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ARTICLE

Pharmacological effect of *Rubus ulmifolius* Schott as antihyperglycemic and antihyperlipidemic on streptozotocin (STZ)-induced albino mice

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Abstract The aim of present study was to evaluate the antihyperglycemic and antihyperlipidemic effects of aerial parts of *Rubus ulmifolius* Schott on streptozotocin (STZ)-induced diabetic albino mice. A total of 48-, 60-day-old either sex (male and female) albino mice were treated with, normal control; 2% Tween-80 suspension (diabetic control); glibenclamide (500 µg/kg/orally); RU methanol extract (150 mg/kg/orally) (RUCrd1); RU methanol extract (300 mg/kg/orally) (RUCrd2); RU chloroform extract (150 mg/kg/orally) (RUC); RU ethyl acetate extract (150 mg/kg/orally) (RUE); and RU butanol extract (150 mg/kg/orally) (RUB) for a period of 15 days. Diabetes was induced in albino mice by single intraperitoneal injection of streptozotocin (50 mg/kg/b/w). After 15 days, group treated with glibenclamide, RUCrd1, RUCrd2, RUC, RUE and RUB exhibited a significant ($P > 0.05$) decrease in blood glucose level as compared to diabetic control groups. The total cholesterol, triglycerides and low-density lipoproteins as well as serum creatinine level, serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and alkaline phosphatase were also significantly ($P > 0.05$) decreased in glibenclamide, RUCrd1, RUCrd2, RUC, RUE and RUB groups albino mice as compared to diabetic control. It was concluded that *Rubus ulmifolius* Schott extract has positive effect as hypoglycemic and antihyperlipidemic on diabetic albino mice.

Keywords Albino mice · Antihyperlipidemia · Antihyperglycemic · *Rubus ulmifolius* Schott · Diabetes mellitus

Abbreviations

LDL	Low-density lipoproteins
HDL	High-density lipoproteins
TGs	Triglycerides
ALP	Serum alkaline phosphatase
SGPT	Serum glutamate pyruvate transaminase
SGOT	Serum glutamate oxaloacetate transaminase
STZ	Streptozotocin

Introduction

Diabetes mellitus is a major metabolic disorder affecting a huge population all over the world. It is a chronic metabolic disorder which is characterized by elevated level of glucose due to deficiency of insulin or insulin receptors [1]. Diabetes also leads to hyperlipidemia which is the leading cause of atherosclerosis and cardiovascular diseases [2]. In addition, diabetic patients experience nephropathy and neuropathy. Currently, the most common therapies used for diabetes are insulin, sulfonylureas, biguanides and thiazolidinediones which have many side effects. The prevalence of diabetes increased with the passage of time, but an

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effective treatment with no side effects is still lacking and needs to develop [3].

Plants have been employed in treating various diseases from the very beginning of human civilization [4]. Rosacea is one of the most economically important plant family in temperate regions and comprises of more than 100 genera and about 3000 species [5]. Phytochemicals like flavonoids, cyanogenic glycosides, phytoestrogens and phenolic compounds present in fruits of Rosaceae are major sources of human nutrition that could potentially promote health and have disease fighting advantages [6, 7]. The genus *Rubus* comprises of almost 740 species, which have been divided into 12–15 subgenera, which makes it the largest genus of the Rosacea family and one of the most diverse among the plant kingdom [8]. Different plant parts of this genus have been used for the treatment for diabetes mellitus, rheumatism, sore throat, hemorrhoid, diarrhea and similar enteric disorders [9, 10]. Many species of this genus such as *ellipticus and fructicosus* were used for the treatment for fever, gastric troubles, diarrhea, dysentery wounds, colic, cough, sore throat, carminative, bleeding gums, anemia, kidneys stone, tonic and aphrodisiac [11, 12]. It has been reported previously that some of these species possessed compounds that act as antibacterial, antiallergic, antiasthmatic and antihyperglycemic agents [13, 14]. Different species of *Rubus* contain high contents of tannins and numerous other polyphenols including flavonoids (quercetin and kaempferol derivatives), anthocyanins and phenolic acids, particularly gallic and ellagic acid [15, 16].

Rubus ulmifolius Schott is a perennial thorny shrub and belongs to family of Rosacea. Fruits of *R. ulmifolius* are edible and have carminative properties. The unripe fruits are used as tonic and aphrodisiac [3, 17]. Leaves and roots are used to treat the skin diseases [11]. *Rubus ulmifolius* in Italian folk medicine has been used for abscesses and ulcers, externally for vaginal lavages and diarrhea [18]. *Rubus ulmifolius* is used in Chilean folk medicine due to its hypoglycemic actions [19]. Previously, many studies were performed on mice/rats to check the antidiabetic effects of different herbs. Mice and rats are widely accepted animal models, and they have resemblance to human diabetes mellitus [20, 21]. The albino mice are used as a diabetic model to conduct many studies [17, 22]. Albino mice were used to conduct in vivo antidiabetic activity tests of *Swertia koutichensis* extracts [23]. A similar, study was conducted on *Piper longum* roots, aerial parts of *Artemisia indica* and *Drynaria quercifolia* Linn rhizome and mung to evaluate the antihyperlipidemic and hyperglycemic effects in diabetic mice and rats [1, 20]. A low concentration of streptozotocin (50 mg/kg b.w) has been reported to induce type II diabetes in mice [3, 20]. Therefore, the current study aims to evaluate the antihyperglycemic and antihyperlipidemic effects of aerial parts of *R. ulmifolius* Schott in streptozotocin (STZ)-induced diabetic albino mice.

Materials and methods

Plant collection and preparation of extracts

Fresh aerial plant parts were collected from near hilly area of Gaddar, Chakdara, Dir lower Khyber Pakhtunkhwa, Pakistan, in the month of July 2016. The plant specimen was deposited in the Pharmacy department, University of Malakand, Khyber Pakhtunkhwa, Pakistan. The aerial parts of *R. ulmifolius* plants were washed with distilled water and dried at 65 °C for 48 h and grinded. 2.5 g of grinding powder was mixed with 500 ml of commercial methanol and kept at room temperature for 3 weeks at room temperature. During soaking the mixture was frequently shaken and filtered by two layers of cheesecloth and Whatman filter paper. The filtrate was concentrated to a semisolid mass using a rotary evaporator under reduced pressure at 45 °C for a final extract. The semisolid mass (789 g) was suspended in 500 ml distilled water. The extract was given orally as it was mixed with water as per body weight (150–300 mg/kg body weight). The same technique was used for ethyl acetate extract (RUE), chloroform extract (RUC) and butanol extract (RUB). These all extracts' combined name is (RUCrd).

Phytochemicals analysis

Preliminary phytochemicals analysis of *R. ulmifolius* Schott (RUCrd) extract indicated the presence of saponins, flavonoids, glycosides, cardiac anthraquinone glycosides, carbohydrates, terpenoids, alkaloids, phenolic compounds, tannins, amino acids and proteins as shown in Table 1.

Table 1 Detection of phytochemicals in *R. ulmifolius*

Phytochemical	Test performed	Results
Tannins	Ferric chloride test	Positive
Glycosides	Legal's test	Positive
Terpenoids	Chloroform	Positive
Carbohydrates	Benedict's test	Positive
Proteins	Ninhydrin test (aq)	Positive
Saponins	Foam test	Positive
Alkaloids	Mayer's test	Mild positive
Flavonoids	NaOH test	Positive
Sterols	Sulfuric acid test	Positive

Chemicals

Streptozotocin was supplied by Sigma-Aldrich, glibenclamide was supplied by Sanofi Aventis Pharma (Pvt.) Ltd, Pakistan, glucose estimation kits were supplied by S.D Chek Gold Korea, normal saline was supplied by Utsoka pharma (Pvt) Ltd., Pakistan, and Tween-80 (2%) was supplied by Daejung Korea, according to the manufacturer's instructions. The different organic solvents and chemicals used for the extraction were purchased from local suppliers of Merck, Germany.

Experimental animals

Standard experimental protocols were followed as per the guidelines of ethical committee of Department of Pharmacy, University of Malakand, Khyber Pakhtunkhwa, Pakistan. A total 48-, 60-day-old either sex (male and female) albino mice, weight range of 18–25 g, were used to evaluate the antidiabetic activity. Albino mice were purchased from the National Institute of Health, Islamabad. After one week of adaptation, the albino mice were divided into eight groups of 6 and treated as follows: normal control; 2% Tween-80 suspension (Diabetic control); glibenclamide (500 µg/kg/orally) (C); RU methanol extract (150 mg/kg/orally) (RUCrd1); RU methanol extract 300 mg/kg/orally) (RUCrd2); RU chloroform extract (150 mg/kg/orally) (RUC); RU ethyl acetate extract (150 mg/kg/orally) (RUE) and RU butanol extract (150 mg/kg/orally) (RUB). The albino mice were maintained in light and dark cycles for 12 h at room temperature (22–25 °C) in laboratory. The albino mice were provided feed and water ad libitum. Blood glucose level and body weight were measured at 1st, 4th, 7th, 10th and 15th day of periods.

Biochemical analysis

Acute toxicity of the RUCrd was determined using either sex (male and female) of albino mice in the weight range of (20–27 g). Acute toxicity study was conducted in two phases, each phase divided into three groups having 6 albino mice. In the first phase, G1 was served as a control, G2 was administered Tween-80 (2%), an oral dose of 250, 500 and 1000 mg/kg/b/w, the animal were constantly observed for any behavioral alteration or death toll with follow-up for 10 days. In the second phase, G3, G4, G5, G6, G7 and G8 received 10, 100, 1000, 1500, 2000 and 2500 mg/kg/b/w an oral dose of RUCrd, respectively. The animals were observed for behavioral changes, physical and pharmacological effects as shown in Fig. 1.

Induction of diabetes

Diabetes was induced in albino mice by STZ, 50 mg/kg b.w, through a single intraperitoneal injection reconstituted in 0.01 M citrate buffer (pH 4.5) after overnight fasting [24]. Blood glucose level was measured after 72 h of STZ administration to check the hyperglycemia condition, with one-touch glucometer strips using SD glucometer (Korea). Mice having fasting blood glucose level more than 300 mg/dl were selected. All the animals were allowed to access free food and water and maintained at room temperature in cages.

Blood collection

Blood samples were taken from the tip of the tail, and the blood glucose level was measured on the 1st, 4th, 7th, 10th and 15th day, with one touch of glucometer strips using SD glucometer (Korea). After completion of antidiabetic assay on 15th day, all animals were anesthetized by pentobarbital sodium (35 mg/kg) and samples of blood were collected through cardiac puncture for the analysis of biochemical parameters including serum alkaline phosphatase (ALP), high-density lipoprotein (HDL), low-density lipoproteins (LDL), total cholesterol (TC), triglycerides (TG) and serum creatinine levels [25, 26].

Statistical analysis

The experiment was a completely randomized design. The data were analyzed using the GLM procedure of SPSS (version 17.0). Means of the significantly affected traits were separated by the Duncan multiple range test [27]. A P value less than 0.05 was considered statistically significant.

Results

Results from the toxicity studies of RUCrd have revealed no mortality when a dose maximum up to 2500 mg/kg/b.w was orally administered. No substantial changes were seen in the animal behaviors in all of the test groups. Hence, 150 and 300 mg/kg doses of RUCrd were selected then to evaluate antidiabetic activities.

The daily oral effects of administered *R. ulmifolius* on blood glucose levels (hypoglycemic and antihyperlipidemic) in STZ-induced diabetic albino mice are shown in Table 2. The fasting blood glucose level in diabetic treated and untreated (G3-G8) albino mice was significantly high. After oral administration of the RUCrd1, RUCrd2, RUC, RUE and RUB at the rate of 150–300 mg/kg significantly ($P < 0.05$) decreased the blood glucose level as observed

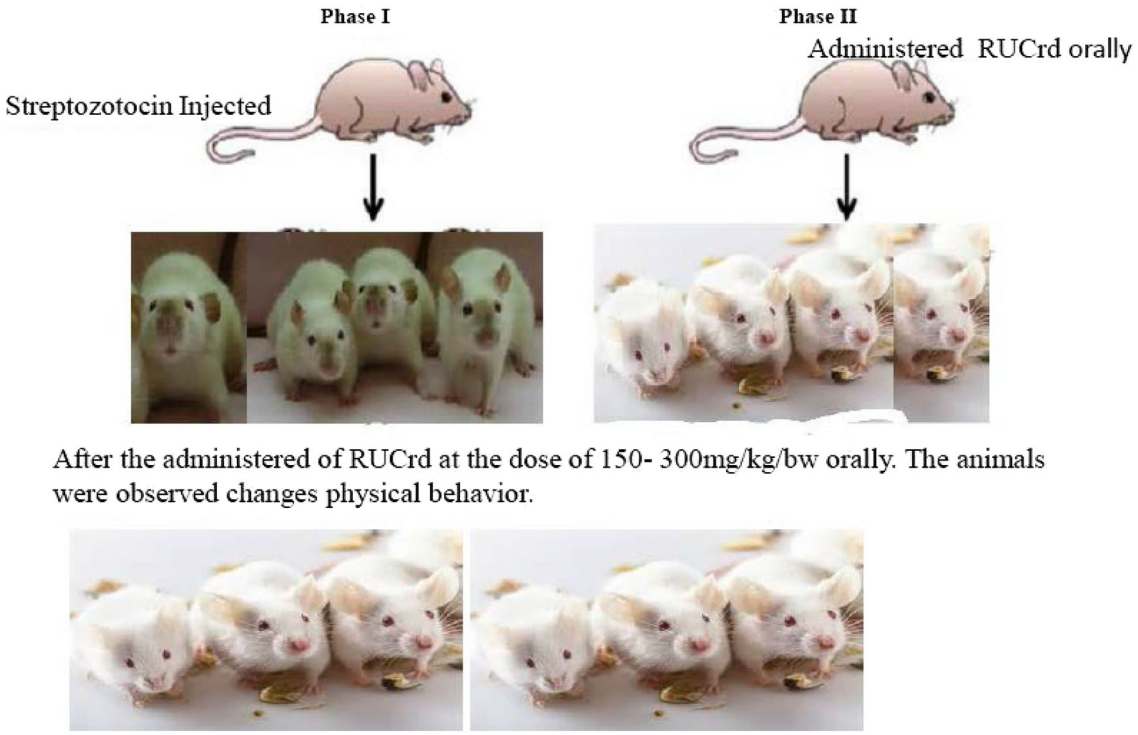

Fig. 1 Acute toxicity analysis

Table 2 Effect of daily oral administered of RUCrd on blood glucose levels (hypoglycemic and antihyperlipidemic) in diabetic albino mice

S. no.	Groups	Dose mg/kg	Day 1st	Day 4th	Day 7th	Day 10th	Day 15th
G1	Normal control	0.4 ml	114.3 ± 4.7	108.3 ± 3.9	104.2 ± 4.5	101.1 ± 5.5	100.3 ± 5.8
G2	Diabetic control	0.4 ml	460.2 ± 5.5	484.3 ± 5.8	493.3 ± 5.8	511.2 ± 4.3	517.3 ± 6.1
G3	Glibenclamide	0.5	466.4 ± 3.5	312.5 ± 4.3*	298.5 ± 4.3*	251.3 ± 4.7**	211.5 ± 4.5**
G4	RUCrd1	150	476.6 ± 5.1	475.2 ± 2.5*	345.1 ± 3.3**	309.5 ± 5.5**	243.4 ± 4.8**
G5	RUCrd2	300	471.4 ± 3.1	489.6 ± 4.7*	368.3 ± 3.5**	254.9 ± 6.1**	241.5 ± 3.5**
G6	RUC	150	467.1 ± 4.3	449.4 ± 3.6*	332.4 ± 4.7**	291.2 ± 4.3**	220.4 ± 4.2**
G7	RUE	150	461.8 ± 2.9	418.4 ± 3.9*	320.5 ± 4.1**	253.1 ± 4.6**	206.5 ± 3.4**
G8	RUB	150	481.9 ± 3.8	460.1 ± 3.3*	464.6 ± 3.2**	398.3 ± 4.3**	390.5 ± 5.1**

Normal control; 2% Tween-80 suspension; glibenclamide (500 µg/kg/overly); *Rubus ulmifolius* (150 mg/kg/orally) (RUCrd1); *Rubus ulmifolius* 300 mg/kg/orally) (RUCrd2); *Rubus ulmifolius* + chloroform (150 mg/kg/orally) (RUC); *Rubus ulmifolius* + ethyl acetate (150 mg/kg/orally) (RUE) and *Rubus ulmifolius* + butanol (150 mg/kg/orally) (RUB)

All values are taken as mean ± SD (* $P < 0.05$, *** $P < 0.01$)

on the day of 1, 4, 7, 10 and 15 as compared to diabetic control.

The effect of *R. ulmifolius* on body weight in STZ-induced diabetic albino mice is illustrated in Table 3. Changes in body weight of control and experimental diabetic albino mice treated with extracts of *R. ulmifolius* and glibenclamide were observed. A significant ($P < 0.05$) reduction in body weight was observed in STZ-induced diabetic albino mice compared to control during the 15 days. After the administration of RUCrd1, RUCrd2, RUC, RUE and RUB extracts, slightly increased body weight was observed on 1st, 4th, 7th, 10th and 15th day on diabetes albino mice.

The effect of RUCrd extracts on lipid profiles in control and experimental STZ-induced diabetes albino mice is shown in Table 4. The TC, TG and LDL increased after induction of STZ as compared to normal control. In addition, there was a substantial decrease ($P < 0.05$) in HDL

Table 3 Effect of RUCrd on the body weight of diabetic albino mice

S. no.	Groups	Dose mg/kg	Day 1st	Day 4th	Day 7th	Day 10th	Day 15th	changes in b.w %
G1	Normal control	0.4 ml	24 ± 2.9	26 ± 3.6	26 ± 3.8	25.6 ± 3.4	25.9 ± 5.1	
G2	Diabetic control	0.4 ml	22.8 ± 4.6	22.3 ± 2.8*	21.9 ± 4.9**	20.4 ± 6.1**	20.1 ± 3.7**	-11.8
G3	Glibenclamide	0.5	21.9 ± 4.5	22.5 ± 3.3*	22.8 ± 4.6*	23.4 ± 5.0**	23.7 ± 3.8**	+8.2
G4	RUCrd1	150	22.0 ± 4.3	22.4 ± 6.0*	23.0 ± 3.9*	23.4 ± 4.6**	23.4 ± 3.4**	+6.3
G5	RUCrd2	300	23.5 ± 3.7	23.7 ± 5.1*	24.1 ± 4.5*	23.8 ± 5.4**	24.4 ± 4.7**	+3.8
G6	RUC	150	20.9 ± 5.1	21.2 ± 4.7*	21.4 ± 4.5**	22.7 ± 3.8*	22.5 ± 5.1**	+7.6
G7	RUE	150	23.5 ± 4.2	23.7 ± 4.6*	23.8 ± 5.1**	24.0 ± 4.2**	24.4 ± 4.7**	+3.8
G8	RUB	150	22.6 ± 3.5	22.1 ± 3.8	22.9 ± 4.7	23.3 ± 4.2	22.9 ± 3.7	+1.3

Normal control; 2% Tween-80 suspension; glibenclamide (500 µg/kg/overly); *Rubus ulmifolius* (150 mg/kg/orally) (RUCrd1); *Rubus ulmifolius* 300 mg/kg/orally) (RUCrd2); *Rubus ulmifolius* + chloroform (150 mg/kg/orally) (RUC); *Rubus ulmifolius* + ethyl acetate (150 mg/kg/orally) (RUE) and *Rubus ulmifolius* + butanol (150 mg/kg/orally) (RUB)

All values are taken as mean ± SD (* $P < 0.05$, *** $P < 0.01$)

Table 4 Effects of RUCrd on lipid profile in STZ-induced diabetic albino mice

S. no.	Groups	Dose mg/kg	Total CH (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
G1	Normal control	0.4 ml	130 ± 6.7	43 ± 4.2	91 ± 4.7	136 ± 7.5
G2	Diabetic control	0.4 ml	196 ± 5.2**	39 ± 3.9*	186 ± 3.9**	179 ± 4.7**
G3	Glibenclamide	0.5	147 ± 6.0**	49 ± 5.2**	99 ± 5.4***	139 ± 3.9**
G4	RUCrd1	150	153 ± 3.8**	48 ± 4.8**	99 ± 4.6***	148 ± 5.3**
G5	RUCrd2	300	149 ± 4.7**	45 ± 5.0**	93 ± 5.0***	146 ± 6.1**
G6	RUC	150	148 ± 4.2*	48 ± 6.2*	92 ± 4.5*	142 ± 4.9**
G7	RUE	150	147 ± 2.9**	43 ± 3.8**	90 ± 4.8**	140 ± 5.6*
G8	RUB	150	154 ± 5.0	52 ± 4.5	101 ± 3.6	151 ± 4.5

Normal control; 2% Tween-80 suspension; glibenclamide (500 µg/kg/overly); *Rubus ulmifolius* (150 mg/kg/orally) (RUCrd1); *Rubus ulmifolius* 300 mg/kg/orally (RUCrd2); *Rubus ulmifolius* + chloroform (150 mg/kg/orally) (RUC); *Rubus ulmifolius* + ethyl acetate (150 mg/kg/orally) (RUE) and *Rubus ulmifolius* + butanol (150 mg/kg/orally) (RUB)

All values are taken as mean ± SD (*$P < 0.05$, ***$P < 0.01$)

cholesterol in diabetic control albino mice as compare to normal control. The administration of the RUCrd1, RUCrd2, RUC, RUE and RUB caused a significant decrease in TC, TG, LDL on the 4th, 7th, 10th and 15th day on diabetes control albino mice. The effect was comparable to that of glibenclamide (500 µg/kg) used as a standard drug. Furthermore, the oral administration of RUCrd1, RUCrd2, RUC, RUE, RUB (150–300 mg/kg) and glibenclamide (500 µg/kg) significantly ($P < 0.05$) increased HDL cholesterol level in diabetic albino mice.

The effects of RuCrd on the activity of hepatic marker enzymes in STZ-induced diabetic albino mice are shown in Table 5. Level of ALP, serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase were significantly decreased in RUCrd1, RUCrd2, RUC, RUE and RUB groups as compare to standard drug glibenclamide and diabetic control albino mice. The serum creatinine was significantly decreased in RUCrd1, RUCrd2, RUC, RUE and RUB groups as compare to standard drug glibenclamide-treated albino mice.

Discussion

Rubus ulmifolius Schott has been used for therapeutic purposes and was considered an important component of herbal medicines. It has also been reported that leaves of *R. ulmifolius* Schott showed hypoglycemic activity and used as an important component of Chilean popular medicines [3, 28]. Previously, no study was reported on aerial parts of *R. ulmifolius* Schott in Pakistan as a potential antidiabetic herb. In the current study, aerial parts of *R. ulmifolius* were used to evaluate the antidiabetic and antihyperlipidemic activities in STZ-induced diabetic albino mice [3, 20]. A similar study reported on *Piper longum* roots and leaves used to evaluate antihyperlipidemic and hyperglycemic effects in diabetic rats [28, 29]. A low concentration of streptozotocin (50 mg/kg b.w) has been reported to induce type II diabetes in mice [20, 24, 26]. RU crude methanolic extracts, chloroform and ethyl acetate fractions showed significant reduction in FBG in STZ-induced diabetic mice (treated) compared to

Table 5 Effect of RUCrd and fractions on serum liver profile in STZ-induced diabetic albino mice

S. no.	Groups	Dose mg/kg	(ALP) IU	(SGPT) IU	(SGOT) IU	Serum creatinine (mg/dl)
G1	Normal control	0.4 ml	189 ± 4.9	19 ± 4. 5	22 ± 5.1	0.59 ± 2.5
G2	Diabetic control	0.4 ml	299 ± 5.5**	59 ± 3.8	46 ± 4.3**	2.9 ± 2.5**
G3	Glibenclamide	0.5	206 ± 6.1**	23 ± 4.7**	26 ± 4.8**	0.70 ± 2.1***
G4	RUCrd1	150	204 ± 6.5**	23 ± 3.9**	22 ± 6.0**	0.89 ± 2.3***
G5	RUCrd2	300	202 ± 6.3**	19 ± 3.9**	20 ± 3.7**	0.68 ± 1.8***
G6	RUC	150	200 ± 5.7**	22 ± 6.1*	21 ± 5.3**	0.65 ± 1.2**
G7	RUE	150	193 ± 4.8**	21 ± 4.7**	19 ± 5.6*	0.59 ± 2.1*
G8	RUB	150	210 ± 5.7	33 ± 3.8	29 ± 6.2	0.99 ± 1.6

Normal control; 2% Tween-80 suspension; glibenclamide (500 μg/kg/overly); *Rubus ulmifolius* (150 mg/kg/orally) (RUCrd1); *Rubus ulmifolius* 300 mg/kg/orally) (RUCrd2); *Rubus ulmifolius* + chloroform (150 mg/kg/orally) (RUC); *Rubus ulmifolius* + ethyl acetate (150 mg/kg/orally) (RUE) and *Rubus ulmifolius* + butanol (150 mg/kg/orally) (RUB)

All values are taken as mean ± SD (** $P < 0.05$, *** $P < 0.01$)

diabetic control (untreated) at a dosage of 150 mg/kg b.w. RUE fraction showed significantly high antidiabetic activity which was similar to standard drug glibenclamide-treated mice. RUB showed comparatively low antidiabetic activity, and this may be due to lack of phytochemicals involved in lowering blood glucose.

Preliminary findings on methanolic extracts of *R. ulmifolius* Schott in the current study of revealed the presence of phenolic compounds, flavonoids, alkaloids and terpenoids. Phytochemicals such as phenolic compounds have been reported as potential agents involved in the prevention of diabetes and cardiovascular disease [29, 30]. Flavonoids are one of the most common polyphenolic compounds which have been used for the treatment for diabetes mellitus since ancient times [31]. It was previously reported that phytochemicals like phenolic, flavonoids, alkaloids and glycosides from various plants such as *Catharanthus roseus*, *Psidium guajava*, *Ephedra sinica* and *Rhizoma pinelliae* showed antidiabetic activity [8, 32]. There are many mechanisms to control the blood sugar level. Inhibition of carbohydrate digestive enzymes especially of α-glucosidase and pancreatic α-amylase is one of the common mechanisms by which these compounds showed blood glucose lowering activity [33]. α-Glucosidase and α-amylase are important enzymes involved in intestinal glucose absorption and the breakdown of starch. The passage of carbohydrates into the blood stream could be slow down due to the inhibition of these enzymes and notably lowering the blood glucose level and play a key role in the management of diabetes [34].

The loss of body weight was observed in diabetic untreated mice (after 15-day treatment) which may be due to muscle wasting and loss of tissue proteins and insufficient utilization of carbohydrates [35]. RU crude methanolic extracts and fractions specifically chloroform and ethyl acetate-treated diabetic mice showed an increase in body weight, but no significant increase in body weight was observed in mice treated with RU butanol fraction. It was also observed that RU butanol fraction possessed very low antidiabetic activity which indicated that this fraction contained no antidiabetic agents.

It was previously reported that some local plants like neem, nayantara, fenugreek and tulsi are traditionally used to treat diabetes mellitus [32]. Similarly, due to common use and medicinal importance of *R. ulmifolius* in Chilean folk medicines the current study was conducted to observe the antidiabetic activity of *R. ulmifolius* (RUCrd) and different solvent fractions in STZ-induced diabetic mice and found very effective in the control of glucose level. Both crude extracts and different solvent fractions including RUE, RUC and RUB of *R. ulmifolius* leaves showed a significant decrease in blood glucose level and hyperlipidemic profile of diabetic in the current study. Since the preliminary phytochemicals analysis showed that *R. ulmifolius* leaves are rich in flavonoids, alkaloids, tannins, phenylpropanoids, terpenoids, carbohydrates and proteins. The antidiabetic and antihyperlipidemic activities of *R. ulmifolius* may be attributed to presence of these phytochemicals, but the exact mechanism is not known yet.

In additions, superoxide, hydrogen peroxide and hydroxyl radicals are reactive oxygen species (ROS), which can cause oxidative damage to cellular structures and functional molecules [36, 37]. There are many reports which showed that oxidative stress is the leading cause of many diseases, including diabetes [38]. Recently, phenolics and flavonoids have been proved to be more effective than vitamin C, E and carotenoids due to stability in structure and hence considered as great antioxidants [36, 39]. Mechanisms responsible for the antioxidant properties of flavonoid and phenolic compounds included scavenging radical species, reactive nitrogen species, suppressing

ROS/RNS formation by inhibiting some enzymes or chelating trace metals concerned with free radical production and protecting antioxidant defense [40]. Plants rich in flavonoids and phenylpropanoid (antioxidants) may ameliorate the effects of these ROS due to their scavenging properties, and their common use may protect from many diseases.

Present study also reported that RUCrd and different solvent extracts of *R. ulmifolius* leaves to be very effective in normalizing liver and kidney profile of mice. This may be due to some potential constituent present in the extracts which may be useful in the treatment for liver- and kidney-related disorders. Further investigation is needed on the potential of effects of *R. ulmifolius* on kidney and liver profiles.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

References

1. Ahmad W, Khan I, Khan MA, Ahmad M, Subhan F, Karim N (2014) Evaluation of antidiabetic and antihyperlipidemic activity of *Artemisia indica linn* (aeriel parts) in streptozotocin induced diabetic rats. J of Ethnopharmacol 151:618–623
2. Sheetz MJ, King GL (2002) Molecular understanding of hyperglycemia's adverse effects for diabetic complications. JAMA 288:2579–2588
3. Ali N, Shaoib M, Shah SWA, Shah I, Shuaib M (2017) Pharmacological profile of the aerial parts of *Rubus ulmifolius* Schott. BMC Complement Altern Med 17:59
4. Balunas MJ, Kinghorn AD (2005) Drug discovery from medicinal plants. Life Sci 78:431–441
5. Dirlewanger E, Cosson P, Tavaud M, Aranzana M, Poizat C, Zanetto A, Laigret F (2002) Development of microsatellite markers in peach [Prunuspersica (L.)Batsch] and their use in genetic diversity analysis in peach and sweet cherry (Prunusavium L.). Theor Appl Genet 105:127–138
6. Macheix JJ, Sapis JC, Fleuriet A, Lee CY (1991) Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. Crit Rev Food Sci Nutr 30:441–486
7. Mazur WM, Uehara M, Wähälä K, Adlercreutz H (2000) Phytooestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. Br J Nutr 83:381–387
8. Deguchi Y, Miyazaki K (2010) Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf extract. Nutr Metab 7:9
9. Guarrera PM (2005) Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). Fitoterapia 76:1–25
10. Patel AV, Rojas-Vera J, Dacke CG (2004) Therapeutic constituents and actions of Rubus species. Curr Med Chem 11:1501–1512
11. Haq F (2012) The ethno botanical uses of medicinal plants of Allai Valley, Western Himalaya Pakistan. Int J Plant Res 2:21–34
12. Gairola S, Sharma J, Bedi YS (2014) A cross-cultural analysis of Jammu, Kashmir and Ladakh (India) medicinal plant use. J Ethnopharmacol 155:925–986
13. Richards RME, Durham DG, Liu X (1994) Antibacterial activity of compounds from Rubus pinfaensis. Planta Med 60:471–473
14. Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR (1990) Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetologia 33:462–464
15. Mertz C, Cheynier V, Günata Z, Brat P (2007) Analysis of phenolic compounds in two blackberry species (Rubus glaucus and Rubus adenotrichus) by high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry. J Agri Food Chem 55:8616–8624
16. Ali L, Alsanius BW, Rosberg AK, Svensson B, Nielsen T, Olsson ME (2012) Effects of nutrition strategy on the levels of nutrients and bioactive compounds in blackberries. Eur Food Res Technol 234:33–44
17. Ahmad N, Anwar S, Fazal H, Abbasi BH (2013) Medicinal plants used in indigenous herapy by people of Madyan Valley in district Swat, Pakistan. Int J Med Aromat Plants 3:47–54
18. Manganelli RU, Tomei PE (1999) Ethnopharmacobotanical studies of the Tuscan Archipelago. J Ethnopharmacol 65:181–202
19. Lemus I, Garcia R, Delvillar E, Knop G (1999) Hypoglycaemic activity of four plants used in Chilean popular medicine. Phytother Res 13:91–94
20. Nabi SA, Kasetti RB, Sirasanagandla S, Tilak TK, Kumar MVJ, Rao CA (2013) Antidiabetic and antihyperlipidemic activity of *Piper longum* root aqueous extract in STZ induced diabetic rats. BMC Complement Altern Med 13:37
21. Yao Y, Chen F, Wang M, Wang J, Ren G (2008) Antidiabetic activity of Mung bean extracts in diabetic KK-Ay mice. J Agri Food Chem 56:8869–8873
22. Singh MP, Pathak K (2015) Animal models for biological screening of anti-diabetic drugs: an overview. Eur J Exp Biol 5:37–48
23. Wan LS, Chen CP, Xiao ZQ, Wang YL, Min QX, Yue Y, Chen J (2013) In vitro and in vivo anti-diabetic activity of Swertia kouitchensis extract. J Ethnopharmacol 147:622–630
24. Gupta S, Kataria M, Gupta PK, Murganandan S, Yashroy RC (2004) Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. J Ethnopharmacol 90:185–189
25. Shepherd J (2005) Does statin monotherapy address the multiple lipid abnormalities in type 2 diabetes. Atheroscler Suppl 6:15–19
26. Shirwaikar A, Rajendran K, Barik R (2006) Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. J Ethnopharmacol 107:285–290
27. Duncan DB (1955) Multiple range and multiple F tests. Biometrics 11:1–42
28. Evans WC (2009) Trease and Evans' pharmacognosy. Elsevier Health Sciences, Amsterdam
29. Shetty K (2001) Biosynthesis and medical applications of rosmarinic acid. J Herbs Spices Med Plants 8:161–181
30. Cotelle N (2001) Role of flavonoids in oxidative stress. Curr Top Med Chem 1:569–590
31. García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA (2009) Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflamm Res 58:537–552
32. Grover JK, Yadav S, Vats V (2002) Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 81:81–100
33. McCue PP, Shetty K (2004) Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. Asia Pac J Clin Nutr 13:101–106

34. Puls W, Keup U, Krause H, Thomas PG, Hoffmeister F (1977) Glucosidase inhibition Naturwissenschaften 64:536–537
35. Chatterjee MN, Shinde R (2002) Jaypee brothers. Medical Publishers, New Dehli
36. Shah AA, Khan MS, Khan S, Ahmad N, Alhidary IA, Khan RU, Shao T (2016) Effect of different levels of alpha tocopherol on performance traits, serum antioxidant enzymes, and trace elements in Japanese quail (*Coturnix coturnix japonica*) under low ambient temperature. Revista Brasileira de Zootec 45:622–626
37. Kusano R, Ogawa S, Matsuo Y, Tanaka T, Yazaki Y, Kouno I (2010) α-Amylase and lipase inhibitory activity and structural characterization of acacia bark proanthocyanidins. J Nat Prod 74:119–128
38. Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408:239–247
39. Halliwell B (1994) Free radicals, antioxidants, and human disease: curiosity, cause, or consequence. The Lancet 344:721–724
40. Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15:7313–7352
41. Chattopadhyay RR (1999) A comparative evaluation of some blood sugar lowering agents of plant origin. J Ethnopharmacol 67:367–372