thoracic aorta was excised and used for isolated vessel preparation and histological study.
Results: The extract produced significant reductions ($p < 0.05$) in the level of low-density lipoprotein (LDL) and triglyceride with concomitant reduction in lipid deposition in the liver. The extract also suppressed markedly ($p < 0.001$) the hypercholesterolemia-induced elevation of malondialdehyde levels both in serum and liver. In hypercholesterolemic rats the endothelium-dependent vasodilatation was improved significantly ($p < 0.05$) by 100 mg/kg/day of the extract. However, in histological study no atherosclerotic lesion was observed.
Conclusion: These results suggest that *S. securidaca* seed in addition to decrease lipid levels and peroxidation, is able to improve vascular endothelium-dependent relaxation in hypercholesterolemia.
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1. Introduction

Atherosclerosis is the major contributor to the pathogenesis of heart and vascular diseases. Elevated blood concentration of cholesterol, especially in LDL, constitutes the primary risk factor for atherosclerosis and endothelial dysfunction (Bonetti et al., 2003). Hypercholesterolemia results in free radical production and thereby elevates lipid peroxides. These free radicals initiate processes involved in atherogenesis (Harrison et al., 2003). Numerous studies have reported that endothelium-dependent vasodilatation is impaired in arteries isolated from hypercholesterolemic and atherosclerotic animals (Moroe and Honda, 2006; Song et al., 2006).

Recently, there has been a resurgence of interest in herbal medicines capable of reducing and/or regulating serum cholesterol and triglyceride levels. Medicinal plants contain a wide array of active components such as flavonoids, polyphenols, tannins, and alkaloids that can explain their hypolipidemic activities (Slowing et al., 2001; Anila and Vijayalakshmi, 2002; El-Beshbishy et al., 2006). *Securigera securidaca* (Fabaceae), also called goat pea, is an annual herb occurring wild in West Asia, Europe and Africa. It is popularly named "Gandeh Talkheh" in Persian (Gharaman, 1993). The seeds of the plant are used in Iranian folk medicine for treatment of various disorders such as hyperlipidemia, diabetes (Hosseinzadeh et al., 2002), and epilepsy (Al-Hachim and Maki, 1969). It is also claimed that extracts from the seeds of *S. securidaca* exert marked chronotropic, diuretic and hypokalamic activities (Ali et al., 1998). Phytochemical studies have revealed the presence of several classes of compounds in the seeds of *S. securidaca*. These include steroidal and pentacyclic triterpenoid type saponins, cardenolides, and flavonoids (Zatula et al., 1966). The naturally occurring flavonoids are believed to possess the ideal chemical structure for scavenging free radicals (Choi et al., 2002). Furthermore, it has been shown that saponins isolated from different plants produce significant hypolipidemic effects mainly by suppression of cholesterol luminal absorption and also by increase of cholesterol secretion

* Corresponding author at: Department of Pharmacology, Tabriz University of Medical Sciences, Tabriz, Iran. Tel.: +98 411 3847908; fax: +98 411 3344798.
E-mail addresses: garjania2002@yahoo.com, garjania@tbzmed.ac.ir (A. Garjani).

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doi:10.1016/j.jep.2009.09.003

through biliary excretion (Francis et al., 2002; Ma et al., 2002). To our knowledge, the hypolipidemic and antioxidant properties of *S. securidaca* (L.) and the effect of its extracts on vascular function in hypercholesterolemia have not been studied. Hence, the present study was carried out to investigate the effects of hydroalcoholic extract of *S. securidaca* (L.) seeds on serum lipid profiles, antioxidant status, and vascular responsiveness in high-fat fed rats.

2. Materials and methods

2.1. Plant materials

Seeds of *S. securidaca* L. were collected from Dezful, Iran. The plant was kindly identified by Dr. Hossein Nazemiyeh; Department of Pharmacognosy, Tabriz, and voucher samples were preserved for reference in the Herbarium of Department of Pharmacognosy, School of Pharmacy, Tabriz. Seeds were dried under shade and then powdered by using a laboratory scale mill.

2.2. Extract preparation

Powdered seeds (400 g) were extracted three times with 1 litre of 70% methanol (MeOH)/H_2O while being macerated at room temperature for 24 h each time. The hydroalcoholic (HAE) extracts were combined and concentrated in vacuo to yield 25 g dried extract. This hydroalcoholic extract was kept in refrigerator for all experiments.

2.3. Phytochemical screening

Phytochemical screening of the extract was performed using the following reagents and chemicals. Flavonoids with the use of Mg and HCl (Markham, 1982), alkaloids with Mayer reagent and tannic acid solution (Harborne, 1973), tannins with $FeCl_3$%5 solution (Trease and Evans, 1989) and saponins by TLC on silica gel 60 F_{254} sheets (Merck, Darmstadt, Germany) with $CHCl_3$:MeOH:H_2O (70:30:4) were used as the mobile phase. After development, the plates were dried and sprayed with a Liebermann–Burchard reagent to visualize the saponin profile and then heated on a hot-plate (Wagner and Bladt, 1996).

2.4. Determination of in vitro total antioxidant activity

For the determination of the antioxidant activity of the hydroalcoholic extract of *Securigera securidaca* seeds, the stable 2,2-di-phenyl-2-picrylhydrazyl hydrate (DPPH, Sigma–Aldrich Química S.A., Madrid, Spain) radical was used. Qualitative [thin-layer chromatography (TLC)] and quantitative (spectrophotometric) methods were employed.

2.4.1. Qualitative assay

A 20 μl aliquot of the extract was spotted on silica gel plate (20–20 cm, silica gel 60 F_{254}, Merck, Darmstadt, Germany) with a solvent system of MeOH/EtOAC/H_2O (70:30:0.5). After developing and drying, TLC plate was sprayed with a 0.2% DPPH solution in methanol and examined for 30 min after spraying. Active antioxidant compounds appeared as yellow spots against a purple background (Cavin et al., 1998).

2.4.2. Quantitative assay

For the quantitative assay, stock solution of the extract was prepared in methanol to achieve a concentration of 1 mg ml^{-1}. Dilutions were made to obtain concentrations of 8–128 μg/ml. Diluted solutions (1.00 ml each) were mixed with DPPH (1.00 ml) and allowed to stand for 30 min for any reaction to occur. The capacity of the extract to scavenge the DPPH radical, which resulted in the bleaching of the purple colour exhibited by the stable DPPH radical, was monitored at an absorbance of 517 nm. The experiments were performed in triplicate and the average absorption was noted for each concentration (Lee et al., 2004). The absorbance of samples was plotted against the concentration and the IC_{50} was calculated. The same procedure was followed for the positive control, L-ascorbic acid (1.25–20 μg/ml).

2.5. Animals

Male Wistar rats (200–220 g) were purchased from Pasture Institute of Iran, Tehran, Iran. The animals were given standard pellet diet and water *ad libitum*. They were housed in the Animal House of Tabriz University of Medical Sciences at a controlled ambient temperature of $25 \pm 2\,^{\circ}C$ with $50 \pm 10\%$ relative humidity and with a 12-h light/12-h dark cycle. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz, Iran (National Institutes of Health Publication No 85-23, revised 1985).

2.6. High-fat diet

A high-fat diet employed by Roberts and coworkers (2001) was used in this study with some modifications. The high-fat pellet diet contained standard Sahand Niroo (Tabriz-Iran) rodent chow powder (62.75%), cholic acid (0.25%), cholesterol (2%), and lard oil (15%), wheat flour (10%), and sucrose (10%).

2.7. Experimental protocol

Animals were allocated into six groups (6 rats for each group), with food and water freely available. The first group received a standard diet (normal control), while the other groups (groups 2–6) were fed with the high-fat diet for 36 days. In addition, group 2 received 1 ml carboxy methyl cellulose (0.5%; CMC) in water per day as vehicle (hypercholesterolemic group: HC), group 3 received lovastatin (10 mg/kg/day); HC + Lovas) as positive control, and groups 4, 5 and 6 received 50, 100, and 200 mg/(kg day) of *S. securidaca* seeds extract (HC + S. Sec), respectively. Lovastatin and the extracts were suspended in 1 ml CMC (0.5%) and were given orally for the last 20 days of the experiments period. At the end of the experiments, the rats were fasted for 16 h and then anaesthetized by intraperitoneal (i.p) injection of ketamin plus xylazine. Blood samples were collected from inferior vena cava and transferred into two centrifuge tubes (one containing EDTA and the other without any additive) and centrifuged to obtain plasma and serum, respectively. The thoracic aorta was rapidly removed and divided into two sections. A section was placed in Krebs–Henseleit solution for isolated blood vessel preparation and the other was used for histological study. The liver was removed immediately by dissection, washed in ice-cold saline, blotted between two filter papers, and divided into two sections. One section was used for histological study and the other was kept at $-80\,^{\circ}C$ for further analysis.

2.8. Serum lipids measurement

Serum concentrations of total cholesterol (TC), HDL, and triglycerides (TG) were determined by enzymatic colorimetric methods using commercially available kits (Randox Laboratories Ltd., UK). The assay was performed according to the manufacturer's instruction. All samples were measured in duplicate. The concentration of LDL was calculated by the following equation:

$$LDL = TC - (HDL + 0.2TG)$$

2.9. Determination of lipid peroxidation in serum and liver

Malondialdehyde (MDA), a thiobarbituric acid reactive substance (TBARS), was measured as a marker for oxidative stress in serum and liver homogenates using the method of Satoh (1978). The lipid peroxide expressed as nanomole MDA production per gram liver and nanomole per milliliter serum, were measured spectrophotometrically.

2.10. Glutathion-S-transferase activity measurement

Glutathione-S-transfrase (GST) activities in the plasma and liver homogenates were measured according to Habig et al. (1974) method. In this method 1-chloro-2,4-dinitrobenzene was used as a substrate and formation of thioester as a product was monitored spectrophotometrically. One unit of enzyme was defined as an amount of GST needed to catalyze formation of 1 nmol of thioester/min and the specific activity is expressed as nmole/min/mg of liver or nmol/min/ml of plasma.

2.11. Measurement of serum paraoxonase activity

In this method phenyl acetate was used as a substrate and serum paraoxonase 1 (PON1) activity was determined as arylesterase activity (Beltowski et al., 2003). Assay mixture included 1 mM phenyl acetate and 0.9 mM $CaCl_2$ in 20 mM Tris–HCl at pH 8. Fresh serum (10 μl) was added into the assay mixture and the absorbance was recorded after 60 and 120 s spectrophotometrically at 270 nm. Non-enzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. One unit of arylesterase was equal to 1 μM/min/ml of hydrolyzed phenyl acetate.

2.12. Histological examination

For histological study, biopsies of aorta and liver tissues ($n = 4$) of all groups were obtained and fixed in 10% neutral-buffered formaldehyde for 48 h, embedded in paraffin and sectioned at 5 μm. The sections were stained with haematoxylin and eosin, and examined by light microscopy (×40 for aorta and ×400 for liver).

2.13. Isolated thoracic aorta preparation

The thoracic aorta was immediately dissected, transferred into a Petri dish containing Krebs solution, cleaned of fat and adhering tissues and was cut into ring segments of approximately 3 mm in length; care was taken to avoid any damage to endothelium. The aortic ring was mounted in 10 ml organ bath containing Krebs solution (37 °C, pH 7.4). Solution was continuously bubbled with a 95% O_2–5% CO_2 gas mixture. In each ring two metallic hooks were inserted through lumen of the ring, one was anchored to the organ bath while the other was vertically attached to a strain gauge force transducers (Lethica Spain), which were connected to a four-channel bridge amplifier (AD Instrument, 4Sp, QUAD Bridge). The isometric force was displayed and recorded on a PowerLab data-acquisition system with a computerized analysis program (Chart 5.4.2, AD Instruments). Aortic rings were allowed to equilibrate for 60 min at a resting tension of 2 g, with the bath medium changed every 15 min (Reil et al., 1999). After the equilibration period, aortic rings were contracted with phenylepherin (10 μM) and exposed to carbachol (0.3–19.2 μM) or sodium nitroprusside (SNP; 5–160 nM) to assess the endothelium-dependent and -independent relaxation.

2.14. Statistical analysis

Data were presented as mean ± SEM. Comparison between groups was made by one-way analysis of variance (ANOVA). If ANOVA analysis indicated significant differences, a Student–Newman–Keuls post-test was performed to compare mean values between treatment groups and control. Differences between groups were considered significant at $p < 0.05$.

3. Results

3.1. Phytochemical screening of total extract of S. securidaca seeds

Preliminary phytochemical screening of hydroalcoholic extract of *Securigera securidaca* seeds indicated the presence of flavonoids, alkaloids, tannins and saponins. The TLC of the extract showed three bands related to triterpenoid saponins with R_f values of 0.78, 0.64 and 0.47.

3.2. In vitro antioxidant activity of S. securidaca seeds extract

The hydroalcoholic extract of *S. securidaca* was tested for its free radical scavenging effect on DPPH and the results are indicated in Table 1. DPPH was reduced concentration dependently by the extract. The free radical scavenging potency of the extract (IC_{50}) was 19.2 μg/ml which was lower than that of L-ascorbic acid (4.25 μg/ml) as a positive reference antioxidant.

3.3. Effect of S. securidaca seeds extract on serum lipid profile

Compared with the normal diet, the high-fat diet for 36 days produced a significant elevation in serum total cholesterol, LDL ($p < 0.001$), and triglyceride by 185%, 350%, and 26%, respectively (Fig. 1). The oral administration of lovastatin (10 mg/kg/day) or total extract of *S. securidaca* seeds (50–200 mg/kg/day) for 20 days to the hypercholesterolemic (HC) rats caused a moderate but not significant decline in serum level of total cholesterol. The extract in doses of 100 and 200 mg/kg/day reduced significantly ($p < 0.05$), even better than lovastatin, the level of LDL from 158 ± 17 mg/dl in HC rats to 107 ± 14 and 100 ± 12 mg/dl in treated groups, respectively (Fig. 1). These declines were accompanied by a significant ($p < 0.05$) reduction of serum triglyceride in all treated groups. There was no significant change in the serum level of HDL either

Table 1
Free radical scavenging effects of hydroalcoholic extract of *Securigera securidaca* seeds on DPPH.

L-Ascorbic acid[a]		*S. securidaca* extract	
Concentration (μg/ml)	Percent inhibition (%)	Concentration (μg/ml)	Percent inhibition (%)
1.25	20.6	8	23.4
2.5	31.8	16	40.3
5	60.8	32	69.4
10	96.9	64	97.5
20	97.4	128	97.8
IC_{50} (μg/ml) = 4.25		IC_{50} (μg/ml) = 19.2	

[a] A reference compound.

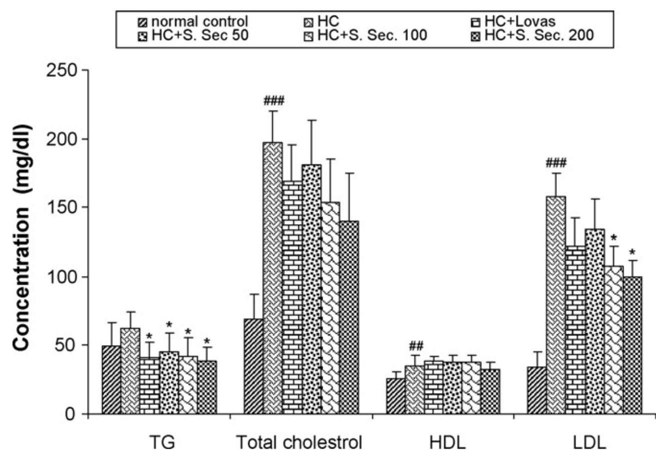

Fig. 1. Effects of oral administration of hydroalcoholic extract of *Securigera securidaca* seeds in hypercholesterolemic rats for 20 successive days (50–200 mg/kg/day) on serum lipid profiles as triglycerides (TG), total cholesterol, HDL, and LDL. Data are expressed as mean ± SEM. Number of rats per group $n = 6$. $^{\#\#}p < 0.001$ and $^{\#\#\#}p < 0.0001$ compared with normal control group. $^{*}p < 0.05$ compared with hypercholestrolemic rats using ordinary ANOVA test. HC = hypercholesterolemic rats; Lovas = Lovastatin, S. sec. = *Securigera securidaca* seeds extract.

in lovastatin (as positive control group) or the extract treated groups.

3.4. Effect of S. securidaca seeds extract on lipid peroxidation and antioxidative enzyme activities

To determine the lipid peroxidation, MDA levels were measured in serum and liver homogenate. Both serum and liver MDA were increased considerably ($p < 0.001$) in the hypercholesterolemic rats in comparison with normal control (Table 2). Similar to lovastatin group, oral administration of 50, 100, 200 mg/kg/day of *S. securidaca* extract to hypercholesterolemic rats diminished the liver MDA level markedly by 64%, 81%, and 72%, respectively ($p < 0.001$). Serum MDA was also decreased significantly ($p < 0.01$; $p < 0.001$) by lovastatin and all three doses of the extract (Table 2). However, the higher dose of 200 mg/kg/day produced less protection against lipid peroxidation than 100 mg/kg/day. Except a slight increase produced by the extract (200 mg/kg/day), glutathione-S-transferase (GST) activities were unaffected in the liver tissue in all studied groups. The serum paraoxonase 1 (PON1) activity, assessed as arylesterase activity, was not changed in all treated groups (Table 2). Compared to the normal control rats the level of PON1 enzyme was moderately but not significantly low in hypercholesterolemic rats.

3.5. Effect of S. securidaca seeds extract on lipid accumulation in aorta and liver

At the end of the experiments, biopsies of thoracic aorta and liver were studied by histological examination for lipid accumulation. A microscopic study of the tissue slices from the aorta did not reveal intimal plaque formation or atherosclerotic changes in all studied groups (Fig. 2). In contrast, lipid deposits as macrovesicular and microvesicular steatosis were abundant in the liver of non-treated (Fig. 3B) and also lovastatin-treated (Fig. 3C) hypercholesterolemic rats. Treatment with all three doses of the extract of *S. securidaca* seeds (50–200 mg/kg/day) reduced the lipid accumulation very noticeably so that, in rats treated with 100 and 200 mg/kg/day of the extract the fatty degenerations and cytoplasm displacements were completely (Fig. 3D–F) vanished.

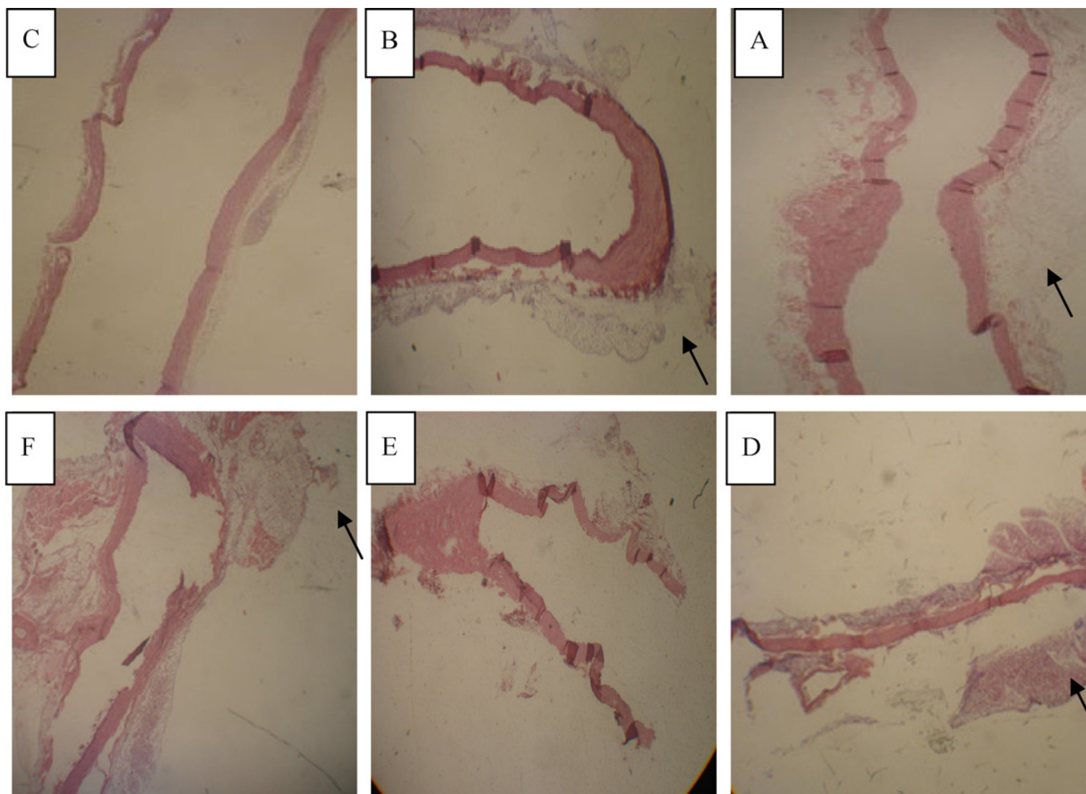

Fig. 2. Microphotography of thoracic aorta slice (haematoxylin and eosin stain) from control normocholesterolemic rat (A), hypercholesterolemic rats (HC; B), lovastatin-treated HC rats (C), and from hypercholesterolemic rats that received the extract (50, 100, and 200 mg/kg/day; D, E, and F, respectively) demonstrate lack of lipid deposition and atherosclerotic plaque in intimal of the vessels. Arrows indicates a slight lipid accumulation outside the wall. Original magnification, 40×.

Table 2
The effects of oral administration of hydroalcoholic extract of *S. securidaca* seeds in hypercholesterolemic rats for 20 successive days (50–200 mg/kg/day) on serum and liver malondialdehyde (MDA) levels, serum paraoxonase (PON) activity, and plasma and liver glutathione-S-transferase (GST) levels.

	Normal control	HC	HC + Lovas	HC + S. sec.50	HC + S. sec.100	HC + S. sec.200
MDA						
Serum (nmol/ml)	2.5 ± 0.3	4.9 ± 0.6$^{###}$	3.2 ± 0.4**	2.8 ± 0.37**	1.3 ± 0.11***	2.05 ± 0.24***
Liver (nmol/gl)	1.85 ± 0.16	6.2 ± 0.4$^{###}$	2.3 ± 0.4***	2.2 ± 0.33***	1.2 ± 0.17***	1.7 ± 0.24***
GST						
Plasma (nmol/min/ml)	43.3 ± 4.5	43.9 ± 3.8	52.3 ± 6.3	40.9 ± 5.4	41.4 ± 2.5	49.7 ± 4.9
Liver (nmol/min/mg)	53.3 ± 5.4	55.1 ± 3.3	62.3 ± 2.7	64.9 ± 5.5	61.4 ± 2.5	69.7 ± 7.2
PON						
Serym (U/ml)	98.7 ± 5.5	80.0 ± 2.3	80.0 ± 3.8	75.6 ± 3.4	82.3 ± 2.3	80.4 ± 1.6

Data are expressed as mean ± SEM. Number of rats per group $n = 6$. $^{##}p < 0.001$ compared with the normal control. $^{**}p < 0.001$, $^{***}p < 0.0001$ compared with the hypercholestrolemic rats using ordinary ANOVA test. HC = hypercholesterolemic rats; Lovas = Lovastatin, S. sec. = *S. securidaca* seeds extract.

3.6. Effect of S. securidaca seeds extract on the relaxation induced by carbachol or SNP in aorta

The arteries from all groups were contracted by phenylepherin (10 μM) and then exposed to different concentrations of either carbachol or sodium nitroprusside (SNP). Compared with the control normal rats the endothelium-dependent relaxation, induced by carbachol, was strongly ($p < 0.001$) decreased in the hypercholesterolemic group (Fig. 4, upper). The maximum relaxation induced by 9.6 μM of carbachol was 95 ± 12% in the control group while, the relaxation induced by 19.2 μM of carbachol was only 34 ± 7% in arteries isolated from the hypercholesterolemic rats. This hypercholesterolemia-induced dysfunction in aorta was improved by all doses of the extract. The maximum relaxation in thoracic aorta isolated from the rats treated by 50, 100, and 200 mg/kg/day of the extract were 72 ± 14%, 82 ± 6.5%, and 73 ± 14%, respectively. The relaxation was increased significantly ($p < 0.05$) in all points in the group treated with 100 mg/kg/day of the extract, in which the relaxation was similar to that of lovastatin-treated group. Lovastatin (10 mg/kg/day), a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor (statin), also significantly increased vasodilatation responses to carbachol in the hypercholesterolemic rats. The carbachol-induced relaxation in lovastatin-treated rats (maximum 88 ± 10%) was similar to that of the normal control group (Fig. 4, upper).

Contrary to the case of endothelium-dependent relaxation, hypercholesterolemia did not affect endothelium-independent vascular relaxation induced by SNP (Fig. 4, lower). In addition, lovastatin and all extract treated groups presented relaxations similar to that of the normal control group.

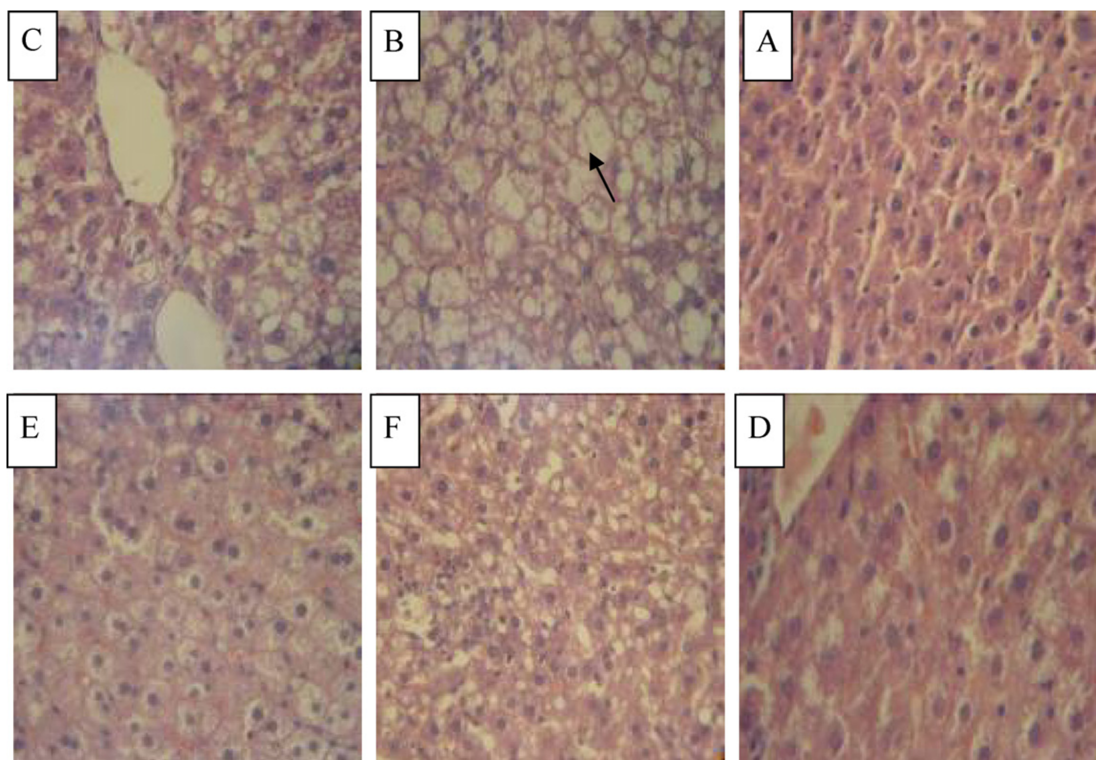

Fig. 3. Liver tissue slice from a control normocholesterolemic rat (A) showing the normal tissue structure. The tissue slices from hypercholesterolemic rats (HC, B) and also lovastatin-treated HC rats (C) show abnormal retention of lipids as steatosis within cells. Lipid accumulation displaced the cytoplasm and the nuclei were distorted. The tissue sections from hypercholesterolemic rats that received the extract (50, 100, and 200 mg/kg/day; D, E, and F, respectively) demonstrate reduced lipid deposition with normal structure. Arrow indicates the lipid vesicles. One representative microphotograph from each of the six experimental groups is shown. Original magnification, 400× and H & E staining.

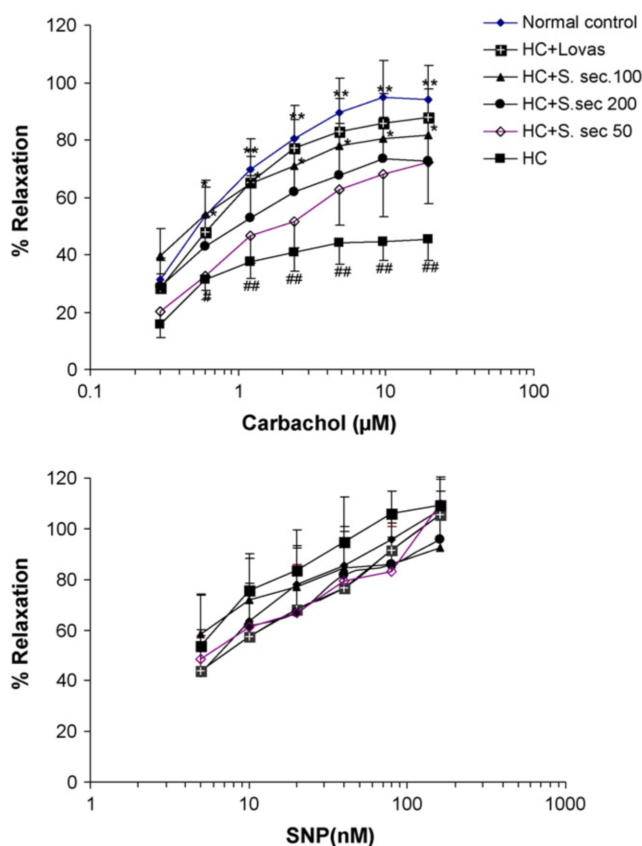

Fig. 4. Relaxation of preconstricted isolated thoracic aorta to carbachol (upper trace) and sodium nitroprusside (SNP; lower trace). Isolated arteries were constricted by 10 μM phenylepherin throughout the experiment. Carbachol or SNP was added cumulatively to construct a vasodilatory concentration–response curve. Data are expressed as mean ± SEM. Number of rats per group $n = 6$. $^{\#}p < 0.01$ and $^{\#\#}p < 0.001$ compared with normal control group. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with hypercholestrolemic rats using ordinary ANOVA test. HC = hypercholesterolemic rats; lovas = lovastatin, S. sec. = *Securigera securidaca* seeds extract.

4. Discussion

Hyperlipidemia is one of the major risk factors of atherosclerosis and endothelial dysfunction (Bonetti et al., 2003). In this study adding cholesterol (2%) and lard oil (15%) to the diet of rats for 36 days led to hypercholesterolemia as indicated by significant increase in serum total cholesterol (185%; $p < 0.001$), LDL cholesterol (350%; $p < 0.001$), and a non significant increase in the level of triglycerides (26%). These results, with some differences, are in agreement with other studies in which an increase in rat dietary cholesterol intake resulted in plasma or serum cholesterol elevation (Zulet et al., 1999; El-Beshbishy et al., 2006). A considerable part of ethnopharmacological research in recent years has been directed toward a better understanding of the hypolipidemic and anti-atherogenic effects of medicinal plants (Slowing et al., 2001; Lee et al., 2004; Choudhary et al., 2005; El-Beshbishy et al., 2006; Lecumberri et al., 2007). In the present study, we investigated the possible hypolipidemic and antioxidative effects of hydroalcoholic extract of *S. securidaca* seeds as well as the effect of the extract on vascular reactivity in hypercholesterolemic rats. Short term (20 days) oral administration of *S. securidaca* seeds extract to hyperlipidemic rats had a significant hypotriglyceridemic (TG) effect, returning serum TG to levels in normal control rats. The extract also produced a partial reduction in serum total cholesterol level, with a considerable decline in LDL cholesterol. The hypolipidemic effect of *S. securidaca* seeds was concomitant with a remarkable reduction of lipid deposition in the liver tissue. Our results also demonstrated that formation of lipid peroxides, caused by high cholesterol diet, was suppressed markedly ($p < 0.001$) in both serum and liver, upon oral administration of the extract. All three doses of the extract (50, 100, and 200 mg/kg/day), similar to lovastatin, significantly ($p < 0.001$; $p < 0.01$) inhibited the rise in MDA level but the effect of 100 mg/kg/day was predominant. The glutathione-S-transferase (GST) family of enzymes comprises a long list of cytosolic, mitochondrial, and microsomal proteins that catalyze the conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centers on a wide variety of substrates (Wang and Ballatori, 1998). This activity is useful in the detoxification of endogenous compounds such as deoxidized lipids (Leaver and George, 1998). The hydroalcoholic extract of *S. securidaca* seeds did not modify GST activity in plasma or liver of the hypercholesterolemic rats. This is in agreement with most studies in which dietary intake of plant material failed to increase the GST activity (El-Demerdash et al., 2003; Yousef et al., 2004; El-Beshbishy et al., 2006; Lecumberri et al., 2007). The result of this study showed that the HDL level was unchanged by administration of the extract. We also found no differences in the serum paraoxonase 1 (PON 1) activity between controls and rats received the extract in the hypercholestrolemic group. PON1 is synthesized in the liver and transported along with HDL in the serum. As an antioxidant; it prevents the oxidation of LDL. In fact, high-fat diet did not affect the antioxidative enzyme activities in this model while, lipid peroxidation was increased highly. It seems that the extract suppressed the lipid peroxidation not through the elevation of antioxidant enzyme activities, but partially by scavenging the free radicals. Although in comparison with ascorbic acid (Vit C) the extract showed a weak in vitro antioxidant activity.

Vascular studies in humans and animal models of hypercholesterolemia/atherosclerosis indicate that endothelial function is impaired during hypercholesterolemia (d'Uscio et al., 2001; Lee et al., 2002; Bourgoin et al., 2008). A study on carotid artery in short-term hypercholesterolemic rabbits has indicated that hypercholesterolemia with no influence on intimal plaque formation attenuates endothelium-derived relaxation (Moroe and Honda, 2006). Although the mechanisms underlying the impairment of endothelium-dependent relaxation have not been fully elucidated, several lines of evidence show that NO inactivation by oxygen-derived free radicals may play a central role (Ohara et al., 1993; Li et al., 2007). The results of the present study showed that responses to carbachol in thoracic aorta isolated from the high-fat fed rats were reduced while; sodium nitroprusside-induced relaxation of the artery did not differ between groups. Histological examination of tissues from hypercholesterolemic rats did not reveal any morphological changes in the aorta. These findings suggest that increased plasma level of cholesterol, even without morphological and atherogenic changes is associated with endothelial dysfunction in aorta and this dysfunction can be reversed by oral administration of the total extract of *S securidaca* seeds. However, similar to the U-shaped decreasing effect of the extract on serum and liver MDA levels, in comparison with the higher dose of the extract (200 mg/kg/day) a low dose of 100 mg/kg/day produced more and significant improvement in endothelium-dependent vasorelaxation. Oxidized LDL affects arterial wall in hypercholesterolemia and play a causal role in the reduction of nitric oxide production thus, impairs NO signaling (Ryoo et al., 2006; Schalkwijk et al., 2007). The effects of the extract as well as lovastatin on improvement of carbachol-induced relaxation in hypercholesterolemic rats may be mediated not only by their lipid-lowering properties but also by an effect on the activity of endothelial nitric oxide synthase (eNOS), by anti-inflammatory, or by the other pharmacological actions which should be elucidated. The discrepancy between the higher effect upon the low dose and the lower effect of the high dose of the extract might be explained by this hypothesis that some of the active constituent(s) of *S. securidaca* at high concentration may

exhibit diverse effect such as pro-oxidant activity. The preliminary phytochemical screening of the extract from seeds of *S. securidaca* in this study showed the presence of saponins, flavonoids, cardenolids. Quantitatively the amounts of saponins were predominant. Flavonoids are antioxidant and reduce the oxidation of LDL cholesterol, therefore suggesting their role in preventing of hyperlipidemic damages in cardiovascular diseases (Sierens et al., 2002). Although, the extract had a weak antioxidant activity but it produced a remarkable reduction in MDA concentration. It is also claimed that flavonoids increase the LDL-receptor number in liver and therefore increasing liver uptake of lipids from blood (Baum et al., 1998). The other main component of the extract was triterpenoid saponins. Saponins are mainly plant-derived surface-active glycosides, occurring as triterpenoid or steroid saponins. A number of studies have shown that saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects (Francis et al., 2002). It is proposed that interaction of saponins with bile acids is responsible for the bile acids increased excretion when saponin-rich foods such as soybean, lucerne and chickpea are consumed (Oakenfull and Sidhu, 1990). The resulting accelerated metabolism of cholesterol in the liver causes its serum levels to go down (Francis et al., 2002). Morehouse et al. (1999) suggested that saponins produced their hyporcholesterolemic effects in luminal too. Also, Han et al. (2000) in a study reported that saponins from aqueous extract of *Platycodi radix* inhibit intestinal absorption of dietary fat by suppression of pancreatic lipase (PL) activity. It is likely that saponins present in *S. securidaca* seeds are responsible for the hypolipidemic and beneficial effects of the extract.

5. Conclusion

The present results demonstrate that hydroalcoholic extract of *Securigera securidaca* seeds in hypercholestrolemic rats exerts a considerable hypolipidemic effects. We suggest that reduction of serum lipid levels, inhibition of lipid peroxidation, and improvement of endothelium-depended function of aorta by the extract can be attributed to flavonoids and mainly saponins. However, these effects probably are not entirely due to direct antioxidant action of the extract or are not mediated by an increase in GST or PON1 activity. It seems that the hypolipidemic effect of the extract is caused partly by direct suppressing of the gastrointestinal absorption of lipids or through the elevation of the bile acid excretion. However, the results of the present study should be taken as a base for further investigation on the exact mode of action of individual constituents of the extract of *S. securidaca* seeds.

Acknowledgments

This study was supported by a grant from the Research Vice Chancellors of Tabriz University of Medical Sciences; Tabriz, Iran.

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