POSSIBLE MECHANISM OF CARDIAC DEPRESSANT ACTIVITY OF BERBERIS ORTHOBOTRYS ROOTS IN ISOLATED RABBIT HEART

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Abstract: Berberis orthobotrys Bien. ex Aitch. (B.o.) has been reported to have antihypertensive effect in different experimental models. The aim of present study was to evaluate the possible antihypertensive mechanism. Aqueous methanolic extract of B.o. roots and its various fractions namely (ethyl acetate, n-butanol or aqueous) in different concentrations (10 ng/mL, 100 ng/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL ) were evaluated in isolated perfused rabbit heart to assess their effect on force of contraction, HR and perfusion pressure. The crude extract of B.o. and its fractions exhibited a significant decrease in heart rate, contractility and perfusion pressure of isolated rabbit heart, however, butanolic fraction produced more prominent effect and was selected for further study. The effects of butanolic fraction were not blocked by atropine (10^-5 M) in isolated perfused heart. However, butanol fraction significantly blocked the effects of adrenaline (10^-5 M). It is therefore conceivable that cardiac depressant activity of B.o. butanol fraction might be due to the presence of certain β-blocking agents which might be responsible for antihypertensive effect. However, further experiments are required to isolate the active compound(s) and elucidate exact mechanism of action.

Keywords: Berberis orthobotrys roots, cardiac depressant, atropine, adrenaline, rabbit

Medicinal plants have always remained a major target for drug development. Most of the traditional medicines used nowadays are from plant origin. In developing countries, medicinal plants provide an alternative therapy, which is cost effective and easily available. It has been estimated that one-third of the world population rely on traditional medicines for their health related needs (1). Traditional medicines though effective yet require evaluation by scientific methods in order to be used to their full extent.

The family Berberidaceae established as “Berberides” is considered one of the most primitive angiosperms (2). Berberidaceae is a small family that contains 15 genera, approximately 650 species worldwide, distributed in temperate regions of northern hemisphere (3). Berberis is represented by 3 genera and 20 species in Pakistan. Most of the species are found in mountainous regions of the country. Berberidaceae is characterized by perennial herbs and shrubs, leaves alternate or basal simple or compound, flowers bisexual and actinomorphic (4).

Berberis orthobotrys (Family: Berberidaceae) commonly known as Ishkeen is a plant indigenous to Pakistan, found mainly in Gilgit Baltistan. Its roots and stem bark has been used in the treatment of wounds, infections, piles, jaundice, liver problems, kidney stones, diabetes, sore throat and uterine tumors (5). In our previous study, antihypertensive activity of B.o. in rats was evaluated (6). This study was therefore conducted to investigate the possible mechanism of antihypertensive action of B.o. in isolated perfused rabbit heart.

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Figure 1. The effect of crude extract of *B. o.* and its fractions on perfusion pressure of isolated perfused rabbit heart (n = 6), where * = (p < 0.05), ** = (p < 0.01), and *** = (p < 0.001) vs. control.

Figure 2. The effect of crude extract of *B. o.* and its fractions on contractility of isolated perfused rabbit heart (n = 6), where * = (p < 0.05) and *** = (p < 0.001) vs. control.

Tracing (1) shows the effect of butanol fraction of *B. o.* on contractility of isolated perfused rabbit heart.
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Figure 3. The effect of crude extract of *B. o.* and its fractions on heart rate of isolated perfused rabbit heart (n = 6), where * = (p < 0.05), ** = (p < 0.01), and *** = (p < 0.001) vs. control.

Tracing (2) shows the effect of butanol fraction of *B. o.* on heart rate of isolated perfused rabbit heart.

Figure 4. The effect of butanol fraction of *B. o.* (10 µg/mL) on various cardiac parameters of isolated perfused rabbit heart both in the absence and presence of atropine (10⁻⁷ M) (n = 6), where *** = (p < 0.001) vs. control.
Tracing (3) shows the effect of butanol fraction of *B.o.* (10µg/mL) on perfusion pressure of isolated perfused rabbit heart in the presence of atropine (10⁻⁵M).

Tracing (4) shows the effect of butanol fraction of *B.o.* (10 µg/mL) on contractility of isolated perfused rabbit heart in the presence of atropine (10⁻⁵M).

Tracing (5) shows the effect of butanol fraction of *B.o.* (10 µg/mL) on heart rate of isolated perfused rabbit heart in the presence of atropine (10⁻⁵M).
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Figure 5. The effect of adrenaline ($10^{-5}$ M) on various cardiac parameters of isolated perfused rabbit heart both in the absence and presence of *B.o.* butanol fraction (10 µg/mL) ($n = 6$), where *** = ($p < 0.001$) vs. control

Tracing (6) shows the effect of adrenaline ($10^{-5}$ M) on perfusion pressure of isolated perfused rabbit heart both in the absence and presence of *B.o.* butanol fraction (10 µg/mL)

Tracing (7) shows the effect of adrenaline ($10^{-5}$ M) on force of contraction of isolated perfused rabbit in the presence of *B.o.* butanol fraction (10 µg/mL)
EXPERIMENTAL

Chemicals and drugs
Methanol, atropine and adrenaline were pur- chased from Sigma Chemicals Co. All the chemicals and drugs used in the experiments were of standard grade.

Animals
Both male and female rabbits of local strain (Oryctolagus cuniculus) weighing 1–1.5 kg were used. All the animals were housed in controlled environment (23–25°C) at animal house of Department of Pharmacy, University of Sargodha, Sargodha. All animals were treated according to the standard procedures and the study protocol was approved by the local ethical committee.

Plant material
The roots of B.o. were collected from district Gilgit, Pakistan during June, 2011 and were identified and authenticated by Dr. Shair Wali, Assistant Professor of 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 B. o. 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% (7).

Fractionation of the extract
Activity directed fractionation of the crude extract of B.o. was carried out by using different organic solvents in order to separate or concentrate the activities in anyone of the corresponding fraction. Fractionation was carried out by using different organic solvents (ethyl acetate, n-butanol, aqueous) based on their polarity order, as ethyl acetate < n-butanol < aqueous.

A known quantify of the crude extract was dissolved in distilled water and mixed with equal volume of organic solvents in a separating funnel, shaken vigorously, with periodical removal of air. The mixture was allowed to separate for about 20–30 min into two layers. The respective layer was removed, the same procedure was repeated twice more times. Then, all the fractions were combined and finally concentrated under reduced pressure on rotary evaporator to obtained the corresponding fraction. Similarly, the remaining layer (aqueous) was further treated with other organic solvents for their respective fractions. Finally, the remaining layer was also evaporated and was considered as aqueous fraction (8).

Effect of crude extract and its fractions on Langendorff perfused isolated rabbit heart
This experiment was carried out in accordance with the Langendorff method (9). The rabbit was
injected with 1000 IU of heparin intravenously through the marginal ear vein. Five min later, a blow on the neck of the rabbit made it unconscious. The chest was opened and the heart was dissected out with about 1 cm of aorta attached, and was quickly washed with oxygenated Krebs-Henseleit solution. The isolated heart was gently squeezed several times to remove as much residual blood as possible. The heart was then transferred to the perfusion apparatus (Radnoti isolated heart system, AD Instrument, Australia) and tied to a glass cannula through the aorta. The perfusion fluid was Krebs-Henseleit solution, which was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide, and was applied at a constant flow mode. The temperature was continuously monitored and kept constant in the range of 36.5–37.5°C.

The heart was allowed to stabilize for 30 min before any drug interventions. The mechanical responses such as force of contraction of spontaneously contracting isolated heart was recorded by attaching one end of a thread to the apex of the heart using a Palmer clip and the other end of the thread to a force transducer (MLT 844; AD Instruments, Australia). Heart rate was calculated indirectly using LabChartpro5 software (AD Instruments, Australia). Perfusion pressure was measured by using pressure transducer. The signals from both pressure and force transducers were filtered and amplified and sent to an analog-to-digital converter (Power Lab data acquisition and analysis system, AD Instruments, Australia) attached to a computer. The signals recorded were saved for later analysis. After stabilization, different doses of the crude extract and fractions (ethyl acetate, n-butanol, aqueous) of B.o. (10 ng, 100 ng, 1 µg, 10 µg, 100 µg/mL) were applied to assess various cardiac parameters i.e., heart rate (beats/min), force of contraction (g) and perfusion pressure (mm Hg) with each heart serving as its own control. In order to explore the possible mechanism of action, the effect of butanol fraction of the extract was assessed both in the absence and presence of atropine 10⁻⁵ M (10). In addition, the effects of adrenaline (10⁻⁵ M) were also assessed both in the absence and presence of butanol fraction of B.o. (11).

### Statistical analysis

The results were expressed as the means ± SEM. Statistical analysis was done by two way ANOVA followed by Bonferroni test using Graph Pad Prism 5.0. Value of p < 0.05 was considered as significant.

### RESULTS

**Effect of crude extract of B.o. and its fractions on perfusion pressure, contractility and heart rate of isolated perfused rabbit heart**

The aqueous methanolic extract of B.o. at all doses produced a significant (p < 0.01–0.001) decrease in force of contraction of isolated heart while a significant (p < 0.05–0.01) decrease in heart rate was observed only at doses from 10 ng/mL to 1 µg/mL. The crude extract (1–100 µg/mL) also exhibited a significant (p < 0.001) decrease in perfusion pressure. Ethyl acetate and aqueous fractions of B.o. demonstrated a significant negative inotropic and chronotropic effect at higher doses only. Similarly, both extracts produced a significant decrease in perfusion pressure merely at 100 µg/mL. Moreover, butanol fraction of B.o. produced a significant (p < 0.05–0.001) decrease in perfusion pressure, heart rate and contractility of isolated heart at all the doses. A prominent effect in all the three cardiac parameters was observed at 10 µg/mL. Butanol fraction was chosen as the most potent of all of the extracts, hence 10 µg/mL of butanol fraction was selected to elucidate its possible mechanism of action. The highest dose of the butanol fraction (100 µg/mL) caused a maximum decrease in contractility and heart rate but it also produced hardening of the heart and stoppage of beating of the heart. Therefore, this dose was not picked to explore its detailed mechanism (Figs. 1, 2 and 3).

**Effect of butanol fraction of B.o. on perfusion pressure, contractility and heart rate of isolated perfused heart in the presence of atropine (10⁻⁵ M)**

In the presence of atropine (10⁻⁵ M), the butanol fraction of B.o. produced a significant (p < 0.001) decrease in perfusion pressure, force of contraction and heart rate of isolated heart. Atropine did not block the negative inotropic and chronotropic effects or reduced perfusion pressure of butanol fraction. The decrease in all the cardiac parameters was quite similar in both presence and absence of atropine (10⁻⁵ M) (Fig. 4).

**Effect adrenaline (10⁻⁵ M) on perfusion pressure, contractility and heart rate of isolated perfused heart in the presence of butanol fraction of B.o.**

To find out the possible β-blocking activity, the effects of adrenaline on various cardiac parameters were studied both in the presence and absence of butanol fraction of B.o. (10 µg/mL). The findings indicated that adrenaline (10⁻⁵ M) produced a significant (p < 0.001) increase in force of contraction,
heart rate and perfusion pressure of isolated heart in the absence of butanol extract of B.o. (10 µg/mL). However, in the presence of butanol extract of B.o. (10 µg/mL), the effects of adrenaline (10^{-5} M) were significantly blocked as there was non-significant increase in all the cardiac parameters of isolated heart (Fig. 5).

DISCUSSION

Herbs were our first source of medicines. No one knows when humans first used plants for medicine, but pollens of at least six medicinal plants were found in a Neanderthal burial site estimated to be at least 60,000 year old and some derivatives, e.g., aspirin, reserpine, and digitalis, have become mainstays of human pharmacotherapy. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets. Several natural product drugs of plant origin have either recently been introduced in the market, including galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials (12, 13). The medicinal values of traditional medicinal plants cannot be disregarded and studies have been carried out in order to explore various active principles of the extracts with intensive follow up studies to establish their exact mechanism of action. One of the most important area in which compounds from plant sources have contributed successfully, is the cardiovascular research.

The present study revealed that aqueous methanolic extract of B.o. exhibited a significant decrease in perfusion pressure, force of contraction and heart rate at most of the doses. Similarly, all the fractions of B.o. produced a significant negative inotropic and chronotropic effect in isolated heart. However, ethyl acetate and aqueous fractions of B.o. showed a significant decrease in perfusion pressure only at 100 µg/mL. Butanol fraction of B.o. was found to be much more potent than other extracts as it exhibited a significant decrease in all the cardiac parameters; this clearly indicates that the active principle(s) responsible for the said effects were more concentrated in this fraction. Hence, butanol fraction of B.o. (10 µg/mL) was selected for a detailed mechanism study.

Previously, it has been reported that negative inotropic and chronotropic effect in isolated heart usually results from a cholinergic/histaminic stimulating activity or by β-receptor or calcium channel blocking effect (14, 15). A decrease in perfusion pressure indicates a coronary vasodilation effect, which is generally caused by three different mediators that include β-receptor antagonist, cholinergic receptor agonist and endothelium derived relaxing factor (9, 15).

In the presence of atropine (10^{-5} M), butanol fraction of B.o. (10 µg/mL) produced a significant decrease in perfusion pressure, heart rate and force of contraction of isolated rabbit heart indicating that the extract did not mediate its actions through cholinergic receptors. In order to investigate the β-blocking effect of butanol fraction of B.o. (10 µg/mL), the effects of adrenaline (10^{-5} M) were studied in isolated rabbit heart. The findings indicated that the butanol extract significantly blocked the positive inotropic and chronotropic effects of adrenaline (10^{-5} M). Similarly, the increased perfusion pressure produced by adrenaline (10^{-5} M) was also significantly antagonized. It has been well established that adrenaline, a sympathomimetic drug, acts directly on β1 receptors and produces an increased heart rate and contractility. Propranolol blocks these receptors and produces a negative inotropic effect and chronotropic effect (16, 17). Hence, the blocking of adrenaline’s pharmacological effects by the butanol fraction of B.o. clearly indicated its β-receptor antagonizing activity on isolated heart. However, the involvement of other receptors cannot be ruled out. Moreover, it has also been reported that β-blockers produce a cardiac inhibitory effect that results in decreased cardiac output and vasodilation, ultimately leading to a fall in blood pressure. Other blood pressure lowering mechanisms of β-adrenergic receptor blocking agents include a decrease in catecholamine release and an antirenin activity (18). It is presumable therefore that this cardiac depressant activity of B.o. in isolated perfused rabbit heart might also be responsible for its antihypertensive effect in rats.

CONCLUSION

It is concluded from this study that cardiac depressant and coronary vasodilating activity of B.o. might be due to its antagonizing effects on β-receptors. Further studies are required to identify and isolate the biologically active compounds in order to determine the exact mechanism of action.

REFERENCES

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