



The effects of ethanol extract of *Berberis vulgaris* fruit on histopathological changes and biochemical markers of the liver damage in diabetic rats

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ABSTRACT

Objective(s): Various studies have shown that the diabetes is associated with liver failure. The objective of this study was determining the effects of *Berberis vulgaris* fruit on histopathological and biochemical markers of liver in diabetic rats.

Materials and Methods: In this experimental study, 60 male Wistar rats weighing 200-250 g with free access to water and *ad libitum* were randomly divided to five twelve-membered groups including healthy control (group 1), diabetic control (group 2, this two groups received distilled water), treated diabetic positive control (group 3) using dose 150 mg/kg/day metformin, and two groups treated with doses 200 (group 4) and 600 (group 5) mg/kg/BW of *B. vulgaris* extracts via gavage feeding for 8 weeks. Diabetes mellitus was experimentally induced by one dose injection of alloxan 120 mg/kg. This pre-clinical study was performed on 120 mg/kg alloxan induced diabetic rats.

Results: The hepatic steatosis status, liver cholestasis and fibrosis were not changed in group 4 and 5. Glycogen deposition changed mildly and polymorphonuclear neutrophils infiltration changed moderately at group 5. Liver hepatitis changed mildly and severely at group 3 as well as group 5, respectively. Glucose, serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase were lower in high dose group compared to other groups.

Conclusion: Results suggested that *B. vulgaris* extract can decrease liver damage by influencing hepatic histopathological and biochemical markers in diabetic rats.

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Introduction

Diabetes mellitus is a common metabolic disease that its prevalence is 6.4 percent worldwide. It is estimated that more than 280 million people have diabetes and it is predicted that over 330 million people will be affected by 2025. Diabetes has several complications such as retinopathy, neuropathy, and nephropathy (1-4).

A number of studies have shown that the diabetes is associated with fibrosis liver disease, non alcoholic fatty liver disease (NAFLD), as well as cirrhosis and hepatocellular carcinoma (HCC). These and some other complications of diabetes cause significant morbidity. Various chemical drugs are used to treat or control the diabetes; however, they are associated with side effects. For example, sulfonylurea drugs cause weight gain due to hyper-insulinemia, biguanides cause weakness, fatigue, lactic acidosis, alpha-glucosidase inhibitors may cause diarrhea while thiazolidinediones may increase

low density lipoprotein (LDL) and cholesterol levels. Alternative therapy for diabetes (5-7) and some other hard curable diseases (8-10). Plants as an alternative are potential source of hypoglycemic drugs, which can manage diabetes more efficiently and safely and are widely used in traditional systems of medicine to prevent or treat diabetes (11-13).

It is well known that *Berberis vulgaris* fruit can cause hypoglycemia and it has cytoprotective effect on hepatocytes. *B. vulgaris* is a shrub in the family Berberidaceae. Previous studies have shown that *B. vulgaris* can inhibit intestinal ion secretion, smooth muscle contraction, ventricular tachyarrhythmia, and inflammation (14). These results suggested that methanol extract of Berberis family such as tinctoria and *vulgaris*/β-cyclodextrin may have potential therapeutic value in the treatment of the liver disorders and also hepatic injury induced by CCl₄ via its antioxidative effect on hepatocytes (15, 16).

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Furthermore, study of Girish *et al* showed that polyherbal liquid formulations (PLFs) can prevent acute liver damage induced by CCl₄ only at a higher dose; therefore, it is suggested that a dose adjustment of these PLFs may be necessary for their optimal effects in human liver diseases (17).

Upwar *et al* in 2011 reported that methanolic extract of *Berberis aristata* significantly reduced the blood glucose, total cholesterol and triglycerides, and also increasing the HDL cholesterol level in diabetic rats; so this extract have very hypoglycemic as well as hypolipidemic activities (18). Results from the Meliani *et al* study demonstrated significant antidiabetic effects of the aqueous extract of *B. vulgaris* that declined significantly biochemical parameters such as blood glucose levels compared to diabetic controls. This researchers also showed that serum cholesterol and serum triglycerides levels were decreased, therefore consequently this plant might be of value in diabetes treatment (19). Thus, it has been also recommended for a number of other diseases like obesity, hyperlipidemia, metabolic syndrome, cholecystitis, and coronary artery disease. Despite the natives' beliefs in the effects of *B. vulgaris*, few investigations have been performed on this species. Therefore, the present study was carried out with the aim of determining the effects of ethanolic extract of *B. vulgaris* on histopathological changes and biochemical markers of liver damage in diabetic rats.

Materials and Methods

Plant collection and extraction

B. vulgaris fruit purchased from a reputable herbal grocery, was evaluated by a plant biologist and recorded at herbarium unit of Medical Plants Research Center of Shahrekord University of Medical Sciences (Shahrekord, Iran) with the accession Code 419. Aqueous ethanol (70:30) extract of *Berberis* fruit was prepared using cold maceration process. The grounded plant material (2 kg) was soaked in 5 l of water-ethanol mixture (70:30) for 72 hr at room temperature. After three days of occasional shaking, the whole material was filtered and the filtrate evaporated under reduced pressure using rotary evaporator. The contents were filtered and the solvent was isolated by a Rotary concentrator at 37°C (IKA®RV 10 digital). The concentrated solution was incubated at 37°C until it was dried. The crude extract was then air-dried to obtain a solid mass. The yield of extract was 30%. Then, the obtained solution was collected from containers and kept in the refrigerator at 4 °C.

Experimental design

In this pre-clinical study, 60 Wistar rats were purchased from the Iran Pasteur institute with a weight range of 250±25 g, and were kept in the animal house of Shahrekord University of Medical Sciences for seven days for acclimatization. The temperature of animals' house was 22± 2 °C with 12

hr of light and 12 hr of darkness. Briefly, 60 male Wistar rats weighing 200-250 g with free access to water and *ad libitum* were randomly divided to five twelve-membered groups including healthy control (group 1), diabetic control (group 2; these two groups received distilled water), treated diabetic positive control (group 3) using dose 150 mg/kg/day metformin, and two groups treated with doses 200 (group 4) and 600 (group 5) mg/kg/BW of *B. vulgaris* extracts via gavage feeding for 8 weeks. Experimental diabetes was induced by a single intraperitoneal injection (IP) with 120 mg/kg alloxan (Sigma Chemical Company, USA) in all groups except healthy control group (20). Animals were deprived of food for 12 hr before alloxan injection. This study was approved by the ethics committee of the medical university of Shahrekord (Code: 91-12-6).

Biochemical assays

At the end of eight weeks, the animals were anesthetized with chloroform, and then blood samples were taken by cardiac puncture for biochemical analysis. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min for the estimation of serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and alkaline phosphatase (ALP) and glucose (21, 22). ALP was determined by enzymatic assays. SGOT and SGPT activities were determined by the methods introduced previously (21, 23).

Histopathological examination

The liver was removed and fixed in 10% formalin solution for histopathological testes. Then it was embedded in paraffin, and stained with Hematoxylin-Eosin. Glycogen deposition, fat deposition (steatosis), hepatic polymorphonuclear neutrophil (PMN) infiltration, inflammation in the liver parenchyma (Hepatitis), accumulation of bile pigments (cholestasis) and fibrosis in the liver parenchyma (fibrosis) were assessed (24).

Statistical analysis

The Mean±SD was used for descriptive statistics. For each variable, the normal distribution was evaluated and confirmed using the one-sample Kolmogorov test. The ANOVA test was used for comparing the variables between groups and LSD test was used for pairwise comparisons. Statistical significance was defined as $P < 0.05$ and analysis was done using SPSS(Version 11; SPSS Inc., Chicago, USA).

Results

Histopathological observations of liver

The histological assessment of liver tissue of control and treated rats with *B. vulgaris* showed mild change in glycogen deposition at group 5 at the end

of the study, however, no change was seen in other groups. Hepatic steatosis status was not changed at any group. Hepatic polymorphonuclear neutrophils (PMN) infiltration changed moderately at group 5 but there was no change in other groups. Liver hepatitis changed mildly and severally at diabetic positive control group and group 5, respectively. Liver cholestasis and fibrosis were not changed at any group (Table 1).

Liver biochemical parameter

As shown in Table 2, the amount of glucose in group 4 was significantly higher than healthy control group and group 5, while the amount of glucose in the group 5 was significantly lower compared to diabetic control, the positive diabetic control and the group 4. The amount of Serum glutamic oxalacetic transaminase (SGOT) was significantly higher in the group 4 compared to the healthy controls, the positive diabetic control and group 5, but it was lower in the group 5 comparing to all groups. The amount of serum glutamic pyruvic transaminase (SGPT) was significantly higher in the group 4 than the healthy control and group 5 while it was lower in group 5 than the positive diabetic control. The alkaline phosphatase (ALP) amount was significantly higher in group 4 compared to the healthy

control group and it in group 5 was significantly lower than diabetic control.

Discussion

An initial objective of this project was to identify the effects of ethanol extract of *B. vulgaris* on histopathological changes of the liver in diabetic rats. The current study found that hepatic steatosis status, liver cholestasis and fibrosis didn't change at any group. Glycogen deposition changed mildly and PMN infiltration changed moderately at group 5. Liver hepatitis changed mildly and severally at diabetic positive control group and group 5 respectively. These results match those observed in earlier studies such as Othman *et al* (25) that berberine (an isoquinoline alkaloid extracted from the barks and roots of several species of the berberis), increased antioxidant protection against HgCl₂-induced toxicity and it may have hepato- and reno-protective effect. Hermenean *et al* have demonstrated that *B. vulgaris*/β-cyclodextrin prevented CCl₄ induced hepatic injury (26). Also in another study performed in diabetic rats it was established that administration of *Berberis integerrima* Bge root before diabetes induction ameliorated liver complications. In another study berberine could protect hepatic tissue destruction (27).

Table 1. Effects of ethanol extract of *B. vulgaris* on liver histopathological changes at the end of study

| Fibrosis | Cholestasis | Hepatitis | PMN infiltration | Steatosis | Glycogen depositon | Groups |
|----------|-------------|-----------|------------------|-----------|--------------------|----------------------------------|
| Neg | Neg | Neg | Neg | Neg | Neg | Healthy control (1) |
| Neg | Neg | Neg | Neg | Neg | Neg | Diab. Control (2) |
| Neg | Neg | Pos+ | Neg | Neg | Neg | Diab. Metformin (3) |
| Neg | Neg | Neg | Neg | Neg | Neg | Diab. <i>B. vulgaris</i> 200 (4) |
| Neg | Neg | Pos ++ | Pos + | Neg | Neg / Pos | Diab. <i>B. vulgaris</i> 600 (5) |

Diab.: Diabetic; *B. vulgaris*: Extract of *Berberis vulgaris*

Healthy and diabetic groups received normal food; Diabetic positive control group received normal food and 150 mg/kg/day metformin; Diab. *B. vulgaris* received 200 mg/kg/day and Diab. *B. vulgaris* received 600 mg/kg of *B. vulgaris*.

Pos.= positive; Neg.= negative; Neg/pos= mild change; Pos += moderate change; Pos +++= sever change

Table 2. Effects of ethanol extract of *B. vulgaris* on biochemical parameters at the end of study

| Groups variable | Healthy Control (1) | Diab. Control (2) | Diab. Metformin (3) | Diab. <i>B. vulgaris</i> 200 mg/kg (4) | Diab. <i>B. vulgaris</i> 600 mg/kg (5) | P value |
|-----------------|---------------------|-------------------|---------------------|--|--|---------|
| Glucose (Mg/dl) | 106.5±8.7 | 526.4±19.3 | 528.8±20.3 | 514.1±12.5 | 404.6±30.9 | P≤0.05 |
| SGOT (U/l) | 138.9±9.3 | 223.6±13.6 | 206.3±8.2 | 220.6±17.9 | 169.5±9.4 | P≤0.05 |
| SGPT (U/l) | 64.6±6.3 | 97.5±10.8 | 107.0±14.3 | 104.0±10.7 | 88.3±7.9 | P≤0.05 |
| ALP (U/l) | 356.4±34.9 | 512.6±36.6 | 489.1±46.7 | 471.1±26.7 | 450.4±24.0 | P≤0.05 |

Diab.: Diabetic; *B. vulgaris*: Extract of *Berberis vulgaris*; SGOT: Serum glutamic oxalacetic transaminase; SGPT: serum glutamic pyruvic transaminase; ALP: alkaline phosphatase and: All results are reported as mean ± standard deviation; P<0.05 was considered statistically significant

†: significant compared with the healthy control group, *: significant compared with the diabetic control group, .: significant compared with the diabetic metformin, a: significant compared with the diabetic *B. vulgaris* received 200 mg/kg and b: significant compared with *B. vulgaris* received 600 mg/kg

This study sought to determine the effects of red Berberis on the biochemical liver markers. The result of the study showed glucose, SGOT, SGPT and ALP were lower in group 5 compared to other groups. These results confirmed previous study in which the levels of SGPT, SGOT, ALP, total proteins, and serum bilirubin decreased in treated animals with the methanol extract of *Berberis tinctoria* (28). While lowering blood sugar, Barberry higher doses (600 mg/kg), had protective effects on the liver. According to previous study the berberis have not side effects or cytotoxicity on animal diabetes models(29). A possible explanation for these results may be the composition of Barberries. Barberries contain organic acids and phenolic compounds such as anthocyanin and carotenoid pigments as well as polyphenolase, phenolase, glycosidase enzymes, and the different alkaloids with an isoquinolinic nucleus such as berbamine, berberine, and palmatine existing in the different parts of this plant. These constituents have antioxidant effects due to their interaction with various molecules. It should be noted that antioxidants have previously confirmed to be effective not only in liver toxicity, but also in a number of other diseases such as kidney disease (30-32) hypertension (33) cancer (34, 35), and infectious diseases (36, 37). In regard to mentioned results, further researches seem necessary to further investigate the effects of this plant in humans. Considering the increasing preclinical studies, berberis seems to be of benefits for both type I and type II diabetes patients (15, 38, 39).

Conclusion

The present study was designed to determine the effects of *B. vulgaris* on histopathological and biochemical markers of the liver in diabetic rats. This research on hepatic histopathological and biochemical markers showed that this plant could delay and moderate liver damage. An implication is the possibility that *B. vulgaris* might be effective on liver damage in humans as well.

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