EFFECT OF BARBERRY FRUIT (BERBERIS VULGARIS) ON SERUM GLUCOSE AND LIPIDS IN STREPTOZOTOCIN-DIABETIC RATS

M.A.R. Hajzadeh[1,2], Z. Rajaei[2]*, S. Shafiee[2], A. Alavinejhad[2], S. Samarghandian[2], M. Ahmadi[2]

[1]Pharmacological Research Center of Medicinal Plants, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

[2]Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Summary

Barberry (Berberis vulgaris) is used in traditional medicine for a number of diseases including diabetes mellitus. The aim of the present study was to evaluate the antidiabetic activity of aqueous extract of *Berberis vulgaris* fruit in streptozotocin-induced diabetic rats. The animals were rendered diabetic by a single intraperitoneal injection of 60mg/kg streptozotocin. Rats received the barberry fruit extract daily in drinking water containing 3.5% and 7.5% from a 100mg/ml of the initial extract, since the day after diabetes confirmation for 6 weeks. The blood glucose and lipids were spectrophotometrically measured in all groups at weeks 0, 3 and 6. The results showed that the aqueous extract of *Berberis vulgaris* fruit at amounts of 3.5 and 7.5% of drinking water did not possess the hypoglycemic and hypolipidemic activity in streptozotocin-diabetic rats during 6-week treatment period. Therefore, the usage of barberry fruit in traditional medicine for the treatment of diabetes may need more investigation.

Key words: Berberis vulgaris, Diabetes mellitus, Hyperglycemia, Hyperlipidemia, Rat.

*Corresponding author: Tel: 98-511-8002221, Fax: 98-511-8828564

E-mail: rajaeiz@mums.ac.ir

Introduction

Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the population. Besides hyperglycemia, several other factors such as dyslipidemia or hyperlipidemia are also involved in the development of cardiovascular complications in diabetes which are the major causes of morbidity and mortality (1-3). Currently available therapy for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, metformin and α-glucosidase inhibitors. Each of the above oral agents suffers from a number of serious adverse effects (4,5). This concern has led to an increase demand for natural products with antidiabetic activity having fewer side effects (6-9).

Barberry (Berberis vulgaris L. family Berberidaceae), which grows in Asia and Europe, is a shrub with yellow wood and obovate leaves, bearing pendulous yellow flowers succeeded by oblong red coloured fruits. The constituents reported in this plant are berberine, berbamine, palmatine, oxyacanthine, malic acid and berberubin (10).

In Iranian traditional medicine, several properties such as antibacterial, antiemetic, antipyretic and antipruritic has been reported for different parts of *Berberis vulgaris* (10-12). Meanwhile, several pharmacological studies on berberine, an isoquinoline alkaloid found in the root, bark and fruit of *Berberis vulgaris*, have demonstrated that it possesses anti-inflammatory, antinociceptive, hypoglycemic and hypolipidemic effects (13-17).

Barberry fruit is extensively used as food additive and its juice is recommended to cure cholecystitis (10). Nevertheless, little pharmacological studies have been performed on barberry fruit. It has been shown that the crude extract of barberry fruit possesses the antihistaminic and anticholinergic activities (18). However, to our best of knowledge, there is no report in the literature on the antidiabetic activity of *Berberis vulgaris* fruit. Therefore, the present study was undertaken to study the effect of *Berberis vulgaris* fruit extract on serum glucose and lipids in streptozotocin-induced diabetic rats.

Materials and Methods

Animals

Male wistar rats, weighing 220-280g were housed in an air-conditioned colony room at $23 \pm 2°C$ 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.

Preparation of the plant extract

Barberry fruits from Ghaenat district (Khorasan, Iran) were obtained from a local store in Mashhad and verrified in Pharmacy Faculty (Mashhad, Iran). For preparation of aqueous extract, the barberry fruits (10g) were macerated in distilled water for 3 hours. Then, they were mixed in a grinder and filtered. Distilled water was added to filtered mixture to reach a volume of 100 ml, thus the initial extract was at a concentration of 100mg/ml. The prepared extract was added to drinking water by 3.5% and 7.5% (v/v) for experimental groups.

Experimental protocol

The overnight fasted rats were rendered diabetic by a single intraperitoneal injection of 60 mg/kg STZ (Axxora, USA) freshly dissolved in cold distilled water. After 72h of the STZ injection, blood samples were collected and serum glucose concentrations was 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. Diabetes was also confirmed by the presence of polyphagia, polydipsia and polyuria. The day on which hyperglycemia had been confirmed was designated as day 0. The rats were randomly allocated and similarly grouped into four groups: control (n=7), diabetic (n=7), diabetics treated with barberry 3.5% in drinking water (diabetic+B3.5, n=7) and diabetics treated with barberry 7.5% in drinking water (diabetic+B7.5, n=8). The animals received the extracts in drinking water since day 0 for 6 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 3 and 6. Blood was allowed to clot and serum separated by centrifugation at 3500 rpm for 10 min. Serum glucose and lipid levels were spectrophotometrically measured using appropriate kits (Parsazmun, Tehran) by Convergys 100 (Germany).

Statistical analysis

The data were expressed as mean ± S.E.M. Statistical analysis was carried out using Student's paired *t*-test, repeated measures and one-way ANOVA followed by Tukey post hoc test. A statistical *p* value less than 0.05 was considered significant.

Results

Measurements of body weight and serum glucose indicated that before diabetes induction, there were no significant differences among animals in each group. Repeated measures ANOVA revealed that the weight of diabetic rats significantly decreased as compared to control rats (Fig.1, $p<0.005$). Treatment with barberry fruit caused no change in the weight of diabetic rats in both experimental groups as compared to untreated diabetics (Fig.1).

Untreated diabetic rats also had an elevated serum glucose level over those of control rats (Fig.2, $p<0.0001$) and treatment of diabetic rats for 6 weeks with the aqueous extract of barberry fruit did not change the serum glucose concentration in comparison to untreated diabetic rats (Fig.2).

Regarding serum lipids, Repeated measures ANOVA revealed that diabetes induction caused a significant increase in triglyceride concentration compared to baseline data (Fig.3, $p<0.05$) and treatment with barberry fruit even increased triglyceride concentration compared to control group; this increase was significant for both diabetic+B3.5 ($p<0.05$) and diabetic+B7.5 ($p<0.0001$) groups compared to control group (Fig.3).

Meanwhile, comparing barberry fruit-treated and untreated diabetic groups showed that there was no difference between the groups after 6 weeks regarding serum total cholesterol and HDL-cholesterol concentrations (Figs. 4,5).
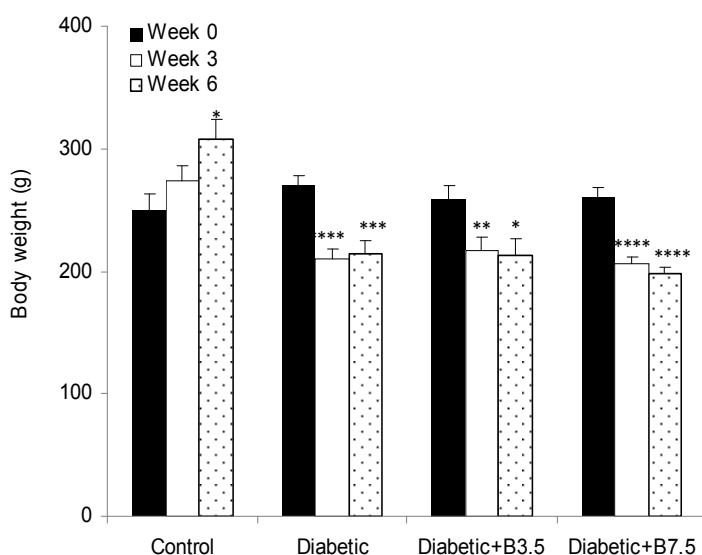

Fig. 1. Comparison of body weight between control, diabetic, diabetic+B3.5 and diabetic+B7.5 groups at week 0 (before diabetes induction) and the end of weeks 3 and 6. Data were expressed as mean ± SEM (n=8 in diabetic+B7.5 , n=7 in other groups). * $p<0.05$, ** $p<0.01$, *** $p<0.005$, **** $p<0.0001$ (as compared to week 0 in the same group).
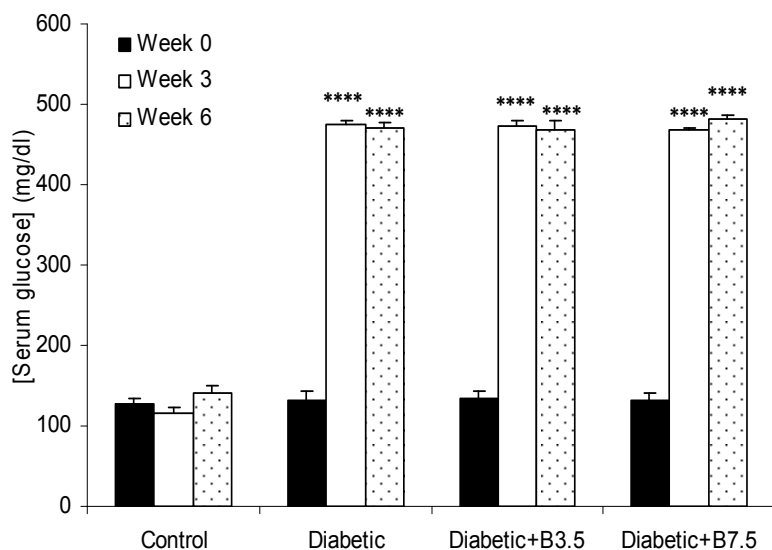
Fig. 2. Comparison of serum glucose concentrations between control, diabetic, diabetic+B3.5 and diabetic+B7.5 groups at week 0 (before diabetes induction) and the end of weeks 3 and 6. Data were expressed as mean ± SEM (n=8 in diabetic+B7.5, n=7 in other groups). **** $p<0.0001$ (as compared to week 0 in the same group).
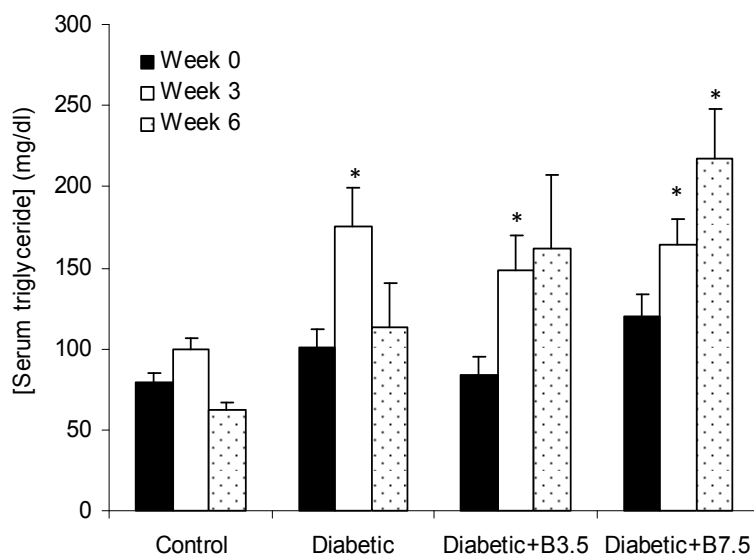

Fig. 3. Comparison of serum triglyceride concentrations between control, diabetic, diabetic+B3.5 and diabetic+B7.5 groups at week 0 (before diabetes induction) and the end of weeks 3 and 6. Data were expressed as mean ± SEM (n=8 in diabetic+B7.5, n=7 in other groups). * $p<0.05$ (as compared to week 0 in the same group).
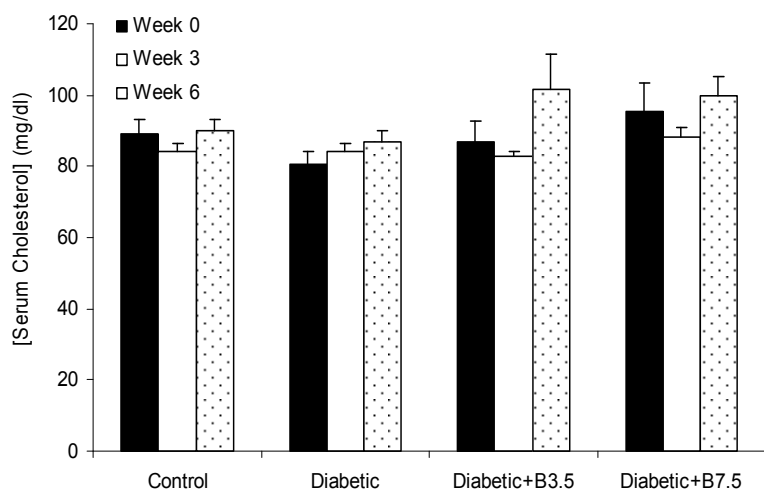
Fig. 4. Comparison of serum total cholesterol concentrations between control, diabetic, diabetic+B3.5 and diabetic+B7.5 groups at week 0 (before diabetes induction) and the end of weeks 3 and 6. Data were expressed as mean ± SEM (n=8 in diabetic+B7.5, n=7 in other groups).
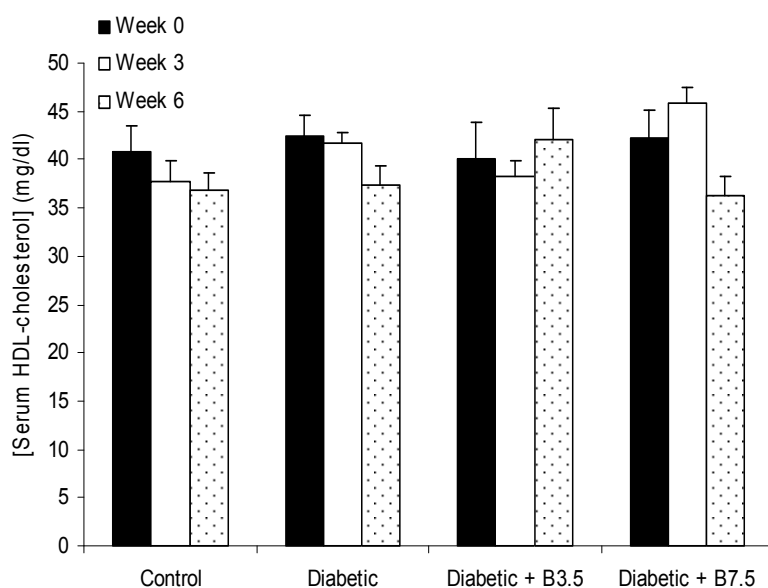

Fig. 5. Comparison of serum HDL-cholesterol concentrations between control, diabetic, diabetic+B3.5 and diabetic+B7.5 groups at week 0 (before diabetes induction) and the end of weeks 3 and 6. Data were expressed as mean ± SEM (n=8 in diabetic+B7.5, n=7 in other groups).

Discussion

In the present study, the effects of barberry fruit on diabetes were assessed using a STZ-induced diabetic rat model. It is well known that injection of a high dose of STZ (>45 mg/kg) significantly damages the ability of pancreatic ß-cells to synthesize and secrete insulin in rats (19). Consequently, these animals develop impaired insulin response to food ingestion and glucose loading, and accordingly, impaired glucose uptake/utilization capabilities (20,21), mimicking human type 1 diabetes mellitus.

The results showed that administration of STZ to rats, as expected, resulted in hyperglycemia and decreased body weight. STZ is taken up by pancreatic β cells via glucose transporter GLUT2. The main cause of STZ-induced β-cell death is alkylation of DNA by the nitrosourea moiety of this compound. However, production of NO and reactive oxygen species may also be involved in DNA fragmentation and other deleterious effects of STZ (19).

Our present data also showed that the aqueous extract of *Berberis vulgaris* fruit at amount of 3.5 and 7.5% of drinking water did not possess the hypoglycemic and hypolipidemic activity in STZ-diabetic rats during the 6-week treatment period.

Different plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances (22) stimulation of ß-cells to produce more insulin (23) and others may increase ß-cells in the pancreas by activating regeneration of pancreatic cells (24,25). The fibers of plants may also interfere with carbohydrate absorption; thereby affecting blood glucose.

Because STZ induces diabetes by damaging ß-cells, the results of the present study suggest that barberry is unable to help the pancreas to recover ß-cell function or regenerate ß-cells in rats experiencing high-dose STZ-induced diabetes under the current experimental conditions.

Another reason for the results is that barberry fruit may contain low berberine. The hypoglycemic and hypolipidemic effects of berberine are supported by the results of previous in vitro (26-28) and in vivo (14-17) studies. Berberine may act as an α-glucosidase inhibitor, which is its main mechanism in diabetes treatment. The inhibitory effect of berberine on diabetes also might be associated with its hypoglycemic effect, modulating lipids metabolic effects and its ability to scavenge free radicals (29). However, the hypoglycemic effect of berberine could also be due to inhibition of intestinal glucose absorption or stimulation of peripheral glucose uptake. Berberine activates AMPK activity in both adipocytes and myocytes, and within these cells type's berberine induces a variety of metabolic effects consistent with AMPK activation. These

include activation of GLUT4 translocation; increased phosphorylation of AMPK, ACC, and p38 MAPK; reduced lipid content in adipocytes; increased expression of genes involved in lipid oxidation; and decreased expression of genes involved in lipid synthesis (30).

Conclusion

In conclusion, this study showed that the aqueous extract of *Berberis vulgaris* fruit at amount of 3.5 and 7.5% of drinking water does not possess the hypoglycemic and hypolipidemic activity in STZ-diabetic rats during the 6-week treatment period. Therefore, the usage of barberry fruit in traditional medicine for the treatment of diabetes may need more investigation.

Acknowledgment

References

1. Peter P, Nuttall SL, Kendall MJ. Insulin resistance--the new goal! J Clin Pharm Ther 2003; 28: 167-74.
2. Taskinen MR. Diabetic dyslipidemia. Atheroscler Suppl 2002; 3: 47-51.
3. Reasner CA. Reducing cardiovascular complications of type 2 diabetes by targeting multiple risk factors. J Cardiovasc Pharmacol 2008; 52: 136–144.
4. Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. Nature 2001; 414: 821-827.
5. Zhang BB, Moller DE. New approaches in the treatment of type 2 diabetes. Curr Opinion Chem Biol 2000; 4: 461-467.
6. Pushparaj P, Tan CH, Tan BKH. Effects of Averrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol 2000; 72: 69–76.
7. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol 1998; 61: 101–110.
8. Singh SK, Kesari AN, Gupta RK, Jaiswal D, Watal G. Assesment of antidiabetic potential of Cynodon dactylon extract in streptozotocin diabetic rats. J Ethnopharmacol 2007; 114: 174-179.
9. Kameswararao B, Kesavulu MM, Apparao C. Evaluation of antidiabetic effect of Momordica cymbalaria fruit in alloxan-diabetic rats. Fitoterapia 2003; 74: 7-13.
10. Zargari A. Medicinal Plants, Tehran University Press, Tehran; 1983. Vol. 1, p. 68.
11. Aynehchi Y. Pharmacognosy and Medicinal Plants of Iran. Tehran University Press, Tehran; 1986. p. 1041.
12. Nafissi A. Foods and Drinks' Properties. Isfahan University Press, Isfahan; 1990. p. 150.

13. Kupeli E, Kosar M, Yesilada E, Husnu K, Baser C. A comparative study on the anti-inflammatory, antinociceptive and antipyretic effects of isoquinoline alkaloids from the roots of Turkish *Berberis* species. Life Sciences 2002; 72: 645–657.

14. Leng SH, Lu FE, Xu LJ. Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. Acta Pharmacol Sin 2004; 25: 496-502.

15. Ko BS, Choi SB, Park SK, Jang JS, Kim YE, Park S. Insulin sensitizing and insulinotropic action of berberine from Cortidis rhizome. Biol Pharm Bull 2005; 28: 1431-7.

16. Pan GY, Huang ZJ, Wang GJ, Fawcett JP, Liu XD, Zhao XC, Sun JG, Xie YY. The antihyperglycaemic activity of berberine arises from a decrease of glucose absorption. Planta Med 2003; 69: 632-636.

17. Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 2004; 10:1344-1351.

18. Shamsa F, Ahmadiani A, Khosrokhavar R. Antihistaminic and anticholinergic activity of barberry fruit (*Berberis vulgaris*) in the guinea-pig ileum. J Ethnopharmacol 1999; 64: 161–166.

19. Szkudelski T. The mechanism of alloxan and streptozotocin action in ß cells of the rat pancreas. Physiol Res 2001; 50: 537-546.

20. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res 2008; 704045: 2008.

21. Ar⸳Rajab A, Ahren B. Long-term diabetogenic effect of streptozotocin in rats. Pancreas 1993; 8: 50-57.

22. Chakravarthy BK, Gupta S, Gambhir SS, Gode KD. Pancreatic beta cell regeneration: A novel antidiabetic mechanism of Petercarpus marsupium. Ind J Pharmacol 1980; 12: 123-128.

23. Ananthan R, Latha M, Pari L, Baskar C, Narmatha V. Modulatory effects of Gymnema montanum leaf extract on alloxan induced stress oxidative in wistar rats. Nutrition 2004; 20: 280-285.

24. Bopanna KN, Kannan J, Gadgil S, Balaraman R, Rathod SP. Antidiabetic and antihyperglycemic effects of Neem seed kernel powder alloxan diabetic rabbits. Ind J Pharmacol 1997; 29: 162-167.

25. Chorvathova V, Bobek P, Ginter E, Klavanova J. Effect of oyster fungus on glycemia and cholesterolemia in rats with insulin-dependent diabetes. Physiol Res 1993; 42: 175-179.

26. Lu SS, Yu YL, Zhu HJ, Liu XD, Liu L, Liu YW, Wang P, Xie L, Wang G. Berberine promotes glucacon-like peptide-1 (7-36) amide secretion in streptozotocin-induced diabetic rats. J Endocrinol 2009; 200: 159-165.

27. Kim SH, Shin EJ, Kim ED, Bayaraa T, Frost SC, Hyun CK. Berberine avtivates GLUT1-mediated glucose uptake in 3T3-L1 adipocytes. Biol Pharm Bull 2007; 30: 2120-2125.

28. Yin J, Hu R, Chen M, Tang J, Li F, Yang Y, Chen J. Effects of berberine on glucose metabolism in vitro. Metabolism 2002; 51: 1439-1443.

29. Tang LQ, Wei W, Chenb LM, Liu S. Effects of berberine on diabetes induced by alloxan and a high-fat⁄high-cholesterol diet in rats. J Ethnopharmacol 2006; 108: 109-115.

30. Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Lee CH, Oh WK, Kim CT, Hohnen-Behrens C, Gosby A, Kraegen EW, James DE, Kim JB. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. Diabetes 2006; 55: 2256-2264.