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Antidiabetic Effect and Antioxidant Potential of *Rosa canina* Fruits

Orhan N, Aslan M*, Hoşbaş S, Deliorman Orhan D

Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330 Hipodrom-Ankara, Turkey

**** Author for Correspondence :*** *M.Aslan e-mail:marslan@gazi.edu.tr*

ABSTRACT

Rosa canina L. fruits (Rosaceae) are used to treat diabetes in Anatolia traditionally. In this study, the ethanol extract of *R. canina* fruits and its fractions were screened for their antioxidant, hypoglycaemic and antidiabetic activities. The ethanol extract that was administered for 7 days possessed a remarkable hypoglycemic effect at 250 mg/kg dose in streptozotocin (STZ) induced diabetic rats. Then it was fractionated through successive solvent extractions to yield $CHCl_3$ Fr., EtOAc Fr., *n*-BuOH Fr. and R-H_2O Fr. respectively. These fractions were administrated to normal plus glucose hyperglycemic rats. Additionally the subacute antidiabetic activities of the fractions were studied in diabetic rats for 7 days. The experimental data indicated that R-H_2O Fr. possessed significant antidiabetic activity (50-62%) in diabetic rats. Also, a minor hypoglycemic effect was observed in normoglycemic plus glucose-hyperglycemic animals treated with R-H_2O Fr. (15%). *In vitro* antioxidant experiments revealed that EtOAc Fr. showed the highest radical scavenging activity on DPPH (79.5±0.4%), whereas $CHCl_3$ Fr. exhibited the maximum reducing power. The highest total phenolic content was observed in $CHCl_3$ Fr. (18.5±0.6% gallic acid equivalent g/g fraction) but no correlation was observed between the antidiabetic activity of fractions and their phenolic contents. Our findings support the traditional usage of *R. canina* fruits as a folk remedy in the treatment of diabetes in Turkey.

KEYWORDS: *Antioxidant, Antidiabetic, OGTT, Phenolic content, Rosa canina, Rosaceae*

INTRODUCTION

Rosa canina L. (Rosaçeae), commonly known as kuşburnu, itburnu, kïpek gülü, has been used as both food and folk remedy in Anatolia. In the German Commission E Monographs, fruits (rose-hips, with seeds) of *R. canina* are reported to possess prophylactic and therapeutic activities against a wide range of ailments, including the inflammatory disorders arthritis, rheumatism, gout, sciatica, for diseases with fever; for colds and infectious diseases including influenza, against gastrointestinal disorders, to aid digestion, prevention of inflammation of the gastric mucosa and gastric ulcer, for gallstones, biliary complaints, as a laxative, for disorders of the kidney and the lower urinary tract, as a diuretic, for dropsy and as an astringent (1). In addition to the effects of the fruits described above, the fruit is known as the most effective remedy against hemorrhoids and diabetes mellitus in Turkish folk medicine. Besides, the roots and leaves of the plant have also been used against bronchitis (2–8). To date, reports on the antioxidant, antiinflammatory, antiulcer, antimicrobial, antimutagenic effects and inflammatory cytokines inhibitory activity of *R. canina* fruits are available (9–20).

Diabetes mellitus is an important metabolic disorder. It is a noncommunicable disease considered to be one of the five leading causes of death world wide (21). About 150 million people around the world have been diagnosed with diabetes and this is likely to increase to 300 million or more by the year 2025 (22). Oxidative stress causing secondary ailments including neuropathy, nephropathy, microangiopathy etc. is a major problem

observed during diabetes. It is important to evaluate both the antioxidant potential and the hypoglycemic activity of antidiabetic drugs. Therefore, antidiabetic researches are still continuous increasingly. Currently available therapeutic options for diabetes mellitus, such as dietary modification, oral antidiabetic agents and insulin, have limitations of their own. Because of the reasons mentioned before, in recent years the searches for new antidiabetic agents have been focused on plants used in traditional medicine.

Streptozotocin (STZ) is a valuable agent for induction of experimental diabetes mellitus (23). In this model, STZ can stimulate free radical generation, which may be one of the most essential causes of β-cell damage and its diabetogenic effect. Therefore, STZ-induced diabetic model is preferred to act diabetes and oxidative stress described above.

The present study has the following objectives: (i) to evaluate the hypoglycemic effects of *R. canina* fruits in normoglycemic plus glucose-hyperglycemic (NG-OGTT) model and STZ-induced diabetic rats, (ii) to investigate *in vitro* antioxidant activity and total phenolic content of ethanolic extract of *R. canina* fruits and its fractions.

MATERIALS AND METHODS

Plant material

Fruits of the plant were collected from Beytepe Campus area of Hacettepe University, Ankara in September 2004 and dried under shade. Voucher specimens were authenticated by Mecit Vural from the Department of Botany, Faculty of Science, Gazi University and deposited in Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey (GUE 2380).

Preparation of the EtOH extract and fractions

The chopped dried fruits of *R. canina* (2 kg) were extracted with 80% ethanol (28 l) on a water-bath adjusted to 40°C for 3 days. The EtOH extract was evaporated under reduced pressure to give "EtOH extract" (670.3 g). The EtOH extract (400 g) was then redissolved in 400 ml of MeOH/H_2O (9:1) and extracted with *n*-hexane (15 × 500 ml). The combined hexane extract was evaporated under reduced pressure to yield "Hexane Fr." (7.27 g). MeOH was evaporated then the remaining extract was diluted with distilled H_2O to 200 ml and further fractionated by successive solvent extraction with chloroform (4 × 500 ml), ethylacetate (4 × 500 ml) and *n*-butanol saturated with H_2O (4 × 500 ml). Each solvent extract as well as the remaining aqueous fraction was evaporated to dryness under reduced pressure to yield "$CHCl_3$ Fr." (3.43 g), "EtOAc Fr." (26.74 g), "*n*-BuOH Fr." (63.02 g) and "Remaining H_2O Fr." (242,35 g).

Determination of total phenolic content

Total phenols were determined by Folin Ciocalteu reagent. Briefly, the samples (0.25 ml, 10 mg ml^{-1}) or gallic acid were put into test tubes; 2.5 ml of Folin-Ciocalteau's reagent and 2 ml of sodium carbonate (1 M) were added. The tubes were vortexed and incubated at room temperature for 15 min. Afterward absorption was measured at 765 nm. The total phenol values are expressed in terms of gallic acid equivalent (24).

In vitro antioxidant activity
DPPH radical scavenging assay

The antiradical activity of the extract, its fractions and the reference was assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical (25). The concentration of DPPH was kept as 6×10^{-5} M. The fractions and reference were dissolved in ethanol (75%). 77 µl of each fraction solution were mixed with 3 ml of DPPH solution and left at room temperature in dark for 15 min. After incubation, decrease in absorption for each solution was measured at 515 nm. The corresponding blank reading was also taken and the remaining DPPH was calculated. Butylated hydroxyanisol (BHA), a widely used synthetic antioxidant for long preservation of food products, was used as reference. Inhibition of free radical DPPH in percent (I %) was calculated in following way:

$I\% = [(A_{blank}/A_{sample}) / A_{blank}] \times 100$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the fractions/reference.

Measurement of chelating activity on metal ions

The chelating activity of sample on Fe^{+2} was measured according to the method of Dinish, Madeira, & Almeida (26). According to the method, the fractions were incubated with 0.05 ml of $FeCl_2$ (2 mM). The reaction mixture was initiated by the addition of 0.2 ml of ferrozine (5 mM) and the total volume was adjusted to 4 ml ethanol. After the mixture had reached equilibrim (10 min), then the absorbance was read at 562 nm. BHA (50 µg ml^{-1}) and EDTA (2.5 and 5 mg ml^{-1}) were used as reference compound. The percentage of inhibition of the ferrozine-Fe^{+2} complex formations was calculated using the formula given below:

Metal chelating activity (%) = $[(A_C-A_S)/A_C] \times 100$, where A_C= absorption of control; A_S= absorption of tested

fractions. The control contained only $FeCl_2$ and ferrozine. Analyses were run in three replicates and averaged.

Reducing power

The reducing power of the fractions was determined according to a modified version of reducing power assay of Oyaizu (27). Different concentrations of the fractions (0.5, 1.0, 1.5 and 2.0 mg ml^{-1}) and BHA (50, 250, and 500 µg ml^{-1}) for comparative purpose were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 min. After the incubation period, 2.5 ml of 10% trichloroacetic acid (TCA) were added and the mixture was vortexed. Following centrifugation, 2.5 ml of the supernatant were mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$, and the absorbance was measured at 700 nm. Analyses were run in three replicates and averaged.

In vivo hypoglycemic activity
Preparation of test samples

The dried ethanol extract (250, 500 mg/kg b.w. [b.w. body weight]), fractions ($CHCl_3$ Fr. 10 mg/kg, EtOAc Fr. 40 mg/kg, *n*-BuOH Fr. 150 mg/kg, R-H_2O Fr. 300 mg/kg b.w.) and the reference drug Tolbutamide (100 mg/kg b.w.) were suspended in 0.5% carboxymethyl cellulose (CMC) prepared in distilled water prior to oral administration to experimental animals (10 ml/kg b.w.). Only CMC suspension was administered to the animals in the control group.

Animals

Male Wistar-albino rats (150–200 g) purchased from the Laboratories of Refik Saydam Central Institute of Health (Ankara, TURKEY) were used in the experiments. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions. The rats were fasted 16 h before the experiments, but allowed free access to water. Six animals were used for each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines (G.Ü.ET-0.5.010) for the care of laboratory animals.

Determination of the blood glucose levels

Blood glucose concentrations (mg/dl) were determined using an Ascensia-Elite commercial test (Serial No. 9123232, Bayer), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns.

Study on normoglycemic and glucose-hyperglycemic rats [NG-OGTT]

After overnight fasting (16 h), the initial blood glucose levels of rats were determined and then the test samples were given immediately. The blood glucose levels were determined in the 30th and 60th min to assess the effect of the samples on normoglycemic animals. Then the rats were loaded orally with 2 g/kg glucose and the blood glucose concentrations were determined 30, 60, 180 and 300 min after the glucose loading (28).

Induction of diabetes

Diabetes was induced by the intraperitoneal (i.p.) injection of streptozotocin at a dose of 55 mg/kg b.w. dissolved in citrate buffer (1 M, pH 4.5) (1 ml/kg). Seven days after the injection, the blood glucose levels were measured and the animals with blood glucose levels higher than 250 mg/dl were considered to be diabetic (29).

Subacute effect of test samples

The EtOH extracts, its fractions, CMC and tolbutamide were administered seven days consecutively. Blood glucose levels were determined at 10:00 a.m. on 1st, 3rd, 5th and 7th days after the administration of test samples. The effect of each test sample on body weight was also monitored in the same days (30).

Statistical analysis

Values are presented as means ± SEM. Statistical differences between the treatments and the controls were tested by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test using the "Instat" statistic computer program. A difference in the mean values of $p< 0.05$ was considered to be statistically significant.

RESULTS

Total phenolic content of the fractions

Total phenol contents of the fractions were determined as gallic acid equivalent (GAE) using Folin-Ciocalteau's reagent. A calibration curve with different concentrations of gallic acid was created as $y= 0.0017\times -0.0016$ ($r^2=0.9995$). Accordingly, the $CHCl_3$ Fr. possessed 18.5±0.6 % GAE g/g fraction of total phenol amount as gallic acid equivalent, followed by the EtOAc (15.2±0.2 % GAE g/g fraction), *n*-BuOH (12.9±0.8 % GAE g/g fraction), and R-H_2O (10.0±0.2 % GAE g/g fraction) fractions.

In vitro antioxidant activity
DPPH radical scavenging activity

The DPPH scavenging activities of different fractions of 80% ethanol extract of fruits of *R. canina* are shown in Table 1. The EtOAc Fr. showed highest DPPH radical scavenging activity of 79.5% at 2 mg ml^{-1} concentration, whereas $CHCl_3$, R-H_2O, and *n*-BuOH fractions showed 69.4%, 62.6%, and 56.3% inhibition, respectively, at the same concentration.

Metal chelating activity

None of the fractions showed metal chelating activity at 2000, 1500, 1000, and 500 µg ml^{-1} as compared with EDTA (Table 1).

Reducing Power Activity

It was noted that $CHCl_3$ Fr. exhibited the maximum reducing power among the fractions. The activity of other fractions was as follows: EtOAc Fr. > *n*-BuOH Fr. > R-H_2O Fr. at the highest concentration tested (Fig. 1).

In vivo hypoglycemic activity
Effect of the fractions on normoglycemic and glucose-hyperglycemic rats [NG-OGTT]

Data obtained from NG-OGTT experiments showed that none of the extract and fractions possessed significant inhibitory activity (except R-H_2O) on blood glucose level of normal and glucose loaded animals (Table 2). A minor hypoglycemic effect was observed in R-H_2O Fr. given group (15%) at the 30th min measurement. Tolbutamide exhibited a potent hypoglycemic effect (35–46%) during all measurements (30–360 min.).

Table 1: DPPH scavenging and metal chelating activities of *R. canina* fractions

Fractions	Concentration (µg ml^{-1})	DPPH scavenging ability (%) [a]	Metal chelating activity (%) [a]
$CHCl_3$	2000	69.4±0.5	- [b]
	1500	54.7±1.6	0.8±0.2
	1000	43.3±0.5	- [b]
	500	43.3±0.7	- [b]
EtOAc	2000	79.5±0.4	1.6±0.1
	1500	63.7±1.6	2.1±1.1
	1000	49.3±1.3	10.0±1.3
	500	54.6±6.7	- [b]
n-BuOH	2000	56.3±4.0	- [b]
	1500	54.3±0.3	3.0±1.0
	1000	46.1±1.5	4.8±0.9
	500	52.7±3.7	1.4±0.6
R-H_2O	2000	62.6±3.6	- [b]
	1500	50.2±0.2	- [b]
	1000	37.0±0.4	3.7±0.5
	500	54.3±0.8	- [b]
BHA	50	67.6±1.0	9.9±0.7
	500	78.0±0.5	nt
	1000	81.6±0.7	nt
	2000	82.9±0.7	nt
Gallic acid	500	91.6±0.1	nt
	1000	92.6±0.1	nt
	2000	93.2±0.1	nt
EDTA	5000	nt	94.8±0.9
	2500	nt	76.7±0.4

a: Values are mean ± SEM of three replicates,
b: No activity nt: Not tested

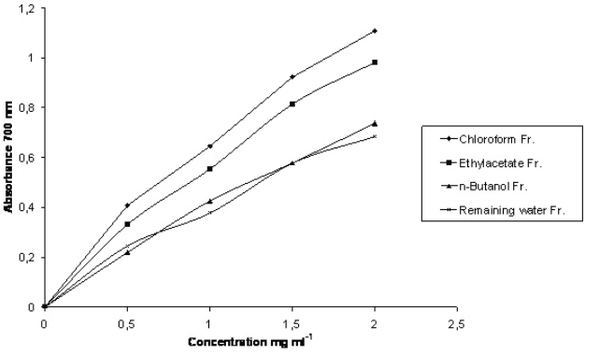

Figure 1: Reducing power of *R. canina* fractions (0.5, 1, 1.5 and 2 mg ml−1). BHA (Abs700 nm; 1.749, 3.569 and 3.677 for 50, 250 and 500 µg ml−1, respectively) was used as reference antioxidant for comparison.

Subacute effect of the fractions in STZ-induced diabetic rats

Antidiabetic activity experiments revealed that the ethanol extract possessed a remarkable hypoglycemic effect at 250 mg/kg dose (Table 3). All of its fractions ($CHCl_3$ Fr., EtOAc Fr., *n*-BuOH Fr. and R-H_2O Fr.) have possessed significant antidiabetic activity except EtOAc Fr. Especially R-H_2O Fr. (50–62%) has shown outstanding antidiabetic effect on the 5th and 7th day measurements. On the other hand, reference drug tolbutamide, used in the experiments did not show any activity.

Subacute effects of R. canina ethanol extract and its fractions on body weight in diabetic rats

In order to monitor the effect of rosehip extract on body weight in diabetic rats during the subacute administration, the body weight of each animal was also recorded on the 1st, 3rd, 5th and 7th days. According to the data demonstrated in Table 4, the body weight of animals in EtOAc Fr. given group increased almost 10%. However, body weight of animals was decreased 10% by administration of *n*-BuOH Fr. No change was observed by the administration of the other extracts and fractions in body weight.

DISCUSSION

R. canina fruits have been used in diabetes mellitus in Turkish folk medicine. It is also commonly consumed

Table 2: Effect of *R. canina* ethanol extract and subfractions on blood glucose levels in normal and 2 g/kg of glucose-loaded hyperglycemic (NG-OGTT) rats.

Test samples	Dose mg/kg	Mean blood glucose concentration ± SEM (mg/dl) (Inhibition %)						
		0. min.	30th min.	60th min. (+ gl. load)	90th min.	120th min.	240th min.	360th min.
Control	-	108.7 ± 2.6	101.5 ±1.6	100.2 ± 2.4	133.3 ± 5.2	132.5 ± 4.9	109.3 ± 1.5	107.6 ± 3.7
Tolbutamide	100	108.0 ±6.6	60.0 ± 3.5*** (41%)	59.0 ±1.4*** (42%)	72.0 ± 4.5*** (46%)	87.0 ± 3.3*** (35%)	61.0 ± 2.1*** (45%)	64.0 ± 4.3*** (41%)
EtOH ext.	250	107.8 ± 3.9	104.2 ± 1.8	114.2 ± 3.6*	121.0 ± 1.4 (10%)	116.7 ± 4.4 (12%)	116.5 ±4.7	105.0 ± 2.9 (3%)
	500	106.3 ± 3.4	104.3 ± 3.0	107.5 ± 5.4	124.2 ± 3.0 (7%)	120.0 ± 4.4 (10%)	106.5 ± 3.7 (3%)	100.5 ± 3.6 (7%)
$CHCl_3$ Fr.	10	108.0 ± 5.8	99.0 ± 3.9 (3%)	103.0 ± 4.6	143.0±5.0	139.5 ± 5.2	114.0±7.0	110.0 ± 3.8
EtOAc Fr.	40	104.9 ± 2.4	90.4 ± 1.6 (11%)	93.4 ± 2.9 (7%)	128.5 ± 6.0 (4%)	128.8 ± 5.4 (3%)	106.3 ± 3.0 (3%)	109.0 ± 3.2
n-BuOH Fr.	150	112.6 ± 2.9	93.2 ± 4.8 (9%)	100.0 ± 2.9	151.4 ± 6.0*	137.0 ± 3.8	113.2 ± 3.1	107.0 ± 2.6
R-H_2O Fr.	300	105.7 ± 2.2	86.8 ± 3.1* (15%)	95.8±0.9 (5%)	126.8±2.9 (5%)	135.7 ± 5.8	104.4 ± 2.8 (5%)	107.0 ± 4.2

Gl: glucose; S.E.M.: standard error mean.

*p< 0.05,

***p< 0.001 significant from control animals

Table 3: Subacute hypoglycemic effect of R. canina ethanol extract and subfractions on STZ-induced-diabetic rats

Test samples	Dose mg/kg	Mean blood glucose concentration ± SEM (mg/dl) (Inhibition %)			
		1st day	3rd day	5th day	7th day
Control	-	377.5±54.2	409.0±22.9	450.0±22.2	434.7±25.5
Tolbutamide	100	364.7±52.7	438.3±22.9	469.0 ±18.3	429.9±21.6 (%2)
EtOH ext.	250	369.6±49.1	351.2±14.7 (15%)	299.4±11.9*** (34%)	390.2±26.7 (11%)
	500	371.3±64.2	380.6 ±63.0 (7%)	425.5±33.8 (6%)	446.0±20.0
$CHCl_3$ Fr.	10	378.0±34.8	381.6±46.6 (7%)	371.8±25.8 (18%)	313.8±20.7** (28%)
EtOAc Fr.	40	354.0±42.5	392.0±26.2 (5%)	456.0±23.7	429.0±16.2 (2%)
n-BuOH Fr.	150	366.0±43.2	460.0±12.2	437.0±21.3 (3%)	280.0±19.5*** (36%)
R-H_2O Fr.	300	364.5±75.0	329.3±13.3* (20%)	229.3±16.1*** (50%)	165.5±22.7*** (62%)

S.E.M.: standard error mean.

***p< 0.001;

**p < 0.01;

*p < 0.05 significant from the control animals.

Table 4 : Subacute effects of *R. canina* ethanol extract and subfractions on body weights in STZ-induced diabetic rats

Test samples	Dose (mg/kg)	Mean body weight±S.E.M. (g)			
		1st Day	3rd Day	5th Day	7th Day
Control	-	190.2±6.8	191.5±8.5	193.2±12.1	186.0±12.1
Tolbutamide	100	174.1±8.1	177.1±8.6	177.4±9.2	183.9±10.6
EtOH ext.	250	212.5±12.8	205.0±10.8	203.0±11.9	198.0±10.8
	500	201.8±16.4	204.5±18.7	204.0±18.3	193.0±18.0
$CHCl_3$ Fr.	10	170.0±3.8	169.3±4.5	170.5±5.7	170.0±5.7
EtOAc Fr.	40	153.4±14.3	159.0±11.1	156.0±10.9	170.0±13.7
n-BuOH Fr.	150	156.4±8.1	141.8±7.4	141.8±6.2	128.4±9.7
R-H_2O Fr.	300	175.5±10.7	172.3±16.1	174.8±19.7	177.5±25.8

S.E.M.: standard error mean

in herbal tea. Previously, Can et al. (31) investigated the effect of the aqueous and ethanol extracts of *R. canina* fruits on blood glucose level of normoglycemic rabbits. But the researchers did not observed any hypoglycemic activity in normal rabbits. In another study, a single oral administration of *trans*-tiliroside, isolated from seeds of *R. canina*, has shown hypoglycemic activity at a dose of 10 mg/kg in normoglycemic mice (32). As seen in the both studies just only normoglycemic animals have been used. However, no research has been conducted on diabetic animals with rose hips so far. Our investigation is the first study to evaluate

the *in vivo* antidiabetic effects of rose hip. The present study demonstrated that R-H_2O Fr. of *R. canina* fruits reduced blood glucose level significantly ($p<0.001$) in STZ-induced diabetic rats (50-62%). On the other hand, minor hypoglycemic effect was observed in R-H_2O Fr. given group (15%) at the 30th min measurement in NG-OGTT model. Other extracts and fractions did not exhibit any inhibitory activity. From the results of the present study, it is an evident that R-H_2O Fr. of *R. canina* fruits possesses the most active antidiabetic principle of the plant. Although the highest phenolic contents were observed in the $CHCl_3$ and EtOAc fractions, there was not a positive correlation between the antidiabetic activity and total phenolic contents of these fractions. It is known that *R. canina* fruits contain biologically active compounds (vitamin B and C, carotene, organic acids, flavonoids, tannins, carbohydrates and pectin), which are responsible for its healing properties (33, 34). In this study, the most polar fraction namely R-H_2O Fr., contains monosaccharides, oligosaccharides, and pectins (35), we presume that they might be active principles contributing to the antidiabetic actions. This is further supported by various reports on the hypoglycemic potential of polysaccharides (36–40).

In conclusion, the results of antidiabetic activity studies support the traditional uses of *R. canina* fruits as a folk remedy in the treatment of diabetes in Turkey. Further studies are necessary to isolate and identify the active hypoglycemic compound/s in *R. canina* fruits as well as elucidating their mechanisms of action.

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