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Original Research Article

Antihypertensive Activity of Aqueous-Methanol Extract of *Berberis Orthobotrys* Bien Ex Aitch in Rats

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Abstract

Purpose: *To investigate the hypotensive potential of Berberis orthobotrys Bien Ex Aitch (Family: Berberidaceae) in both normotensive and hypertensive rats.*
Methods: *Aqueous-methanol (70:30) extract of Berberis orthobotrys at doses of 25, 50, 75 and 100 mg/kg was evaluated for its effect on blood pressure and heart rate using non-invasive blood pressure measuring apparatus. After initial screening, 100 mg/kg dose that produced a maximum effect was selected for the antihypertensive study. Median lethal dose (LD_{50}) and sub-chronic toxicity of the extract were also determined. Various biochemical parameters and organ weight were measured using standard procedures.*
Results: *The extract produced a significant ($p < 0.01$) decrease in systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP) and heart rate of normotensive rats at all test doses with maximum effect at 100 mg/kg. Similarly, a significant antihypertensive and negative chronotropic effect was observed in both hypertensive models. LD_{50} of the extract was 200 mg/kg in mice. The extract also exhibited a reduction ($p < 0.05$) in serum alanine transaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), triglycerides and low density lipoprotein (LDL) levels while a significant ($p < 0.05$) increase in high density lipoproteins (HDL) level was observed.*
Conclusion: *It seems that the aqueous-methanol extract of Berberis orthobotrys possesses active compounds which may be responsible for the antihypertensive and negative chronotropic effects in rats.*

Keywords: *Berberis orthobotrys, Antihypertensive, Egg feed diet, Blood lipids*

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INTRODUCTION

Cardiovascular diseases have become a leading cause of morbidity and mortality throughout the world. Hypertension constitutes a major risk factor for several cardiovascular diseases such as atherosclerosis, heart failure, renal insufficiency, coronary artery disease and stroke. The risk factor increases with the age in both sexes. According to World Health Organization (WHO), about one-third of the world's population suffers from hypertension and the incidence has been increasing at a rapid rate due to life style modification [1]. In Pakistan, one in every four middle-aged adults suffers from high blood pressure [2].

Many synthetic drugs have been developed for the treatment of hypertension because of the severity and prevalence of the disease. Most of

these drugs have demonstrated better efficacy but possess a number of side effects. Herbal medicines therefore, have been regaining importance because of their ease of availability, less side effects and cost effective [3]. Recent attention has been focused on the herbal preparations as alternative agents for the treatment and prevention of cardiovascular problems. Ethnobotanical surveys of various medicinal plants indicate their vast use in the treatments of cardiovascular disorders. For example, plants like *Syzygium guineense, Passiflora nepalensis* Wall, *Ginko biloba*, *Stephania tetandra* and *Uncaria rhynchophylla* have been used for the treatment of hypertension [4,5].

Despite the fact that traditional medicines have been used in the modern world, very little work has been done in Pakistan to determine their possible mode(s) of action, side effects, toxicity and interactions. Hence biological evaluation of most of these traditional medicines remains yet to be elucidated [6].

Berberidaceae is an important family with several medicinal uses. Berberis species also contain certain active compounds that have been reported to have strong antihypertensive effects [7,8,9. *Berberis orthobotrys* (Family: Berberi-daceae) is a plant indigenous to Pakistan, that is found mostly in Gilgit Baltistan, Pakistan where local peoples commonly use this for the treatment of hypertension. Since no pharmacological data are available on this plant, to the best of our knowledge, a study was conducted to evaluate the possible antihypertensive effect of this plant.

EXPERIMENTAL

Chemicals and drugs

Glucose and methanol were purchased from Sigma Chemicals Co. All other chemicals and drugs used were of analytical grade.

Animals

Sprague Dawley rats and albino mice weighing 200-350g and 30-40g respectively were used for this study. All the animals were housed in controlled environment (23-25°c) and received human care in accordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals. The study protocol was approved by the institutional Animal Ethics committee Faculty of Pharmacy, University of Sargodha (Approval No.20-A12 IEC UOS). Experiments performed complied with the rulings of National Research Council [10].

Plant material

The root of *Berberis orthobotrys* were collected from district Gilgit, Pakistan during the month of June, 2011 and was identified and authenticated by Dr. Shair Wali , Assistant Professor Botany, Karakurum international University Gilgit Baltistan, Pakistan. A voucher (no. BO-15-12) has been deposited in the herbarium, Faculty of Pharmacy, University of Sargodha for future reference.

Preparation of extract

Aqueous methanolic (70:30) extract of *Berberis orthobotrys* was prepared using cold maceration process. The grounded plant material (2 kg) was soaked in 5 L of water-methanol mixture (70:30) for 72 h at room temperature. After three days of occasional shaking, the whole material was filtered and the filtrate evaporated under reduced pressure using rotary evaporator. The crude extract was then air-dried to obtain a solid mass with a yield of 15 %. [11].

Determination of hypotensive activity in normotensive rats

Normotensive rats of either sex were randomly assigned into four groups (n = 5). Group 1 and 2 received 25 and 50 mg/kg of the aqueous methanolic extract of *Berberis orthobotrys* while animals in group 3 and group 4 received 75 and 100 mg/kg of same extract, respectively. Animals in each group served as their own control. Basal blood pressure and heart rates were measured at 0 h (Before extract administration).. After intra-peritoneal administration of various doses of the extract, blood pressure and heart rate of each of these groups were determined from the tails of rats at 0, 2, 4 and 6 h using non-invasive blood pressure (NIBP) measuring apparatus (IN125, AD Instruments, Sydney, Australia). Each rat was placed in NIBP restrainer and appropriate cuff with sensor was then mounted on its tail and warmed to about 33 – 35 °C. The tail cuff (MLT 125/R) was inflated to a pressure well above the expected systolic blood pressure, i.e., 200 mm Hg, and slowly released during which the pulses were recorded by using Power Lab data acquisition system and computer running Lab Chart 5.0 software (Ad Instruments, Sydney, Australia). Systolic blood pressure (SBP), Mean blood pressure (MBP) and heart rate were measured directly using pulse tracing while diastolic blood pressure (DBP) was calculated from SBP and MBP as in Eq 1 [12].

DBP = (3MBP - SBP)/2 …… (1)

Determination of antihypertensive activity in hypertensive models

Egg feed-induced hypertensive rat model

Sprague Dawley rats of either sex were divided into two groups (n = 5). Group 1 received orally a special prepared egg feed diet (12 egg yolk mixed with 500 g of normal rat diet) for 21 consecutive days in order to produce cholesterol-induced hypertension. Animals in group 2 received egg feed diet and aqueous methanol extract of *Berberis orthobotrys* (100 mg/kg) for the same time period. All animals received saline instead of tap water *ad libitum.* Blood pressure and heart rate of each of these groups were measured on week 0, 1, 2 and 3 by the method described above [12,13].

Glucose-induced hypertensive rat model

Sprague Dawley rats of either sex were randomly assigned into two groups (n = 5). Group 1 received 10 % glucose solution instead of tap water for 21 consecutive days. Animals in group 2 were given 10 % glucose solution and an aqueous methanolic extract of *Berberis orthobotrys* (100 mg/kg) for same time period. Animals were fed on standard diet. Blood pressure and heart rate of these groups were measured on week 0, 1, 2, and 3 by the above mentioned method [12,13]

Acute toxicity study

Albino mice of either sex were randomly divided into five groups (n = 2). Group 1 served as control and received normal saline (10 ml/kg) while other groups (2, 3, 4 and 5) were administered the extract in doses of 30, 50, 100 and 200 mg/kg, respectively. Mortality rate was observed for 24 h. All the doses were administered by intra-peritoneal route. The highest dose, which did not kill any animal, and the lowest dose, which killed only one animal, were noted. LD_{50} was calculated from the mean of these two doses [14].

Sub-chronic toxicity assessment

Sprague Dawley rats of either sex were randomly divided into two groups (n = 6). The first group received normal saline (10 ml/kg body weight p.o.) that served as control and the animals in group 2 received 100 mg/kg body weight of the extract daily for 28 days. Food and water intake of animals were observed during this period. On the 29th day, blood was collected from overnight fasted rats of each group by cardiac puncture for the determination of serum biochemical parameters. The rats were sacrificed by cervical dislocation for the study of various organs (heart, liver and kidney) weights [15].

Evaluation of biochemical parameters

For the estimation of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol, triglycerides, low-density lipoprotein (LDL) and high density lipoprotein (HDL), blood samples were collected in clot activator gel tubes. The serum was separated by centrifuging the blood samples at 2000 rev/min for 10 minutes. Serum biochemical parameters were then measured by using commercially available reagent kits (Abbott Laboratories, USA) [15].

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM) and statistical analysis was carried out by Student's t-test using Graph Pad Prism 5.0. Differences were considered significant at $p < 0.05$.

RESULTS

Hypotensive activity in normotensive rats

The aqueous methanol extract of *Berberis orthobotrys* produced a significant decrease in SBP, MBP and DBP of normotensive rats at the doses of 25 and 50 mg/kg. The extract at the doses of 75 and 100 mg/kg also exhibited a highly significant ($p < 0.001$) decrease in blood pressure when compared to control (0 h). The maximum hypotensive effect was observed at 100 mg/kg. Similarly, the extract also produced a significant ($p < 0.01$) decrease in heart rate at all the doses with maximum effect at 100 mg/kg (Figure 1 and Table 1).

Egg feed-induced hypertension

The extract, at a dose of 100 mg/kg, produced a significant ($p < 0.01$) antihypertensive effect after three weeks of treatments. The extract also demonstrated a remarkable decease in heart rate of egg feed induced hypertensive rats at week 1. At the end of weeks 2 and 3, a significant ($p < 0.01$) decrease in heart rate of hypertensive rats was observed, compared to egg feed control group (Figure 2 and Table 2).

Glucose-induced hypertension

In glucose-induced hypertensive rats, the extract, at a dose of 100 mg/kg, produced a significant ($p < 0.001$) antihypertensive effect after 3 weeks of treatment. Similarly, a significant negative chronotropic effect was observed after weeks 2 and 3 treatments (Figure 3 and Table 2).

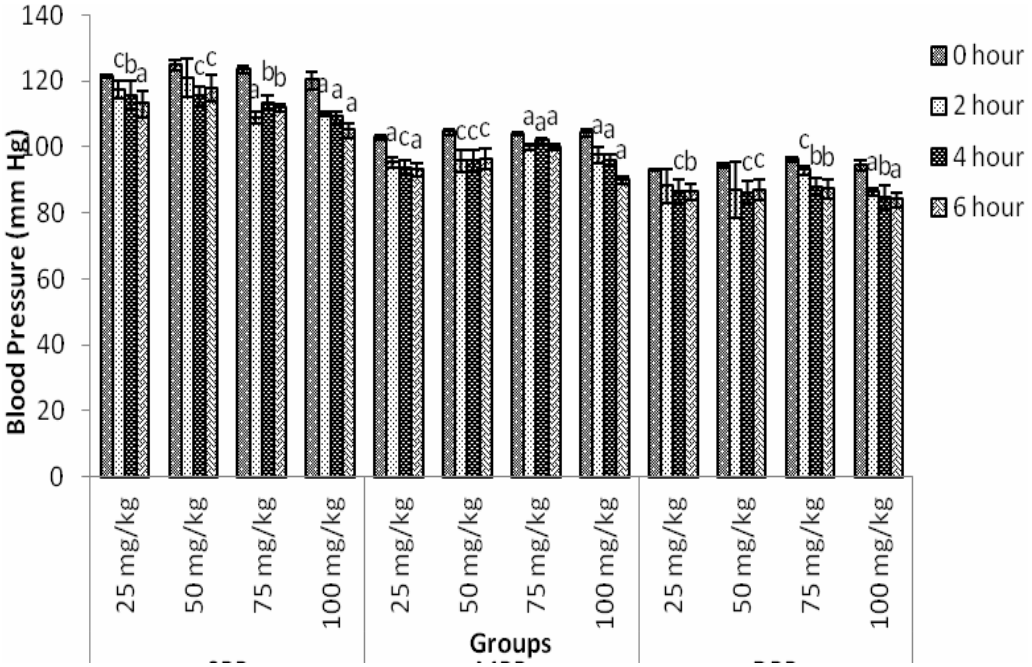

Figure 1: Hypotensive effect of *Berberis orthobotrys* in normotensive rats. ***Key:*** a = ($p < 0.001$), b = ($p < 0.01$) and c = ($p < 0.05$), compared to control (0 h); control = basal blood pressure at 0 h (i.e., before extract admisinstration)

Table 1: Effect of *Berberis orthobotrys* on the heart rate of normotensive rats

Normotensive rats (heart beats/min ± SEM)				
	Treatment			
Time	***25mg/kg***	***50mg/kg***	***75mg/kg***	***100mg/kg***
0 h	376.0 ± 3.58	361.1 ± 3.59	412.6 ± 2.76	380.2 ± 3.99
2 h	377.6 ± 2.10	357.4 ± 6.40	375.0 ± 5.65[a]	360.6 ± 7.99[a]
4 h	371.0 ± 8.94	355.4 ± 7.37	365.8 ± 8.21[b]	348.4 ± 6.27[b]
6 h	367.0 ± 3.93[a]	344.8 ± 5.52[b]	358.0 ± 6.91[b]	330.0 ± 6.56[b]

Note: *a = p < 0.05 and b = p < 0.01, compared to control*

Table 2: Effect of *Berberis orthobotrys* on the heart rate of hypertensive rats

Hypertensive rats (Heart beats/min, ± SEM)				
	Treatment			
Time	***Egg feed control***	***Extract 100mg/kg***	***Glucose control***	***Extract 100mg/kg***
0 week	374.8 ± 6.1	384.2 ± 6.1	377.0 ± 7.3	382.2 ± 6.9
1 week	410.4 ± 10	358.8 ± 7.0[a]	399.6 ± 7.5[a]	363.6 ± 8.7[a]
2 week	433.4 ± 10	357.2 ± 10[b]	449.0 ± 9.31	353.4 ± 8.9[b]
3 week	465.0 ± 9.2	350.0 ± 6.5[b]	456.0 ± 8.11	351.4 ± 5.4[b]

Note: *a = p < 0.05 and b = p < 0.01, compared to control*

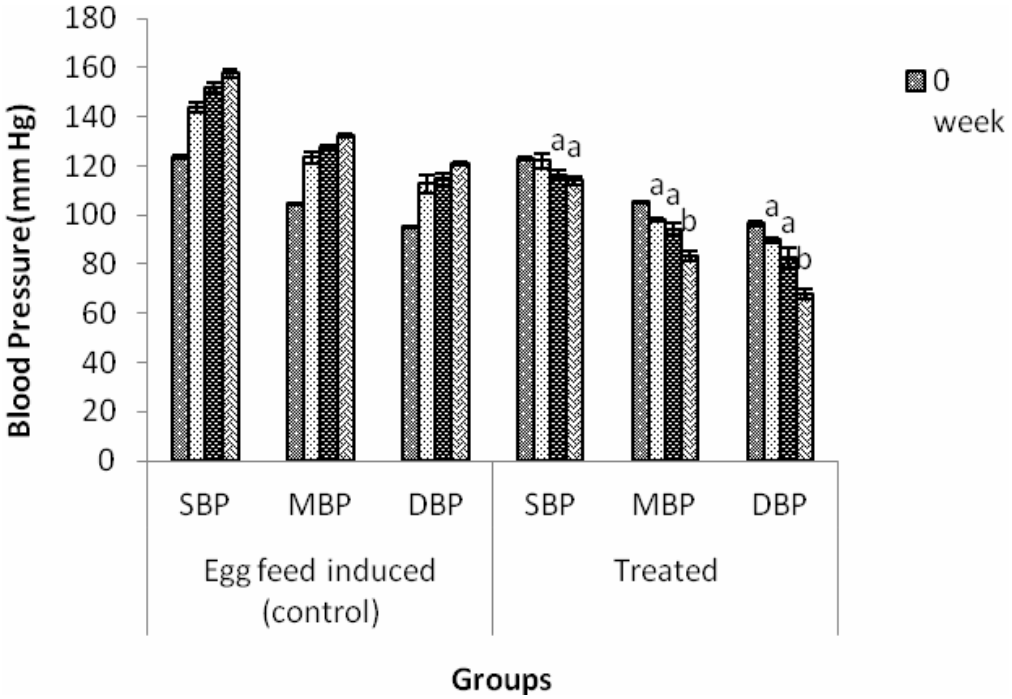

Figure 2: Antihypertensive activity of *Berberis orthobotrys* aqueous methanol extract in egg feed-induced hypertensive rats. ***Key:*** a = ($p < 0.001$) and b = ($p < 0.01$), compared to control

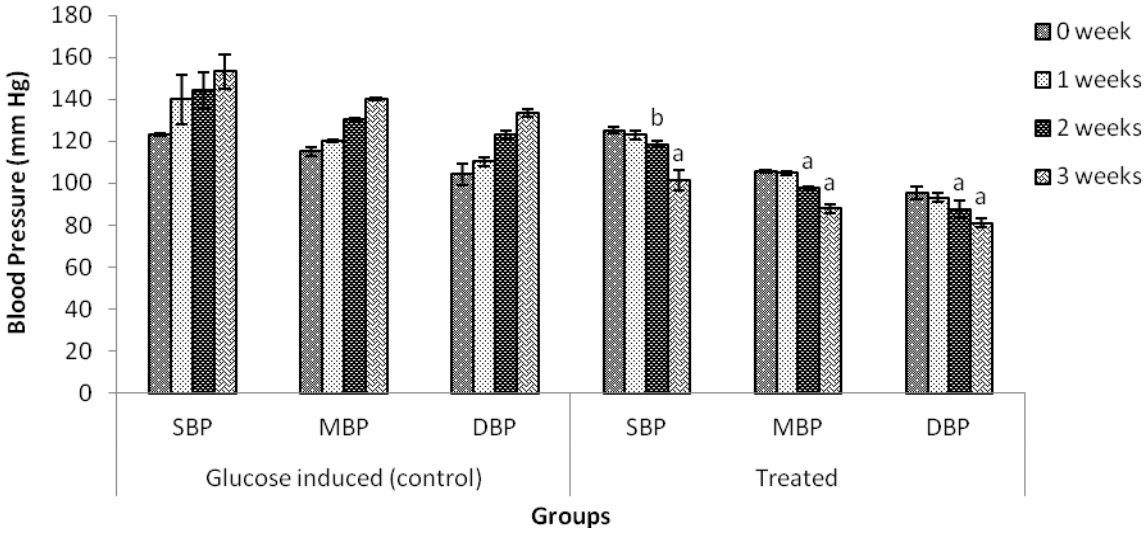

Figure 3: Antihypertensive activity of *Berberis orthobotrys* aqueous methanol extract in glucose-induced hypertensive rats. ***Key:*** a = ($p < 0.001$) and b = ($p < 0.01$), compared to control
Note: *a = p < 0.05 and b = p < 0.01, compared to control*

Acute and sub-chronic toxicity of extract

The median lethal dose (LD_{50}) of the extract in mice was 200mg/kg. All the animals showed signs of respiratory depression and convulsion before death.

In sub-chronic toxicity studies, the extract did not cause any significant alteration in food and water intake, body weight and organs weight of rats. The results also revealed that the extract, at a dose of 100 mg/kg, produced significant reduction in serum ALT, AST and ALP levels. The extract exhibited significant ($p < 0.01$) decrease in total cholesterol, triglycerides and LDL levels but increase in HDL levels was observed (Table 3).

Table 3: Effect of *Berberis orthobotrys* aqueous methanol extract on body weight, organ weight and other biochemical parameters of rats

Parameter	Control (Normal saline)	Extract (100 mg/kg)
Liver (g)	5.86 ± 0.10	5.85 ± 0.12
Kidney (g)	1.39 ± 0.14	1.38 ± 0.11
Heart (g)	1.47 ± 0.11	1.46 ± 0.12
Body Weight (g)	280 ± 5.11	281 ± 4.50
ALT (IU/L)	40.1 ± 2.29	36.9 ± 1.69[a]
AST (IU/L)	90.1 ± 1.06	80.2 ± 1.09[a]
ALP (IU/L)	70.0 ± 2.01	58.2 ± 1.94[a]
Triglycerides (mg/dl)	89.2 ± 1.92	75.0 ± 1.74[a]
Cholesterol (mg/dl)	60.5 ± 1.56	50.0 ± 1.02[b]
LDL (mg/dl)	20.09 ± 2.07	14.20 ± 1.09[b]
HDL (mg/dl)	33.4 ± 1.18	46.5 ± 2.02[a]

Values are expressed in means ± SEM (n=6) where a = $p < 0.05$ and b = $p < 0.01$, compared to control

DISCUSSION

It is noteworthy that the aqueous methanol extract of *Berberis orthobotrys* produced a significant reduction in SBP, MBP, DBP and heart rate of normotensive rats indicating a hypotensive effect. Maximum decrease in blood pressure and heart rate was observed at 100 mg/kg. Hence this dose was selected for antihypertensive study in both egg feed- and glucose-induced hypertensive rats. Reduction in blood pressure produced by the extract was much higher in hypertensive rats than in normotensive rats. This is in agreement with previous findings that hypertensive rats appear to have a stronger response to hypotensive agents [16].

In rats, egg feed-induced hypertension has been reported to be largely due to increased cholesterol level while glucose-induced hypertension has been associated with increased reactive oxygen species production as well as reduction in nitric oxide levels [13,17,18]. Cholesterol rich diet and high glucose intake are linked to dyslipidemia which is considered a major risk factor for hypertension [8,12]. Furthermore, increased sympathetic activity has also been associated with raised blood pressure and a positive chronotropic effect [8]. Thus, the antihypertensive effect of *Berberis orthobotrys* in egg feed- and glucose-induced hypertensive rats might be due to certain hypolipidemic, antioxidant, vasodilator and depressor constituents of the plant extract.

Previously, it has been well documented that various Berberis species contain certain alkaloids, phenols and flavonoids that exhibit antihypertensive activity [19,20]. Alkaloids and phenolic compounds also show antioxidant effects which reduce endothelial dysfunction through increased nitric oxide formation, decreased LDL formation, increased prostacyclin formation and enhanced EDHF mediated vasorelaxation [19,21]. Therefore, the antihypertensive activity of *Berberis Orthobotrys* extract might be linked to the presence of these antioxidant principles, especially as berberine, an important alkaloid of *Berberidiaceae* family has been reported to exert antihypertensive and vasorelaxation effects [21].

In acute toxicity study, all the animals showed convulsions and respiratory depression before death which suggest some effect of the extract on rat nervous system [22]. Sub-chronic toxicity studies in rats indicate that the extract is safe as no significant signs of toxicity were observed. Food and water intake, body weights and organ weights remained unaltered during the 28 days of treatment with the extract. However, biochemical parameters related to hepatic functions such as ALT, AST and ALP significantly decreased. Reduction in the levels of these enzymes indicate that the extract did not have any toxic effects on both liver and heart tissues [22]. The extract also revealed a significant decrease in serum cholesterol, triglyceride and LDL levels, and increase in HDL levels in rats thus indicating possible reduction in cardiovascular risk factor that could lead to the death of animals, such as the anti-hyperlipidemic drugs like statins [23].

CONCLUSION

The aqueous methanolic extract of *Berberis orthobotrys* most likely contains certain active principles that may be responsible for its antihypertensive and negative chronotropic effects. Further studies are required to isolate these phytochemical constituents and elucidate their possible mechanisms of action.

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