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Rom J Diabetes Nutr Metab Dis. **23**(3):239-245
doi: 10.1515/rjdnmd-2016-0028

THERAPEUTIC EFFECTS OF HYDROALCOHOLIC AND AQUEOUS EXTRACTS OF BERBERIS VULGARIS FRUITS IN STREPTOZOTOCIN INDUCED TYPE 1 DIABETES MELLITUS RATS

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received: May 22, 2016 *accepted*: August 11, 2016
available online: September 15, 2016

Abstract

Background and Aims: *To evaluate and compare the beneficial effects of aqueous and hydroalcoholic extracts of Berberis vulgaris fruit in streptozotocin (STZ) induced type I diabetes mellitus (DM).* ***Materials and Methods:*** *Forty male rats were allocated into 4 groups as negative control (NC), positive control (PC), aqueous extract (AE), and hydroalcoholic extract (HE). DM was induced with streptozotocin (STZ) and three days later, the AE and HE groups received 200 mg/kg extracts orally for 30 days. The serum liver enzymes plus glucose level and concentration of HbA1c three days after STZ injection and at day 30 and liver histopathology at the end of the study were analyzed.* ***Results:*** *PC and both treatments groups had higher serum glucose levels in comparison to NC group ($P<0.05$). Higher serum alanine aminotransferase activities in both AE and HE groups in comparison to PC group ($P=0.005$ and $P=0.045$, respectively) and lower percentage of HbA1c in NC and HE groups in comparison to PC group ($P=0.001$ and $P=0.014$, respectively) were detected.* ***Conclusion:*** *Noticing the beneficial effects of B. vulgaris, the extract of this plant can be a good choice attenuating the side effects of DM.*

key words: *Diabetes mellitus, Berberis vulgaris, aminotransferase, histopathology.*

Background and Aims

Diabetes mellitus (DM) is a metabolic disease characterized by high blood sugar levels over a prolonged period. This is the most common endocrine disease, resulting from defects in insulin secretion, action or both. International Diabetes Federation in 2015 indicated a diabetes prevalence of approximately 9%, with a total of near 415 million diabetic

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subjects worldwide. [1]. Long term DM is accompanied by serious complications such as cardiovascular disorders and renal, neuropathic and eye related diseases which cause early disability and death. Also, the quality of life is severely affected in this disease and imposes health care costs to individuals and society [2]. Pattern of global incidence and prevalence of diabetes mellitus shows that the disease is more frequent in developing countries and in the low socio-cultural peoples of developed countries [2]. Therefore, applying prevention and therapeutic strategies in these groups are highly needed.

In recent years, use of traditional medicinal plant and their derivatives as replacement or supplementary treatments in different diseases in human and animal models received more attention by scientists [3-8]. *Berberis vulgaris* (simply called Barberry), is a plant in the family of Berberidaceae. The genus of Berberis contains about 500 species worldwide but 3 of them (*B. orthobotrys*, *B. khorassanica*, and *B. vulgaris*) are mainly found and cultured in northern, eastern and south-east of Iran. The South Khorasan province is the world's largest barberry cultivation area [9]. Several therapeutic effects such as antihistaminic, anticholinergic and anti-inflammatory were reported for *B. vulgaris* [10]. Such effects are related to its contents include berberine, berbamine, alkaloids, tannins and polyphenolic antioxidants [11]. The number of studies about the beneficial effects of *B. vulgaris* in treatment of DM is scarce. For instance, Taheri and colleagues in 2012 reported that hydroalcoholic extract of root of *B. vulgaris* compensated the hyperchlostrolemia related elevation of liver enzymes due to its antioxidant contents [12]. Also, Shidfar and collaborators found that hydroalcoholic extract of *B. vulgaris* could normalize serum lipoproteins, apoB, and apoA-I in type 2 diabetic patients [13].

We previously reported the beneficial therapeutic effects of some medicinal plants in experimental DM models [3,4,7]. There are no reports about the effects of hydroalcoholic and aqueous extracts of *B. vulgaris* fruits in type 1 DM. Therefore, we focused on the evaluation of liver transferase enzymes and liver histopathology as well as blood hemoglobin A1c (HbA1c) and serum glucose level in male rats with DM induced by streptozotocin (STZ).

Materials and Methods

Ethics statement

This study was undertaken at the Center of Comparative and Experimental Medicine and was approved by the Ethical Committee of Shiraz University of Medical Sciences, Shiraz, Iran and AJA University of Medical Sciences, Tehran, Iran. All efforts were made to minimize suffering during the experimental period. Also, we included the lowest number of animals per each group in order to receive approval from animal welfare but still reach statistical significances.

Animals

Forty male adult Sprague Dawley rats (180 ± 10 g) were obtained from the Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences. The animals were housed in standard cages, 5 rats per cage, and maintained in experimental conditions (12-h light/dark cycles, temperature of 22 ± 2 °C, and 50% relative humidity), with free access to food and water. Rats were left undisturbed to acclimatize for 3 days before the experiments.

Plant materials and extracts preparation

B. vulgaris fresh fruits were purchased from local market. These fruits were harvested from Birjand, South Khorasan, Iran (GPS coordinates: Latitude: 32.864904 | Longitude: 59.226247).

Taxonomic identification of the plant material was confirmed by the local research laboratory and also matched to the digital herbarium of Botanical Garden and Botanical Museum Berlin-Dahlem, Freie University, Berlin (http://herbarium.bgbm.org/object/BW06922010). The fruits were checked for any fungal contaminations, washed carefully with warm water and then were air-dried at room temperature in the shade for three years. The fruits were grounded to a fine powder and were used for preparation of hydroalcoholic and aqueous extracts based on protocols which reported previously [4,6]. Both extracts were then stored at 4 °C until use for gavage administration.

Interventions

In a double-blind animal-based controlled randomized clinical trial, rats were randomly allocated into four equal independent groups. The feed treatments were applied to each group as follow:

Group 1 (negative control, NC): Neither DM induction, nor treatments

Group 2 (positive control, PC): DM induction without treatments.

Group 3 (aqueous extract, AE): DM induction and dietary treatment with 200 mg/kg aqueous extract for 30 consecutive days.

Group 4 (hydroalcoholic extract, HE): DM induction and dietary treatment with 200 mg/kg hydroalcoholic extract for 30 consecutive days.

DM was induced by administration of 60 mg/kg STZ, intraperitoneally, after 24 h off feeding. Treatments were applied using gavage needle at the 8.00 AM of each day by an expert technician who was familiar with animal working. Also, the two control groups received similar volumes of normal saline.

Biochemical measurements

Blood sampling was performed at 3 days after DM induction (as day 0) and at the end of the study (day 30). The serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) plus glucose level were measured by autoanalyzer BT-3000 (Biotecnica Instruments, Italy) using commercial kits and reagents (ParsAzmoon Co, Iran). The concentration of HbA1c was evaluated using high performance liquid chromatography (HPLC) by ion exchange column.

Pathological evaluations

At the end of the experiment, the animals were anesthetized with ether and at deep anesthesia were killed by cervical dislocation. The liver was immediately removed and fixed in 10 % buffered formalin for histopathologic examination. Formalin-fixed tissues were processed routinely and embedded in paraffin. Blocks were cut at 5 μm sections and then were stained with hematoxylin-eosin. All sections were studied and photographed by a light photomicroscope [14].

Statistical analysis

All data were expressed as mean and SD and analyzed using SPSS version 21. The between group comparison was performed using one way analysis of variance (ANOVA). The Tukey test was used as Post hoc test. P value lower than 0.05 was considered as significant difference.

Results

The mean and SD of serum activity of liver enzymes and glucose levels plus percentage of HbA1c in the beginning of the study (day 3) and at the end of the study (day 30) are presented in Table 1. As presented, only the serum glucose level was affected in related to DM and positive control and both treatments groups had higher serum glucose levels in comparison to negative control group at day 3 ($P<0.05$). At the end of

the study, only significant differences were detected in ALT activity and percentage of HbA1c. These include higher serum ALT activities in both aqueous and hydroalcoholic extract groups in comparison to positive control group (P=0.005 and P=0.045, respectively) and lower percentage of HbA1c in negative control and hydroalcoholic extract groups in comparison to positive control group (P=0.001 and P=0.014, respectively).

Table 1. Comparison of mean and standard deviation (SD) of serum activity of liver enzymes and glucose concentration plus percentage of HbA1c at the beginning of the study (day 3) and in the end of the study (day 30).

	Liver enzymes activity (IU/L)				Blood glucose	HbA1c
Groups	ALT	AST	ALP	GGT	(mg/dl)	(%)
Day 3						
NC	37.30±2.18^{a}	3.30±0.30^{a}	2469.70±84.41^{a}	3.68±0.33^{a}	416.70±66.08^{a}	4.09±0.17^{a}
PC	36.80±3.18^{a}	3.60±0.48^{a}	2459.10±91.11^{a}	2.91±0.57^{a}	788.60±37.66^{b}	4.24±0.19^{a}
AE	45.70±3.05^{a}	3.10±0.18^{a}	2810.60±235.11^{a}	2.84±0.75^{a}	688.30±29.57^{b}	4.25±0.10^{a}
HE	41.40±2.88^{a}	4.70±0.82^{a}	2777.60±364.41^{a}	3.09±0.38^{a}	738.50±29.95^{b}	4.36±0.14^{a}
Day 30						
NC	36.60±9.00^{AB}	5.20±1.74^{A}	2179.40±491.68^{A}	2.29±1.08^{A}	535.60±92.71^{A}	4.10±0.18^{A}
PC	19.22±8.20^{B}	4.11±1.45^{A}	3057.22±650.91^{A}	3.77±2.07^{A}	630.11±122.08^{A}	8.05±0.87^{B}
AE	60.00±6.81^{A}	3.10±1.51^{A}	2181.00±499.69^{A}	1.88±0.92^{A}	425.70±94.74^{A}	6.12±0.75^{AB}
HE	50.00±7.16^{A}	6.70±1.89^{A}	2169.60±514.17^{A}	2.68±0.83^{A}	476.30±99.19^{A}	5.07±0.61^{A}

NC, negative control; PC, positive control; AE, aqueous extract; HE, hydroalcoholic extract. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase. Significant differences in each factor indicated by different superscript lower and upper case letter in each column for day 3 and 30, respectively.

Histopathological features of the liver in the different groups are presented in Figure 1. The histopathological evaluations demonstrated that several lesions exist in the liver based on the grouping. These include lack of any pathological changes in NC, hepatocytes confluent necrosis and massive necrosis with abscess formation in PC group (Figure 1A), mild portal inflammation, mild and focal spotty necrosis and granuloma formation in AE group (Figure 1B), mild portal inflammation and mild and focal spotty necrosis in HE group (Figure 1C). As shown, the severity and extent of the lesions were higher in PC group than AE group and in AE group than HE group.

Discussion

In the present study, the therapeutic effects of 30 days oral consumption of aqueous and hydroalcoholic extract of *B. vulgaris* in STZ induced type 1 DM male rats were evaluated using measurement of liver histopathology, serum hepatic enzymes activity and glucose concentration plus blood HbA1c percentage. Totally, we found that both extracts, especially hydroalcoholic form, prevented severe DM related hepatic injuries, and decreased the percentage of HbA1c.

Use of traditional medicinal plants has obtained great interest by scientists. In this study, administration of STZ to rats resulted in hyperglycemia as expected. Indeed, STZ is absorbed by pancreatic β cells and induced cell death in them by alkylation of DNA, production of nitric oxide (NO) and reactive oxygen species (ROS) [15]. Therefore, STZ inhibits insulin secretion and elevate the serum glucose level. It has been reported that *B. vulgaris* contains an effective alkaloid, berberine, that shows antidiabetic effects. This is an organic cation that penetrates cell membrane and bands to DNA molecule [16], and induces DNA conformational

changes and alters gene expression [17]. In some of the previous studies, the hypoglycemic or anti-hyperglycemic effects of some species of Berberis such as *B. aristata* [18], *B. lyceum* [19], *B. integerrima* [20], and *B. vulgaris* [13,21,22] were reported.

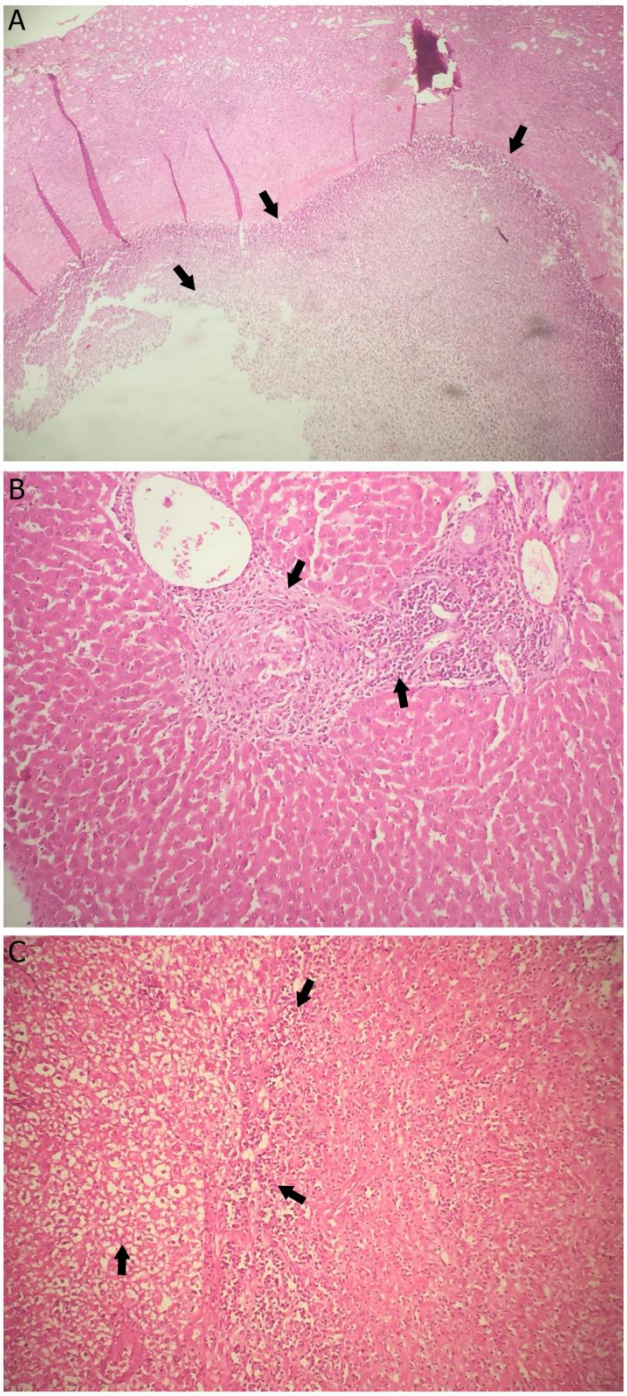

Figure 1. *Histopathological changes of the liver. A, massive necrosis and abscess formation in PC group (H&E, ×100); B, inflammatory cell infiltration of a portal tract with granuloma formation AE group (H&E, ×250); C, necrosis of hepatocytes with acute and chronic inflammatory cells infiltration in HE group (H&E, ×400). The related lesions indicated by black arrows.*

B. vulgaris contains several alkaloids and effective substances that were reviewed by Mokhber-Dezfouli and colleagues [9]. Among them, berberin is better known for its therapeutic effects. There are some reports which express the beneficial effects of berberine on the liver functions, bile secretion and lowering serum low density lipoprotein (LDL) concentration [23]. Berberine can act similar to metformin in reducing serum glucose level by aldolase reductase inhibition, glycolysis induction, and prevention of insulin resistance through enhancing of insulin receptor expression [23,24]. Also, berberine decrease the serum blood glucose due to protein kinase B activation and increasing of glucose uptake due to 5' AMP-activated protein kinase (AMPK)-38 molecular pathway [19]. It seems that the beneficial effects for *B. vulgaris* aqueous and hydroalcoholic extracts in lowering serum glucose level and HbA1c percentage described in our experiment are related to the berberin content of this plant.

Our findings about the hypoglycemic effects of aqueous and hydroalcoholic extracts of *B. vulgaris* are in line with those found by Hemmati and collaborator [25], but opposite with those found by Hajzadeh et al [26]. Hajzadeh and colleagues reported that the aqueous extract of *B. vulgaris* fruit at amounts of 3.5 and 7.5% of drinking water did not possess hypoglycemic and hypolipidemic activity in STZ-diabetic rats during 6-week treatment period [26]. The main reasons for this discrepancy is the lower dose of the plant extract in comparison to our dose and also use of only aqueous extract against the hydroalcoholic extract which we used. Shidfar et al in 2012 reported that the intake of 3 g per day of aqueous extract of *B. vulgaris* for 3 months may have beneficial effects on glycemic control in type 2 diabetic patients. They found no

significant effects on the percentage of HbA1c but found significant decline in the serum glucose level [13]. About the hepatic effects of this plant, Taheri and colleagues reported that hydroalcoholic extract of root of *B. vulgaris* in hyperlipidemic rat can significantly reduce the serum activities of ALT and ALP, without any changes in serum AST activity [12]. Preventive effects of aqueous extract of *B. integerrima* root on liver injury in type 1 DM rats were evaluated by Ashraf and Zare in 2015. They reported that administration of aqueous extract of *B. integerrima* root before diabetes induction resulted in enhanced amelioration of liver complications compared to the group receiving it after induction, indicating that it can play a preventive role in this animal model [20]. Their results are in line with our findings except in lack of any changes in the serum activity of AST and ALP which can be related to difference in the time and dose of the administration. Despite of our findings, this study suffered from two limitations. These include no measurement of serum insulin level and lack of histopathological changes of the pancreas.

Conclusion

The present study demonstrated that consumption of aqueous and hydroalcoholic extracts of *B. vulgaris* for 30 consecutive days as dose of 200 mg/kg can beneficially affect the hepatic liver enzymes, especially ALT, due to the liver regeneration and providing the source of the liver enzyme. Also, these extracts beneficially impact liver histopathology and percentage of HbA1c. Performing other research to overcome the limitations of this study and also performing molecular evaluation of results which we found in this study are highly recommended in the future.

Acknowledgment and Duality of interest. Authors wish to thanks the staff of Gastroenterohepatology Research Center and Pathology Department of Shiraz University of Medical Science, Shiraz, Iran for their kindly scientific helps. Authors declared that there are no conflict of interests.

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