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A pharmacological study on *Berberis vulgaris* fruit extract

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Abstract

Berberis vulgaris fruit (barberry) is known for its antiarrhythmic and sedative effects in Iranian traditional medicine. The effects of crude aqueous extract of barberry on rat arterial blood pressure and the contractile responses of isolated rat aortic rings and mesenteric bed to phenylephrine were investigated. We also examined effect of the extract on potassium currents recorded from cells in parabrachial nucleus and cerebellum rejoins of rat brain. Administration of the extract (0.05–1 mg/100 g body weight of rat) significantly reduced the mean arterial blood pressure and heart rate in anaesthetized normotensive and desoxycorticosteron acetate-induced hypertensive rats in a dose-dependent manner. Concentration–response curves for phenylephrine effects on isolated rat aortic rings and the isolated mesenteric beds in the presence of the extract were significantly shifted to the right. Application of the extract (1–50 μg/ml) shifted the activation threshold voltage to more negative potentials, leading to an enhancement in magnitude of the outward potassium current recorded from cells present in rat brain slices of parabrachial nucleus and cerebellum. This effect on potassium current may explain the sedative and neuroprotective effects of barberry. The present data support the hypothesis that the aqueous extract of barberry has beneficial effects on both cardiovascular and neural system suggesting a potential use for treatment of hypertension, tachycardia and some neuronal disorders, such as epilepsy and convulsion.
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1. Introduction

Berberis vulgaris L. var. *asperma* Don (Berberidaceae) is a bush with yellow to brown coloured bark. The plant has obovate leaves, bearing pendulous yellow flowers in spring succeeded by oblong red coloured fruits (barberry). Various parts of this plant including its root, bark, leaf and fruit have been used as folk medicine for long in Iran. In Iranian traditional medicine several properties, such as antibacterial, antipyretic, antipruritic and antiarrhythmic activities for different parts of *Berberis vulgaris* have been reported with unknown mechanisms of actions (Zargari, 1983; Aynehchi, 1986; Nafissi, 1990). As it is increasingly believed

now that traditional medicines become more popular worldwide, there is accumulating evidence suggesting medicinal plants are unlimited reservoirs of drugs. The amazing structural diversity among their active components makes them a useful source of novel therapeutic compounds. Researchers with interest in natural products have intensified their efforts towards scientific evaluation of traditional medicines. Previous pharmacological studies on berberine, an isoquinoline alkaloid found in root and bark of *Berberis vulgaris*, demonstrated that it possessed potent vasodilatory and antiarrhythmic activity, and prolonged the action potential duration in Purkinje fibres and ventricular muscles (Chiou et al., 1991; Ricciopo, 1993; Kathleen, 2000). There is some evidence for antiinflammatory and antinociceptive effects of isoquinoline alkaloids found in *Berberis vulgaris* (Kupeli et al., 2002).

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Barberry is extensively used as food additive and its juice is recommended to cure cholecytitis (Zargari, 1983). Nevertheless, little pharmacological analysis has been performed on barberry. It has been shown that the crude extract of barberry has antihistaminic and anticholinergic activities (Shamsa et al., 1999). There are no reports based on scientific observation in the literature on the hypotensive activity of *Berberis vulgaris* fruit. Therefore, in the present study, the effects of *Berberis vulgaris* fruit extract on rat heart rate, rat blood pressure, contractility of rat aortic ring and perfusion pressure in rat isolated mesenteric bed were studied. Cardiovascular studies were performed on both normotensive and hypertensive rats to verify whether the extract had the ability to modify cardiovascular functions under pathological conditions. Also, since it has recently been reported that berberine blocked potassium currents in acutely isolated CA1 pyramidal neurons of rat hippocampus (Wang et al., 2004), it became of our interest to examine possible effect of the extract on potassium currents recorded from cells in parabrachial nucleus (PBN) and cerebellum regions of rat brain in vitro. To determine if barberry affects the heart via the CNS, brain slices including the PBN were used. We chose the PBN because it is a key interface between the brainstem and forebrain structures involved in autonomic regulation (Saleh and Connell, 1997, 2003). Cerebellum has its importance due to controlling motion and motor activity. The main objective for this study was to elucidate whether experimental observations in animal models and isolated tissues could support the positive health claims regarding barberry's therapeutic properties.

2. Materials and methods

2.1. Preparation of the extract

Berberis vulgaris fruit was collected from farms in the Birjand area of Khorasan, Iran and authenticated at the Herbarium of Mashhad School of Pharmacy (voucher no. 2003B15). Some 10 g dried barberry was extracted in boiling water (100 ml, for 5 min). The filtered aqueous extract was concentrated in a rotary vacuum evaporator and dried by exposure to hot air to yield 522 mg solid material. The stock solution of the extract (10 mg/ml) was prepared from this solid material on the day of experiment.

2.2. Induction of experimental hypertension

The experiments were performed in accordance with Animal (scientific procedures) Act of 1986 (Britain). For cardiovascular experiments, male Sprague–Dawley rats (purchased from Razi Institute, Mashhad, Iran) weighing between 200 and 250 g were used. Animals were housed in temperature- and humidity-controlled, light-cycled quarters and randomly divided into two groups. One group received saline injection (0.5 ml/kg, twice weekly, for 5

weeks, s.c., $n = 25$) whereas the other group were injected with desoxycorticosteron acetate (DOCA)-salt (20 mg/kg, twice weekly, for 5 weeks, s.c., $n = 25$) and NaCl (1%) was added to their drinking water. Using this protocol, hypertension was induced in the second group of rats. This model of hypertension (DOCA-salt-induced hypertension) has been used by several investigators (e.g. Somers et al., 2000; Fareh et al., 2000; Fatehi-Hassanabad et al., 2004).

2.3. Measurement of blood pressure and heart rate

Five weeks after the first saline or DOCA injection, rats were anaesthetised with sodium thiopental (30 mg/kg, i.p.) The right common carotid artery was catheterized for the measurement of blood pressure and heart rate, right and left jugular veins were cannulated for the administration of drugs and the extract, respectively. The trachea was cannulated and the animals were allowed to breathe spontaneously. Body temperature was recorded using a rectal thermistor probe and was maintained at $37 \pm 1\,^{\circ}\text{C}$ using an incandescent lamp placed over the abdomen. After 20 min stabilization period, arterial blood pressure (systolic, diastolic and mean pressure) and heart rate were measured.

2.4. Isolated aortic rings

The descending thoracic aorta was excised and trimmed free of adhering fat and connective tissues (Cordellini, 1999). The aorta was cut into rings (2 mm width) and vertically mounted in a 10 ml tissue bath containing Krebs solution of the following composition (in mM): 118.4 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$ and 11.1 glucose which was maintained at about $37\,^{\circ}\text{C}$ and bubbled with a mixture of 95% O_2 and 5% CO_2. The preparations were allowed to equilibrate for at least 1 h under a resting tension of 1 g. Changes in tension were recorded with an isometric transducer and displayed on a Washington recorder. Cumulative concentration–response curves for phenylephrine (0.001–1 μM) were constructed using tissues removed from normotensive and hypertensive rats in the absence and presence of the extract.

2.5. Isolated and perfused mesenteric beds

The abdominal cavity was opened by a mid-line incision through the linea alba and the mesenteric bed was excised using the procedure described previously (Fatehi-Hassanabad et al., 1995). The isolated mesenteric beds were placed on a Petri dish mounted in a heated water-bath (about $37\,^{\circ}\text{C}$) and perfused at a constant rate (5 ml/min; Gilson Minipuls 2) with Krebs solution bubbled with a mixture of 95% O_2 and 5% CO_2. The preparations were allowed to equilibrate for 30 min before recording the perfusion pressure. The cumulative concentration–response

curves for phenylephrine (0.001–1 μM) were constructed using the tissues removed from normotensive and hypertensive rats in the absence and presence of the extract.

2.6. Brain slice preparation

Male Sprage–Dawley rats (Charles River, Montreal, PQ, Canada) weighing 100–150 g were deeply anaesthetized with exposure to isoflurane (Abbott Laboratories, Saint-Laurent, PQ, Canada) and decapitated. After quick removal, the brain was immersed in ice-cold (2–3 °C) artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 126 NaCl, 2.5 KCl, 11 D-glucose, 18 NaHCO$_3$, 1.2 NaH$_2$PO$_4$, 1.3 MgCl$_2$, 2.5 CaCl$_2$ (pH 7.4), which was continuously bubbled with 95% O$_2$–5% CO$_2$. Coronal slices (300–400 μm thick) were prepared from tissue blocks of the brain containing parabrachial nucleus and cerebellar regions kept in the cold carbogenated aCSF with the use of a vibratome (model 1000 plus, TED PELLA Inc., Redding, CA, USA). Slices were incubated in aCSF at room temperature (22–25 °C) for at least 45 min prior to recording. A single slice was then transferred to a 500 μl recording chamber and submerged in a continuously flowing extracellular solution (2–3 ml/min) gassed with 95% O$_2$–5% CO$_2$.

2.7. Electrophysiology

Whole-cell patch-clamp recordings were performed on cells in PBN and cerebellum regions using an EPC-10 amplifier (Heka Elektronik) controlled by pulse software (Heka Elektronik). All electrophysiological experiments were carried out at room temperature (22–25 °C). A tight gigaohm seal on cells was obtained using micropipettes (4–6 MΩ) pulled from thin-walled (outer diameter, 1.5 mm) borosilicate glass capillaries (World Precision Instruments) by a vertical puller (model PIP5; Heka Elektronik). Composition of the intracellular (pipette-filling) solution (in mM) was: 130 K-gluconate, 6 NaCl, 10 HEPES, 2.5 Na-ATP, 0.1 Na-GTP, (pH adjusted to 7.2 by KOH). The extracellular solution contained (in mM) 145 NaCl, 2.5 KCl, 10 D-glucose, 26 NaHCO$_3$, 1.2 NaH$_2$PO$_4$, 1.3 MgCl$_2$, 2.5 CaCl$_2$ (pH adjusted to 7.4 HCl). Sodium and calcium currents were blocked, respectively, by adding tetrodotoxin (1 nM) and CdCl$_2$ (2 mM) into the extracellular solution. Electrophysiological data acquisition and analysis were performed using pulse and pulsefit softwares (Heka Elektronik), respectively.

2.8. Drugs

Deoxycorticosterone acetate (Iran-Hormone Co., Iran), phenylephrine-HCl and heparin sodium (Sigma Laboratories) and thiopental sodium (Biochemie GmbH, Viennea, Austria) were used in this study. All other chemicals used

for patch-clamp experiments were obtained from Merck Laboratories and Fisher Scientific, respectively, unless otherwise indicated. Phenylephrine solution was freshly prepared in normal saline. All stock solutions were kept at −20 °C.

2.9. Statistical analysis

All results are expressed throughout as means ± S.E.M. For comparison of responses to phenylephrine in aortic and mesenteric preparations, means were analysed by one-way analysis of variance (ANOVA) followed by a Tukey–Kramer multiple comparison test. Results expressed as percentages of control were considered to be non-parametric data and analysed by employing the Mann–Whitney U-test. Statistical significance was determined at $P < 0.05$.

3. Results

3.1. Effects of the extract on arterial blood pressure and heart rate

Systolic, diastolic and mean arterial blood pressures were significantly higher in DOCA-treated (hypertensive) rats (Table 1 and Fig. 1). The mean arterial pressure and heart rate in normotensive rats before injection of the extract were 99 ± 8 mmHg and 398 ± 9 beats per minute, respectively. In hypertensive rats, the corresponding values were 166 ± 5 mmHg and 509 ± 9 beats per minute, respectively. Within seconds after administration (i.v.) of the extract (0.005–1 mg/100 g body weight), a significant reduction in

Table 1
Haemodynamic effects of the extract

Dose of extract (mg/100 g BW)	Systolic pressure (mmHg)	Diastolic pressure (mmHg)
(A) Normotensive group		
0	115 ± 7	87 ± 6
0.001	111 ± 8	84 ± 6
0.005	91 ± 5*	72 ± 4*
0.010	67 ± 9**	35 ± 6**
0.1	46 ± 7**	31 ± 5**
1	42 ± 4**	26 ± 5**
(B) Hypertensive group		
0	186 ± 6	146 ± 5
0.001	183 ± 7	141 ± 9
0.005	141 ± 8*	123 ± 7*
0.010	109 ± 9**	93 ± 5**
0.1	61 ± 7**	44 ± 4**
1	52 ± 6**	27 ± 8**

Measured values for systolic and diastolic blood pressure before and after intravenous administration of various doses of the extract in normotensive and hypertensive (DOCA-treated) rats are expressed as mean ± S.E.M. of five experiments below.
* $P < 0.05$.
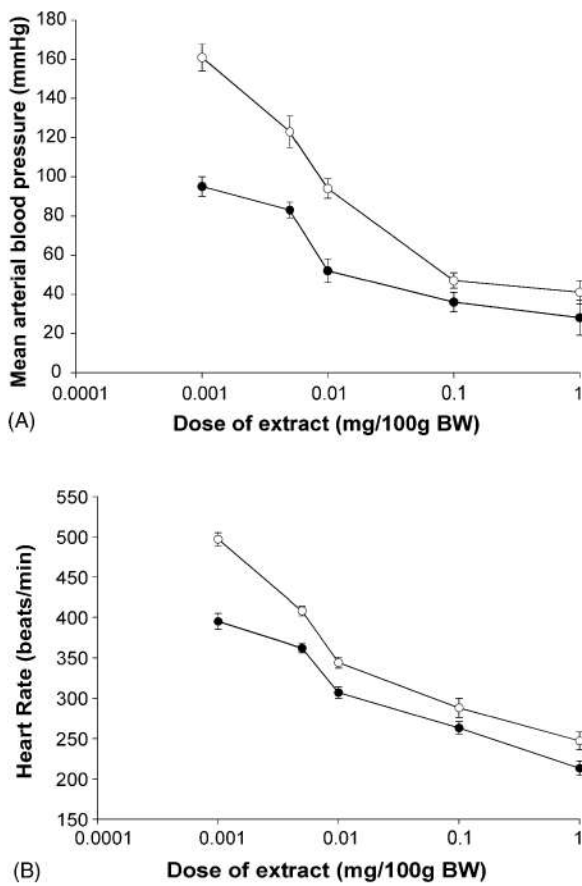** $P < 0.01$ vs. basal values (before extract injection).

Fig. 1. Decrease in mean arterial blood pressure (A) and in heart rate (B) in response to various doses of the extract in normotensive (●, $n = 5$) and experimentally hypertensive (○, $n = 5$) rats. All responses to doses >0.005 of the extract were significantly different from baseline values ($P < 0.01$).

these values compared to the baseline values were observed (Table 1 and Fig. 1).

3.2. Effects of the extract on aortic responses to phenylephrine

Prior to exposure of the aortic rings to phenylephrine, response of each preparation to 40 mM KCl was recorded. Then the contractile responses of these preparations to various concentrations of phenylephrine were measured as a percentage of response to 40 mM KCl. Addition of phenylephrine (0.001–10 μM) to the bath where the aortic rings were incubated in normal Krebs solution produced a concentration-dependent contraction (Fig. 2). After constructing concentration–response curve for phenylephrine, the preparations were incubated in Krebs solution containing the extract (50 μg/ml for 5 min) and exposed to several concentrations of phenylephrine. As shown in Fig. 2, incubation of the isolated aortic rings in the Krebs solution containing the extract caused a rightward shift in concentration–response curves in tissues isolated from normotensive and hypertensive rats.
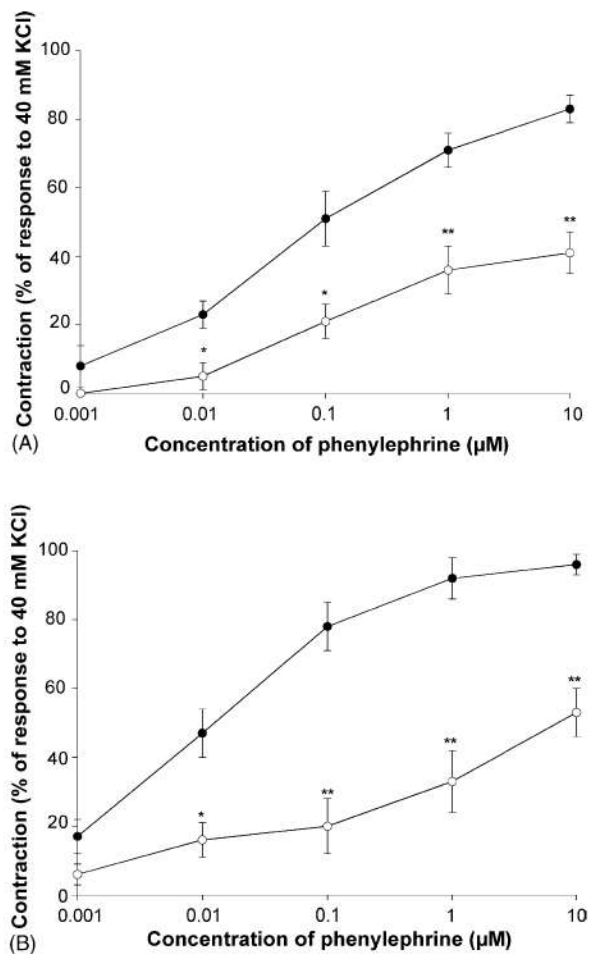

Fig. 2. Contractile responses (as percentage of contraction to 40 mM KCl) of the isolated aortic rings removed from saline-treated (A) and DOCA-salt treated (B) rats in the absence (●) and presence of 0.01 mg/ml extract (○) to various concentrations of phenylephrine (0.001–10 μM). One-way ANOVA performed on aortic responses to phenylephrine at all concentrations showed a significant difference between pre-treated and non-treated tissues with the extract $^{*}P < 0.05$, $^{**}P < 0.01$. Each value is the mean ± S.E.M. of five observations.

3.3. Effects of the extract on mesenteric vascular responses to phenylephrine

The contractile responses to various concentrations of phenylephrine in mesenteric vascular beds removed from DOCA-treated rats were similar to those of tissues removed from normotensive rats (Fig. 3). In the presence of the extract (50 μg/ml) responses of the mesenteric vascular beds removed from both normotensive and hypertensive rats to phenylephrine were significantly reduced. This inhibitory effect of the extract was also reflected in the perfusion pressure (Fig. 3). For instance, in the absence of the extract, the changes in basal perfusion pressure in response to phenylephrine (1 μM), in tissues removed from normotensive and hypertensive rats were about 95 mmHg. However, preincubation of the tissues with the extract (50 μg/ml for 5 min) decreased the magnitude of change in perfusion pressure
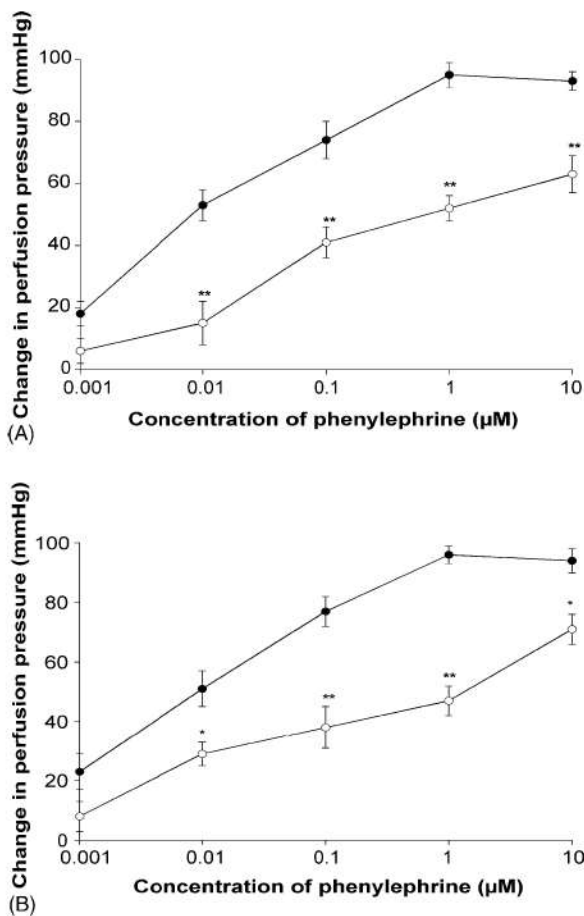
Fig. 3. Changes in perfusion pressure (mmHg) in response to various concentrations of phenylephrine (0.001–10 μM) recorded from the isolated mesenteric beds removed from saline-treated (A) and DOCA-salt treated (B) rats in the absence (●) and presence of 0.01 mg/ml extract (○). One-way ANOVA performed on responses to phenylephrine at all concentrations showed a significant difference between pre-treated and non-treated tissues with the extract ($^*P < 0.05$, $^{**}P < 0.01$). Each value is the mean ± S.E.M. of five observations.

significantly (about 50 and 40 mmHg in normotensive and hypertensive preparations, respectively, $P < 0.001$).

3.4. Effects of the extract on potassium currents

A series of experiments were performed to examine the effect of several concentrations of the extract on potassium outward currents. Voltage-clamp recordings were made from cells located in two different regions of rat brain slices, parabrachial nucleus and cerebellum.

Current–voltage curves were constructed under control conditions and following superfusion of the brain slices with the extract (0.5–50 μg/ml). Application of the extract (1–50 μg/ml) had a significant effect on the outward potassium currents recorded from cells in rat cerebellum and parabrachial nucleus. First, exposure to the extract (5–50 μg/ml) caused a marked increase in potassium current amplitude across a range of test potentials from −40 to +20 mV (Figs. 4 and 5). Second, application of the extract

(5–50 μg/ml) resulted in a hyperpolarizing shift in the threshold for current activation, from about −40 to −60 mV (Fig. 4). Fig. 5 summaries the effect of several concentrations of the extract on the amplitude of the currents elicited by a step depolarization from −70 mV (holding potential) to +20 mV (test potential).

4. Discussion

The present study was designed to evaluate the cardiovascular effects of the extract of *Berberis vulgaris* fruit and to investigate if it could affect cell excitability. Here, we have demonstrated that this extract had a potent hypotensive effect and was an opener of potassium channels activated by cell membrane depolarization.

As food additive, 0.5–1 g dried barberry per an adult person in a week is the common amount to be eaten. However, in pathologic conditions, such as hypertension, extract of 1 g dried barberry per day for at least 5 consecutive days is recommended (personal communication with some herbal medicine specialists in Iran). Our results concerning the efficacy of the extract obtained from in vivo experiments are comparable with dosages recommended in folk medicine. The extract caused a significant dose-dependent decrease in mean arterial blood pressure in normotensive and hypertensive rats. This effect was immediate upon intravenous administration of the extract. The immediate reduction in blood pressure suggests that both heart function and blood vessels contractility are affected by the extract. This is strongly supported by our observations on the effects of the extract on heart rate, contractile responses of the aortic rings and the isolated mesenteric beds to phenylephrine. This also suggests a central effect since both blood pressure and heart rate have been decreased in a parallel pattern.

Hypertension induced by DOCA treatment causes an endothelial dysfunction both in the isolated aortic rings and perfused mesenteric bed (Fatehi-Hassanabad et al., 2004). Since the extract did not discriminate between normotensive and hypertensive tissues for the vasodilator effect, our data clearly demonstrated that this effect was not endothelium-dependent. A similar inhibitory effect of tetrandrine and berbamine, alkaloids found in *Berberis vulgaris* bark, on responses of perfused rat mesenteric artery to phenylephrine has been reported (Kwan, 2002). Based on this study, it was proposed that their inhibitory effect on responses to phenylephrine might be due to alpha-adrenoreceptor antagonism. It has been shown that berbamine also prevented ventricular fibrillation probably through inhibition of sodium and calcium overload (Zhang et al., 1992). Augmentation of potassium currents caused by the extract may contribute to its vasodilatory and antiarrhythmic effects. There is evidence that phenolic compounds are present in barberry (Pozniakovskii et al., 2003). It has been shown that phenolic compounds increase potassium channels activity (Kim et al., 2000; Lin et al., 2004). Vasorelaxation induced by some polyphenolic com-
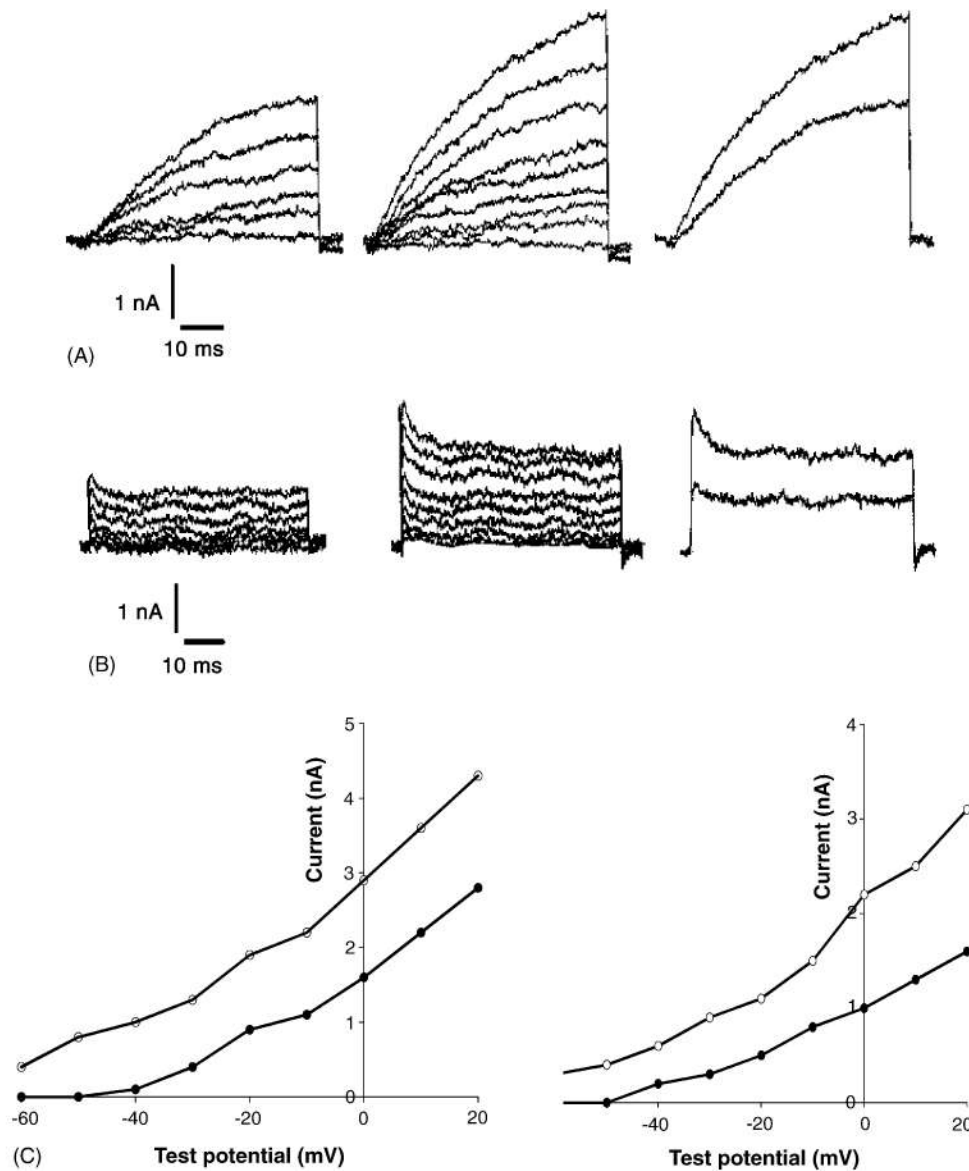
Fig. 4. Effects of the extract on potassium currents recorded from cells located in two different regions of rat brain slice. (A) Whole-cell potassium currents recorded from a cell located in parabrachial nucleus evoked by voltage-clamp depolarizing steps from −30 to +20 mV (holding potential: −70 mV) in control conditions (left panel) and in the presence of 50 μg/ml extract (middle panel), the superimposed traces of maximum currents (control and after exposure to the extract) (right panel). (B) Whole-cell potassium currents recorded from a cell located in cerebellum, currents were evoked by voltage-clamp depolarizing steps from −50 to +20 mV (holding potential: −70 mV). (C) Current–voltage (I–V) curves for experiments shown in (A) and (B) (left panel and right panel, respectively) in control conditions (●) and in the presence of the extract (○). Note the augmentation of potassium currents at all test potentials and a shift to the left of I–V curves for both cells.

pounds were inhibited by potassium channel blockers (Kim et al., 2000). However, other mechanisms of action such as blockade of ryanodine-sensitive calcium channels and antagonizing adrenoreceptors cannot be excluded from underlying mechanisms involved in the extract cardiovascular effects.

Although recently, it was shown that berberine, an alkaloid isolated from bark and root of *Berberis vulgaris*, exerted an anxiolytic effect in mice (Peng et al., 2004), to the best of our knowledge, no experimental evidence exists for sedative effects of the *Berberis vulgaris* fruit extract. Enhancement of potassium currents recorded from PBN and cerebellar

regions of rat brain induced by the extract may suggest an inhibitory effect on cell excitability. There is some evidence that anticonvulsant drugs may act through activation of potassium channels (Main et al., 2000). Further investigation is required to determine whether the hypotensive effect of the extract is solely due to its effect on potassium channels activity or to some other antagonistic action on adrenoreceptors or serotonergic receptor. In conclusion, our data suggest an endothelium-independent hypotensive effect and an inhibitory action on cell excitability for the extract. The functional studies on cardiovascular system and
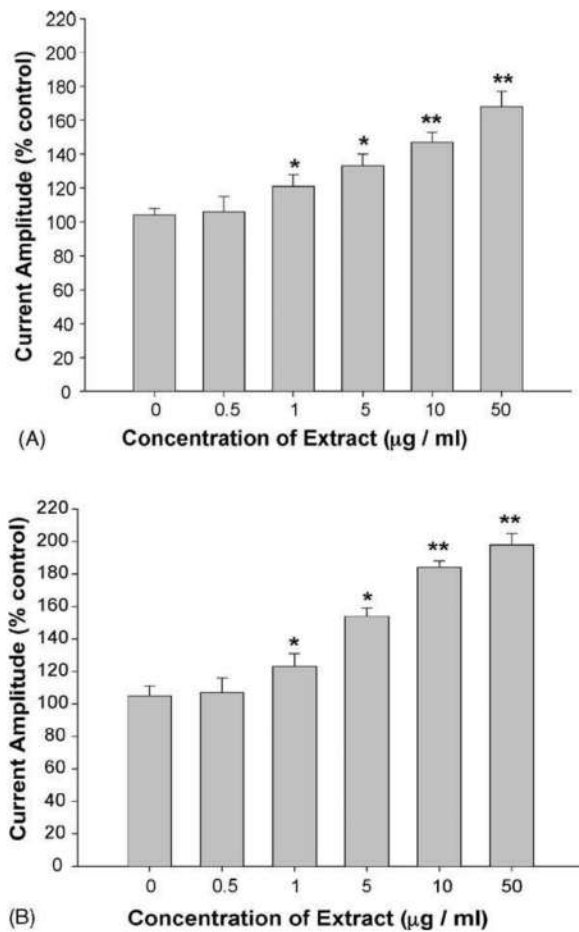
Fig. 5. Concentration–response relations for the effects of extract on maximum potassium currents recorded from cells located in parabrachial nucleus (A) and cerebellum (B) of rat brain slices evoked by a step depolarizing pulse from the holding potential ($-70\,mV$) to a test potential of $+20\,mV$, $n = 5$ cells for each concentration, $^*P < 0.05$ and $^{**}P < 0.01$ vs. control, respectively.

potassium channel activity reported here could be supportive information for growing interest in *Berberis vulgaris* fruit in Iranian folk medicine.

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