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BERBERRY (BERBERIS VULGARIS) SUPPLEMENTATION TO DIABETIC RATS IMPROVES SERUM AND HEPATIC OXIDATIVE STRESS MARKERS

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ABSTRACT

Diabetes is one of the most common metabolic disorders and is interrelated to oxidative stress-induced diseases. According to the role of dietary antioxidants in control and prevention of diabetes, this study was aimed to evaluate the effect of berberry extract on serum glucose levels and serum and hepatic total antioxidant capacity (TAC) and lipid (MDA) in diabetic rats. Experimental diabetes in rats was induced by intraperitoneal injection of streptozotocin (55 mg/Kg). Alcoholic extract of berberry (100, 200 mg/Kg) was given by oral gavage to normal and diabetic rats for 4 weeks. Finally, serum glucose and serum and hepatic levels of MDA and TAC were measured and analyzed statistically. Data showed that berberry extract at dose of 200 mg/Kg significantly decreased the serum glucose levels, serum and hepatic MDA concentration and increased the total antioxidant capacity in diabetic

rats ($p < 0.05$). Berberry supplementation also increased hepatic TAC in normal rats ($p < 0.05$). The antihyperglycemic and antioxidative features of berberry make it an attractive candidate for the prophylactic treatment of diabetes, although further investigation is needed to determine exact dose and duration of supplementation

Keywords: Diabetes, Berberry, Berberins, Oxidative stress

INTRODUCTION

Diabetes mellitus that is characterized by hyperglycemia is growing at an alarming rate and the number of individuals suffering from this disease throughout the world is predicted to reach 366 million by the year 2030 (1).

There is considerable evidence that reactive oxygen species (ROS), generated as results of hyperglycemia, lead to many of the secondary complications of diabetes and oxidative damage to peripheral tissues (2, 3).

Several observations confirmed the antioxidative, anti-inflammatory and anti-diabetic activities of some phytochemicals such as polyphenols (4). Therefore, a promising approach for hyperglycemia and its complications might be a combination therapy utilizing dietary polyphenols and hypoglycemic drugs at a suboptimal dosage to minimize any potential adverse side effects.

The fruit of *Berberis vulgaris* is used often in traditional Iranian medicine. Two studies examine barberry fruit extract in relation to possible antihistaminic, anticholinergic and antiarrhythmic effects. Positive dose dependent results were also found in the response to aqueous extract of barberry fruits on isolated guinea-pig ileum. The outcome supporting the use of barberry fruits as antihistaminic and anticholinergic agents (5). The other in vivo study demonstrated that after taking a crude aqueous extract of Barberry fruit, the measurement of rat arterial blood pressure was significantly reduced, as was heart rate. There is a possible correlation of the extract effects on potassium currents and traditional sedative and neuroprotective effects of Barberry. The study is supportive of the hypothesis that barberry fruit extract is potentially beneficial in the treatment of hypertension, tachycardia, epilepsy and convulsion (6).

Berberine sulfate was used to determine possible antimicrobial action. The in vitro study found that berberine sulfate interferes with the adhesion of LTA (lipoteidoic acid, a ligand responsible for the adherence of *Streptococci* to epithelial cells). The effect was the blockage of adherence of *Streptococci* to the host cell. At 30 g/mL berberine sulfate completely inhibited the

growth of *Streptococci*. The complexion of LTA with fibronectin and the dissolution of such complexes if formed were also inhibited by berberine sulfate (7). A combination of berberine and geranium leaf extract, in an oral dose, has been shown to inhibit diarrhoea in vivo. Use of berberine in the clinical treatment of cholera has been supported in the outcome of an in vivo trial. Oral administration of berberine was found to be effective in the cessation of diarrhoea resulting in prolonged survival. The study concluded that berberine is an effective antidiarrhoeal drug (8). The effects of the alkaloid constituents are primarily responsible for the historical use of Berberidaceae species extracts in inflammatory conditions (9). Berberine and oxycanthine alkaloids from *Berberis vulgaris* were administered in acute inflammation (paw oedema). Oxycanthine was less effective than berberine in the tests used (10). An in vivo study using Turkish Berberis species demonstrated that all alkaloids (from this species of *Berberis*) inhibited inflammation with dose dependent activity. Berberine, palmatine and berbamine were the most effective in topical and oral administration (11). Berberine has inhibitory effects on potassium and calcium currents in isolated rat hepatocytes, which may be involved in hepatoprotection. These effects of berberine on ion channels of isolated rat hepatocytes were extrapolated from an *in vivo* study. The study examined potassium and calcium ion channels. The role of potassium channels has been linked to regulation of metabolic processes in the liver, including gluconeogenesis, amino acid transport and cell functions (12). This study found that berberine could block potassium channels and decrease the extracellular potassium, resulting in regulation of the metabolic processes in the liver. Hepatocyte damage is linked to increased calcium ion concentration in the cytoplasm. The hepatoprotective action of berberine can therefore be partly linked to its inhibitory effects on potassium and calcium channels (13). In the search for cancer chemoprotective agents, ethanol extract of *Berberis vulgaris* root bark has been studied using a hydroxyl radical scavenging assay (14). Three phenolic compounds were isolated N-(p-trans-coumaroyl) tyramine, cannabisin G and lyniresinol. The results of the in vitro study demonstrate that cannabisin G and lyniresinol are in part responsible for the antioxidant activity of *Berberis vulgaris* root bark (15). In another study using human cell lines, *Berberis vulgaris* fruit extract was tested for its possible antioxidant and chemoprotective effects. The outcome of the study found that

Barberry fruits extract exhibited antioxidant properties, resulting in cytotoxic effects (16,17).

As a result, the present study is based on the hypothesis that the bioactive compounds found in berberry (*Berberis vulgaris*) have anti-diabetic and antioxidant activities. Hypoglycemic and antioxidant effects of berberry have been shown before. However, there are limited evidences about its effect on tissue biomarkers of oxidative stress and very little is known about its mechanisms. The purpose of this study was to investigate the effect of two doses of berberry extract (100, 200 mg/Kg) on serum glucose levels, as well as, serum and hepatic biomarkers of oxidative stress (MDA and TAC) in streptozotocin-induced (STZ) diabetic rats.

EXPERIMENTAL

Preparation of berberry extract

In this study, berberry (*Berberis vulgaris*) leaves were collected from North region of Iran in 2010. Dried berberry leaves were identified by a pharmacognosist in herbarium of school of pharmacy, Ahvaz Jundishapur University of Medical Sciences. Briefly, the dried berberry leaves were powdered by electrical miller. In order to prepare the extract, 150 g of greentea powder was mixed with 1000 mL of 95% ethanol (1:10 w/v) and shacked constantly for 48 h. The suspension was filtered through Whatman No. 1 filter paper and the residue was extracted again and the pooled berberry extract was vacuumed and evaporated in a rotary evaporator. The dried extracts were stored at 4°C until being used. In the present study, each 100 g of dried plant yielded about 15 g of dried extract powder (extraction efficiency = 15%.).

Animals

In this assay, forty-eight male albino rats of wistar strain (180-230 g), aged 5-7 weeks, were obtained from Physiology Research Center of Tabriz University of Medical Sciences. The animals were housed in the steel cages in an air condition room (22 ± 3°C, 55 ± 5% humidity and a 12-h light/dark cycle) and were maintained with free access to water and ad libitum standard laboratory diet.

Study design

The experimental animals were divided randomly into six groups (n = 8) and received the following treatment: Group 1: Non-diabetic control rats; Group 2: Non-diabetic rats treated with 100 mg/Kg berberry extract; Group 3: Non-diabetic rats treated with 200 mg/Kg berberry extract; Group 4: Diabetic control rats; Group 5: Diabetic rats treated with 100 mg/Kg berberry extract; Group 6: Diabetic rats treated with 200 mg/Kg berberry extract. A single intraperitoneal injection of

55 mg/Kg streptozotocin (STZ) (Sigma, Aldrich, USA) dissolved in citrate buffer (0.1 M, PH: 4.6) was used for the induction of diabetes. Diabetes was confirmed through the measuring of fasting blood glucose levels 4 days after STZ injection from tail vein. Rats with fasting blood glucose ≥ 250 mg/dL with glycosuria were considered diabetic. One week after the injection of STZ, berberry extract was administered orally by gavage tube for 4 weeks. In this study, DMSO 10% was used to prepare various concentrations of berberry extract and final volume of administration was 1 mL in all groups. Animals in control groups received DMSO 10% as vehicle. During the intervention, animals were carefully monitored and weighed daily. *Sample preparation*

At the end of the study, after an overnight fasting, animals were anesthetized by light ether and sacrificed by cervical dislocation and then, blood samples were collected directly from the heart. Serum was obtained by centrifuging the blood samples at 3000 rpm for 15 min. The livers of animals were removed, weighed and rapidly washed in cold saline (0.9%) and then placed in ice-cold isotonic potassium chloride solution (1.15% KCl w/v) containing 0.1 mM EDTA. The livers were then chopped into 4-5 volumes of 50 mM phosphate buffer (pH = 7.4) and homogenized by a homogenizer fitted with a Teflon pestle. The homogenate was then centrifuged at 3000 g for 10 min, the lipid layer was carefully removed and the resulting supernatant fraction was further centrifuged at 15,000 g for 60 min at 4°C. The supernatant was stored at - 80°C until use.

Biochemical analysis

Serum glucose levels were determined enzymatically using standard methods by autoanalyzer SA1000.

MDA concentration in serum and liver was assayed as a biomarker of lipid peroxidation. Briefly, 0.5 mL serum was shaken with 2.5 mL of 20% trichloroacetic acid (TCA) in a 10 mL centrifuge tube. One mL of 0.67% TBA was added to the mixture, shaken, and warmed for 60 min in a boiling water bath followed by rapid cooling. Then, it was shaken into a 4 mL of *n*-butanol layer in a separation tube and the malondialdehyde (MDA) content in the serum was determined at 532 nm by spectrophotometer against *n*-butanol.

The total antioxidant capacity of serum and liver samples were assayed by commercially available kits (Randox labs, Grumlin, UK). The assay principle was based on the ability of antioxidants to quench the absorbance of the radical cation that is formed by the reaction of a chromogen with the peroxide and H2O2 (18).

Statistical analysis

All data were expressed as mean ± SD. The statistical significance was evaluated by independent sample t-test and one-way analysis of variance (ANOVA) using the SPSS (version 17.0) program followed by post-hoc Tukey HSD test. Values were considered statistically significant when $p < 0.05$.

RESULTS

Table 1 illustrates the effects of berberry extract on serum glucose levels in experimental groups. As it is obvious, the administration of 100 mg/Kg berberry extract to diabetic rats did not significantly reduce the serum levels of glucose (5%), but a profound reduction of glucose levels (39%) was observed in diabetic group treated with 200 mg berberry when compared to diabetic control group ($p = 0.04$). The results also showed that the oral administration of 200 mg/Kg of berberry extract in diabetic rats even reaches the serum glucose levels to the normal values and the difference between the mean of glucose concentration in this group and non-diabetic control group was not statistically significant. However, following the treatment of normal rats with two dosage of berberry extract (100 and 200 mg/Kg), the serum glucose concentration did not significantly change.

In diabetic control group, the hepatic levelsof MDA, as a biomarker of lipid peroxidation, were statistically higher than those of normal control group ($p = 0.000$). However, the serum MDA concentration in diabetic control rats was not significantly different from that of normal control rats (Table 2). Only oral administration of 200 mg/Kg berberry extract to diabetic rats induced a significant reduction in serum and liver MDA concentrations after 4 weeks ($p < 0.05$ and $p < 0.001$, respectively).

In non-diabetic groups, the effect of berberry extract on serum and liver levels of MDA was not statistically significant ($p > 0.05$). In this study, there was a strong positive correlation between serum levels of glucose and MDA ($r = 0.406$, $p = 0.01$). Table 3 gives the serum and hepatic total antioxidant capacity of experimental groups. At the end of the study, the serum and hepatic total antioxidant capacity in diabetic control rats was significantly lower than those of normal control rats ($p = 0.000$) and following the treatment of these animals with 200 mg/Kg berberry extract, a significant increase in serum and hepatic total antioxidant capacity was observed ($p = 0.04$ and $p = 0.02$, respectively). However, the total antioxidant capacity was not significantly changed in diabetic group supplemented with 100 mg/Kg berberry.

Table 1. Effect of berberry extract supplementation on serum glucose levels.

Groups	Glucose (mg/dL)	%Change
Normal control	152.15 ± 11.62	-
Normal+berberry (100mg/Kg)	159.15 ± 19.87	+ 4.46%
Normal+berberry (200mg/Kg)	151.5 ± 10.53	- 0.25%
Diabetic control	298.13 ± 82.10	-
Diabetic+berberry (100 mg/Kg)	283.10 ± 88.13	- 5%
Diabetic+berberry (200 mg/Kg)	181.49 ± 40.10*	- 38%*

Table 2: Effect of berberry extract supplementation on serum and hepatic MDA levels.

Groups	Serum MDA (µmol/L)	Change%	Hepatic MDA (nmol/mg)	Change%
Normal control	1.31 ± 0.16	-	10.18 ± 1.73	-
Normal+berberry (100 mg/Kg)	1.20 ± 0.13	- 7.6%	8.73 ± 1.35	- 13%
Normal+berberry (200 mg/Kg)	1.23 ± 0.21	- 5.5%	9.38 ± 1.30	- 7.66%
Diabetic control	1.67 ± 0.14	-	18.13 ± 1.10#	-
Diabetic+berberry (100 mg/Kg)	1.52 ± 0.14	- 8.31%	15.12 ± 2.76	- 15%
Diabetic+berberry (200 mg/Kg)	1.41 ± 0.13*	- 14.5%*	12.27 ± 2.14**	- 30.6%**

a: All values are expressed as mean ± SD (n = 8). Independent sample t-test was used for statistical analysis. *: Indicates p < 0.05; **: Indicates p < 0.001 *vs.* diabetic control group; #: Indicates p < 0.001 *vs.* normal control group.

Table 3: Effect of berberry extract supplementation on serum and hepatic TAC levels.

Groups	Serum TAC (µmol/L)	Change%	Hepatic TAC (nmol/mg)	Change%
Normal control	2.24 ± 0.14	-	0.40 ± 0.06	-
Normal+berberry (100 mg/Kg)	2.32 ± 0.20	+ 3%	0.47 ± 0.06	+ 12.6%
Normal+berberry (200 mg/Kg)	2.57 ± 0.38	+ 13%	0.67 ± 0.16#	+ 51.8%#
Diabetic control	1.40 ± 0.16#	-	0.13 ± 0.06#	-
Diabetic+berberry (100 mg/Kg)	1.50 ± 0.17	+ 5.8%	0.36 ± 0.13	+ 33%
Diabetic+berberry (200 mg/Kg)	2.00 ± 0.14*	+ 31.3%*	0.44 ± 0.43*	+ 60.6%*

a: All values are expressed as mean ± SD (n = 8). Independent-sample t-test was used for statistical analysis. *indicates p < 0.05 and ** indicates p < 0.001 vs. diabetic control group; and # indicates p < 0.001 vs. normal control group.

A volume of 200 mg/Kg berberry extract supplementation was also able to significantly increase the hepatic total antioxidant capacity in non-diabetic rats (p = 0.006). A significant inverse correlation is also shown between the total antioxidant capacity and glucose concentration in this study (r = -0.449, p = 0.04).

DISCUSSION

The current study was conducted to determine whether feeding 2 doses of berberry extract (100, 200 mg/Kg) have beneficial effect on serum glucose levels and serum and hepatic oxidative stress biomarkers (TAC and MDA) in STZ-induced diabetic rats.

Our findings clearly showed that the oral administration of berberry extract at dose of 200 mg/Kg improved the glycemic control and even reached the serum glucose levels to the normal values. The results are in agreement with other studies (19, 20 and

21). Berberry aqueous extract significantly alleviated hyperglycemia (resulting from type 1 and 2 diabetes) induced by alloxan or cholesterol-rich diet in rats (22). In one prior study increasing in insulin-stimulated glucose uptake, inhibition of the intestinal GLUT system and decrease in expression of genes that control gluconeogenesis are the possible mechanisms proposed for the anti-hyperglycemic effect of berberry (23). However in another study the supplementation of berberry berberins does not change the blood glucose concentration in normal rats, which is consistent with our finding in normal group (24). This property of berberry (*Berberis vulgaris*) and its major polyphenol constituents (berberins) in normal state could be considered as an advantage for this medicinalplant. Although the elevated levels of glucose in the circulation could give rise to diabetes and possibly other metabolic disorders (25), the excessive lowering of the glucose level in the circulation, hypoglycemia, might also contribute to several adverse effects (26).

In current study, we also found that the higher concentration of glucose was interrelated to the higher lipid peroxidation and the lower total antioxidant capacity. This result confirmed a direct relationship between diabetes and oxidative stress condition. Glucose oxidation, protein glycation, formation of advanced glycation end-products and the polyol pathways are some of the major mechanisms involved in elevated oxidative stress biomarkers in diabetes (27).

The metabolic effects of berberine were investigated in an *in vivo* study of two animal models of insulin resistance. In the first group, berberine reduced body weight and resulted in improved glucose tolerance. Similarly, in the second group of high fat fed rats, berberine reduced body weight and plasma triglycerides and improved insulin action. The action of berberine on adipocytes was examined, the outcome being strongly inhibited triglyceride accumulation (28). The mode of action is due to, in part, the activation of Adenosine monophosphate kinase (AMPK) in multiple cell types. The overall results of this study suggest that berberine may have a major application as a new treatment for obesity and/or insulin resistance in humans. Berberine acts by changing the expression of metabolic genes in fat and muscle *in vivo* (29).

Numerous studies have demonstrated that berberry and its constituent phytochemicals (berberins) possess antioxidative properties as they improve total antioxidant capacity, suppress destructive oxygen free radicals and prevent oxidative stress damage (30 ,31). One prior study demonstrated that

berberry consumption significantly increases the plasma total antioxidant capacity and reduces the lipid peroxidation products, malondialdehyde (MDA) and malondialdehyde+4-hydroxy-2(E)-nonenal (MDA+4-HNE), after 4 weeks in healthy human (32). In one reported study, consumption of berberry for 2 weeks decreases serum malondialdehyde-modified LDL (MDA-LDL) concentrations in healthy male non-smokers (33). Furthermore,one prior study reported that berberry extract lowers the lipid peroxidase activity in heart and aorta of diabetic rats (34). There is no evidence about the effect of berberry on hepatic biomarkers of oxidative stress until now, and the present study is the first one in this regards.

In this investigation, we observed a significant increase in serum and hepatic total antioxidant capacity and decrease in MDA concentration, following the treatment of diabetic rats with 200 mg/Kg berberry extract. It is important to note that the berberry exert mostly its antioxidant effects in diabetic rats rather than normal rats. In this study, lower dose of berberry extract (100 mg/Kg) could not significantly compensate the abased total antioxidant capacity or the elevated level of MDA concentration in diabetic rats. However, in current assay, we used only two doses of berberry extract, so further studies are required to distinct the effective dose of it.

In conclusion, the administration of 200 mg/Kg berberry extract has antihyperglycemic and antioxidative properties, so, berberry can be effective in preventing diabetes complications. However, despite evidences representing promising effects of berberry in rodents, human studies are deficient and inconsistent. Therefore, this plant should be considered as an excellent candidate for future human studies on diabetes.

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