HYPOTENSIVE ACTIVITY OF METHANOLIC EXTRACT OF BERBERIS VULGARIS (ROOT PULP AND BARK)

AISHA AZMAT\textsuperscript{a}, MUHAMMAD AHMED\textsuperscript{b}, NAVAID UL ZAFAR\textsuperscript{c} AND S.I. AHMAD\textsuperscript{d}

\textsuperscript{a}Umm Al-Qura University, Faculty of Medicine, Department of Physiology, P.O. Box 7607 Makkah, Saudi Arabia
\textsuperscript{b}Umm Al-Qura University, Faculty of Pharmacy, Department of Pharmacology, P.O. Box 7607. Makkah, Saudi Arabia
\textsuperscript{c}Hamdard Labs(WAQF), Pakistan, Karachi.74600.
\textsuperscript{d}Dr. HMI Institute of Pharmacology and Herbal Sciences, Hamdard University, Karachi-74600, Pakistan

ABSTRACT

Many plants have been recommended in alternative systems of medicine for hypotensive activity although few systematic studies have been done. In this study, the hypotensive activity was studied in normotensive albino rats at different doses of BRPM and BB. The receptor activity was assessed by the drugs Ach on rat heart. Administration of different methanolic extract (BRPM, BB) showed significant reduction in blood pressure comparable to its respective control. Both methanolic extract completely blocked the Ach receptors in heart. This study suggests that the methanolic extract (BRPM, BB) have significant Ach receptor blocking and hypotensive activity and may be recommended for further investigation.

Keywords: Berberis vulgaris, Hypotensive, methanolic extract of root pulp (BRPM), methanolic extract of root bark (BB).

INTRODUCTION

Each year heart disease (hypertension) kills twice as many people as cancer and eight times as many people as car accidents or infections (Bapist, 2003). According to Survey, hypertension and other “vascular diseases” such as stroke and kidney failure cause 43 percent of all deaths recorded each year (Saleem et al., 2003). While in the United State, Hypertension accounts for more deaths, one million people die annually or more than 2500 die daily (Rose and Treadway, 1999). This disease is responsible for over 50 percent of all death in Pakistani males of working age and about 90 percent of all sudden deaths. Because it kills a person in the prime of his carrier, the economic and social burden of the disease is immense (Debakey and Gott, 1997). Control of blood pressure in patient with hypertension is necessary for cardiovascular morbidity and mortality (Walker et al., 2002) because high blood pressure if untreated, ultimately leads to the most common heart diseases like angina pectoris, stroke, heart attack, congestive heart failure and atherosclerosis (Ibarrola, 1996).

Therefore the present work have been planned to see/study the effect of plant on blood pressure using new and sensitive technologies and statistical analysis. Earlier the aqueous extract from Berberis vulgaris fruit was tested to evaluate its antihypertensive effects on DOCA-induced hypertension in the rats (Fatehi, et al., 2005). Present studies led to the determination of hypotensive activity of methanolic extract of Berberis vulgaris root.
MATERIALS AND METHODS

Plant Material
The roots of *Berberis 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
1. Uncrushed root pulp of *Berberis vulgaris* was extracted with methanol four times at room temperature. The extracts were combined together and evaporated on rotavapour. At the end yellowish brown colored residue (BRPM) was collected and used during the whole study.

2. Root bark of *Berberis vulgaris* was extracted with methanol four times at room temperature. The extracts were combined together and evaporated on rotavapour. A blackish brown and thick (BB) matter was settling down in the flask. That was used during the whole study.

All these extracts (BRPM and BB) and chemical were dissolved in distilled water for in vivo experiments. All extracts and chemicals were freshly prepared before each experiment.

Animals
Adult Sprague dawley rats (200-225g) 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 12h light/dark cycle. Each experimental group consisted of six animals. All animals were housed in an air-conditioned room at 23±1°C during the quarantine period.

Instruments for the extraction and recording of blood pressure parameters
Rotavapour (R114 Buchi) was used for the extraction of BRPM and BB. 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 (200-250 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. Drugs were injected (vol. 0.2-0.25 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, 60-3003) 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 (Adebouye 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 + 2.31% (mean + 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 BRPM and BB.

Methods for the determination of receptor activity
Normotensive Sprague-Dawley rats of either sex (200-250 g) were anaesthetized and their blood pressure was recorded through
carotid artery as described earlier. Atropine 10−4M/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 BRPM and BB administered one by one and change in blood pressure was monitored.

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

I. Effect of various doses of BRPM on Various Blood Pressure Parameters
The effect of intravenous administration of various doses of methanolic extract of root (BRPM) on various blood pressure parameters has been presented in Table 1.

Table 1
Effect of methanolic extract of root (BRPM) obtained at different doses on various BP Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0.5mg/kg</th>
<th>1mg/kg</th>
<th>10mg/kg</th>
<th>30mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Systolic BP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>109 ± 8.63</td>
<td>76.83 ± 12.3</td>
<td>122.75 ± 7.11</td>
<td>69.25 ± 9.04</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>103.83 ± 8.71</td>
<td>72 ± 11.93</td>
<td>121.75 ± 7.11</td>
<td>66.25 ± 10.18</td>
</tr>
<tr>
<td>MABP</td>
<td>105.53 ± 8.68</td>
<td>73.61 ± 12.05</td>
<td>122.08 ± 7.11</td>
<td>67.25 ± 9.79</td>
</tr>
</tbody>
</table>
Hypotensive Activity of Methanolic Extract of Berberis vulgaris

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significant (p<0.005) (Fig. 1). Hypotensive effect at 10mg/kg remained effective for a longer time period of 977.20±237.42 sec. It tries to return to control values but failed and the blood pressure dropped to 10.53±2.54%.

d) Effect of 30mg/kg BRPM on various Blood Pressure Parameters

BRPM at the dose of 30mg/kg was found to reduce the systolic pressure (126.14±9.24 to 77±11.34), diastolic (123.43±9.09 to 71.71±11.81) and mean arterial blood pressure (124.33±9.14 to 73.48±11.63) that was 40%, 43% and 42% respectively in comparison with their controls as shown in Fig. 1. Decreases in various blood pressure parameters were statistically significant (p<0.005). At the dose of 30mg/kg hypotensive effect remained for 851±441.17 sec than it tries to return to normal but failed and the blood pressure dropped to 11±4.16%.

In all dosage of BRPM tested maximum reduction in various blood pressure parameters was observed at 10mg/kg (Fig. 1) being the optimal dose.

II. EFFECT OF VARIOUS DOSES OF BB ON VARIOUS BLOOD PRESSURE PARAMETERS

The effect of intravenous administration of Methanolic extract of root bark (BB) on various blood pressure parameters has been presented in Table 2.

Table 2

Effect of methanolic extract of root Bark (BB) obtained at different doses on various BP Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>10mg/kg</th>
<th>30mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>116.33±2.48</td>
<td>77.33±0.41</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>115±2.83</td>
<td>74.33±0.41</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>MABP</td>
<td>115.44±2.71</td>
<td>75.33±0.24</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

The values have been presented as mean±S.E.M (n)

In all the dosage of BRPM tested maximum reduction in various blood pressure parameters was observed at 10mg/kg (Fig. 1) being the optimal dose.

Table 2

Effect of methanolic extract of root Bark (BB) obtained at different doses on various BP Parameters

<table>
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<td>(6)</td>
</tr>
</tbody>
</table>

The values have been presented as mean±S.E.M (n)
parameters were statistically significant (p<0.005) in comparison with their respective controls (Fig. 2). Hypotensive effect at 10mg/kg remained effective for a time period of 50.5±6.36 sec.

III. Result of receptor activity

The receptor activity was determined in rats results demonstrate that BRPM (10mg/kg), and BB (10mg/kg) has been found to decrease the mean arterial blood pressure that was 50% and 34% respectively when compared with their respective controls (Fig. 1, 2). While, on pre-treatment with Atr (10^-4 M) the BRPM (10mg/kg), and BB (10mg/kg) did not produce any reduction in Blood pressure. These behavior matches exactly to that of Acetylcsholine. The use of Ach (10^-6 M) has resulted in significant fall in MABP that was 46% than its respective control. Further, on pre-treatment with Atr (10^-3 M) the Ach (10^-6 M) did not show decline in MABP as shown earlier without Atr (10^-3 M) pre-treatment.

DISCUSSION

In present study methanolic extracts of Berberis vulgaris has been tested for its hypotensive effects in rats. The acute intravenous administration of different extract of Berberis vulgaris (BRPM, BB) 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. Physiologically, the reduction in blood pressure has been reported to be either due to their direct or indirect effect on pumping ability of heart (Kimura et al., 1986; Ebeigbe and Ezimokhai, 1988) or associated with decrease in peripheral resistance (vascular muscle tone leading to vasodilation) (Katzung, 1998). On these basis it is difficult to pin point the exact mechanism of extracts. Earlier it is suggested by Kamanyi et al., (1993) that the fall in blood pressure immediately after the administration of the plant extract was due to a possible direct relaxing effect of extract on vascular smooth muscle. It is reported that Ach cause a generalized vasodilation that is an indirect effect mediated by release of nitric oxide from vascular endothelial cells (Furchgott and Zawadzki 1980). That’s why 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 Berberis vulgaris, when administered alone have reduced the blood pressure. On the other hand the use of Ach and different extracts of Berberis 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 Berberis vulgaris is mediated through same receptor and mechanism as established for Ach indicating a role of cardiac muscarinic receptor in this response.

It is suggested that different extracts of Berberis vulgaris stimulates the muscarinic receptors located on the endothelial cells of the vasculature. This stimulation results in the release of endothelium-derived relaxing factors (EDRFs) or nitric oxide (Moncada et al 1991), that diffuse to smooth muscle cells and
cause their relaxation (Furchgott 1983; Vanhoutte et al 1995). It may therefore be concluded that muscarinic M2 receptors on cardiac muscles as well vascular dilation by endothelium derived relaxing factor may contribute to the cholinergic hypotensive effect of these extracts.

The present study suggests that different extracts of Berberis vulgaris possess significant hypotensive.

**CONCLUSION**

From the above discussion it is concluded that BRPM and BB is a physiologically and pharmacologically active drug because systolic, diastolic and mean arterial blood pressure is influenced by BRPM and BB used in the present study. Different methanolic extract of berberis vulgaris used in this study caused hypotensive effect by stimulating non-selective muscarinic receptors.

**REFERENCES**


