ORIGINAL ARTICLE

Veterinary Research Forum. 2021; 12 (4) 459 – 465
doi: 10.30466/vrf.2020.113744.2707
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Effects of *Ribes khorasanicum* hydro-ethanolic extract on streptozotocin-induced diabetic complications in rats

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Article Info

Article history:

Received: 03 September 2019
Accepted: 09 June 2020
Available online: 15 December 2021

Keywords:

Diabetes
Hyperglycemia
Hyperlipidemia
Oxidative stress
Ribes khorasanicum

Abstract

The anti-diabetic effects of *Ribes khorasanicum* as a traditional remedy were investigated in diabetic rats. Thirty-five male rats were divided into five groups: control, diabetic, diabetic treated with metformin (300 mg kg^{-1}; D+Met), diabetic treated with 250 and 500 mg kg^{-1} of *Ribes khorasanicum* hydro-ethanolic extract (D+Rib250 and D+Rib500). After six weeks of treatment, sera of overnight fasted animals were collected and used for measurement of glucose, insulin, lipid profile, urea, creatinine, and hepatic enzymes levels. Moreover, liver and kidney of rats were removed and used for measurement of oxidative stress including malondialdehyde (MDA), thiol content, and the activity of catalase (CAT) and superoxide dismutase (SOD). Streptozotocin (STZ)-induced diabetes increased the levels of serum glucose, triglycerides (TG), total cholesterol (TC), and LDL-C, urea, creatinine, hepatic enzymes, and kidney and liver oxidative stress markers, while decreased insulin and HDL-C when compared to control group. In all treated groups serum levels of glucose, TC, LDL-C, TG, and urea were decreased, while liver SOD activity was increased compared to the diabetic group. The D+Rib500 group had lower Serum glutamic pyruvic transaminase (SGPT), creatinine, and kidney MDA levels, but higher insulin, HDL-C levels, liver CAT activity, and kidney thiol content, and CAT activity compared to diabetic group. In D+Met group, serum levels of serum glutamic-oxaloacetic transaminase (SGOT), creatinine, and MDA of liver and kidney were decreased, while liver SOD activity was increased compared to the diabetic group. Based on our findings, treatment with *Ribes khorasanicum* improved diabetic complications, while the effect of a higher dose of the extract was comparable to metformin's.

Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by high blood glucose levels (hyperglycemia) which may be induced by insulin deficiency or resistance to insulin or both. This chronic disease might cause several long term disabling complications which decrease the patients' quality of life and increase the morbidity and mortality rate.[1] This metabolic disease is considered as one of the major health problems in both developed and undeveloped countries because of its high prevalence and costly treatment.[2] In 2017, the number of diabetic individuals (18-99 years old) was estimated to be about 451 million and is expected to increase to 693 million by the year of 2045.[3] Metabolic disturbance and chronic hyperglycemia in patients with diabetes can induce macro and micro vascular damages resulting in cardiovascular dysfunction, nephropathy, retinopathy and neuropathy. These complications can lead to blindness, kidney failure, heart attacks, stroke, lower limb amputation and premature death in diabetic patients.[4,5]

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Different kinds of synthetic anti-diabetic drugs have been produced and are in use for the treatment of diabetic patients. Many of these drugs are too expensive (especially for patients in low-income countries), and have various undesirable side effects which cannot effectively slow down the complications related to the development of diabetes.[6] Therefore, herbal remedies with their high anti-hyperglycemic activities have been proposed and examined in various experimental and clinical studies.[7,8]

Ghareghat with the scientific name of *Ribes khorasanicum* (*R. khorasanicum*) is a native and endemic medicinal plant spices on the north of Khorasan Razavi province of Iran which belongs to a family of Grossulariaceae.[9] In Iran, the genus of *Ribes* has four identified taxonomic herbs spices including the *Vaccinium arctostaphylos L.*, *Ribes biebersteinii* Berland., *R. khorasanicum* F.Saghafi and Assadi, *Ribes orientale* Desf.[10] The locals use *R. khorasanicum* is for treatment of gastrointestinal toxicity, for lowering blood pressure and blood glucose. In traditional medicine, the Ghareghat's fruit seeds and leaves are recommended as an herbal remedy for diabetes, hyperlipidemia and hypertension,[11] however few experimental studies have been performed to study these effects. Phytochemical evaluation of the plant flowers and their ripe and unripe fruits has revealed the presence of phenolic, flavonoid, saponins, tannins, and alkaloid compounds with effective antimicrobial properties.[12] Recently, total flavonoid, phenolic and anthocyanin and soluble and insoluble hydrocarbons contents as well as the antioxidant activity of *R. khorasanicum* were measured. The main antioxidant phenolic compound in *R. khorasanicum* was found to be anthocyanin.[13] Some studies have demonstrated the protective roles of flavonoids and anthocyanins against diabetes, insulin resistance, hyperlipidemia, cardio-vascular disease and cancer previously.[14-16]

The anti-diabetic and anti-hyperlipidemic effects of other taxonomic identified spices of Ghareghat had been demonstrated in both animal experimental models and human studies.[17] However, to the best of our knowledge, the anti-diabetic properties of *R. khorasanicum* has not be evaluated previously. Therefore, the aim of this study was to investigate the metabolic and antioxidant effects of *R. khorasanicum* hydro-ethanolic extract in diabetic rats.

Materials and Methods

Preparation of extracts. *Ribes khorasanicum* shrubs grow land stretches from Kalat to Dargaz (37° 26′ 40″ N 59° 06′ 29″ E), Laein to Ors Nahalestan (37° 03′ 42″ N 59° 24′ 05″ E), and to heights of Hezar Masjed (36° 58' 15" N 59° 21' 34" E) in the north and northeast of Khorasan Razavi province of Iran. The dried plant fruits were purchased from a local market. The plant specimen was identified by botanists in the herbarium of Mashhad School of Pharmacy, Mashhad, Iran (specimen No. 13233). To prepare the hydro-ethanolic extract, 50.00 g of chopped *R. khorasanicum* dried fruit was mixed with 1,400 mL of 50.00% ethanol for 72 hr at 40.00 ˚C. Then, the solvent (ethanol + water) was totally removed by rotary evaporation under reduced pressure, resulting in the extraction yield being 34.11%. This process was then repeated four times during the study. The dried fruit and the extract were stored at 4.00 ˚C in a dark closed container.

Animals. Thirty-five male Wistar rats (250.00 ± 25.00 g) were purchased from the Animal House of Mashhad University of Medical Sciences. During the experimental period, animals were kept under standard conditions (at 22.00 ± 2.00 ˚C temperature with 12 hr:12 hr of light: dark cycles) with free access to food and water. The study was carried out in accordance with the ethical principles of the Committee on Animal Research of Mashhad University of Medical Sciences (Ethical No. IR.MUMS.REC.1396.54).

Diabetes induction. Diabetes was induced by a single freshly prepared streptozotocin (STZ) injection (60.00 mg kg^{-1}, intraperitoneally) to overnight fasted rats. For each animal STZ was weighed and dissolved in 0.50 mL of 10.00 mM sodium citrate (Merck KGaA, Darmstadt, Germany), pH 4.50, with 0.90% saline solution. Then to confirm diabetes induction, the rats' blood glucose levels (samples were obtained from animal's tail) were determined after 72 hr by Easygluco glucometer (Infopia Co., Anyang, South Korea) and the animals with glucose levels of more than 250 mg dL^{-1} were considered as diabetic and were included in this study.

Experimental design. Rats were randomly divided into five different groups (n = 7) and designated as the following: The control (C), diabetic (D), diabetic rats treated daily with metformin (300 mg kg^{-1}; D+Met), diabetic rats treated with 250 mg kg^{-1} of *R. khorasanicum* hydro-ethanolic extract (D+Rib250) and the diabetic rats treated with 500 mg kg^{-1} of *R. khorasanicum* hydro-ethanolic extract (D+Rib500).[11] There was no data about the toxicity or LD_{50} and ED50s of *R. khorasanicum*, however, the LD_{50} of *Vaccinium arctostaphylos* fruit extract as a similar spices to *R. khorasanicum* was higher than 15.00 g kg^{-1}.[18] The treatments were given by oral gavage for six weeks, while the control and diabetic animals received saline solution. The body weights of the animals were measured at the beginning followed by weekly measurements during the experimental periods. The blood samples were collected from retro-orbital plexus of overnight fasted rats under deep anesthesia with urethane (1.60 g kg^{-1}; Merck KGaA). At the end of experiment, the animals were euthanized by bilateral thoracotomy and the abdomen was cut open and kidney and liver tissues were removed. The isolated sera and tissues were kept at – 20.00 ˚C for further analyses.

Biochemical assessment and chemicals. The enzyme immunoassay kits (Cayman Chemical Co., Pittsfield Township, USA) were used for measuring the plasma insulin levels, while the kits that were used for determination of glucose, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) levels were purchased from Pars Azmoon Co. (Tehran, Iran). The measurement was done according to the kits manufacturer's instructions. In order to measure the oxidative stress, the tissues were homogenized with a phosphate buffer solution (PBS; pH 7.4). The homogenates were centrifuged at 1,500 rpm for 10 min, then submitted for oxidative stress measurement. The content of total thiol (SH), malondialdehyde (MDA) level, superoxide dismutase (SOD) and catalase (CAT) activities were determined in both the liver and kidney tissues. The absorbance of all the samples of biochemical evaluation were read by microplate reader (Biotek, Winooski, USA). The STZ was purchased from Sigma Chemical Co. (St. Louis, USA). Disodium salt of ethylene diamine tetra-acetic acid (EDTA), 5, 5-dithiobis-(2-nitro-benzoic acid) (DTNB) and 1-chloro-2, 4-dinitrobenzene (CDNB) were obtained from Merck Company.

Measurement of oxidative stress. The MDA, was measured based on the MDA reaction with thiobarbituric acid (TBA), which produced a pink complex with a peak absorbance at 535 nm.[19] Total thiol content was measured by the method of Ellman.[20] The SH groups produced a yellow complex which had a peak absorbance at 412 nm. The SOD activity was measured based on a procedure described by Madesh and Balasubramanian.[21] The procedure involved the production of superoxide through auto-oxidation of pyrogallol and the inhibition of superoxide-dependent reduction of 3- 4, 5-dimethyl-thiazol-2-yl -2, 5-diphenyl tetrazolium bromide (MTT) conversion to formazan.[21] The activity of CAT was measured according to the method of Aebi, based on the rate of decomposition of hydrogen peroxide by catalase using a spectrophotometer at 240 nm.[22]

Statistical analysis. Data were presented as mean ± SEM. Data were analyzed using InStat Software (version 3.05; GraphPad software Inc., San Diego, USA). One-way analysis of variance followed by Tukey's post-hoc test was used for further statistical analysis. Statistical significance was considered at $p < 0.05$.

Results

Effect on bodyweight changes. The bodyweight of the diabetic and treated animals with *R. khorasanicum* extract and metformin were decreased significantly compared to the control group ($p < 0.001$ for all groups).

The percentage of bodyweight was decreased in the diabetic animals treated with both doses of *R. khorasanicum* extract and was significantly less than the diabetic group (both $p < 0.01$). There were no significant differences among bodyweight changes of D+Rib250, D+Rib500 and D+Met groups (Fig. 1).

Effect on fasting serum glucose levels. Six weeks after the induction of diabetes, measurement of the serum glucose level showed a significant level of hyperglycemia in the diabetic animals and *R. khorasanicum* extract treated groups compared to the control group ($p < 0.05$ and $p < 0.001$). However, the serum glucose levels in *R. khorasanicum* extract and metformin treated groups were significantly lower compared to those of the diabetic group ($p < 0.001$ for all cases). There were no significant differences among serum glucose levels of the D+Rib250, D+Rib500 and D+Met groups (Fig. 2A).

Effect on serum insulin levels. Serum insulin levels of the diabetic, D+Met, D+Rib250 and D+Rib500 groups were significantly decreased compared to the control group ($p < 0.001$). At the end of the experimental period, the serum level of insulin in the D+Rib500 group was significantly higher than that of the diabetic group ($p < 0.05$). There were no significant differences among the serum insulin levels in the diabetic animals treated with either metformin or the two different doses of *R. khorasanicum* extract (Fig. 2B).

Effect on serum lipid profile. In the diabetic group, there was a significant increase in the serum levels of TG, TC, and LDL-C ($p < 0.01$ and $p < 0.001$), however, a significant decrease in the HDL-C was also observed compared to the control group ($p < 0.001$). On the other hand, in the D+Met, D+Rib250 and D+Rib500 groups, serum levels of TG, TC, and LDL-C were decreased significantly compared to the diabetic rats ($p < 0.01$ and $p < 0.001$). Moreover, the serum levels of HDL-C were significantly increased in the D+Rib500 group compared to the diabetic group ($p < 0.05$), while the HDL-C serum level changes in the D+Met and the D+Rib250 groups were not significantly different from those of the diabetic group (Table 1).

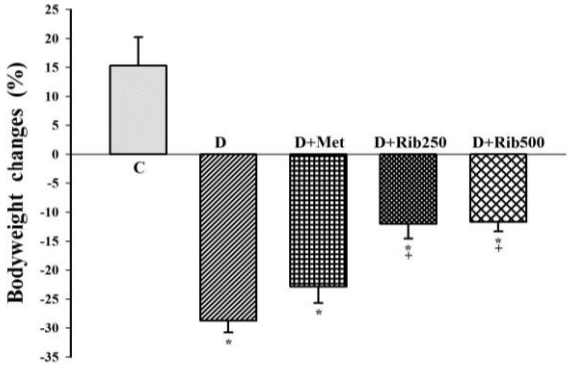

Fig. 1. Effect of *R. khorasanicum* extract and metformin treatment on body weight changes (%) in control (C), diabetic (D), diabetic+metformin (D+Met), diabetic+250 mg kg^{-1} extract (D+Rib250), and diabetic+500 mg kg^{-1} extract (D+Rib500) groups. * $p < 0.001$, compared to the control; + $p < 0.01$, compared to the diabetic group.

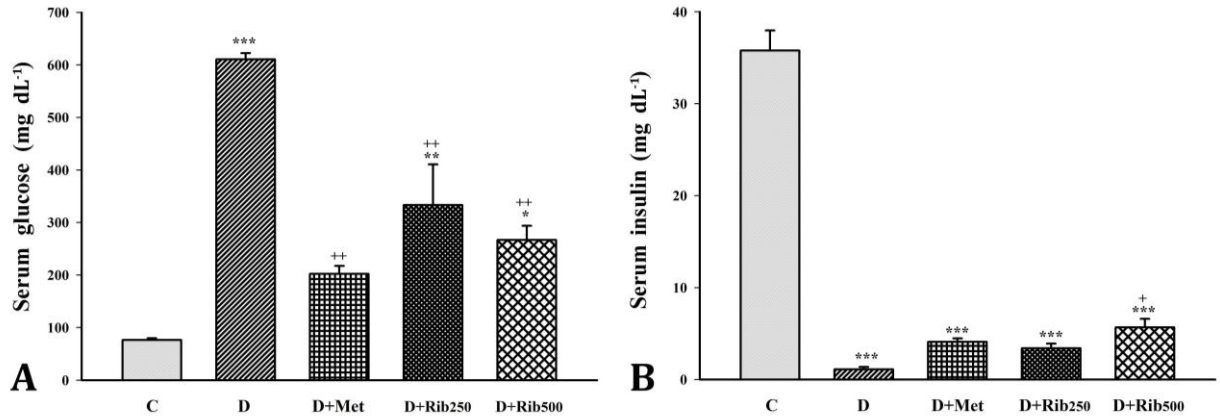

Fig. 2. Effect of *R. khorassanicum* extract and metformin treatment on **A)** serum glucose and **B)** serum insulin levels of control (C), diabetic (D), diabetic+metformin (D+Met), diabetic+250 mg kg^{-1} extract (D+Rib250), and diabetic+500 mg kg^{-1} extract (D+Rib500) groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to control; $^{+}p < 0.05$, $^{++}p < 0.001$, compared to diabetic group.

Table 1. The serum lipid profile values and biochemical markers of liver and kidney function of different groups. Values are expressed as mean ± SEM.

Variables	Control	Diabetic	D+Met	D+Rib250	D+Rib500
Triglycerides (mg dL^{-1})	48.48 ± 1.70	178.29 ± 10.50***	54.28 ± 3.49 $^{+++}$	94.29 ± 12.60 $^{**\,+++\,\#}$	68.00 ± 8.40 $^{+++}$
Total cholesterol (mg dL^{-1})	69.00 ± 8.10	113.32 ± 8.77**	58.29 ± 5.63^{+++}	74.86 ± 3.41^{++}	69.43 ± 8.03^{++}
HDL-cholesterol (mg dL^{-1})	57.87 ± 1.40	40.57 ± 2.98***	47.98 ± 2.25	50.14 ± 2.73	51.00 ± 2.57 $^{+}$
LDL-cholesterol (mg dL^{-1})	17.51 ± 0.57	28.57 ± 1.63**	16.83 ± 1.01^{++}	17.71 ± 3.64 $^{++}$	15.29 ± 1.69 $^{+++}$
SGOT (IU L^{-1})	95.81 ± 4.58	210.63 ± 8.40***	129.14 ± 6.80^{++}	190.69 ± 27.59$^{***\,\#}$	156.06 ± 14.63
SGPT (IU L^{-1})	58.17 ± 3.59	140.63 ± 10.58***	117.75 ± 10.19***	127.25 ± 8.89***	101.38 ± 10.64^{*+}
Urea (mg dL^{-1})	48.30 ± 1.69	92.38 ± 5.25***	54.41 ± 4.97^{+++}	69.38 ± 3.37$^{*\,++}$	62.81 ± 5.88^{+++}
Creatinine (mg dL^{-1})	0.60 ± 0.01	0.89 ± 0.02***	0.74 ± 0.03^{*+}	0.78 ± 0.05**	0.73 ± 0.05 $^{+}$

D+Met: diabetic+metformin, D+Rib250: diabetic+250 mg kg^{-1} extract, and D+Rib500: diabetic+500 mg kg^{-1} extract groups. * $p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to control; $^{+}p < 0.05$, $^{++}p < 0.001$, $^{+++}p < 0.001$, compared to diabetic group; $^{\#}$ $p < 0.05$, compared to D+Met group. SGOT: serum glutamic-oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase.

Effect on liver and kidney oxidative balance and function. Table 1 summarizes the serum levels of serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), urea and creatinine which reflect the liver and renal functions. The serum SGPT levels of the diabetic, D+Met, D+Rib250 and D+Rib500 groups and also the SGOT levels of the diabetic and D+ Rib250 groups were significantly increased compared to the control group ($p < 0.05$ and $p < 0.001$). Treatment of diabetic animals with metformin significantly decreased the serum SGOT level compared to that of the diabetic group ($p < 0.01$). Moreover, the serum SGOT level was significantly higher in the D+Rib250 group compared to the D+Met group ($p < 0.05$). Treatment of diabetic animals with *R. khorasanicum* extract (dose of 500 mg kg^{-1}) significantly decreased the serum SGPT levels compared to that of the diabetic group ($p < 0.05$). The serum levels of urea and creatinine of the diabetic group were significantly higher than the control group ($p < 0.001$). The serum creatinine level of the D+ Met and the D+Rib250 groups were significantly lower than the control group ($p < 0.05$ and $p < 0.01$ respectively). Treatment of the diabetic rats with both metformin and either doses of *R. khorasanicum* extract reduced the serum level of urea compared to that of the diabetic group ($p < 0.01$ and $p < 0.001$). Moreover, the serum creatinine levels of the D+Met and the D+Rib500 groups were decreased significantly compared to the diabetic group (both $p < 0.05$). However, there were no significant differences between the serum urea and creatinine levels of the D+Met and *R. khorasanicum* extract treated groups (Table 1). In the liver tissue total thiol content, SOD and CAT activities were significantly lower while the MDA concentration was significantly higher in the diabetic group compared to the control group ($p < 0.05$ and $p < 0.001$). The MDA concentration of the D+Rib250 was significantly higher and the SOD activity of both the D+Met and D+Rib250 groups was significantly lower than the control group ($p < 0.05$ and $p < 0.01$). Treatment of diabetic rats with metformin decreased the MDA concentration but increased the CAT and SOD activities in liver tissue compared to the diabetic group ($p < 0.05$ and $p < 0.001$). The liver tissue CAT and SOD activities of the D+Rib500 group and the SOD activity of the D+Rib250 group were significantly higher than the diabetic group ($p < 0.05$ and $p < 0.001$). MDA concentration of the D+Met group was significantly lower than the D+Rib250 group ($p < 0.05$), (Table 2).

Table 2. Oxidative stress markers and antioxidants in liver and kidney tissue of different groups. Values are expressed as mean ± SEM.

Variables	Control	Diabetic	D+Met	D+Rib250	D+Rib500
Liver malondialdehyde (nmol g^{-1})	8.17 ± 0.60	27.83 ± 3.83***	10.00 ± 0.36++	23.48 ± 2.23** #	16.37 ± 4.40
Liver total thiol (µmol g^{-1})	2.98 ± 0.03	1.87 ± 0.05*	2.59 ± 0.09	2.26 ± 0.37	2.40 ± 0.34
Liver superoxide dismutase activity (U g^{-1})	32.42 ± 2.47	17.00 ± 1.63***	21.17 ± 1.27*+++	20.88 ± 2.26** ++	24.95 ± 2.96+++
Liver catalase activity (U g^{-1})	17.38 ± 2.78	6.92 ± 0.82**	10.65 ± 1.26	9.75 ± 1.45*	13.17 ± 1.35
Kidney malondialdehyde (nmol g^{-1})	8.56 ± 0.80	30.25 ± 1.53***	12.98 ± 1.20+++	18.10 ± 2.30**	12.50 ± 1.20+++
Kidney total thiol (µmol g^{-1})	4.93 ± 0.30	0.63 ± 0.15***	1.30 ± 0.13***	0.96 ± 0.06***	1.59 ± 0.22***+
Kidney superoxide dismutase activity (U g^{-1})	23.50 ± 4.32	6.33 ± 0.77***	11.83 ± 1.70*	9.12 ± 2.26**	12.80 ± 1.25*
Kidney catalase activity (U g^{-1})	46.33 ± 2.10	20.83 ± 1.97***	27.67 ± 3.22***	23.00 ± 2.98***	32.33 ± 2.56**+

The control (C), diabetic (D), diabetic+metformin (D+Met), diabetic+250 mg kg^{-1} extract (D+Rib250), and diabetic+500 mg kg^{-1} extract (D+Rib500) groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to control; + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$, compared to diabetic group; # $p < 0.05$, compared to D+Met group.

In the kidney tissue total thiol content, and SOD and CAT activities were significantly lower while the MDA concentration was significantly higher in the diabetic and the D+ Rib250 groups compared to the control group ($p < 0.01$ and $p < 0.001$). Kidney tissue thiol content, SOD and CAT activities of both the D+Met and the D+Rib500 groups were significantly lower than the control group ($p < 0.05$ and $p < 0.01$). Treatment of the diabetic rats with metformin and the higher dose of *R. khorasanicum* extract decreased the MDA concentration in kidney tissues compared to that of the diabetic group (both $p < 0.001$). Kidney tissue thiol content and CAT activities of the D+Rib500 group were significantly higher than the diabetic group (both $p < 0.05$), (Table 2).

Discussion

Low insulin production or insulin resistance in diabetes was shown to induce different metabolic disturbances including hyperglycemia, hyperlipidemia, ketoacidosis, and overproduction of ROS.[23,24] In this study the STZ injection-induced diabetes in rats was confirmed by the significant increase in FBS, TG, TC, and LDL-C serum levels, and the decrease in animals' weight gain, serum insulin, and HDL-C levels that were in agreement with other studies.[7,25] Treatment of diabetic rats with both doses of *R. khorasanicum* extract improved animals' weight gain and the serum FBS, while only the higher dose of the extract significantly increased serum insulin levels. These results showed the ameliorative effect of the plant extract on diabetes-induced metabolic disturbances. To the best of our knowledge, there was no experimental study on the effect of *R. khorasanicum* in diabetes. Phenolic content of *R. khorasanicum* was determined as 33.60 mg g^{-1} dry weight and its anthocyanin content was shown as 62.90 mg g^{-1} dry weight of plant fruit, however, the type and chemical structure of anthocyanins was not determined.[13] The anthocyanins and phenolic compounds of this plant fruit extract might have natural α-amylase and α-glucosidase inhibitory activities which reduced and delays glucose absorption from the intestine by slowing starch hydrolysis.[15,26,27] It was reported that other fruit plants of Ribes had α-amylase and α-glucosidase inhibitory activities,[26,28] however, there was no data on *R. khorasanicum*. Additionally, our data showed a significant increase in serum insulin levels of diabetic rats that were treated with a higher dose of *R. khorasanicum*. This result may indicate the stimulatory effect of this plant extract on β cells insulin secretion. Treatment of diabetic animals with both doses of this plant extract alleviated STZ-induced hyperlipidemia and reduced liver enzymes which revealed the hepatoprotective and antihyperlipidemic capabilities of this plant. Several animal and human studies have indicated the antidiabetic and antihyperlipidemic effects of *Vaccinium arctostaphylos* (*V. arctostaphylos*, another member of the Grossulariaceae family) which had similar constituents to *R. khorassanicum.*[17,29-31] In a randomized controlled trial study, four weeks of treatment of diabetic patients with *V. arctostaphylos* leaf extracts (300 mg per day) decreased FBS, serum levels of ALT, AST, GGT and CRP.[32] The *R. khorasanicum* has flavonoid, phenolic, and anthocyanin constituents.[13] Anthocyanin was shown to exert hypoglycemic activity through several mechanisms including an α-glucosidase and pancreatic α-amylase inhibitor effect, therefore, enhancing the glucose uptake by increasing sensitivity to insulin in muscle and adipose tissues.[16] Also, both *in vivo* and *in vitro* experiments have indicated that anthocyanin could increase lipolytic enzyme activity and decrease lipogenic factors.[15,33] In a clinical study, anthocyanin supplementation alleviated diabetic patients hyperlipidemia and decreased their serum levels of LDL cholesterol, triglycerides, apolipoprotein (apo) B-48 and apoC-III while it increased HDL and cholesterol, improved antioxidant capacity and reduced insulin resistance.[34]

Diabetes induced metabolic disturbance and elevation of ROS might lead to hepatic injury which is manifested by elevation of liver enzymes and oxidative stress markers.[24] In the present study, there was a marked increase in serum levels of SGOT and SGPT, plus hepatic MDA levels as well as a decrease in the total hepatic thiol content, and SOD and CAT activities in the diabetic group. Treatment of the diabetic rats with a higher dose of *R. khorasanicum*

increased hepatic SOD and catalase activity and reduced serum levels of SGPT indicating the hepatoprotective and antioxidant effects of the extract. The *R. khorasanicum* is rich in antioxidant constituents such as flavonoid and phenolic (anthocyanins),[13] which could balance the overproduction of ROS and protect the liver from oxidative injury. Improvement of renal function indices and oxidative status in the kidney tissues also could be attributed to those antioxidant components of the extract. Besides, anthocyanins have shown to have an ACE inhibitor activity[35] which might lead to improvement of renal blood flow and function.

Regarding the hypoglycemic, hypolipidemic and cardioprotective effects of metformin, it is still one of the best synthetic drugs for alleviating metabolic disturbances of type 2 diabetes.[36] Our findings showed that hypoglycemic, hypolipidemic, and antioxidant effects of the higher dose of the extract were comparable to those of metformin.

In conclusion, the results of this study confirmed the hypoglycemic, anti-hyperlipidemic, hepatoprotective and renoprotective properties of the hydro-ethanolic extract of *R. khorasanicum*. Further evaluation of these effects in future clinical trials could provide more supporting evidence and lead to the possible development of a *R. khorasanicum* based new remedy for the management of type 2 diabetic complications in the future.

Acknowledgments

The authors would like to thank the Vice Presidency of Research of Mashhad University of Medical Sciences for financial support (grant No. 951356).

Conflict of interests

The authors declare no conflicts of interest in the present study.

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