

Full Length Research Paper

Hypotensive activity, toxicology and histopathology of different extracts of *Berberis vulgaris*

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A number of plants have been used widely in the traditional system of medicine or Tibb-e-Unani (Unani medicine) in the management of many diseases but these mostly, have not been investigated for their described effects. In this study, the hypotensive, toxicology and histological activities were studied in normotensive albino rats at different doses of ethanolic extract of root pulp (BRE) and aqueous extract of root pulp (BRD). The receptor activity was assessed by using the drugs acetylcholine (Ach) and atropine (Atr) on rat heart. Administration of different extracts (BRE and BRD) showed significant reduction in blood pressure comparable to its respective control. While on pre-treatment with Atr (10^{-4} M) the BRE (10 mg/kg) and BRD (20 mg/kg) did not produce any reduction in blood pressure. These behavior matches exactly to that of acetylcholine (1 μ g/kg). The results confirmed that oral and intraperitoneal administration of BRE does not indicate any structural and functional disturbance of liver, heart and kidney up to the dose 100 mg/kg. While, 1000 mg/kg appeared as lethal dose (LD) and all mice died at the interval of 24 h. In conclusion, different extract of BRE used in this study caused hypotensive effect by stimulating non-selective muscarinic receptors. The toxicological, hematological and histopathological results further confirm the safety of BRE up to the dose of 100 mg/kg.

Key words: *Berberis vulgaris*, hypotensive, ethanolic extract of root pulp (BRE), aqueous extract of root pulp (BRD).

INTRODUCTION

The commonest cardiovascular infection affecting the adult population is hypertension (high blood pressure). Hypertension and other "vascular diseases" such as stroke and kidney failure cause 43 percent of all deaths recorded each year (Saleem et al., 2003). World Health Organization (WHO) has identified hypertension as the leading cause of cardiovascular mortality and suggests

that more than 50% of the hypertensive populations worldwide are unaware of their condition (Chockalingam, 2007). Control of blood pressure in patient with hypertension is necessary for cardiovascular morbidity and mortality (Walker et al., 2002). Drugs used in the treatment of cardiovascular diseases are very expensive and beyond the reach of a common man. The American

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Heart Association estimated the direct and indirect costs of high blood pressure in 2010 as \$76.6 billion (Lloyd-Jones et al., 2010), while on the other hand these drugs produce mild to severe side effects; so side effects associated with these drugs restricted the people to use them.

Barberries have reported as hepatoprotective, hypoglycemic and as a herbal remedy for the treatment of a variety of complaints including diabetes, liver dysfunction (Jaundice), gallbladder pain, gall stone diarrhea, indigestion and urinary tract diseases (Hermenean et al., 2012; Meliani et al., 2011; Jellin et al., 2000; Chevallier, 1996; Gruenwald, 1998). Earlier the aqueous and methanolic extract from *Berberis vulgaris* fruit and root was tested to evaluate its antihypertensive effects on DOCA-induced hypertension in the rats (Fatehi et al., 2005b; Azmat et al., 2009). Present studies led to the determination of hypotensive activity and toxicity of different ethanolic extract of *B. vulgaris* root and root bark.

MATERIALS AND METHODS

Plant

The roots of *B. vulgaris* were yellowish brown, cylindrical, more or less knotty, hard and tough. With the bark intact they are cut into pieces of varying length and a maximum diameter of 45 mm. The bark is internally dark brown and soft. The root in powdered form is bright yellow with a slight odour and a bitter taste.

Extraction

Root (500 gm) of *B. vulgaris* was extracted with ethanol for four times at room temperature. The extracts were combined together and evaporated on rotavapour. At the end yellowish brown colored residue (BRE) was collected and used during the whole study. Another extract (decoction) BRD was prepared by boiling the *Berberis* root in distilled water.

Animals

Adult NMRI mice (20 to 25 g) and Sprague dawley rats (200 to 225 g) of either sex were obtained from Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences (Dr. HMIIPHS) and were housed in groups of 6 per cage for seven days prior to experimentation with free access to food and tap water *ad libitum* and kept on a 12 h light/dark cycle. Each experimental group consisted of six animals. All animals were housed in an air-conditioned room at $23 \pm 1^\circ\text{C}$ during the quarantine period. The experimental procedures were performed according to Guidelines for Care and Use of Laboratory Animals (National research council, 2011). All experimental procedure was approved by review board of departmental research committee.

Chemicals and drugs

Different chemicals and drugs used in the present study were,

acetylcholine and sodium chloride from E. Merck (Germany), atropine sulfate from Boehringer Ingelheim (Germany). Acetylcholine (10^{-6} M) used as positive control while saline (0.9% NaCl) as negative controls, while atropine (10^{-4} M) used for receptor activity. Heparin (Leo Pharmaceutical Denmark) and Pentothal sodium from Abbott Karachi (Pakistan) were used as anaesthetic agent and anticoagulant, respectively.

Instruments for the extraction and recording of blood pressure parameters

Rotavapour (R114 Buchi) was used for the extraction of BRE. The arterial blood pressure was recorded by using research grade blood pressure transducer (Harvard, 60-3003) coupled with four channels Harvard Universal Oscillograph (Curvilinear, 50-9307) (United kingdom).

Hypotensive activity

Normotensive Sprague-Dawley rats of either sex (200 to 250 g) were anaesthetized with Pentothal sodium (40 mg/kg i.p.) as described by Ulicna et al. (2003). Then the trachea was exposed and cannulated with polyethylene cannula to facilitate spontaneous respiration (Ogochukwu et al., 2009). Drugs were injected (vol. 0.2 to 0.25 ml) through a polyethylene cannula inserted into the external jugular vein followed by a saline flush (0.2 ml). The arterial blood pressure was recorded from the carotid artery via arterial cannula connected to a research grade blood pressure transducer (Harvard, 60 to 3003) coupled with four channels Harvard Universal Oscillograph (Curvilinear, 50-9307) (UK). The temperature of the animals was maintained at 37°C by using the overhead lamp. Animals were allowed to equilibrate for at least 15 min before administration of any drug.

Measurements

Mean arterial blood pressure was calculated as sum of the diastolic blood pressure plus one-third-pulse width (Adeboye et al., 1999). Changes in blood pressure were expressed as the percent of control values, obtained immediately before the administration of test substance (Saleem et al., 2003). Acetylcholine used as positive control caused $57.61 \pm 2.31\%$ (mean \pm SEM, $n = 15$) fall in mean arterial blood pressure as the dose of 10^{-6} M/kg. The hypotensive studies were carried out on different doses of BRE and BRPD.

Methods for the determination of receptor activity

Normotensive Sprague-Dawley rats of either sex (200 to 250 g) were anaesthetized and their blood pressure was recorded through carotid artery as described earlier. Atropine 10^{-4} M/kg were injected through a polyethylene cannula inserted into the external jugular vein followed by a saline flush (0.2 ml), to block the muscarinic receptors.

The arterial blood pressure was continuously monitored from the carotid artery via arterial cannula connected to a research grade blood pressure transducer (Harvard, 60-3003) coupled with four channels Harvard Universal Oscillograph (Curvilinear, 50-9307) (UK). Animals were allowed to equilibrate for at least 5 min than acetylcholine was administered to check the blockade of receptor than BRD and BRE administered one by one, and change in blood pressure was monitored.

Toxicological/safety evaluation studies in mice

Five groups of NMR-I mice (25 to 30 g) containing twelve animals in each group (six male, six female) were used in this study. All animals were treated orally once daily for 14 consecutive days.

1. Group I was treated with saline served as control.
2. Group II was treated with BRE (100 mg/kg), administered orally (p.o.).
3. Group II was treated with BRE (1000 mg/kg) administered orally (p.o.)
4. Group IV was treated with BRE (100 mg/kg), administered intraperitoneally (i.p.).
5. Group V was treated with BRE (1000 mg/kg) administered intraperitoneally (i.p.).

Animals were weighed daily before the administration of dose. All the animals were kept under observation for nearly two hours after the administration of dose, for any change in behavior or physical activities. Numbers of expired animals were noted at the end of study period. At the end of 7th day all survived mice were anaesthetized with pentothal sodium (40 mg/kg) and autopsied.

Toxicological studies in rats

Two groups of Sprague dawley rats (225 to 250 g) containing twelve animals in each group (six male, six female) were used in this study. All animals were treated intraperitoneally once daily for fourteen consecutive days.

1. Group I was treated with BRE (100 mg/kg).
2. Group II was treated with distilled water.

Autopsy

At the end of 14th day all survived mice and rats were anaesthetized with pentothal sodium 40 mg/ml i.p (Ulicna et al., 2003) and autopsied.

Estimation of different biochemical parameters

At the end of 14th day all survived rats were anaesthetized with pentothal sodium 40 mg/ml i.p and the blood samples approximately (4 to 8 ml) were withdrawn from cardiac puncture before dissecting the animals with sterile disposable syringe from all treated and control rats, were left at room temperature for 20 min, then incubated at 37°C for 30 min and centrifuged separately in (BHG) Hermle Z230 (Germany) at the speed of 3,000 rpm for 20 min. Supernatants (Serum) were separated out and the residue was discarded. Serum obtained (1 to 3 ml) was subjected for the study of the following parameters: Bilirubin, SGPT, gamma glutamyl transferase (γ GT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), CK, aspartate amino transferase (ASAT, SGOT), total protein, albumin, urea uric acid, blood urea nitrogen (BUN), creatinine, high density lipoprotein (HDL), cholesterol, triglycerides (TG), glucose. All tests were performed by using commercial assay kits. Kits used were: Mercko test[®] for bilirubin, Ecoline[®] 25 for SGPT. Ecoline[®] S+ by Szasz method for γ GT, Ecoline[®] 25 for Alkaline Phosphatase, Ecoline[®] 125 for LDH, Ecoline[®] 125 for ASAT (GOT), Ecoline[®] 125 for CK-NAC, Ecoline[®] S+ by biuret method for Total Protein, Ecoline[®] 100 by UV test, GIDH method for Urea, Ecoline[®] 100 by UV test, GIDH method for

BUN, Ecoline[®] S+ 100 by bromocresol green method for Albumin, Ecoline[®] 100 for Uric Acid (TBHBA), Ecoline[®] S+ by Jaffé method for Creatinine, Ecoline[®] 125 for Triglycerides GPO, diagnostica Merck by CHOD-PAP method for HDL-Cholesterol, Ecoline[®] 125 for Cholesterol, Ecoline[®] 1000 by GOD-PAP method for glucose. All these kits were purchased from diagnostica Merck (Germany). U-2000 spectrophotometer (Hitachi) was used to measure the absorbance of light.

Histology

Heart, liver, spleen and kidneys were fixed in 10% formalin. After usual processes of dehydration, clearing and infiltration, tissues were embedded in paraffin wax and sectioned into 7 μ m slices through Leica RM 2145-rotation microtom. The tissues were stained with hematoxylin and eosin. The slides were studied and photographed through nikon advance trinocular research microscope OPTIPHOT model X2T-21E equipped with Nikon Microphotography system; model UFX-DX-35 and phase contrast N plan.

Statistical analysis

Changes in blood pressure and serum biochemical levels were compared using student's t-test. Values of $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered to be significant.

RESULTS

Effect of various doses of BRE on various blood pressure parameters

The effect of intravenous administration of various doses of ethanolic extract of root (BRE) on various blood pressure parameters has been presented in Table 1.

Effect of 10 mg/kg BRE on various blood pressure parameters

BRE at the dose of 10 mg/kg was found to reduce the systolic, diastolic and mean arterial blood pressure that was 44, 48 and 47% in comparison with their controls, respectively as shown in Figure 1. Decreases in various blood pressure parameters were statistically significant ($p < 0.005$).

Effect of 20 mg/kg BRE on various blood pressure parameters

Intravenous administration of 20 mg/kg dose of BRE showed significant ($p < 0.005$) hypotensive effect. It was found to decrease the systolic, diastolic and mean arterial blood pressure by 43, 47 and 46%, respectively as shown in Figure 1. These reductions in various blood pressure parameters were statistically significant ($p <$

Table 1. Effect of ethanolic extract of root (BRE) obtained at different doses on various BP parameters.

Parameter	10 mg/kg		20 mg/kg	
	Before administration	After administration	Before administration	After administration
Systolic BP	133±4.41(6)	73.67±4.55*(6)	126.5±2.12(6)	71.50±3.54*(6)
Diastolic BP	129.67±1.08(6)	66.33±6.53*(6)	123±2.01(6)	65±5.66*(6)
MABP	130.7±8.53(6)	68.78±5.86*(6)	124.14±0.71(6)	67.17±4.95*(6)

The values have been presented as mean±S.E.M (n). *Represents significant difference after the administration of extracts.

Table 2. Effect of decoction of root (BRD) obtained at different doses on various BP parameters.

Parameter	10 mg/kg		20 mg/kg	
	Before administration	After administration	Before administration	After administration
Systolic BP	139.13±5.9 (10)	107.63±10.16* (10)	130.5±5.86 (10)	71.70±4.88* (10)
Diastolic BP	137.5±5.74 (10)	105.63±10.16* (10)	128.4±5.89 (10)	69.60±4.9* (10)
MABP	138.04±5.79 (10)	106.29±10.06* (10)	129.10±5.88 (10)	70.30±4.89* (10)

The values have been presented as mean±SEM (n). *Represents significant difference after the administration of extracts.

0.005) when compared to its control as shown in (Table 1).

Effect of various doses of BRD on various blood pressure parameters

The effect of intravenous administration of various doses of decoction of root (BRD) on various blood pressure parameters has been presented in Table 2.

Effect of 10 mg/kg BRD on various blood pressure parameters

Intravenous administration of BRD at the dose of 10 mg/kg was found to reduce the systolic pressure, diastolic and mean arterial blood pressure that was 23% in comparison with their controls as shown in Figure 2. Decreases in various blood pressure parameters were statistically significant ($p < 0.005$). At the dose of 10 mg/kg hypotensive effect remained for 91 ± 14.29 s.

Effect of 20 mg/kg BRD on various blood pressure parameters

BRD at the dose of 20 mg/kg was found to reduce the systolic pressure, diastolic and mean arterial blood pressure that was 45% in comparison with their controls as shown in Figure 2. Decreases in various blood pressure parameters were statistically significant ($p < 0.005$) which remained effective for 381 ± 203 s.

Result of receptor activity

The receptor activity was determined in rats results demonstrated that BRE (10 mg/kg) and BRD (20 mg/kg) has been found to decrease the mean arterial blood pressure that was 47 and 45%, respectively when compared with their respective controls (Figures 1 and 2). While, on pre-treatment with Atr (10^{-4} M) the BRE (10 mg/kg) and BRD (20 mg/kg) did not produce any reduction in blood pressure. These behavior matches exactly with that of acetylcholine (1 µg/kg). The use of Ach (10^{-6} M) has resulted in tremendous fall in MABP { $124.63 \pm 4.93(6)$ to $67.77 \pm 4.11(6)$ } that was 46% than its respective control. Further, on pre-treatment with Atr (10^{-3} M) the Ach (10^{-3} M) did not show decline in MABP as shown earlier without Atr (10^{-3} M) pre-treatment.

Toxicological studies in mice and rats

Toxicological studies of BRE were carried out in mice. Oral administration of BRE (100 mg/kg) did not show any change in physical behavior of mice while oral administration of higher dose of BRE (1000 mg/kg/d) caused decreased motor activity, corner sitting, hind limb abduction and palpebral ptosis in early two hours after dosing (Table 3). None of the groups showed any significant change.

Intraperitoneal administration of BRE (100 mg/kg) i.p. showed a lot of symptom like Abdominal cramps, ataxia, decreased motor activity, corner sitting, hind limb abduction and localized paralysis, while 1,000 mg/kg appeared as lethal dose (LD) and all mice died at the

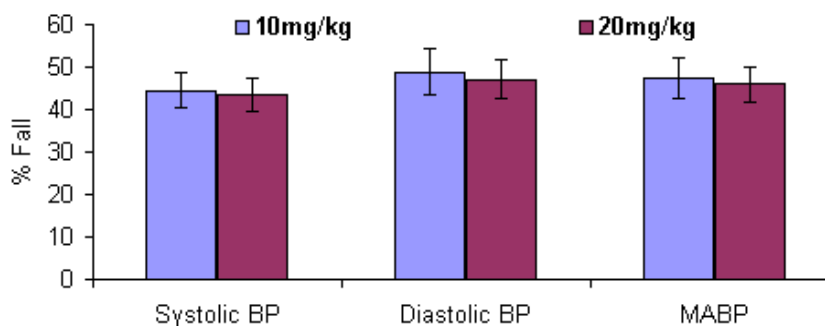


Figure 1. Effect of different doses of BRE on various BP parameters.

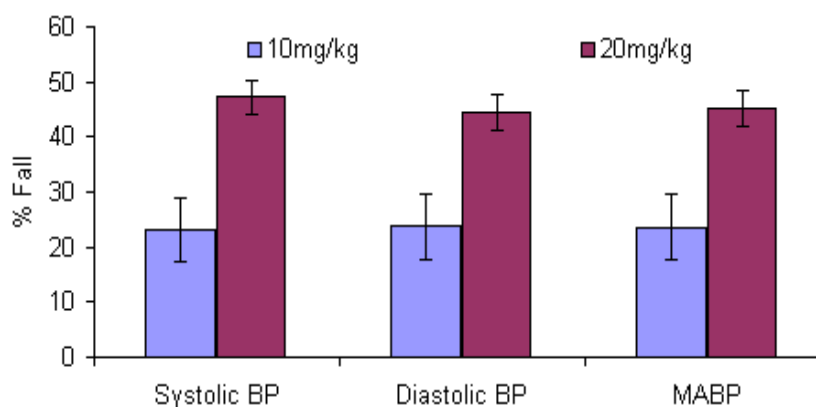


Figure 2. Effect of BRD on various BP parameters.

interval of 24 h (Table 3). Control group did not show any mortality or significant change in their general behavior or physical activities (Table 3). Male and female rats treated with BRE (100 mg/kg-body weight) did not show any mortality as shown in Table 3. None of these animals showed any sign of toxicity but some physical behavioral changes were observed in first 2 h after dosing like decreased motor activity and corner sitting (non quantified observation) as shown in Table 3 and the animal returned to normal within 2 h.

Autopsy

Autopsy revealed after the administration of BRE (100 mg/kg), no gross changes were observed in organs like liver, spleen, heart and kidney. Drug did not cause any internal body hemorrhage.

Effect of BRE on different biochemical parameters

The effects of BRE observed on different biochemical parameters of rat. Oral administration of 100 mg/kg dose

of BRE for 14 consecutive days was not found to alter the liver enzyme significantly. It reduces the serum bilirubin, γ GT and ALP with respect to its control, although this reduction was statistically non significant ($p > 0.05$). BRE (100 mg/kg) produced slight but non-significant increase in the serum SGPT but this increase was also statistically non significant ($p > 0.05$) as shown in Table 4. Heart profile showed that It reduced the serum CK, LDH and SGOT in comparison to control rats, although these reductions were statistically non significant ($p > 0.05$) as shown in Table 4. It prevented the elevation of lipid profile significantly. It reduces the serum cholesterol and TG with respect to its control, although this reduction was statistically non significant ($p > 0.05$), while an increase was observed in serum HDL level after the administration of BRPM (100 mg/kg) for 14 consecutive days as shown in Table 4. A non-significant decrease in the serum total protein, albumin, urea, uric acid, creatinine and BUN was observed in the BRE (100 mg/kg) treated rats in comparison to control rats. Although these reductions were statistically non significant ($p > 0.05$). A non-significant decrease was also observed in blood glucose level.

Table 3. Toxicological study of different extracts of *Berberis vulgaris* in mice and rats.

S/no	Extract	Animal	Dose	ROA	No of animals	Days	Mortality	Symptoms
1	Saline	Mice	0.5 ml	I.P	6 Male 6 Female	14	Nil	Nil
			100 mg/kg	Oral	6 Male 6 Female	14	Nil	Nil
	BRE		1000 mg/kg	Oral	6 Male 6 Female	14	Nil	Decreased motor activity, corner sitting, hind limb abduction
			100 mg/kg	I.P	6 Male 6 Female	14	Nil	Abdominal cramps, ataxia, decreased motor activity, corner sitting, hind limb abduction and localized paralysis
2	Saline	Rats	1000 mg/kg	I.P	6 Male 6 Female	14	12	Convulsion and then death
			0.5 ml	Oral	6 Male 6 Female	14	Nil	Nil
	BRE		100 mg/kg	Oral	6 Male 6 Female	14	Nil	decreased motor activity and corner sitting

ROA = Route of administration.

Histopathology

A histopathological study was performed on the organs of surviving rats, which were sacrificed at the end of experiment. Histology of hearts, kidneys, liver and spleen of most of the treated animals examined seems unaffected.

DISCUSSION

In the present study, different ethanolic and aqueous extracts of *B. vulgaris* has been tested for its hypotensive effects in rats. The acute intravenous administration of different extract of *B. vulgaris* (BRE, BRD) as well as Ach used as positive reference drug (Lahlou et al., 2002) in this study, all exerted immediate and significant fall in systolic, diastolic and mean arterial blood pressure (MABP) in normotensive anaesthetized rats.

Earlier, the methanolic extract of *B. vulgaris* was reported to be hypotensive by Azmat et al. (2009). It is hypothesized that these extracts might be acting like Ach and keeping these assumptions, the receptor activity of extracts has also been tested in the present study by using cholinergic agonist Ach and cholinergic competitive antagonist Atr. The results demonstrate that Ach and different extracts of *B. vulgaris*, when administered alone have reduced the blood pressure. On the other hand, the use of Ach and different extracts of *B. vulgaris*, on Atr pre-treated animal did not show such decline in blood pressure as shown earlier without atropine pre-treatment. These results clearly indicate that effect of *B. vulgaris* is mediated through same receptor and mechanism as established for Ach. Ach and muscarinic receptor agonists can cause vasodilation of most blood vessels, resulting in a decrease in total peripheral resistance (Harvey,

2012). It may therefore be concluded that muscarinic responses may contribute to the cholinergic hypotensive effect of these extracts.

The present study suggests that different extracts of *B. vulgaris* possess significant hypotensive activity. Keeping this view in mind, the toxicological studies were carried out. *B. vulgaris* root extract (BRE) was found to be safe because the results obtained from toxicological studies suggested that after oral administration of BRE at the dose of 100 and 1000 mg/kg and intraperitoneal administration at the dose of 100 mg/kg, no mortality was observed. But intraperitoneal administration at the dose of 1000 mg/kg/day was found as lethal dose (LD₁₀₀), killing all mice. It is reported that barberry is generally considered safe when consumed orally and appropriately for medicinal purposes, but due to its moderately toxic properties cannot be recommended for consumption in quantities over

Table 4. Effect of BRPM (100 mg/kg) on different biochemical parameters.

Parameter	Control	BRPM treated	P value
Bilirubin	1.36±0.1	1.16±0.19	P>0.05
Alanine aminotransferase (ALAT:SGPT)	14.77±5.98	15.55±1.99	P>0.05
Gamma glutamyl transferase (γGT)	4.24±0.85	3.72±0.67	P>0.05
Alkaline phosphatase (AP)	76.4±16.63	63.26±7.01	P>0.05
Lactate dehydrogenase (LDH)	196.99±16.38	190.23±24.8	P>0.05
Creatine kinase (CK)	32.86±6.41	21±5.56	P>0.05
Aspartate amino transferase (ASAT:SGOT)	77.52±4.75	63.02±6.76	P>0.05
Total protein (TP)	6.4±0.211	6.26±0.13	P>0.05
Albumin	3.595±0.09	3.42±0.1	P>0.05
Urea	30.97±7.32	30.68±5.46	P>0.05
Uric acid	3.66±0.152	3.49±0.24	P>0.05
Blood urea nitrogen (BUN)	18.918±2.88	17.45±2.93	P>0.05
Creatinine	1.02±0.03	0.875±0.07	P>0.05
Cholesterol	87.91±2.76	82.167±3.19	P>0.05
High density lipoproteins (HDL)	46.67±1.68	54.76±2.17	P>0.05
Triglycerides (TG)	119.7±2.32	109.33±2.03	P>0.05
Glucose	113.27±4.16	101.7±4.23	P>0.05

All values are presented as mean±SEM (n=12).

500 mg (Jellin et al., 2000).

Biochemical studies showed that there were non-significant effects on liver, kidney heart and diabetic profile in BRE (100 mg/kg) treated rats. The non-significant data collected after 14 days were interpreted as biological variability normally observed in rats. Different enzyme tested for liver function, cardiovascular functions and kidney function because these levels rise faster in cholestatic, obstructive disease, hepatocellular damage, atherosclerosis, muscular dystrophy and acute myocardial infarction. Results suggest that after the administration of BRE showed non-significant changes in serum level of bilirubin, γGT, alkaline phosphatase, SGPT, CK, SGOT, LDH, TG, HDL, total protein, albumin, urea, uric acid BUN, creatinine level. The histopathological study confirmed that BRE at the dose of 100 mg/kg did not produce any change in liver, heart, kidney and spleen.

The results confirmed that oral and intraperitoneal administration of BRE does not indicate any structural and functional disturbance of liver, heart and kidney up to the dose 100 mg/kg.

Conclusion

From the discussion, it is concluded that BRE is a physiologically and pharmacologically active drug because systolic, diastolic and mean arterial blood pressure is influenced by BRE used in the present study. Different extract of BRE used in this study caused hypotensive

effect by stimulating muscarinic receptors. The toxicological, hematological and histopathological results further confirm the safety of BRE at the dose of 100 mg/kg.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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ABBREVIATIONS

BRE, Ethanolic extract of root pulp; **BRD**, aqueous extract of root pulp; **DrHMIIPS**, Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences; **p.o.**, oral administration; **i.p.**, intraperitoneally.

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