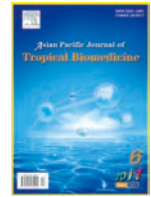




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## Hypoglycaemic effect of *Berberis vulgaris* L. in normal and streptozotocin–induced diabetic rats

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## ABSTRACT

**Objective:** To achieve a primary pharmacological screening contained in the aqueous extract of *Berberis vulgaris* (*B. vulgaris*) and to examine the hypoglycaemic effect and biochemical parameters of aqueous and saponins extract on groups of rats rendered diabetic by injection of streptozotocin. **Methods:** The phytochemical tests to detect the presence of different compounds were based on the visual observation of color change or formation of precipitate after the addition of specific reagents. Diabetes was induced in rats by intraperitoneal (*i.p.*) injection of streptozotocin (STZ) at a dose of 65 mg/kg bw. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips (Dextrostix, Bayer Diagnostics). Blood samples were taken by cutting the tip of the tail. Serum cholesterol and serum triglycerides were estimated by enzymatic DHBS colorimetric method. **Results:** Administration of 62.5 and 25.0 mg/kg of saponins and aqueous extract respectively in normal rats group shows a significant hypoglycemic activity (32.33% and 40.17% respectively) during the first week. However, diabetic group treated with saponin extract produced a maximum fall of 73.1% and 76.03% at day 1 and day 21 compared to the diabetics control. Also, blood glucose levels of the diabetic rats treated with aqueous extract showed decrease of 78.79% on the first day and the effect remains roughly constant during 3 week. Both extracts also declined significantly biochemical parameters (20.77%–49.00%). The control in the loss of body weight was observed in treated diabetic rats as compared to diabetic controls. **Conclusions:** These results demonstrated significant antidiabetic effects and showed that serum cholesterol and serum triglycerides levels were decreased, significantly, consequently this plant might be of value in diabetes treatment.

### 1. Introduction

*Berberis vulgaris* (*B. vulgaris*) Linn which is commonly known as Barberry belongs to the family Berberidaceae. *Berberis* is the genus of spiny deciduous evergreen shrubs, with yellow wood and yellow flowers, and comprises 190 species. In traditional medicine the extracts of various *Berberidaceae* (*Berberis aquifolium*, *Berberis vulgaris* and *Berberis aristata*) are used for rheumatic and other types of chronic inflammations[1]. Some authors demonstrated that these extracts have a significant activity against bacteria, viruses, fungi, protozoa, helminthes and chlamydia[2]. Studies carried out on the properties and chemical composition of the extracts show that their principal activity is due to their alkaloid constituents with an isoquinolinic

nucleus such as berberine, oxyacanthine, berbamine and palmatine[2]. It has been shown that berberine has febrifugal, hypotensive, immuno–stimulating, anti-inflammatory, antiarrhythmic antimicrobial properties, and it prolonged the action potential duration in Purkinje fibres. There are on–going studies regarding a possible anti-tumour activity of berberine[1–5].

Diabetes mellitus is a major illness of the human race implicated with numerous clinical manifestations. It is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. According to World Health Organization projections, the diabetes population is likely to increase to 300 million or more by the year of 2025[6,7]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides,  $\alpha$ –glucosidase inhibitors and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation[8]. Many of these oral antidiabetic agents have a number of

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serious adverse effects<sup>[9]</sup>. Thus, the management of diabetes without any side effects is still a challenge. Approximately 80% of the world population is almost entirely dependent on traditional medicines<sup>[10]</sup>. The ethnobotanical details and the antidiabetic potential of several plants are known at present<sup>[11–14]</sup>, but there is very little information about plants which possess hypoglycemic properties. The hypoglycaemic activity could be due to the existence of saponins, polyphenols and alkaloids<sup>[4,15]</sup>. A survey conducted by herbalists has shown that, in folk medicine, aqueous extracts made from the root bark of *B. vulgaris* are recommended in the management of diabetes. Considering the wide use of this herb in folk therapeutics for the treatment of diabetes, the present study investigates the action of saponins and aqueous extract of root bark of *B. vulgaris* in rats to ascertain the scientific basis for the use of this plant in the treatment of diabetes.

## 2. Materials and methods

### 2.1. Plant material

Root barks of the plant material *B. vulgaris* were purchased from a herborist from city Tlemcen, Algeria during May 2008. The plant was authenticated by Prof. N Benabadi, of the Botanical Laboratory, Biology, Tlemcen, Algeria. The voucher specimen (B.B 125) has been deposited in the Department of biology, Aboubekr Belkaïd University, Tlemcen, Algeria.

### 2.2. Phytochemical prospecting

The phytochemical tests to detect the presence of saponins, tannins, flavonoids, sterols, anthraquinones, emodols and alkaloids were used by simple qualitative methods<sup>[16, 17]</sup>. The tests were based on the visual observation of color change or formation of precipitate after the addition of specific reagents. The results for the extracts studied revealed the presence of tannins, alkaloids, saponins, sterols and anthraquinones.

### 2.3. Preparation of aqueous extract

The aqueous extract was prepared by cold maceration of 150 g of powdered root barks in 500 mL of distilled water for 48 h. Then the extract was filtered, concentrated, dried in vacuo (yield 10 g) and the residue was stored in a refrigerator at 2–8 °C for use in subsequent experiments.

### 2.4. Extraction of crude saponins

The powdered sample was defatted by petroleum ether for 3 h at 40 °C. After filtering the petroleum ether, the sample was extracted with methanol for 3 h with mild heating. The combined methanol extract was concentrated and methanol extract of the sample was obtained. In order to get the crude saponins extract the sample was dissolved in methanol and acetone was added (1: 5 v/v) to precipitate the saponins. The precipitate was dried under vacuum, turning to a whitish

amorphous powder named as crude saponin extract (CSE)<sup>[15]</sup>.

### 2.5. Animals

Experiments were performed on adult male Wistar rats at weights ranging from 190 to 230 g. Animals were housed in animal room at 23–25°C and maintained with free access to water and libitum feeding of which per 100 g comprises 13 g water, 20 g proteins, 49 g glucides, 8 g lipids, 7 g fibres, 310 kcal energy.

### 2.6. Experimental induction of diabetes

Diabetes was induced in rats by intraperitoneal (*i.p.*) injection of streptozotocin (STZ) at a dose of 65 mg/kg bw, dissolved in 0.1M cold citrate buffer (pH = 4.5)<sup>[18]</sup>. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips (Dextrostix, Bayer Diagnostics). Blood samples were collected by cutting the tip of the tail.

### 2.7. Experimental protocol

All the animals were randomly divided into six groups with six animals in each group. Preliminary *i.p* injection LD<sub>50</sub> doses of saponins and aqueous extract of *B. vulgaris* in mice were found to be 175 and 625 mg/kg, respectively. Group A (NC): normal rats receiving distilled water served as a control group. Group B (DC): diabetic rats receiving distilled water served as control diabetic rats. Group C (DTAE): diabetic rats treated with aqueous extract in one–tenth of LD<sub>50</sub> doses (62.5 mg/kg). Group D (DTSE): diabetic rats treated with saponins extract in one–seventh of LD<sub>50</sub> doses (25 mg/kg). Group E (NAE): normal rats receiving the aqueous extract in one–tenth of LD<sub>50</sub> doses (62.5 mg/kg). Group F (NSE): normal rats receiving the saponins extract in one–seventh of LD<sub>50</sub> doses (25 mg/kg). 10 days after STZ–injection, rats with plasma glucose levels of > 300 mg/dL were included in the study. Treatment with saponins and aqueous extract were started ten days after STZ–injection. Blood samples were drawn at weekly intervals till the end of study (*i.e.* 3 weeks). Fasting blood glucose estimation and body weight measurement were done on day 1, 7, 14 and 21 of the study<sup>[19]</sup>. On day 21, Serum cholesterol and serum triglycerides were estimated by enzymatic DHBS colorimetric method<sup>[20,21]</sup>.

### 2.8. Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of the mean (Mean ± SEM) and analyzed for ANOVA and post hoc Dunnet's *t*–test. Differences between groups were considered significant at *P* < 0.01 levels.

## 3. Results

### 3.1. Changes in body weight

As shown in Table 1, normal control animals were found

**Table 1**

Effect of 3-week treatment with various extracts of *B. vulgaris* on body weight and blood glucose level of STZ (65 mg/kg *i.p.*)-induced diabetes rats (Mean±SEM).

Gp (n=6)	Treatment	Dose (mg/kg)	Average body weight (g)				Blood glucose concentration (mg/dL)			
			Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
A	NC	1 mL <sup>a</sup>	210.40 ± 2.80	212.83 ± 1.60	213.10 ± 1.60	214.83 ± 1.80	78.10 ± 14.90	101.30 ± 4.10	89.50 ± 6.60	90.60 ± 5.50
B	DC	1 mL <sup>b</sup>	215.50 ± 4.80	185.00 ± 2.50*	170.43 ± 3.20*	158.73 ± 1.70*	106.30 ± 8.80	193.70 ± 10.50	303.40 ± 12.20	328.20 ± 14.50
C	DTAE	62.5	217.12 ± 2.50	202.12 ± 2.80	198.21 ± 1.30	195.32 ± 2.20	102.20 ± 7.10***	98.50 ± 3.50***	102.50 ± 6.30***	105.30 ± 7.80***
D	DTSE	25	219.55 ± 2.40	212.50 ± 1.90	210.20 ± 4.20	201.33 ± 4.40	88.20 ± 4.70***	71.10 ± 2.50***	71.20 ± 2.10***	78.60 ± 8.80***
E	NAE	62.5	217.76 ± 2.30	220.00 ± 1.40	221.17 ± 2.20	222.10 ± 4.90	80.10 ± 3.20	54.20 ± 9.10**	64.50 ± 5.30**	89.30 ± 7.60
F	NSE	25	216.23 ± 1.60	219.26 ± 1.30	222.15 ± 3.60	225.26 ± 2.70	76.60 ± 3.60	61.30 ± 2.50**	77.20 ± 1.40	94.40 ± 4.30

a: distilled water; b: STZ single dose of 65 mg/kg *i.p.* on day 0;

\*  $P < 0.01$  when compared to normal control on corresponding day; \*\*  $P < 0.001$  when compared with normal control; \*\*\*  $P < 0.001$  when compared with diabetic control.

to be stable in their body weight but diabetic rats treated by saponins and aqueous extract showed a little reduction in body weight by 8.30% and 10.04% respectively. While normal rats treated by saponins and aqueous extract were showed a negligible increased in the body weight by 4.17% and 1.20%. The body weight of diabetic control group decreased by 26.34% during 21 days.

### 3.2. Hypoglycaemic effect of aqueous and saponins extract

The hypoglycaemic effect of the saponins and aqueous extract on the fasting blood sugar levels of diabetic rats is shown in Table 1. The results clearly indicated that the saponins and aqueous extract shows a significant hypoglycaemic activity in normoglycemic rats by 32.33% and 40.17% respectively in the first week of treatment, however, at the end of 21 day it increases to achieve normal blood sugar levels, that is (94.4 ± 4.3) mg/dL and (89.3 ± 1.6) mg/dL respectively. Administration of STZ (65 mg/kg, *i.p.*) led to 3.5-fold elevation of fasting blood glucose levels. However one injection of aqueous extract (62.5 mg/kg) showed a 68.79% decrease in blood glucose level on day 1 of the treatment in comparison with diabetic control. The effect remains roughly constant during 3 weeks. Of the same way the saponins extract at the dose of 25 mg/kg produced a maximum fall of 73.10% and 76.03% at day 1 and day 21 respectively.

**Table 2**

Effect of various extracts of *B. vulgaris* on serum profile in STZ (65 mg/kg, *i.p.*)-induced diabetic rats after 21 days of treatment (Mean±SEM) (mg/kg).

Group (n=6)	Treatment	Dose	Serum cholesterol	Serum triglycerides
A	NC	1 mL <sup>a</sup>	96.54 ± 3.90	131.21 ± 8.60
B	DC	1 mL <sup>b</sup>	153.60 ± 6.90	154.10 ± 7.50
C	DTAE	62.5	78.33 ± 2.60*	99.22 ± 5.10*
D	DTSE	25	83.54 ± 2.90*	68.69 ± 3.80*
E	NAE	62.5	72.67 ± 1.50*	99.36 ± 4.80*
F	NSE	25	76.48 ± 1.80*	94.17 ± 4.10*

a: distilled water; b: STZ single dose of 65 mg/kg *i.p.* on day 0;

\*  $P < 0.01$  diabetic control was compared with the normal control and extract treated groups were compared with the diabetic control.

### 3.3. Effect on serum lipid profile

Table 2 shows the effect of saponins and aqueous extract of root bark of control and experimental groups. The

hyperlipidemic parameters like serum triglycerides and serum cholesterol were elevated in diabetic groups in comparison to the control. However, both these parameters were decreased significantly in the diabetic groups treated with saponins and aqueous extracts (about 55.42% and 35.61% for triglycerides respectively) and (about 46.61% and 49.00% for cholesterol respectively) compared to diabetic rats. On the other hand normal rats group treated by saponins and aqueous extracts for 3 weeks caused a little significant reduction on both parameters (about 20.77% and 24.72% for cholesterol respectively) and (about 28.22% and 24.27% for triglycerides respectively) compared to normal rats (Table 2).

## 4. Discussion

Many years ago, diabetes was treated orally with several medicinal plants or their extracts based on traditional folk medicine[22]. Actually, more than 150 plant extracts and their active principles are used to treat diabetes mellitus[23,24]. However, insulin substitutes from medicinal plant sources should be scientifically studied and tested. In the present study we examined the plasma glucose lowering effect of root bark of *B. vulgaris*. Single *i.p.* administration of saponins and aqueous extracts during the first day to STZ-diabetic rats resulted in a significant reduction in blood glucose level and lipid profile. The saponins extract of root bark displayed a greater activity than aqueous extract. These observations may be attributed to the nature of biological active components. It has been documented that saponins are plants metabolites that is well known for antidiabetic activity[25,26]. The aqueous extract also produced significant hypoglycaemic effects. The activity of this fraction could be due to the presence of tannins, saponins, flavonoids, alkaloids and steroids components. Different mechanisms to reduce blood glucose levels with the help of plant extracts already exist. From the results it is assumed that the root bark extracts could be responsible for stimulation of insulin release and the observed restoration of metabolic activities. Also these results suggest that the extracts possess active phytochemicals principles with either cytoprotective functions on the pancreatic beta cells or insulin-protective properties. A number of other plants have also been shown to exert hypoglycaemic activity through stimulation of insulin release[22,27]. Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide.

They reduce blood glucose in normoglycemic animals[28,29].

In conclusion, the saponins and aqueous extracts of root bark of *B. vulgaris* exhibited significant anti-hyperglycaemic activities in STZ-induced diabetic rats. The results suggest that the hypoglycaemic effect was due to the presence of saponins which may have stimulating effect on the remnant beta cells. However, further experiments are required to elucidate the exact mechanism of action. These extracts also showed improvement of parameters like lipid profile and so might be of value in diabetes treatment.

### Conflict of interest statement

We declare that we have no conflict of interest.

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