

Acute hypotensive and diuretic activities of *Berberis vulgaris* root bark aqueous extract in normal rats

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The aim of this study is to investigate the effectiveness of intravenous administration of *Berberis vulgaris* root bark aqueous extract (BRBD) on the cardiovascular and renal functions of healthy normotensive rats. The different doses of BRBD 1, 10 and 20 mg/kg were administered intravenously (i.v) in normal rats. Blood pressure, diuretic activity and serum renal profile were analyzed. Intravenous injection of BRBD at the different doses of 1, 10 and 20 mg/kg showed a dose-dependent reduction in mean arterial blood pressure ($P < 0.001$). At different doses of 1, 10 and 20 mg/kg, the hypotensive effect remained for more than one hour. Single dose administration of BRBD at doses of 10 and 20 mg/kg caused a significant increase in urine output ($P < 0.001$) as compared to the control rats. Serum renal profile test (albumin, Urea, Uric Acid, creatinine and BUN) did not show any significant alteration. The authors conclude that the BRBD is a potent hypotensive and possesses diuretic potential

Keywords: *Berberis vulgaris* root bark/ aqueous extract (BRBD). Mean arterial blood pressure (MABP). Diuresis. Serum renal profile test

INTRODUCTION

Since ancient times, numerous species of plant have been used in folkloric medicine to manage different ailments due to their medicinal properties. The World Health Organization claims that more than 80% of the world's population confide in plants and plant-based herbal medicines for healthcare (Gurib-Fakim, 2006). In the list of non-communicable diseases, the management of cardiovascular diseases, especially hypertension, without any side effects is still a hard task for health professionals. For that reason, research on medicinal plants (for the management of cardiovascular disorders) has become a necessity nowadays. Different plants and their extracts reported for their hypotensive activity include *Artemisia herba alba* (Zeggwagh, Michel, Eddouks, 2014), *Tropaeolum majus L* (Gasparotto *et al.*, 2011), *Berberis orthobotrys Bien Ex Aitch* (Alamgeer *et al.*, 2013), *Hibiscus sabdariffa L*. (Allison *et al.*, 2013),

Berberis vulgaris (Azmat *et al.*, 2009) and *Moringa stenopetala* (Baker f.) Cufod. Leaves (Geleta *et al.*, 2016). For the treatment of cardiovascular disorders especially hypertension, different antihypertensive therapies are used with diuretics (Zeggwagh, Michel, Eddouks, 2014). For this management, the economic impact of hypertension is enormous because cost-effective antihypertensive medicine with diuretic potentials is currently unavailable. Earlier studies have reported the diuretic effect of several plant extracts on normal and hypertensive rats (Amonkan *et al.*, 2013; Hernández-Luis *et al.*, 2014). Furthermore, the observed diuretic activity was associated in several cases with a reduction in arterial blood pressure (Kazama *et al.*, 2012). Taking in consideration these prospects, the present study was planned to observe the Acute Hypotensive and Diuretic Activities of *Berberis vulgaris* Root Bark Aqueous Extract in Normal Rats.

MATERIAL AND METHODS

Plant

The roots bark of *B. vulgaris L.* is dark brown and soft. The root bark in powdered form is a dark brown color. The

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roots of *B. vulgaris* were collected from the Gilgit-Baltistan region (Khan, Khan, Rehman, 2014) in Pakistan in June 2003 and transported safely to Karachi, Pakistan. The root bark was then separated from the root pulp in the research lab with the help of a sharp garden knife, and the bark was powdered in a mill.

Extraction (Preparation of Decoction)

BRBD was prepared by boiling the root bark powder in distilled water for ten minutes, then cooling it for fifteen minutes and filtering. For the preparation of the decoction, 100mg root bark powder was boiled with 10ml of distilled water for ten minutes and later it was considered that

100 mg	10 mL
10 mg	1 mL
1 mg	0.1 mL

A freshly prepared decoction was used to test for hypotensive and diuretic activity.

Animals

Rats (Sprague Dawley: 220 to 240 g) were selected from Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences (Dr. HMIIPHS) 6 days before experimentation and kept in optimal conditions (food and tap water *ad libitum*: 12 h light/dark cycle). The experimental procedures were performed according to the Guidelines for Care and Use of Laboratory Animals (National Research Council, 2011). All experimental procedures were approved by a review board of the departmental research committee.

Chemicals and drugs

In the present study, the following drugs were used.

Acetylcholine (10 ⁻⁶)	E. Merck (Germany)	positive control
Sodium chloride (0.9%)	E. Merck (Germany)	negative control
Heparin	Leo Pharmaceutical (Denmark)	anticoagulant
Pentothal sodium	Abbott Karachi (Pakistan)	anesthetic agent

Hypotensive activity

Just prior to the experiment for the measurement

of mean arterial blood pressure (MABP), Pentothal sodium (40 mg/kg i.p.: Azmat, Ahmed, 2014) was used as the anesthetic. First, the trachea and external jugular vein both were both cannulated for smooth respiration (Feng *et al.*, 2015) and intravenous drug administration. The arterial blood pressure (ABP) was monitored from the carotid artery via a heparinized tube connected to a transducer (Harvard, 60 to 3003). During experimentation, body temperature was controlled at 37 °C by using the overhanging lamp.

Mean arterial blood pressure (MABP) was determined as DBP+1/3 Pulse pressure or pulse width (Adeboye *et al.*, 1999). Any variation in blood pressure (BP) was noted as a percent of control values (Azmat *et al.*, 2009). Acetylcholine (ACh: positive control) was used to check the normal cardiac functions. A single dose of ACh (10⁻⁶) caused 47.61 ± 3.31% (mean ± SEM, n = 6) drop in MABP. The hypotensive studies were performed on different doses of BRBD.

Diuretic activity

The three groups of rats (each with six rats) were used in this study to measure diuretic activity. The rats were catheterized as described earlier.

The first group of six rats served as control (saline solution, 0.9%), whereas the second and third groups received i.v. BRBD, 10 and 20 mg/kg respectively. The experimental phase for each rat was 4 h. During this 4hour period, excreted urine samples were collected and measured.

Biochemical estimation (Renal Profile)

At the end of 4hours the samples of blood (5 to 8 mL) were collected from the jugular vein, and were used for the detection of different parameters:

Serum Total Protein	Ecoline® S+ by biuret method
Albumin	Ecoline® S+ 100 by bromocresol green method
Urea	Ecoline® 100 by UV test, GIDH method
Uric Acid	Ecoline® 100
Blood Urea Nitrogen (BUN)	Ecoline® 100 by UV test, GIDH method
Creatinine	Ecoline® S+ by Jaffè method

Statistical analysis

Variation in blood pressure and serum level of renal profile after i.v administration of BRBD was analyzed by using student's t-test. Values of $P < 0.05$ were considered to be significant.

RESULTS

Effect of various doses of BRBD on various blood pressure parameters

The results regarding the intravenous administration of BRBD on various blood pressure parameters are shown in Figure 1. Three different doses of BRBD initiated an immediate significant reduction in MABP (Figure 1). The maximal reduction was observed at the dose of 20mg/kg ($P < 0.001$). This observed reduction in MABP remained for more than one hour and returned to baseline after 106 ± 12.36 min and 193 ± 13.94 min at 10 mg/kg and 20 mg/kg BRBD respectively. However, in the negative control group, the saline administration did not affect arterial blood pressure. On the other hand, ACh (10^{-6}) caused a 46% fall in MABP, but it returned to baseline after 35 ± 5 s.

Diuretic activity

Effect on urine volume

Results of diuretic activity are shown in Fig. 2. Intravenous administration of BRBD (10 mg/kg) showed marked diuresis during the 4 h of the test (BRBD

2.961 ± 0.436 mL versus control 1.52 ± 0.681 mL; $P < 0.05$). At the dose of 20 mg/kg, BRBD also showed marked diuresis during the 4 h of the test (BRBD 5.361 ± 0.23 mL versus control 1.52 ± 0.681 mL; $P < 0.05$).

Effect of BRBD on serum renal profile

Non-significant changes in the serum total protein, albumin, urea, uric acid, creatinine and BUN were monitored in the BRBD (10 mg/kg), intravenous treated rats in comparison to their respective control rats.

DISCUSSION

Berberis is widely used in folkloric medicine to treat various ailments (Javadzadeh, Ebrahimi, 2013). This study assessed the hypotensive and diuretic effects of intravenous injection of aqueous *Berberis vulgaris* root bark extract on normal rats at the doses of 1, 10 and 20 mg/kg, and arterial blood pressure was recorded through a catheter implanted in the carotid artery. Usually, this method is used for blood pressure measurement to screen the effect of drugs on the cardiovascular system (Kurtz *et al.*, 2005). The results demonstrated that i.v administration of BRBD showed an immediate and significant decrease in mean arterial blood pressure (MABP). Our findings are in agreement with the previous finding that ethanolic and methanolic extract of *B. vulgaris* root pulp possesses hypotensive activity (Azmat *et al.*, 2009). Another study also revealed that extract of barberry root reduced the blood pressure in cats for several hours (Javadzadeh, Ebrahimi, 2013). In our present study, the hypotensive effect persisted

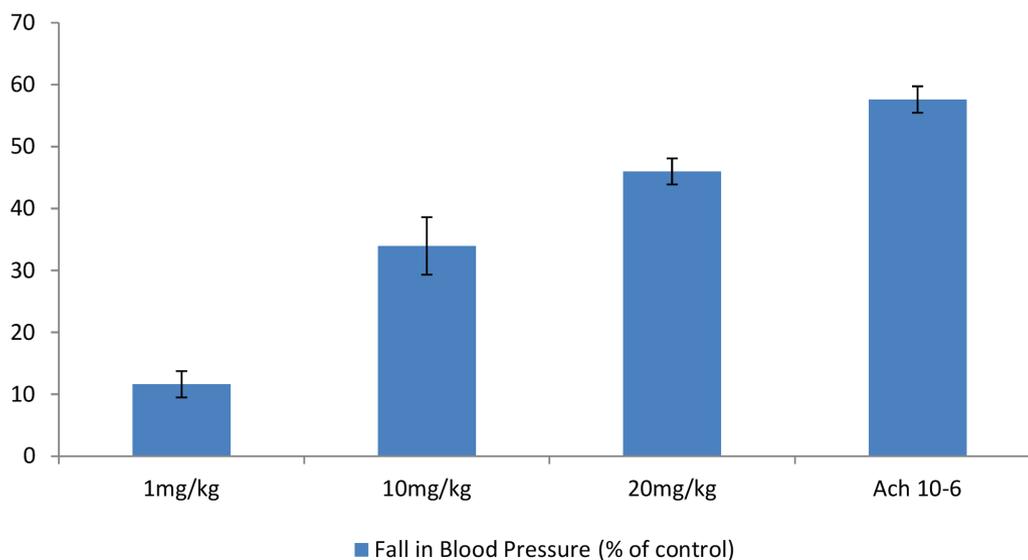


FIGURE 1 - Hypotensive activity of BRBD in normotensive rats.

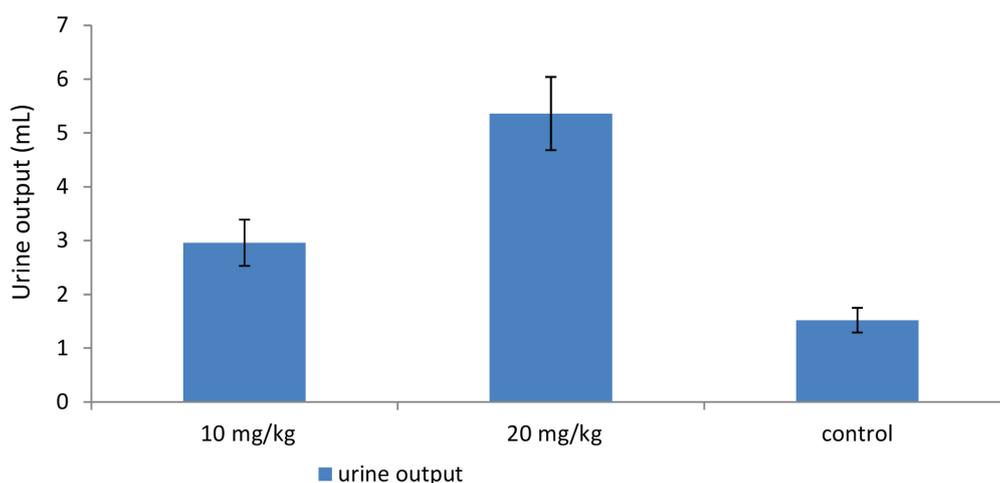


FIGURE 2 - Diuretic activity of BRBD in rats.

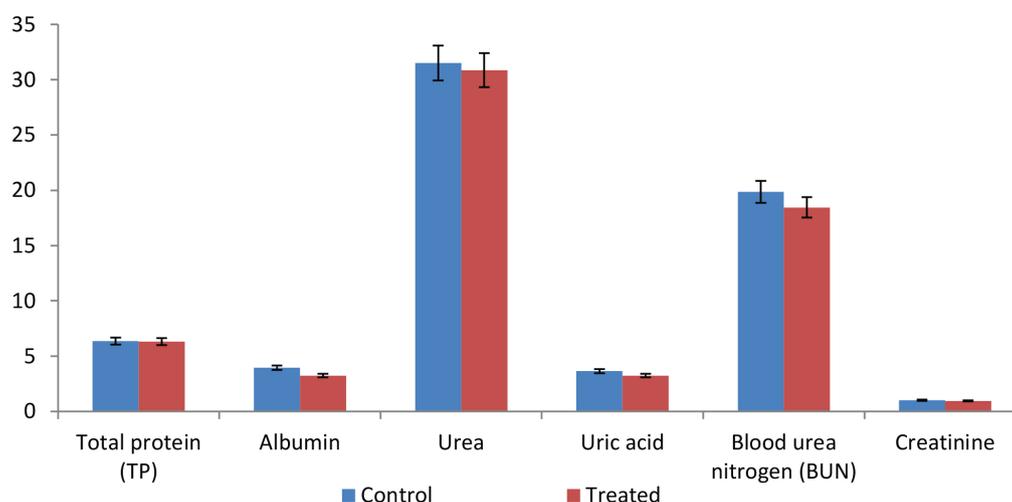


FIGURE 3 - Effect of BRBD (20mg/kg) on Serum Renal Profile.

for an extended period. Different studies proposed a different mechanism of action, suggesting that Berberine increased contractile force, decreased peripheral vascular resistance and lowered blood pressure (Lau *et al.*, 2001; Shen, 1997; Harvey, 2012). For diuretic activity, BRBD was administered intravenously (10 and 20 mg/kg) and Urine volume was measured to assess the renal effect of BRBD. The results demonstrated that BRBD extract increased diuresis significantly. Earlier research has shown that the presence of organic acids in barberry is responsible for the diuretic activity of medicinal plants (Javadzadeh, Ebrahimi, 2013). Serum renal profile showed non-significant changes in serum albumin, total protein, uric acid, urea, blood urea nitrogen (BUN) and creatinine level indicating nephroprotective effects. It is concluded that the BRBD possesses a dose-dependent hypotensive and diuretic effect upon i.v administration in rats.

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