Research in Pharmaceutical Sciences, February 2018; 13(1): 22-29
Received: July 2017
Accepted: October 2017

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

The effects of hydroalcoholic extract of *Allium elburzense* Wendelbo bulb on dexamethasone-induced dyslipidemia, hyperglycemia, and oxidative stress in rats

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Abstract

Recent evidences have suggested the beneficial cardiovascular effects of some plants belonging to the genus *Allium*. The present study is an attempt to investigate the effects of hydroalcoholic extract of *Allium elburzense* bulb on dexamethasone-induced dyslipidemia in rats. Total phenolic content of *A. elburzense* bulb hydroalcoholic extract was determined using Folin-Ciocalteu method. Thirty-six male Wistar albino rats in 6 groups were studied. Group 1 (dyslipidemic control) received dexamethasone (10 mg/kg/day, s.c.) for 7 days, groups 2-4 (treated) received dexamethasone and simultaneously treated orally with 100, 200, or 400 mg/kg of *A. elburzense* extract, group 5 (normal control) received a single daily injection of normal saline (1 mL/kg, s.c.) and the vehicle orally, and group 6 (reference) received dexamethasone and atorvastatin (40 mg/kg) orally. At the end of experiment, blood glucose, lipid profile, and malondialdehyde (MDA) levels were assessed in serum samples. Livers were processed for histopathological examination. Total phenolic content of *A. elburzense* extract was estimated to be 33.52 ± 1.3% mg gallic acid equivalent/g of the dried plant extract. The plant extract significantly reduced serum blood glucose, triglyceride, total cholesterol, low-density lipoprotein-cholesterol, and MDA levels and increased the high density lipoprotein-cholesterol level and also improved liver steatosis compared to the dyslipidemic control group. These results suggest the hydroalcoholic extract of *A. elburzense* bulb has anti-dyslipidemic, anti-hyperglycemic, and antioxidant effects on rats receiving high doses of dexamethasone.

Keywords: Allium; Hyperlipidemias; Lipid peroxidation; Dexamethasone

INTRODUCTION

Hyperlipidemia is an acquired or genetic disorder elucidated by the increase in one or more of the plasma levels of lipids such as triglycerides, cholesterol, cholesteryl esters, and/or plasma lipoproteins (1). Abnormal levels of plasma lipids especially high levels of total cholesterol and low levels of high density lipoprotein (HDL)-cholesterol are important risk factors for developing atherosclerosis. This inflammatory and stenotic disorder of arteries is a leading cause of various cardio- and cerebro-vascular diseases with significant morbidity and mortality worldwide (2).

Oxidative stress as an imbalance between generation of reactive oxygen species (ROS) and antioxidant defense system also plays a principal role in chronic inflammation and development of cardiovascular disorders (3). Recently, herbal medicines have been interested for finding new effective cholesterol lowering agents with antioxidant properties and therefore reducing the risk of certain chronic diseases such as atherosclerosis (4).

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Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.220964

The world ethnobotanical surveys reported a number of medicinal herbs including *Allium* plants which are used for managing hyperlipidemia and its potential complications (5).

Allium is the primary genus of Amaryllidaceae family with about 800 species around the world. This genus is a rich source of various bioactive compounds such as flavonoids, saponins, and sulphuric compounds, and some species such as onion and garlic have lengthily been used as foods or medicine (6). Plants belonging to the genus *Allium* are popular for their helpful effects in cardiovascular diseases including cholesterol and lipid-lowering effects, antithrombotic, anti-platelet aggregatory and fibrinolytic activities, blood pressure-lowering effects, and strong antioxidant properties (7). *Allium elburzense (A. elburzense)* Wendelbo is an endemic Iranian plant which grows in Elburz Mountains. This edible vegetable is locally called "Valak", and its aerial parts are used as food and also as antidiabetic, antihelminthic, antirheumatic, and aphrodiasic agent in folk medicine. Some pharmacological properties including anti-hyperglycemic, antispasmodic, fibrinolytic, and antioxidant effects have been reported for *A. elburzense* (8-10). The aim of this study was to investigate the beneficial effects of hydroalcoholic extract of *A. elburzense* bulb on dexamethasone-induced alterations of serum lipid profile, glucose level, and oxidative status as well as hepatic weight and histology in rats.

MATERIALS AND METHODS

Plant material and preparation of the extract

The plant was purchased from a local market in Damavand (Tehran province of Iran) in March 2016. It was identified as *A. elburzense* by a botanist and a voucher specimen (No. 1145) was deposited at the Herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran. For preparation of hydroalcoholic extract, 1200 g of the air-dried ground bulbs were extracted with 5 liter of ethanol:water (70:30) using percolation method for 48 h at room temperature. The hydroalcoholic extract was then filtered and concentrated using a rotary evaporator. The residue (330 g) was freeze-dried and kept in refrigerator at -20º C until use. The plant extract was dissolved in water for oral administration in animals.

Determination of total phenolic content

The total phenolic content of *A. elburzense* extract was determined using Folin-Ciocalteu method. Briefly, sodium carbonate (20%) was added to the plant samples. The mixture was then treated with diluted Folin-Ciocalteu reagent. After 2 h, the absorbance was measured by a spectrophotometer (Bio-Tek, PowerWave XS, USA) at 765 nm. Results were obtained using a standard curve plotted based on different concentrations of gallic acid (0-5500 µg/mL) and expressed as milligram of gallic acid equivalent (GAE)/g of the dried plant extract (11).

Animals

Male Wistar albino rats weighing 200 ± 20 g were obtained from the animal house of the School of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran. The animals had free access to water and standard rodent diet and were kept under standard laboratory condition including room temperature of 20-25 ºC and a 12 h light/12 h dark cycle. Rats were acclimatized for 1 week before the experiment. All animal experiments were approved by the Animal Research Ethics Committee of Isfahan University of Medical Sciences (ethical approval ID: IR.MUI.REC.1394.3.703) and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Induction of dyslipidemia

For induction of dyslipidemia, dexamethasone (Darou Pakhsh Pharmaceutical Co., Iran), a synthetic member of glucocorticoids was injected 10 mg/kg body weight (s.c.) for 7 days in rats. The animals' body weights were measured every other day. At the end of the experimental period, the blood samples of overnight fasted rats were taken from orbital sinus plexus with heparinized capillary tubes under light ether anesthesia. The serum was collected for biochemical analysis (12). After scarification

of animals, the heart, kidneys and the liver were separated and weighed out. The livers were kept immersed in 10% formalin and were further processed for pathological examinations.

Experimental protocol

Animals were randomly divided into 6 groups of 6 rats each as follows: Group 1, the dyslipidemic control group which received dexamethasone (10 mg/kg/day, s.c.) for 7 days; Groups 2-4, received dexamethasone and simultaneously treated orally with 100, 200, or 400 mg/kg of *A. elburzense* hydroalcoholic extract using an intragastric tube (8); Group 5, the normal control group receiving daily injection of normal saline (1 mL/kg, s.c.) and oral administration of the vehicle; and Group 6, the reference group received dexamethasone and atorvastatin (40 mg/kg, orally). Atorvastatin was dissolved in water using carboxymethyl cellulose 1% and administered as a standard treatment for 7 days (13).

Biochemical analysis

Blood glucose levels based on glucose oxidase method and serum lipid profiles including triglycerides, total cholesterol and HDL-cholesterol were estimated enzymatically using the respective biochemical kits (Pars Azmoon Co., Iran). Low-density lipoprotein (LDL)-cholesterol was calculated by the following formula (14):

$$LDL = Cholesterol - (Triglyceride / 5) - HDL \quad (1)$$

The malondialdehyde (MDA) concentration was measured in serum samples as an indicator of lipid peroxidation. In brief, serum was mixed with trichloroacetic acid (20%) and the precipitate was dispersed in H_2SO_4. Then thiobarbituric acid solution (0.67%) in Na_2SO_4 was added to the mixture. After heating in a boiling water bath for 1 h and then rapid cooling, it was mixed with n-butanol, and the absorbance of the chromogen formed between the end product of lipid peroxidation and thiobarbituric acid was measured at 532 nm by a spectrophotometer and expressed as MDA equivalents in nmol/mL. Standard curve was generated using different concentrations of MDA tetrabutyl ammonium.

Histopathological analysis

The formalin fixed liver samples were processed and routinely embedded in paraffin blocks. Sections of 5 μm thickness were stained with hematoxylin and eosin and were observed microscopically for histopathological changes.

Statistical analysis

Data are presented as mean ± standard error of the means (SEM). One-way analysis of variance (ANOVA) followed by Tukey post-hoc test using the Statistical Package for the Social Sciences (SPSS software V. 18.0) were performed for statistical analysis. P values < 0.05 were considered as the significant level.

RESULTS

Total phenolic content

The yield of the *A. elburzense* bulb hydroalcoholic extract was 27.5% (w/w). It was standardized based on its total phenolic content. The calibration curve was determined by linear regression of different concentrations of gallic acid. The regression equation was:

$$y = 0.001\ x + 0.0053 \quad (2)$$

where, x is the concentration of galic acid in sample (μg/mL) with the correlation co-factor R^2 = 0.9924. Total phenolic content of *A. elburzense* extract was estimated as 33.52 ± 1.3% mg GAE/g of the dried plant extract.

Effect of A. elburzense on body and organ weight changes

The results showed significant reduction in body weight of dexamethasone-induced dyslipidemic rats when compared to normal control group (P < 0.001). Administration of atorvastatin and different doses of *A. elburzense* extract could not prevent body weight loss induced by dexamethasone. The weight of the heart and kidneys was unaffected, however, significant increase was observed in the liver weight in dyslipidemic rats compared to the normal rats (P < 0.05). Administration of *A. elburzense* extract prevented liver weight gaining at the doses of 100 and 400 mg/kg (Table 1).

Table 1. Effect of hydroalcoholic extract of *A. elburzense* bulb on body and organ weight in dexamethasone-induced dyslipidemia.

Groups	BW changes (g)	Heart (BW%)	Kidneys (BW%)	Liver (BW%)
Normal control	+4.31 ± 1.52	0.40 ± 0.07	0.82 ± 0.05	4.72 ± 0.61
Dexamethasone-induced hyperlipidemic control	-40.83 ± 1.91$^{\#\#\#}$	0.38 ± 0.07	0.81 ± 0.07	5.63 ± 0.63$^{\#}$
Dexamethasone + atorvastatin (40 mg/Kg)	-44.72 ± 3.31$^{\#\#\#}$	0.51 ± 0.04	1.02 ± 0.05	5.61 ± 0.52$^{\#}$
Dexamethasone + *A. elburzense* (100 mg/Kg)	-46.12 ± 4.50$^{\#\#\#}$	0.36 ± 0.05	0.79 ± 0.06	4.12 ± 0.41**
Dexamethasone + *A. elburzense* (200 mg/Kg)	-37.24 ± 1.93$^{\#\#\#}$	0.50 ± 0.06	1.01 ± 0.04	5.92 ± 0.33$^{\#\#}$
Dexamethasone + *A. elburzense* (400 mg/Kg)	-31.64 ± 2.71$^{\#\#\#}$	0.44 ± 0.05	0.83 ± 0.03	4.15 ± 0.43**

Values are expressed as means ± SEM (n = 6). Tukey post hoc analysis, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus normal control, and $^{**}P < 0.01$ versus dexamethasone-induced dyslipidemic control. BW, body weight.

Table 2. Effect of hydroalcoholic extract of *A. elburzense* bulb on serum biochemical parameters in dexamethasone-induced dyslipidemia.

Groups	BG (mg/dL)	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal control	155.2 ± 11.5	131.5 ± 9.1	108.2 ± 4.3	43.1 ± 3.1	39.2 ± 5.5
Dexamethasone-induced hyperlipidemic control	182.4 ± 13.2$^{\#\#}$	169.4 ± 14.6$^{\#\#}$	149.2 ± 7.1$^{\#\#\#}$	27.1 ± 3.2$^{\#\#\#}$	68.5 ± 5.6$^{\#\#\#}$
Dexamethasone + atorvastatin (40 mg/Kg)	169.1 ± 10.3*	140.2 ± 8.2**	115.3 ± 3.2***	36.2 ± 2.2*	49.3 ± 2.1**
Dexamethasone + *A. elburzense* (100 mg/Kg)	176.5 ± 9.6	145.3 ± 9.2*	120.5 ± 3.3	36.1 ± 4.3*	54.4 ± 5.1
Dexamethasone + *A. elburzense* (200 mg/Kg)	172.3 ± 6.1*	141.4 ± 6.3**	110.1 ± 5.2***	39.2 ± 3.2**	51.3 ± 4.2**
Dexamethasone + *A. elburzense* (400 mg/Kg)	158.2 ± 4.2**	134.3 ± 3.1**	107.2 ± 4.3***	40.1 ± 2.1***	43.2 ± 6.3***

Values are expressed as means ± SEM (n = 6). Tukey post hoc analysis, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus normal control, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus dexamethasone-induced dyslipidemic control. BG, blood glucose; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein.

Effect of A. elburzense on biochemical parameters

As shown in Table 2, administration of dexamethasone resulted in a significant increase in serum blood glucose, triglyceride, total cholesterol, and LDL level and a significant decrease in HDL level ($P < 0.001$).

Atorvastatin significantly reduced serum blood glucose (7.3%), triglyceride (17.1%), total cholesterol (22.7%), and LDL level (28.0%) and also increased the HDL level (33.6%) compared to the dyslipidemic control group.

Administration of hydroalcoholic extract of *A. elburzense* bulb decreased serum blood glucose and atherogenic lipid markers. There was a reduction of 13.3% in blood glucose, 20.7% in triglyceride, 28.1% in total cholesterol, 36.9% in LDL level, and an elevation of 35.3% in HDL level after administration of *A. elburzense* extract at the dose of 400 mg/kg. The serum concentration of MDA was significantly increased in dexamethasone-induced dyslipidemic rats compared to the normal control group ($P < 0.001$).

There was significant reduction in this marker of lipid peroxidation after administration of atorvastatin and different doses of *A. elburzense* bulb extract (Fig. 1).

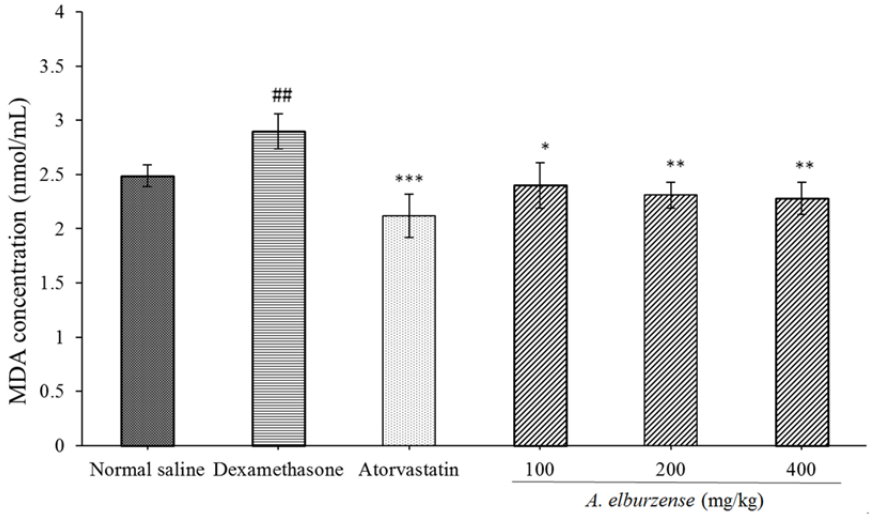

Fig. 1. Effect of hydroalcoholic extract of *A. elburzense* bulb on serum MDA concentration in dexamethasone-induced dyslipidemia. Values are means ± SEM (n = 6). $^{\#\#}P < 0.01$ versus normal control, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus dexamethasone-induced dyslipidemic control. MDA, Malondialdehyde.

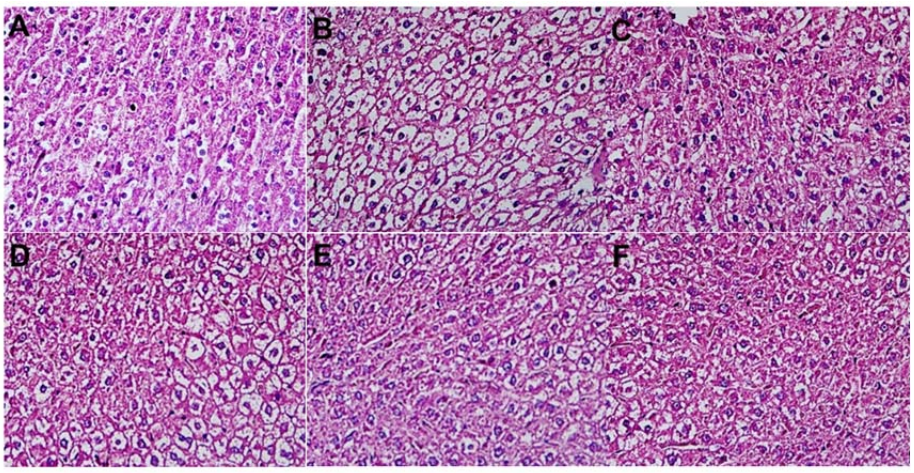

Fig. 2. Representative hematoxylin and eosin histological sections of the liver tissue of (A), normal control group showing normal hepatocytes architecture; (B), dexamethasone-induced dyslipidemic group showing cellular swelling, fatty degeneration, and diffused vesicular steatosis; (C), atorvastatin treated group showing nearly normal hepatic architecture; *A. elburzense* extract treated groups with doses of (D), 100 mg/kg showing moderate vesicular steatosis, (E), 200 mg/kg, and (F), 400 mg/kg showing mild vesicular steatosis, ×40 magnification.

Effect of A. elburzense on liver histopathology

Histopathological examination of liver section of dexamethasone-treated animals showed cellular swelling, fatty degeneration and diffused steatosis compared to the normal architecture of the hepatocytes in normal control group (Figs. 2A and 2B). Microscopical examination of liver section of atorvastatin-treated group exhibited nearly normal hepatic architecture (Fig. 2C). *A. elburzense* extract-treated groups at the dose of 100 mg/kg (Fig. 2D), 200 mg/kg (Fig. 2E), and 400 mg/kg (Fig. 2F) showed partially reduction in micro- and macro-vesicular steatosis.

DISCUSSION

In the present study, the antihyperlipidemic, antihyperglycemic, and antioxidant effects of hydroalcoholic extract of *A. elburzense* bulb as a plant with limited pharmacological information was screened in dexamethasone-induced dyslipidemia. Dexamethasone is a powerful synthetic member of glucocorticoids

that is widely used for the treatment of inflammation, autoimmune disorders, and preventing rejection in organ transplant recipients. Administration of high doses of glucocorticoids particularly during long-term treatment may lead to hyperglycemia, dyslipidemia, and metabolic syndrome through glucose intolerance, insulin resistance, and imbalance in lipid metabolism (15). Accumulation of fat in the liver occurs due to increased lipogenesis and insufficient synthesis of triacylglycerol. Secretion of very low density lipoprotein by the liver is increased due to reduced activity of lipoprotein lipase. Inhibitory effect on the catabolism and uptake of LDL and therefore elevated plasma LDL, increased total cholesterol level because of reduction in the activity of lecithin cholesterol acetyl transferase, and elevation of free fatty acids due to increased lipolysis in the adipose tissue are some other mechanisms involving in glucocorticoid-induced dyslipidemia (15,16). Moreover, the obesity and alterations in lipogenesis is supposed to be associated with abnormalities in the hypothalamic-pituitary-adrenal axis and subsequently increase in leptin production (17).

Oxidative stress is also considered as one of the main issues involved in the pathogenesis of cardiovascular disorders induced by glucocorticoid excess (18). Over-production of free radicals and hepatic oxidative damage has been reported in dexamethasone-induced dyslipidemia (19).

In this study, significant alterations were observed in the body and liver weights, the liver architecture, the blood sugar and lipid profile, and also in the serum lipid peroxidation index in animals treated with dexamethasone. Treatment with *A. elburzense* bulb extract significantly improved liver histopathological changes, the level of blood glucose, lipid markers, and MDA concentration. *A. elburzense* was effective in preventing liver weight gaining at the doses of 100 and 400 mg/kg but not 200 mg/kg. Administration of dexamethasone is also associated with significant decrease in body weight in some animal models such as hyperlipidemia and hypertension in rats (20). Because of severe body weight loss in the present model, treatment with atorvastatin and different doses of *A. elburzense* extract could not prevent body weight declining.

Many studies have reported hypoglycemic, hypolipidemic, and anti-atherosclerotic activities from several *Allium* members such as garlic (*A. sativum*), onion (*A. cepa*), *A. tuberosum*, *A. hirtifolium*, *A. porrum*, *A. chinense*, etc. In the study of Al-Numair significant reduction in total cholesterol (33.2%), triglyceride (34.1%), and LDL (37.7%) and significant increase in HDL level (12.6%) was observed after 3 months of garlic administration in hyperlipidemic rats (21). In an experimental model of atherosclerosis, 50% reduction in atheromatous lesions has been observed after long term supplementation with garlic (22).

Vidyavati, *et al*. also reported hypolipidemic activity of *A. cepa* by reduction of serum cholesterol (23%) and LDL (37%) in hypercholesterolemic rats (23).

In some clinical studies, significant decrease in the level of total cholesterol (12.4%) and LDL (16.3%) has been observed by garlic (24). A recent meta-analysis has reported 17 ± 6 mg/dL decrease in cholesterol and 9 ± 6 mg/dL in LDL level with slightly improvement in HDL and no effect on triglyceride level in hypercholesterolemic patients after 2 months treatment with garlic (25).

Various mechanisms including evoking the insulin production and secretion, influencing gene expression of glucose-regulating enzymes, inhibiting cholesterol absorption, inhibiting hepatic cholesterol biosynthesis, increasing cholesterol turnover, inhibiting development of arteriosclerotic plaques in arterial walls, anticoagulant, and fibrinolytic effects may contribute in these cardio-protective activities of *Allium* plants (26,27).

Strong antioxidant properties through improving the cellular antioxidant enzymes, increasing glutathione storage, decreasing the lipoprotein susceptibility to oxidation, and scavenging the free radicals have also been established for plants belonging to *Allium* genus (28).

In our study, administration of the highest dose of *A. elburzense* extract (400 mg/kg) resulted in a significant decrease in blood glucose (13.3%), triglyceride (20.7%), total

cholesterol (28.1%), LDL (36.9%), and MDA level (24.1%) and increase in HDL level (35.3%). A recent study has also showed the hypoglycemic effect of hydroalcoholic extract of *A. elburzense* bulb on blood glucose level of normal and streptozotocin-induced diabetic rats at similar doses (8).

The major bioactive constituents found in *Allium* plants including saponins such as alloside B, polyphenols, and flavonoids such as quercetin and organo-sulphur compounds such as allicin are recognized as responsible agents for their beneficial cardiovascular effects (25,26). Our previous studies have shown the strong hypoglycemic, antioxidant, and fibrinolytic activities for *A. elburzense* extract which may participate in its preventive effects on atherosclerosis (8,9). The presence of similar biochemicals such as sulfuric compounds, flavonoids and high amounts of saponins which distinguishes *A. elburzense* from other *Allium* members may suggest its therapeutic activities in atherosclerotic disorders (9,10,29). *A. elburzense* is rich in saponins and some sapogenins and new saponins namely elburzensoides have been found in this plant which may propose strong hypolipidemic effect through inhibition of cholesterol absorption from gastrointestinal tract and also affecting cholesterol metabolism (30).

CONCLUSION

In conclusion, this study showed that hydroalcoholic extract of *A. elburzense* bulb has anti-dyslipidemic, anti-hyperglycemic, and antioxidant effects in an animal model of dyslipidemia. Further experiments are required to elucidate the precise mechanism of action and to prove the clinical worth of this herbal medicine in human dyslipidemic disorders.

ACKNOWLEDGEMENTS

The content of this paper is extracted from a Pharm.D thesis No. 394703 submitted by Sajad Karimi which is financially supported by Vice Chancellor of Research, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

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