Indian J Physiol Pharmacol 2015; 59(2) : 223–230

Original Article

Effects of Hydroalcoholic Extract of Watercress (*Nasturtium Officinale)* Leaves on Serum Glucose and Lipid Levels in Diabetic Rats

Mousa-Al-Reza Hadjzadeh[1], Ziba Rajaei[2]*, Reyhaneh Moradi[1] and Ahmad Ghorbani[3]

[1]Neurocognitive Research Center and Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[2]Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

[3]Pharmacological Research Center of Medicinal Plants, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Objective: Hyperlipidemia is a known complication of diabetes mellitus and predisposes to coronary heart disease. The lowering of total cholesterol and low density lipoprotein (LDL)-cholesterol should reduce the incidence of coronary disease. The aim of the present study was to examine the antihyperlipidemic and antidiabetic effects of the hydroalcoholic extract of watercress (*Nasturtium officinale*) leaves in streptozotocin-induced diabetic rats.

Methods: Female Wistar rats were randomly divided into 4 groups: control, diabetic and diabetic rats treated with the extract of watercress (*Nasturtium officinale*) at doses of 100 and 200 mg/kg. Diabetic rats received the watercress extract daily in drinking water for 4 weeks since the day after diabetes confirmation. The levels of serum glucose and lipids were spectrophotometrically measured in all groups at weeks 0 (before diabetes induction), 2 and 4.

Results: There was a significant increase in serum glucose, triglycerides, total cholesterol, and LDL-cholesterol in streptozotocin-induced diabetic rats, accompanied by a decrease in high density lipoprotein (HDL)-cholesterol. The treatment of diabetic rats with hydroalcoholic extract of watercress (*Nasturtium officinale*) leaves over a 4-week period significantly reduced serum glucose, total cholesterol and LDL-cholesterol in comparison with diabetic untreated rats.

Conclusion: Our findings demonstrated that a 4-week treatment with watercress extract at a dose of 200 mg/kg has hypoglycemic and hypolipidemic effects in streptozotocin-diabetic rats. This implies that the consumption of watercress leaves can be helpful in reducing the complications of hyperglycemia and dyslipidemia associated with diabetes.

Key words: *Nasturticum officinale,* Streptozotocin, Diabetes, Hyperglycemia, Hyperlipidemia, Rat.

***Corresponding author :**

Ziba Rajaei, PhD, Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, Tel: 98-31-37922433, Fax: 98-31-36688597
E-mail: rajaeiz@med.mui.ac.ir

(Received on March 14, 2014)

Introduction

Diabetes mellitus is a metabolic disorder characterized by high levels of glucose in the blood resulting from defects in insulin secretion, insulin

action or both (1). Besides hyperglycemia, the levels of plasma lipids are usually elevated in diabetes mellitus causing a risk factor for coronary heart disease (2). Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications. 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 (3-6).

Watercress *(Nasturtium officinale),* a member of Brassicaceae family, is a hardy perennial native to Europe and Asia. It is usually consumed with salads, juices or other dishes as an ingredient, flavor or garnish. The plant contains a high concentration of glucosinolates, as well as carotenoids, polyphenols, vitamin C, vitamin A and α-tocopherol (7-9). Watercress *(Nasturtium officinale)* leaves are traditionally used as a stomachic, depurative, diuretic, expectorant, hypoglycemic, odontalgic and stimulant (10, 12). It also cures pain, ulcers, jaundice and fever (10, 11). Meanwhile, it has been used to treat asthma, bronchitis, scurvy, tuberculosis and urinary tract infection and calculi (10-11). The plant possesses antimicrobial, antioxidant (13), antiestrogenic, anticarcinogenic activities (14). It is also considered as an excellent functional food for the prevention of cancer (15). Watercress (*Nasturtium officinale)* has also been used as an antidiabetic agent in Iranian traditional medicine (11). The objective of the present investigation was to ascertain the scientific basis of its use in treatment of diabetes. It has already been shown that the ethyl acetate extract of aerial parts of *Nasturtium officinale* decreases the blood glucose levels in diabetic rats after 2 months treatment (16). However, there is no report about the hypolipidemic activity of watercress in experimental diabetes. Therefore, we examined the influence of hydroalcoholic extract of watercress (*Nasturtium officinale)* leaves on serum glucose and lipid levels in streptozotocin-induced diabetic rats.

Materials and Methods

Preparation of the hydroalcoholic extract of watercress

Aerial parts of the plant were collected from Mashhad (Razavi Khorasan, Iran) and graciously identified by Ferdowsi University herbarium (Mashhad, Iran). The plant was dried in shadow and then powdered. The powdered plant (110 g) were macerated in 1500 ml of 70% ethanol/H_2O for 72 h. Then the hydroalcoholic extract was filtered and concentrated in an oven at 40-45°C for 72 h. The resulted extract after drying gave 30.3 g (i.e. 27.5% yield) of brownish extract. The plant extract was dissolved in water for pharmacological experiments.

Animals

Female Wistar rats, weighing 200-230g were housed in an air-conditioned colony room at 23±2°C on a standard pellet diet and tap water at libitum. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the study was approved by Mashhad University of Medical Sciences.

Induction of diabetes

The overnight fasted rats were rendered diabetic by a single intraperitoneal injection of 55 mg/kg streptozotocin (Enzo Life Sciences, USA) freshly dissolved in cold distilled water (17). After 72 h of the streptozotocin injection, serum glucose levels were measured using a glucometer (Glucocard, Japan). Only those animals with serum glucose higher than 250 mg/dl were selected as diabetics for the following experiments. The day on which hyperglycemia had been confirmed was designated as day 0. Diabetes was also confirmed by the presence of polyphagia, polydipsia and polyuria during the experiment.

Experimental design

Rats were randomly allocated and similarly grouped

into four groups: control (n=8), diabetic (n=7), diabetics treated with the extract of watercress (*Nasturtium officinale*) in drinking water at doses of 100 (watercress 100 mg/kg, n=9) and 200 mg/kg (watercress 200 mg/kg, n=8). The animals received the watercress extracts in drinking water since day 0 for 4 weeks. Changes in body weight, food consumption and water intake were regularly recorded during the experimental period. For blood sampling, rats were fasted overnight and blood samples were obtained from retro-orbital plexus before diabetes induction (week 0) and at the end of weeks 2 and 4. Blood was allowed to clot and serum separated by centrifugation at 3500 × g for 10 min.

Biochemical parameters

Serum concentrations of glucose, triglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL)-cholesterol were determined by enzymatic colorimetric methods using commercially available kits (Pars Azmun, Iran) by Convergys 100 (Germany). The assay was performed according to the manufacturer's instructions. Very low density lipoprotein (VLDL) cholesterol was calculated as TG/5 and low density lipoprotein (LDL)-cholesterol was estimated by using Friedewald et al. (1972) (18) formula as follows : LDL (mg/dl) = TC – (HDL + VLDL).

Statistical analysis

The data were expressed as Mean±S.E.M. Statistical analysis was carried out using one-way ANOVA followed by LSD post hoc test. A statistical P value less than 0.05 was considered significant.

Results

Effects of watercress extract on serum glucose levels

As shown in Fig. 1, there was no significant difference in serum glucose levels among animals in the experimental groups before diabetes induction (week 0). Diabetic rats showed a significant increase in serum glucose levels at weeks 2 and 4 as

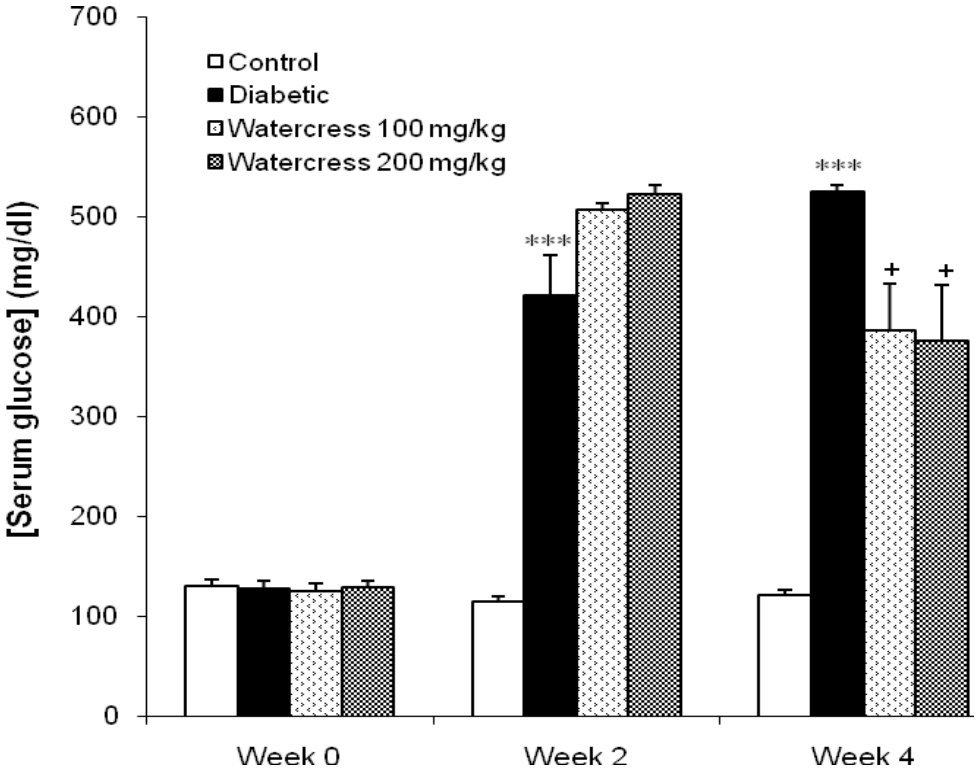

Fig. 1 : Effect of hydroalcoholic extract of Nasturtium officinale on serum glucose levels in STZ-induced diabetic rats at week 0 (before diabetes induction) and at the end of weeks 2 and 4. Data are mean±SEM. ***P<0.001 vs control group, +P<0.05 vs diabetic group.

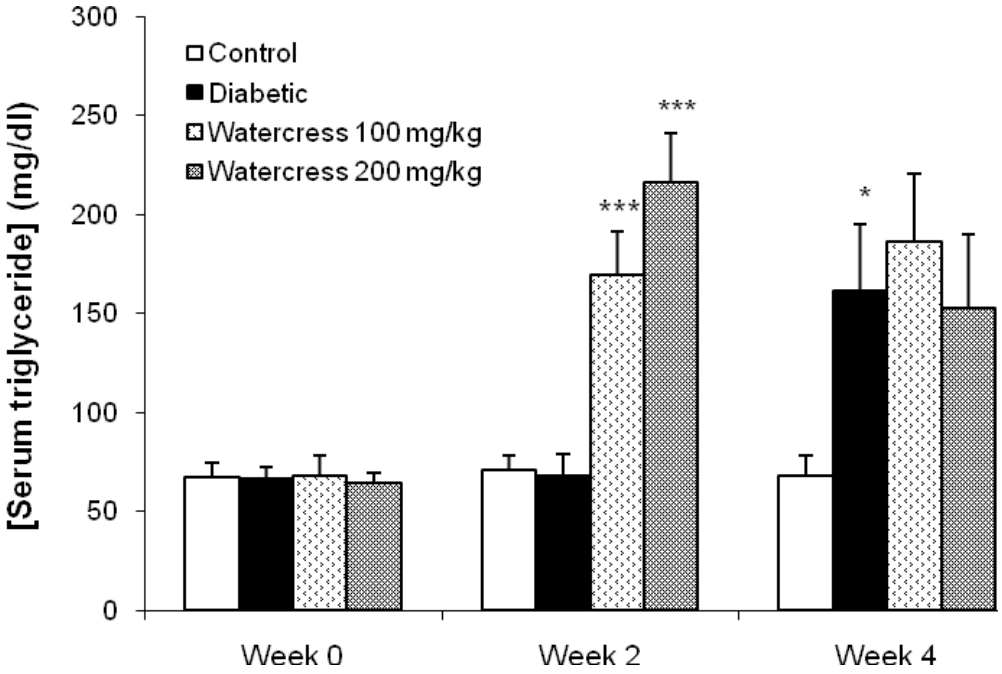

Fig. 2 : Effect of hydroalcoholic extract of Nasturtium officinale on serum triglyceride levels in STZ-induced diabetic rats at week 0 (before diabetes induction) and at the end of weeks 2 and 4. Data are mean±SEM. *P<0.05, ***P<0.001 vs control group.

compared to control rats (Fig. 1, P<0.001). Treatment of diabetic rats for 4 weeks with watercress (*Nasturtium officinale*) extract at doses of 100 and 200 mg/kg significantly decreased their serum glucose levels (Fig. 1, P <0.05).

Effects of watercress extract on serum lipid profile

Diabetic rats showed a significant increase in serum triglyceride levels at week 4 as compared to control rats (P<0.05, Fig. 2) and treatment of diabetic rats with watercress extract had no effect on triglyceride levels (Fig. 2).

The levels of total cholesterol and LDL-cholesterol were significantly increased (P<0.001, Figs. 3, 4), in diabetic rats as compared to the control rats at week 4. Treatment of diabetic rats with watercress extract at dose of 200 mg/kg for 4 weeks significantly reduced the levels of total cholesterol and LDL-cholesterol (P<0.01, Figs. 3, 4) as compared to diabetic animals. In addition, the levels of HDL-cholesterol were significantly decreased (P<0.001, Fig. 5) in diabetic rats at week 4 as compared to the controls, and treatment of diabetic rats with watercress extract at doses of 100 and 200 mg/kg for 4 weeks did not change the HDL-cholesterol levels as compared to diabetic animals (Fig. 5).

Discussion

Diabetes is a complex metabolic disorder characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism. This abnormal metabolism leads to an increased generation of reactive oxygen species (19). In the present study, streptozotocin-induced diabetic rat model was used to assess the effects of watercress extract on diabetes. Diabetogenic effect of streptozotocin is due to excess production of reactive oxygen species leading to toxicity in pancreatic cells which reduces the synthesis and the release of insulin (20). Accordingly, in our study streptozotocin treatment induced a diabetic state characterized by a hyperglycemia, and treatment with watercress extract at doses of 100 and 200 mg/kg significantly reduced the blood glucose level. These findings are in agreement with those results reported by Hosseini et al. (2009), who showed that ethyl acetate extract of aerial parts of watercress at a dose of 100 mg/kg decreased the blood glucose levels in diabetic animals after 2 months treatment (16). However, their results also showed that the

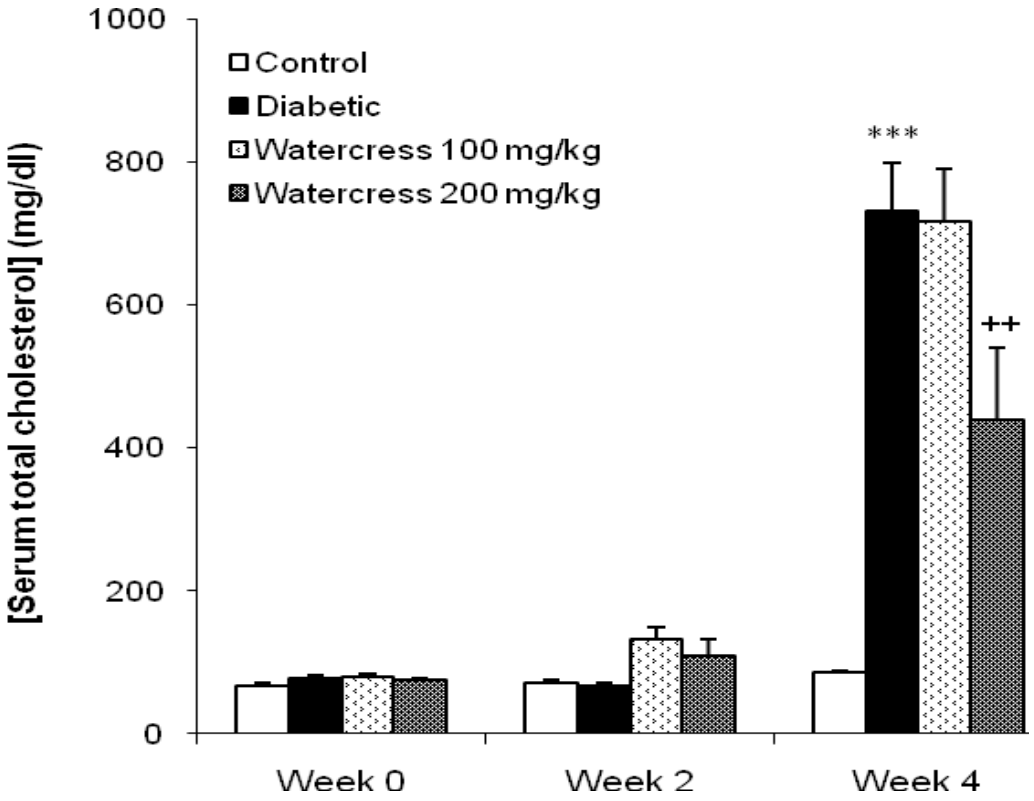

Fig. 3 : Effect of hydroalcoholic extract of Nasturtium officinale on serum total cholesterol levels in STZ-induced diabetic rats at week 0 (before diabetes induction) and at the end of weeks 2 and 4. Data are mean±SEM. ***P<0.001 vs control group, ++P<0.01 vs diabetic group.

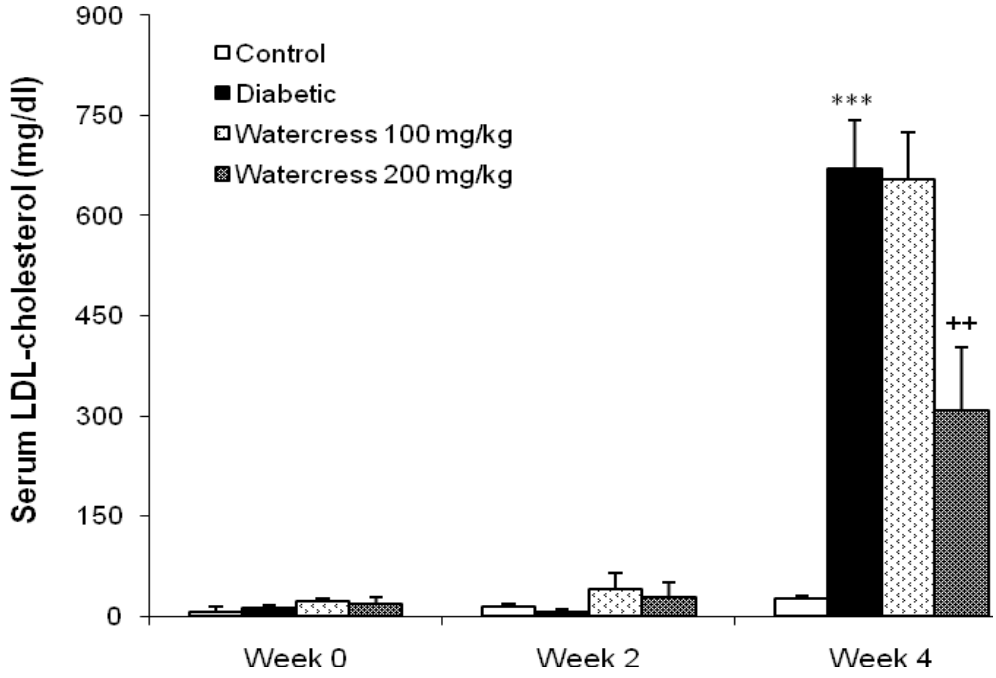

Fig. 4 : Effect of hydroalcoholic extract of Nasturtium officinale on serum LDL-cholesterol levels in STZ-induced diabetic rats at week 0 (before diabetes induction) and at the end of weeks 2 and 4. Data are mean±SEM. ***P<0.001 vs control group, ++P<0.01 vs diabetic group.

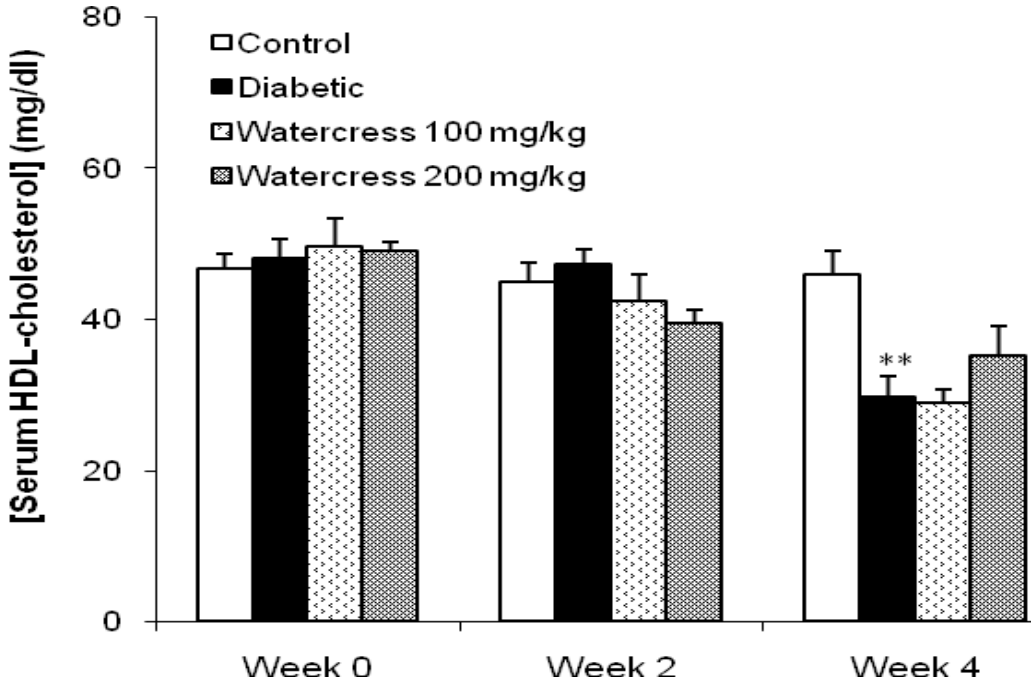

Fig. 5 : Effect of hydroalcoholic extract of Nasturtium officinale on serum HDL-cholesterol levels in STZ-induced diabetic rats at week 0 (before diabetes induction) and at the end of weeks 2 and 4. Data are mean±SEM. **P<0.001 vs to control group.

aqueous and methanolic extract of aerial parts of watercress at doses of 800 and 1000 mg/kg had no effect on blood glucose levels after 1 week treatment (16). This discrepancy between our results and their study could be due to the type of the extract and duration of the treatment.

The possible mechanism by which watercress extract exerts its hypoglycemic action may be due to a stimulation of Langerhans islets, an improvement of peripheral sensitivity to remnant insulin, and to the antioxidant properties of watercress. The antioxidant activity of watercress has been reported in several studies. Ozen et al. (2009) showed that watercress extract acts as an antioxidant to reduce cellular lipid peroxidation, reducing power, superoxide anion and free radical scavenging activities (13). Gill et al. (2007) have also reported that watercress supplementation in diet reduced lymphocyte DNA damage and altered blood antioxidant status in healthy adults (7). Therefore, hypoglycemic effect of watercress extract, at least in part, could be due to its antioxidant properties and to prevent streptozotocin-induced oxidative stress, so that watercress protects β-cells resulting in an increased insulin secretion, and decreases elevated blood glucose levels.

The levels of plasma lipids are also usually raised in diabetes mellitus and such elevation represents a risk factor for coronary heart diseases (2). Hypercholesterolemia and hypertriglyceridemia in streptozotocin-induced diabetic rats are well documented (21). In the present study, we recorded a significant increase in the serum total cholesterol, triglycerides, and LDL-cholesterol as well as a decrease in the HDL-cholesterol levels at diabetic rats. The abnormally high concentration of plasma lipids is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots (22).

The results also demonstrated that the hydroalcoholic extract of watercress produced a significant decrease in serum total cholesterol and LDL-cholesterol levels on repeated oral administration in streptozotocin diabetic rats. The lowering of total cholesterol and LDL-cholesterol would reduce the incidence of coronary disorders. To our knowledge, this is the first study reporting the hypolipidemic activity of watercress extract in streptozotocin-induced diabetes.

The lipid lowering activity of watercress could be due to a decrease in cholesterol absorption from the intestine, its binding with bile acids within the intestine and increasing bile acids excretion (23, 24) and/or a decrease of cholesterol biosynthesis (25, 26) and/or enhanced uptake of LDL by increasing LDL receptors (27).

Phytochemical investigation of watercress has revealed the presence of antioxidants; polyphenols, glucosinolates, carotenoids, vitamin C and α-tocopherol in the aerial parts of the plant (7-9, 28). Several studies have reported that the plant flavonoids, phenolic compounds and glycosides have hypolipidemic and hypocholesterolemic effects (4, 29, 30). Hence it may be concluded that the hypolipidemic effect produced by the extract may be due to the presence of polyphenols and glycosides.

Conclusion

Based on this study, we can conclusively state that the hydroalcoholic extract of watercress (*Nasturtium officinale*) ameliorates diabetic hyperglycemia and hyperlipidemia. This finding provides a scientific rationale for the use of watercress (*Nasturtium officinale*) as an anti-diabetic plant in Iranian folk medicine. Further investigations are needed to elucidate the mechanism(s) of the antidiabetic and hypolipidemic effect in watercress and the active constituent(s) of the extract.

Acknowledgments

The results presented in this work have been taken from a student's thesis. This study was supported by the Council of Research, Mashhad University of Medical Sciences.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. American Diabetes Association: Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013; 36: S67–S74.
2. Kannel WB, Mc Gee DL. Diabetes and cardiovascular risk factors: the Framingham study. *Circulation* 1979; 59: 813.
3. Slowing K, Ganado P, Sanz M, Ruiz E, Tejerina T. Study of garlic extracts and fractions on cholesterol plasma levels and vascular reactivity in cholesterol fed rats. *J Nutr* 2001; 131: 994S–999S.
4. Anila L, Vijayalakshmi NR. Flavonoids from Emblica officinalis and Mangifera indica: effectiveness for dyslipidemia. *J Ethnopharmacol* 2002; 79: 81–87.
5. El-Beshbishy HA, Singab ANB, Sinkkonen J, Pihlaja K. Hypolipidemic and antioxidant effects of Morus alba L. (Egyptian mulberry) root bark fractions supplementation in cholesterol-fed rats. *Life Sci* 2006; 78: 2724–2733.
6. Rajaei Z, Hadjzadeh MA, Moradi R, Ghorbani A, Saghebi A. Antihyperglycemic and antihyperlipidemic effects of hydroalcoholic extract of *Securigera securidaca* seeds in streptozotocin-induced diabetic rats. *Adv Biomed Res* 2015; 4; 33.
7. Gill C, Haldar S, Boyd L, Bennett R, Whiteford J, Butler M, et al. Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy adults. *Amer J Clin Nutr* 2007; 85: 504–510.
8. Mazandarani M, Momeji A, Zarghami Moghaddam P. Evaluation of phytochemical and antioxidant activities from different parts of *Nasturtium officinale* R. Br. in Mazandaran. *Iran J Plant Physiol* 2013; 3: 659–664.
9. Casanova NA, Ariagno JA, López Nigro MM, Mendeluk GR, Gette MA, Petenatti E, et al., *In vivo* antigenotoxic activity of watercress juice (Nasturtium officinale) against induced DNA damage. *J Appl Toxicol* 2012; Apr 4. doi: 10.1002/jat.2746.
10. Zargari A. Medicinal Plants. 8th ed., vol. 1. University Publication, Tehran, 2011.
11. Mirheidar H. Encyclopedia of Medicinal Plants of Iran. 6th ed., vol. 1. Islamic Culture Press, Tehran, 2004.
12. Bahramikia S, Yazdanparast R. Effect of hydroalcoholic extracts of *Nasturtium officinale* leaves on lipid profile in high-fat diet rats. *J Ethnopharmacol* 2008; 15: 116–121.
13. Ozen T. Investigation of antioxidant properties of *Nasturtium officinale* (watercress) leaf extracts. *Acta Pol Pharmacol* 2009; 66: 87–93.
14. Tamayo C, Richardson MA, Diamond S, Skoda I. The chemistry and biological activity of herbs used in Flor-Essence herbal tonic and Essiac. *Phytother Res* 2000; 14: 1–14.
15. Potter JD, Steinmetz K. Vegetables, Fruit and Phytoestrogens as Preventive Agents, vol. 139. IARC scientific publications, 1996.
16. Hosseini HF, Gohari AR, Saeidnia S, Shahabimajd N, Hadjiakhoondi A. The effect of *Nasturtium officinale* on blood glucose level in diabetic rats. *Pharmacologyonline* 2009; 3: 866–871.
17. Rajaei Z, Hadjzadeh MA, Nemati H, Hosseini M, Ahmadi M, Shafiee S. Antihyperglycemic and antioxidant activity of crocin in streptozotocin-induced diabetic rats. *J Med Food*

2013; 6: 206–210.

18. Friedewald WT, Levy RI, Fredrickson DS, In: Tietz (Ed.), Determination of LDL cholesterol. Text Book of Clinical Biochemistry, New York, 1972; p. 874–898.
19. Rajasekaran S, Kasiappan R, Karuran S, Sorimutha S. Beneficial effects of *Aloe Vera* Leaf Gel extract on lipid profile status in rats with streptozotocin diabetes. *Clin Exp Pharmacol Physiol* 2006; 33: 232–237.
20. Szkudelski T. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas. *Physiol Res* 2001; 50: 536–546.
21. Pushparaj P. Tan CH, Tan BKH. Effects of Averrhoabilimli leaf extract on blood glucose and lipids in streptozotocin diabetic rats. *J Ethnopharmacol* 2000; 72: 69–76.
22. Ahmed I, Lakhani MS, Gillett M, John A, Raza H. Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced diabetic rats. *Diabates Res Clin Prac* 2001; 51: 155–161.
23. Kritchevsky D. Fiber, lipids and atherosclerosis. *Amer J Clin Nutr* 1978; 31S: 65–74.
24. Kelly JJ, Tsai AC. Effect of pectin, gum Arabic and agar on cholesterol absorption, synthesis and turnover in rats. *J of Nutr* 1978; 108: 630–639.
25. Kedar P, Chakrabarti CH. Effects of bittergourd (*Momordica charantia*) seed and glibenclamide in streptozotocin induced diabetes mellitus. *Indian J Exp Biol* 1980; 20: 232–235.
26. Sharma SB, Nasir A, Prabhu KM, Murthy PS, Dev G. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits. *J Ethnopharmacol* 2003; 85: 201–206.
27. Slater HR, Packard CJ, Bicker S, Shephered J. Effects of cholestyramine on receptor mediated plasma clearance and tissue uptake of human low density lipoprotein in the rabbit. *J Biol Chem* 1980; 255: 10210–10213.
28. Aires A, Marques E, Carvalho R, Rosa EA, Saavedra MJ. Evaluation of Biological Value and Appraisal of Polyphenols and Glucosinolates from Organic Baby-Leaf Salads as Antioxidants and Antimicrobials against Important Human Pathogenic Bacteria. *Molecules* 2013; 18: 4651–4668.
29. Chan PT, Fonf WP, Cheung YL, Huang Y, Ho WK, Chen ZY, Jasmine green tea epicatechins are hypolipidemic in hamsters fed a high fat diet. *J Nutr* 1999; 129: 1094–1101.
30. Guimaraes PR, Galavao AMP, Batista CM, Azovedo GS, Oliveira RD, Lamounier P, et al. Eggplant (*Solanum melongena*) infusion has modest and transitory effect on hypercholesteremic subjects. *Braz J Med Biol Res* 2000; 33: 1027–1036.