

Effects of hydro-ethanolic extract of *berberis vulgaris* fruit on rabbit isolated heart

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Received 9 Jan 2006; Revised 18 April 2006; Accepted 23 May 2006

ABSTRACT

Several therapeutic effects including antimicrobial, antidiarrhea, hepatoprotection and cardiostimulant for *Berberis vulgaris* have been described. In the present study, the effects of hydro-ethanolic extract of *Berberis vulgaris* on the rate and contractility of isolated heart were examined. The heart mounted on a modified Langendorff apparatus and circulation was perfused through aorta. Heart rate and contractility were determined in the presence of four concentrations of hydro-ethanolic extract (0.5, 1.0, 2.0 and 5.0 mg/100ml) and diltiazem, a calcium channel blocker (0.1, 1, 10 and 100 μ M) in comparison with baseline values in two different groups of experiments as follows: 1) Perfused heart with normal Krebs solution (group 1 experiments, n=10). 2) Perfused heart with calcium free Krebs solution (group 2 experiments, n=9). In group 1 only 3 highest concentrations of diltiazem showed significant reduction in heart rate ($p<0.05$ to $P<0.001$). However, 3 highest concentrations of diltiazem showed significant decrease and the last 2 concentrations of hydro-ethanolic extract increased heart contractility significantly ($p<0.01$ to $P<0.001$). In group 2 only the last concentration of diltiazem showed significant reduction in heart rate and contractility ($p<0.05$). The relationship between concentrations of hydro-ethanolic extract and heart rate in both group were negative ($p<0.01$ to $p<0.001$). However, there was positive correlation between concentrations of hydro-ethanolic extract and heart contractility. These results showed that of hydro-ethanolic extract of *Berberis vulgaris* has strong effect on heart contractility. The results of the present study may also indicate an activation of the calcium channel of isolated heart by the extract.

Keywords: *Berberis vulgaris*, Calcium channel blocker, Isolated heart, Rabbit.

INTRODUCTION

Heart disorders have become of serious medical concern, and are increasing throughout the world (1). Several drugs that are of therapeutic value in congestive heart failure, and those with positive inotropic effect such as cardiac glycosides and phosphodiesterase inhibitors (PDEI) have several side effects that limit their clinical usefulness. Therefore it is necessary to develop new and effective drug with fewer side effects (2). *Berberis vulgaris* L. (from berberidaceae family) is a shrub with 1 to 3 meters in height that grows in many area of world, including Iran (especially Khorasan). *Berberis vulgaris* contains berberine, oxyacanthine and other alkaloids such as berbamine, columbamine, malic acid, palmatine, jatrorrhizine and berberubine (3). Fruit, leaf and stem of this plant have been used for medical purposes including: hepatoprotection (4,5), cardiostimulant (6) and anti-microbial activity (7, 8). Recent studies demonstrated several cardio-vascular

effects of berberine and its derivatives such as positive inotropic on isolated guinea pig atria (6), negative chronotropic activity (9), antiarrhythmic activity (10), heart failure improvement in rat (11) and human (12), anti-hypertensive (13), vasodilator (14), neuroprotective (15), lowering resistance of peripheral vessels (16) and lowering cholesterol (17,18).

In the present study the effects of hydro-ethanolic extract from this plant on isolated rabbit heart was examined.

MATERIAL AND METHODS

Plant and extracts

Berberis vulgaris L. Fruits were collected from Ghaem (north-east of Iran) in autumn and identified by botanists in the herbarium of Ferdowsi University of Mashhad. Fruits were dried at room temperature in shadow. The hydro-ethanolic extract was prepared as follows: 50 g of fruits were crushed and then macerated in 80% aqueous-

ethanol for 72 h with occasional shaking. The was then removed at 35°C by rotary evaporation under reduced pressure. A total of 5 g of dried extract was obtained (10%), which was then dissolved in distilled water to obtain the concentration of 10% w/v.

Preparation of the isolated hearts

Rabbits of either sex were purchased from Razi Institute, Mashhad, Iran, with a body weight of 2.0 ± 0.3 kg and killed by a blow on the neck. The hearts were removed rapidly and immersed in ice-cold solution in an oxygenated petri dish. The heart was mounted on a modified Langendorff apparatus at a constant perfusion pressure of 70 mm Hg and coronary circulation was perfused through aorta (19, 20). The heart circulation was perfused through aorta with Krebs - Henseleit buffer solution (37°C, pH 7.4, aerated with 95% O₂ and 5% CO₂). The Krebs buffer solution contained the following ingredients (mmol/L): NaCl 118, NaHCO₃ 25.0, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, and glucose 11.0. The heart was first perfused with Krebs solution for stabilization in the Langendorff apparatus for 30 minutes and then effects of extracts of *Berberis vulgaris* and diltiazem were examined.

Protocol of experiments

The effects of four different concentrations of hydro-ethanolic extract of *Berberis vulgaris* (0.5, 1.0, 2.0 and 5.0 mg/100ml) and diltiazem (0.1, 1.0, 10 and 100 µM) on heart rate and contractility were examined. Each concentration was given for one-minute intracoronary infusion and the heart rate and contractility were recorded in the last 30 sec. During the experiments each heart served as its own control before injection of each solution. In the absence of pharmacological intervention, both heart rate and contractility were reproducible. The effects of different solutions were tested in 2 different experimental designs as follows:

1. Perfused heart with normal Krebs solution (group 1 experiments, n=10).
2. Perfused heart with calcium free Krebs solution (group 2 experiments, n=9).

The effects of the extract and diltiazem in two groups of experiments were examined in two different series of animal hearts with a 30-min resting period for heart between examination of the effect of each two solution while circulation was perfused with normal Krebs. In all experiments, the heart rate and contractility were recorded on a kymograph (ET8 G-Boulitt, Paris) and measured after fixation. The local Animal Research Committee of Mashhad University of Medical Sciences approved the experimental

extract was filtered and solvent of the extract procedures of the present study.

Statistical analysis

The data of the heart rate and contractility are expressed as mean \pm SEM and were compared using ANOVA test in each group. The effect of each concentration of hydro-ethanolic extract and diltiazem between two groups was compared using unpaired *t* test. The effect of hydro-ethanolic extract and diltiazem were related to the concentrations of the solutions using least square regression. Significance was accepted at $p < 0.05$.

RESULTS

Effect of hydro-ethanolic extract on heart rate

In group 1 experiments, the last 3 concentrations of diltiazem significantly reduced heart rate of rabbits compared to baseline values ($p < 0.05$ to $p < 0.001$, Table 1) but hydro-ethanolic extract did not change it significantly. In group 2 experiments, the last concentration of diltiazem and highest concentration (5.0 mg/100ml) of hydro-ethanolic extract reduced heart rate of rabbits significantly compared to baseline values ($p < 0.05$ Table 1)

Effect of hydro-ethanolic extract on heart contractility

In group 1 experiments, diltiazem in concentrations of 1.0, 10 and 100 µM reduced heart contractility of rabbit significantly compared to the baseline values ($p < 0.01$ to $p < 0.001$, Table 2). However, hydro-ethanolic extract in concentrations of 2.0 mg/100ml and 5.0 mg/100ml increased heart contractility ($p < 0.05$ to $p < 0.001$, Table 2) significantly. In group 2 experiments only diltiazem in concentration of 100 µM significantly reduced heart contractility of rabbit compared to baseline values ($p < 0.05$, Table 2).

Differences between the effect of diltiazem and hydro-ethanolic extract

In group 1, the effects of all concentrations of diltiazem on heart rate were significantly higher than hydro-ethanolic extract ($P < 0.01$ to $p < 0.001$, Fig. 1). In this group the effect of the last 3 concentrations of hydro-ethanolic extract on heart contractility were significantly greater than those of diltiazem ($P < 0.01$ to $p < 0.001$, Fig. 3).

In group 2, the effects of the last 2 concentrations of hydro-ethanolic extract on heart contractility were significantly greater than that of diltiazem ($P < 0.05$, Fig. 4).

Differences in the effects of extract between two groups of experiments

The baseline of the heart rate prior to addition of diltiazem and hydro-ethanolic extract in group 2

were significantly lower than those of group 1 ($p<0.001$ for all cases, Table 1). In addition the **Table 1.** The effect of four different concentrations of hydro-ethanolic extract from *berberis vulgaris* and diltiazem on heart rate of isolated rabbit hearts in two groups of experiments.

Experimental design	Group1	St. Dif vs B.	Group 2	St. Dif vs B.	St. Dif vs G.1
Baseline	207.51±11.42		106.34±8.12		$p<0.001$
0.1 μ M	178.5±12.31	NS	98.77±8.20	NS	$p<0.001$
1.0 μ M	143.60±13.37	$P<0.01$	91.38±8.96	NS	$P<0.001$
Diltiazem 10 μ M	89.39±11.88	$P<0.001$	65.43±13.72	NS	NS
100 μ M	46.45±6.30	$P<0.001$	57.15±12.49	$P<0.05$	NS
Baseline	207.51±11.42		106.34±8.12		$p<0.001$
0.5 mg%	200.36±13.16	NS	94.88±9.43	NS	$P<0.001$
Hydro-ethanolic 1.0 mg%	195.40±15.38	NS	83.02±9.25	NS	$P<0.001$
2.0 mg%	180.68±18.63	NS	66.50±11.04	NS	$P<0.001$
extract 5.0 mg%	162.45±22.38	NS	41.40±10.45	$P<0.05$	$P<0.001$

Values are presented as mean± SEM. Group 1: experiments on isolated rabbit's heart in the presence of normal Krebs solution (n=10). Group 2: experiments on isolated rabbit's heart in the presence of calcium free Krebs solution (n=9). St. Dif: Statistical difference, NS: non-significant difference, B: baseline.

heart rate in the presence of the first 2 concentrations of diltiazem and all concentrations of hydro-ethanolic extract were significantly lower than those of group 1 ($p<0.001$, Table 1). Baseline heart contractility prior to addition of diltiazem and hydro-ethanolic extract in group 2 were also significantly lower than those of group 1 ($p<0.01$ to $p<0.001$, Table 2). The heart contractility in the presence of 3 lower concentrations of diltiazem (0.1, 1.0 and 10 μ M) and all concentrations of hydro-ethanolic extract in group 2 were also significantly lower than those of group 1 ($p<0.01$ to $p<0.001$, Table 2).

Relationship between concentration and the effect of hydro-ethanolic extract and diltiazem

There was significant negative correlation between

heart rate and concentrations of diltiazem and hydro-ethanolic extract and positive correlation with concentrations of extract on heart contractility ($P<0.05$ to $p<0.001$, Table 3).

DISCUSSION

The results of the present study show that the effects hydro-ethanolic extract of *Berberis vulgaris* in the presence of normal Krebs solution is a concentration dependent but non-significant decrease in heart rate and a significant increase in heart contractility (group 1 experiments).

In group 1, the last 3 concentrations of diltiazem significantly reduced heart rate. In this group, the last 2 concentrations of hydro-ethanolic extract in contrast to diltiazem, which significantly inhibited the heart contractility increased it. The results of

Table 2. The effect of four different concentrations of hydro-ethanolic extract from *berberis vulgaris* and diltiazem on contractility of isolated of rabbit hearts in two groups of experiments.

Experimental design	Group1	St. Dif vs B.	Group 2	St. Dif vs B.	St. Dif vs G.1
Baseline	2.36±0.31		0.93±0.15		$p<0.001$
Diltiazem					
0.1 μ M	2.05±0.28	NS	0.88±0.12	NS	$p<0.001$
1.0 μ M	1.39±0.22	$p<0.01$	0.80±0.13	NS	$p<0.001$
10 μ M	0.93±0.13	$p<0.001$	0.74±0.09	NS	$p<0.01$
100 μ M	0.45±0.07	$p<0.001$	0.64±0.08	$p<0.05$	NS
Baseline	2.36±0.31		0.93±0.15		$p<0.001$
Hydro-ethanolic extract					
0.5 mg%	2.65±0.37	NS	0.91±0.18	NS	$p<0.01$
1.0 mg%	3.10±0.45	NS	1.00±0.20	NS	$p<0.001$
2.0 mg%	3.80±0.48	$P<0.05$	1.11±0.20	NS	$p<0.001$
5.0 mg%	4.40±0.49	$p<0.001$	1.20±0.30	NS	$p<0.001$

For abbreviations see Table 1.

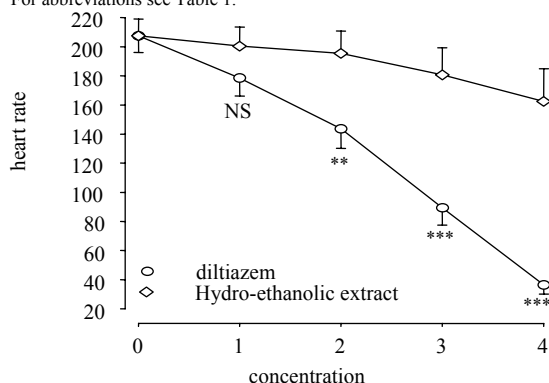


Figure 1. Concentration response curves of hydro-ethanolic extract from *berberis vulgaris*, and diltiazem, on rate of isolated rabbits heart in group 1 (in the presence of normal Krebs solution, n=10). Tested concentrations for extract were 0.5, 1.0, 2.0 and 5.0 mg% and for diltiazem 0.1, 1.0, 10 and 100 μ M. Statistical differences between the effect of extracts with that of diltiazem; NS: non-significant difference, *, p<0.05, **, p<0.01, ***, p<0.001.

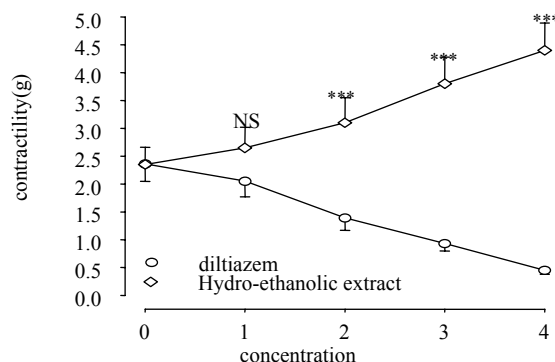


Figure 3. Concentration response curves of hydro-ethanolic extract from *berberis vulgaris*, and diltiazem, on contractility of isolated rabbits heart in group 1 (in the presence of normal Krebs solution, n=10). Tested concentrations for extract were 0.5, 1.0, 2.0 and 5.0 mg% and for diltiazem 0.1, 1.0, 10 and 100 μ M. Statistical differences between the effect of extracts with that of diltiazem; NS: non-significant difference, *, p<0.05, **, p<0.01, ***, p<0.001.

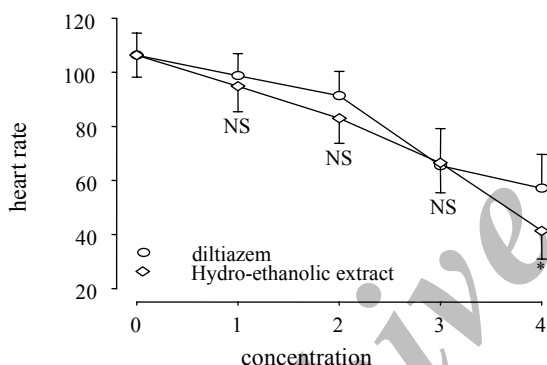


Figure 2. Concentration response curves of hydro-ethanolic extract from *berberis vulgaris*, and diltiazem, on rate of isolated rabbits heart in group 2 experiments (in the presence of calcium free Krebs solution, n=9). Tested concentrations for extract were 0.5, 1.0, 2.0 and 5.0 mg% and for diltiazem 0.1, 1.0, 10 and 100 μ M. Statistical differences between the effect of extracts with that of diltiazem; NS: non-significant difference, *, p<0.05, **, p<0.01, ***, p<0.001.

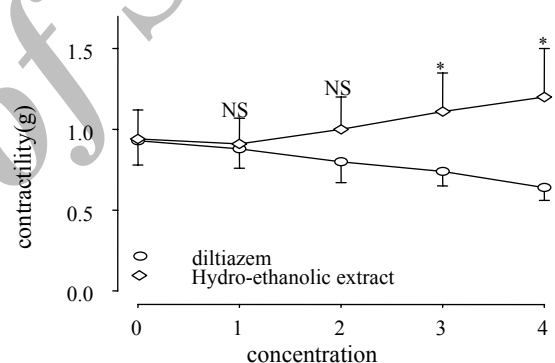


Figure 4. Concentration response curves of hydro-ethanolic extract from *berberis vulgaris*, and diltiazem, on contractility of isolated rabbits heart in group 2 experiments (in the presence of calcium free Krebs solution, n=9). Tested concentrations for extract were 0.5, 1.0, 2.0 and 5.0 mg% and for diltiazem 0.1, 1.0, 10 and 100 μ M. Statistical differences between the effect of extracts with that of diltiazem; NS: non-significant difference, *, p<0.05, **, p<0.01, ***, p<0.001.

group 1 indicated a small inhibitory effect of hydro-ethanolic extract of *Berberis vulgaris* on heart rate but potent and concentration dependent effect on heart contractility. The effect of hydro-ethanolic extract on heart rate was similar but on heart contractility was different from diltiazem. To explore effects of this plant extract on calcium channels, their effects on the heart rate and contractility of isolated heart were re-examined in the presence of calcium free Krebs solution (group 2 experiments). In this group similar to the effect of diltiazem only the last concentration of hydro-ethanolic extract reduced heart rate but caused a very small increase in heart contractility. In group

2, the effects of hydro-ethanolic extract on heart rate were similar to those of group 1, but different on contractility.

These findings indicated that hydro-ethanolic extract of *berberis vulgaris* might have effects on calcium channels of isolated rabbit heart. The significant negative correlation between concentrations of different solutions and their effects on heart rate and positive correlation of extract on heart contractility supports strongly concentration dependent effects of hydro-ethanolic extract and diltiazem. Therefore the results of the present study indicated that hydro-ethanolic extract of *berberis vulgaris* has a potent inotropic effect but

Table 3. Correlation (r) between the effects of hydro-ethanolic extract from *berberis vulgaris* on heart rate (HR) and contractility (Cont) of isolated rabbits heart with concentrations in two groups of experiments.

Experimental Groups	Hydro-ethanolic extract		Diltiazem	
	r	P value	r	P value
Group 1				
HR	-0.238	NS	- 0.442	P<0.001
Cont	+ 0.468	P<0.001	- 0.048	P<0.001
Group 2				
HR	- 0.545	P<0.001	- 0.236	P<0.05
Cont	+0.014	NS	- 0.359	P<0. 01

does not have significant chronotropic effect. The possible mechanisms of action of inotropes on heart are β -adrenoceptor stimulatory, calcium channel opening activity or effects on intracellular cAMP (2). If the hydro-ethanolic extract stimulate β -adrenoceptor, it should clearly increase both heart rate and contractility which was not observed in this study. In addition the lack of β -adrenoceptor stimulatory effect has been reported for the plant (2). The other possible mechanisms of action of the plant is an increase in cAMP levels, like phosphodiesterase III inhibition (21) or forskolin-like action (22), due to the positive inotropic effect of *berberis vulgaris*. On the other hand calcium sensitizing activity which may exerts a negative chronotropic action could be involved which blunts the positive chronotropic effect induced by elevation of cAMP (23-25).

The most possible mechanism of action of the extract might be calcium channel opening effect, because in the presence of calcium free Krebs,

extract did not increase heart contractility significantly.

Such a profile is novel and may represent a pharmacological action that is attractive under various conditions of cardiac impairment. In the case of the lack of activator of calcium (e.g: hypocalcemia) (26,27) which results in low systolic intracellular free calcium concentration, *berberis vulgaris* would increase the contractile response, thereby improving cardiac pumping function. Further work will be devoted to isolate and identify the active ingredient(s) in the fruits of *berberis vulgaris*.

In conclusion this study showed a novel and potent inotropic effect for *Berberis vulgaris* on isolated rabbit heart without tachycardia.

ACKNOWLEDGEMENT

This study was financially supported by the Vice Presidency of Research of Mashhad University of Medical sciences.

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