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Original Article

Evaluation of mechanism for antihypertensive and vasorelaxant effects of hexanic and hydroalcoholic extracts of celery seed in normotensive and hypertensive rats

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ABSTRACT

Celery (*Apium graveolens* L., Apiaceae) is one of the popular aromatic vegetables and part of the daily diet around the world. In this study, aqueous-ethanolic and hexane extracts of celery seed were prepared and the amount of *n*-butylphthalide, as an active component, was determined in each extract. Then the effects of hexanic extract on systolic, diastolic, mean arterial blood pressure and heart rate were evaluated in an invasive rat model. The vasodilatory effect and possible mechanisms of above mentioned extracts on aorta ring were also measured. High performance liquid chromatography analysis revealed that hexanic extract contains significantly higher amounts of *n*-butylphthalide, compared to aqueous-ethanolic extract. The results indicated that hexanic extract significantly decreased the systolic, diastolic, mean arterial blood pressure and heart rate in normotensive and hypertensive rats. Our data revealed that celery seed extract exerts its hypotensive effects through its bradycardic and vasodilatory properties. Moreover, the active components in celery seed extracts could induce their vasodilatory properties through Ca^{2+} channel blocking activity in endothelial and non-endothelial pathways and particularly by interference with the extra or intracellular calcium.

Introduction

Nowadays, high blood pressure (BP), also called hypertension (HTN), is one of the most common causes of early death in symptom-free adults. Uncontrolled HTN increases the risk of serious health problems and is considered a major risk factor for stroke, heart attack or myocardial infarction (MI), ischemia or brain atrophy, blindness and kidney disease (Zimmet et al., 2001). The increase in blood pumps from the heart and the narrowing of the arteries normally lead to HTN. Moreover, there is a direct relationship between systolic blood pressure (SBP) and hemorrhagic stroke (Kanter et al., 2004). In fact, the risk of hemorrhagic stroke and heart attack is higher when SBP increases by more than 175 mmHg. Furthermore, BP increases over the years and may affect all individuals while, by controlling high blood pressure, some disorders such as dyspnea, erectile dysfunction, and nocturia could be treated (Tanner and Tanner, 2001).

Due to different mechanisms involved in etiology of HTN, several antihypertensive chemical groups have been applied in controlling the BP in patients. Unfortunately, no clear treatment protocol has been introduced for management of HTN. The chemical agents are able to only control the HTN disorder in patients. Regarding the several side effects of pharmacologic agents such as hypotension, orthostatic hypotension, reduced glucose tolerance, increased plasma cholesterol and sexual dysfunction, herbal medicines have been recently much more considered for HTN and cardiovascular diseases management. Consequently, researchers are motivated to find some traditional herbal extracts or active ingredients with lower side effects and toxicities than chemical drugs for controlling hypertension. Herbal plants have been widely used in folk medicine for treatment of many disorders such as cardiovascular diseases, diabetes, jaundice, hypertension, and cancer, especially in eastern countries (Wiyakrutta et al., 2004; Modak et al., 2007; Chatterjea and Shinde, 2012).

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Therefore, many studies have focused on the effects of herbal medicines and their active components such as green tea (Bogdanski et al., 2012), garlic (Reinhart et al., 2008), *Nigella sativa* (Dehkordi and Kamkhah, 2008) and pepper (Kwon et al., 2007) in HTN treatment. Celery (*Apium graveolens* L.) species belong to the family of Apiaceae have some beneficial effects on hypertension (Moghadam et al., 2013). Celery leaves are odd-pinnate with dentate leaflets of 3–6 cm long and 2–4 cm broad on a central stem (Zhou et al., 2009). Celery has many constituents, one of them is n-butylphthalide (NBP) along with sedanolide which provide the aroma and taste of celery, respectively (Sowbhagya, 2014). Some researchers demonstrated NBP, extracted from other herbal plants, had some antihypertensive effects in animal models (Dimo et al., 2003; Zhu et al., 2015). According to the previous studies, the most content of active compounds were found in the celery seeds, compared to other parts of the plant (the roots and leaves) (Fazal and Singla, 2012). Other workers have demonstrated that celery seeds and its active ingredients have different therapeutic properties such as hepatoprotective activity (Niaz et al., 2013), cognitive enhancement (Peng et al., 2010), neuroprotective effects (Peng et al., 2012), anti-inflammatory activity (Powanda and Rainsford, 2011) and antioxidant properties (Momin and Nair, 2002).

Admittedly, vasodilation means the widening of blood vessels by smooth muscle cells relaxation within the vessel walls. In the other words, blood pressure reduction results from dilation in arterial blood vessels (mainly the arterioles). The response may be arise from local processes in the surrounding tissue (intrinsic) or originated from hormones or the nervous system (extrinsic). On the other hands, different drugs have different functions to reduce the BP. Some intracellular stimuli result in blood vessels vasodilation. Different mechanisms are involved in performing vasodilatory effects. Some of them are nitrovasodilators that leads to vasodilation by nitric oxide donation like nitroglycerine (Moncada et al., 1991). While some others act as calcium channel blockers that change the cell's membrane potential and make it more negative by hyperpolarization (Pei et al., 2000). Furthermore, some drugs such as prostacyclin are acted by cAMP-mediated (Li et al., 2004). Angiotensin converting enzyme inhibitors and angiotensin II receptor blockers are from other antihypertensive drugs (Bickerton and Buckley, 1961). Some drugs may induce their antihypertensive effects through diuretic activities (Golalipour et al., 2011) and some antihypertensive agents are from beta receptor blockers (Bristow, 2000). According to our previous study, the celery seed hexanic and hydroalcoholic extract decrease the BP and heart rate (HR) while there was no evidence to demonstrate what the main mechanism of this reduction is.

In the present study, antihypertensive effects of celery seed extracts were investigated in an invasive model of normotensive and hypertensive rats. Vasodilatory properties of celery seed extracts and their related mechanisms were also evaluated in isolated aorta artery. The results were promising and indicated that celery seed extracts can be considered as an antihypertensive agent in future clinical trial studies.

Materials and methods

Chemicals

The celery seeds (*Apium graveolens* L., Apiaceae) were purchased from Imam Pharmacy (Mashhad, Iran) identified and authenticated by Dr. Abbas Zojaji (Pharm. D., PhD) in herbarium of Department of Pharmacology, Mashhad University of Medical Sciences. A voucher specimen (293-0107-18) has been deposited at the herbarium of the Department of Pharmacology in Mashhad University of Medical Sciences. Methanol, ethanol (96%), liquid paraffin, dimethyl sulfoxide (DMSO), potassium chloride, sodium hydrogen phosphate, D-glucose, sodium chloride, sodium bicarbonate, calcium chloride dihydrate, magnesium sulfate heptahydrate and *n*-hexane were obtained from Merck Company (Darmstadt, Germany). Acetylcholine, L-NAME, nifedipine, indomethacin and phenylephrine hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO). Xylazine and ketamine obtained from Loughrea Corporation (Galway, Irland). Normal saline (NS) (0.9%) and heparin (5000 IU/ml) were purchased from Samen Corporation (Iran, Mashhad) and Caspian Tamin Pharmaceutical Corporation (Rasht, Iran), respectively. Formalin and distilled water were obtained from Hakim Pharmaceutical Corporation (Tehran, Iran).

Preparation of celery seed extracts

Dry celery seeds (50 g) were powdered and suspended in the solvent (250 ml) [water–ethanol (20/80, v/v) or hexane] at room temperature. Then each solvent was shaken for 48 h in the darkness and filtered through the cotton filter to separate the coarse particles, whereas the clear and green suspension was centrifuged ($3000 \times g$ for about 15 min). The supernatant was separated and evaporated to dryness in the darkness at room temperature. Finally, the aqueous-ethanolic extract (dry powder) and hexanic extract (green oil) were remained and kept in refrigerator.

High performance liquid chromatography (HPLC) analysis

Chromatographic analysis of NBP was performed using a Younglin Acme 9000 system (South Korea), consisting of an SP930D solvent delivery module, SDV50A solvent mixing vacuum degasser, column oven CTS30, UV730 dual wavelength UV/VIS detector and ODS C18, 4.6 mm × 150 mm, 5 μm column. The data analysis was performed by Autochro 3000-software. The UV detection was carried out at 230 nm. The flow rate was 1 ml/min, the volume of injection was 20 μl and the column temperature was fixed at 30 °C. A gradient method was applied in which the mobile-phase composition was changed from 20% methanol in water to 80% in 20 min run time. A fixed concentration of all extracts (50 μg/ml) were prepared in methanol and applied for HPLC analysis. The concentrations of NBP were measured from the area under curve (AUC) by comparing them to an NBP standard solution.

In vivo *studies*

Thirty male Wistar rats (180–220 g) were obtained from the animal facilities of the Pharmaceutical Research Center, Bu Ali Research Institute, Mashhad University of Medical Sciences. The animals were housed in cage with a 12 h light/12 h dark cycle at 21 ± 2 °C and had free access to food and water. The experimental procedures for all animals were in accordance with the Ethics Committee Acts of Mashhad University of Medical Sciences (protocol number 920129).

The rats were randomly divided equally into four groups (Table 1). Two groups (12 rats) were normotensive and two groups (12 rats) were hypertensive in this study. To induce hypertension,

Table 1
Classification of experimental groups: *in vivo* study.

Groups	DOCA	Hexanic extract (2.5, 5, 7.5, 10, 12.5 mg/kg)	Nifedipine (1, 2, 4 mg/kg)
1	*	*	–
2	*	–	*
3	–	*	–
4	–	–	*

1% NaCl was added into the drinking water and deoxycorticosterone acetate (DOCA) (20 mg/kg), as a synthetic mineralocorticoid derivative, was injected (subcutaneously) into each rat two times a week for one month. The normotensive rats received tap drinking water without DOCA injection. All animals were fed standard laboratory rat chow. Systolic blood pressure (SBP) was measured weekly and before surgery by the standard tail-cuff method (pneumatic transducer SP844, PowerLab 8/30, ADInstrument ML870, Australia) in conscious restrained rats. An invasive model was applied for evaluation of hypotensive study of extracts in male rat. Approximately 20 min before experimental procedure, the animal was placed and relaxed at room temperature (25 °C). A mixture of ketamine (60 mg/kg) and xylazine (6 mg/kg) was injected intraperitoneally for anesthetizing the animals. Briefly, after a midline neck incision, the right common carotid artery and then the left jugular vein were cannulated with heparinized polyethylene catheter for BP monitoring and drug administration, respectively. The surgical method was in accordance with the technique applied in our previous published article (Mohajeri et al., 2011). The effect of each vehicle was explored before administration of drugs and extracts. For this purpose, 50 μl of each vehicle (DMSO and paraffin) was tested after intrajugular administration. Each dose was dissolved and adjusted in 50 μl vehicle and injected intrajugularly. Nifedipine samples were prepared in DMSO, whereas hexanic extract was prepared in paraffin. The tubes and transducer were heparinized to prevent blood clotting. Afterwards, the cannulated right common carotid artery was connected to the pressure transducer of PowerLab system (PowerLab 8/30, ADInstrument ML870), data acquisition system, and all the data were viewed and recorded by Chart 7 computer software. The heparinized (50 IU/ml) polyethylene tube (PE-50) was applied for vessel cannulation. The tube was connected to a pressure transducer (SP844, Capto) for sending and monitoring blood pressure signals. The instrument was first calibrated before drug administration. HR, SBP, DBP, and mean arterial blood pressure (MABP) (1/3 SBP plus 2/3 DBP) were determined every 10 min intervals between different doses of experiment. The measurement was performed 20 min after surgery to obtain the most accurate results.

Ex vivo *studies*

Male Wistar rats were anesthetized with intraperitoneal injection of a mixture of ketamine (60 mg/kg)/xylazine (6 mg/kg) and their chests were opened to isolate the thoracic aorta. Then the aorta superficial connective tissue or adhering fat was cleaned and cut into 4–5 mm long ring segments. All rings were placed immediately under 2 g resting tension on stainless steel hooks in standard 20 ml organ baths filled with Krebs–Henseleit solution (KHS). The Krebs solution was prepared freshly just before use in the cold deionized water with composition of: 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4{\cdot}2H_2O$, 1.2 mM $KH_2PO_4{\cdot}2H_2O$, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$ and 11.1 mM glucose, continuously aerated with a mixture of 95% O_2, 5% CO_2 and kept at 37 °C with a pH of 7.4. Krebs solution provided appropriate factors and environment for survival of tissue. Tension was measured isometrically by a force transducer and recorded continuously by a transducer amplifier (AD Instruments).

The rings were equilibrated and pre-contracted for 60 min at a resting tension with 0.1 ml of phenylephrine 10^{-6} M, and then the changes in tension were measured after cumulative addition of different doses of aqueous-ethanolic (0.250, 0.5, 1 mg/ml) or hexanic (0.25, 0.125, 0.0625 mg/ml) celery seed extracts to the organ bath chambers. Hexanic extract doses was prepared in DMSO-NS (30/70, v/v) and emulsified with tween 80 in organ bath. The involvement of endothelial dependent vasodilation pathways were evaluated by adding different ingredients such as potassium chloride (80 mM), indomethacine (10^{-5} M) and L-NAME (10^{-5} M) to the organ chamber with presence or absence of phenylephrine (10^{-6} M) and different cumulative concentrations of aqueous-ethanolic or hexanic extracts. Finally, the potency and efficacy of celery seed extracts were compared to nifedipine as a positive control. In other words, 10^{-6} M phenylephrine was applied to make tension in the aorta rings and then vasorelaxant effects of nifedipine (4×10^{-6}–4×10^{-2} mg/ml) and celery seed extracts were evaluated.

Non-endothelial dependent vasodilation pathway was studied after mechanically removing the endothelium in aorta rings, by gently rubbing the lumen with the tips of fine forceps and induction of tension by phenylephrine (10^{-6} M) to the tissue. Acetylcholine (10^{-6} M) was added to the bath solution and possible vasorelaxation was tested. No relaxation by acetylcholine in pre-contracted tissue (by phenylephrine) indicated successful destruction of the endothelium layer in the vessel. The modified tissue with no endothelium was exposed to the suitable tension after immersing in the freshly made Krebs solvent for 20 min, phenylephrine (10^{-6} M) was added and different doses of aqueous-ethanolic (0.250, 0.5, 1 mg/ml) or hexanic extracts (0.250, 0.125, 0.0625 mg/ml) were cumulatively added.

Statistical analysis

In vivo statistic evaluation was performed using one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test, whereas the results obtained in isolated aorta experiments were compared by *t*-student test. The data was reported as mean ± SEM and *p* value less than 0.05 was considered as a significant difference between groups.

Results

Amounts of NBP in celery extracts

The chromatographic analysis indicated that NBP contents in hexanic aqueous-ethanolic (20/80, v/v) were 0.42% and 0.09% of extract, respectively (Fig. 1). NBP is an oily compound which could be dissolved in nonpolar solvents such as hexane. Thus its concentration in hexanic extract would be higher than that in aqueous-ethanolic (20/80, v/v) extract. According to this data

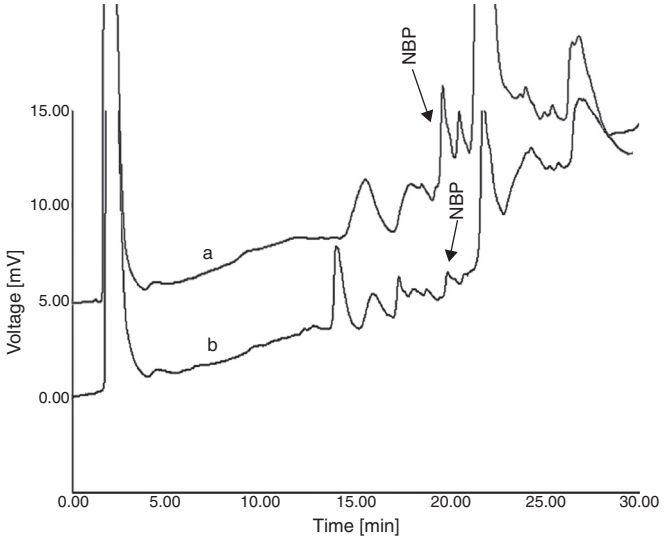

Fig. 1. Chromatograms of hexanic extract (50 μg/ml) (a) and aqueous-ethanolic extract (50 μg/ml) (b) of celery seeds.

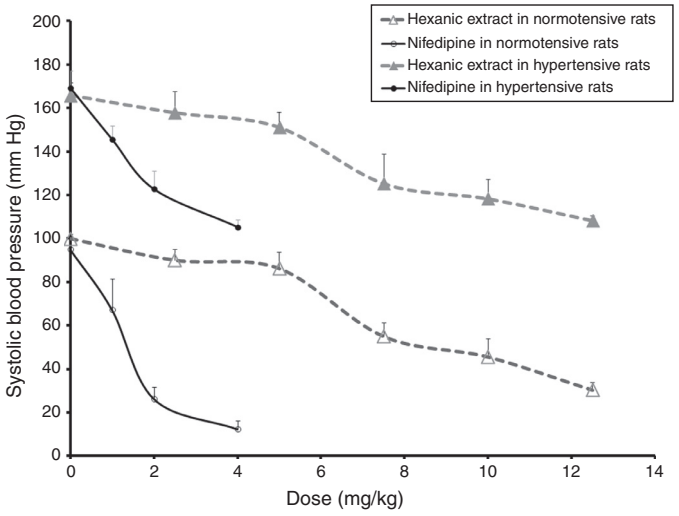

Fig. 2. Systolic blood pressure (SBP) after intra jugular administration of different doses of hexanic celery seed extract and nifedipine in an invasive model of normotensive and hypertensive rats. Each point is mean ± SEM ($n = 6$).

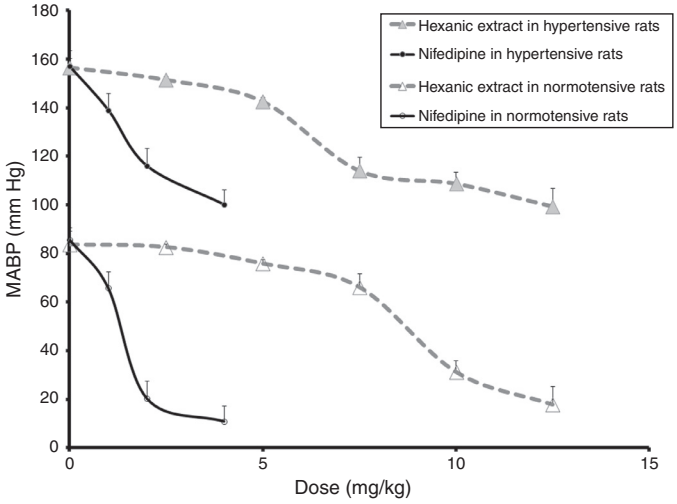

Fig. 4. Mean arterial blood pressure (MABP) after intra jugular administration of different doses of hexanic celery seed extract and nifedipine in an invasive model of normotensive and hypertensive rats. Each point is mean ± SEM ($n = 6$).

other nonpolar agents would be significantly higher than those in aqueous-ethanolic (20/80, v/v) extract.

Effect of celery seed extract on BP and HR

Intrajugular administration of vehicles (50 μl) did not significantly change the BP and HR at all groups ($p > 0.05$). Figs. 2–5 reveal that *n*-hexane extract, particularly at 7.5, 10 and 12.5 mg/kg, significantly decreased SBP, DBP, MABP and HR in normotensive and hypertensive rats. Nifedipine as a calcium blocker agent and positive control reduced SBP, DBP and MABP, whereas increased HR in both normotensive and hypertensive animals. Administration of hexanic extract of celery seed at 12.5 mg/kg made a 69.5%, 80%, 79.2% and 25% reduction in SBP, DBP, MABP and HR, respectively in normotensive rats, whereas in hypertensive animals the reduction values were 34.6%, 37.6%, 36.5% and 33% in SBP, DBP, MABP and HR ($p < 0.001$). The efficacy of the hexanic extract in reducing the BP was comparable to that of nifedipine as a positive control, whereas its potency was significantly less than nifedipine. For example, 4 mg/kg nifedipine made a 36.5% reduction in MABP in hypertensive rats which is similar to the hypotensive effects of 12.5 mg/kg hexanic extract administration.

Effect of celery seed extracts on rat isolated aortic rings

After vasoconstriction induction by phenylephrine (10^{-6} M) and 80 mM KCl, celery seed extract doses were added cumulatively to the organ bath and the vascular relaxation percent was measured. Although, both hexanic and hydroalcoholic extracts made 100% relaxation in aorta ring, the potency of hexanic extract was significantly more than that of hydroalcoholic extract (Fig. 6). Maximum relaxation was observed with 0.25 mg/kg hexanic extract, whereas the similar response was obtained with 1 mg/kg hydroalcoholic extract of celery seed. In order to block the prostaglandins and nitric oxide production pathways, the tissue was incubated with indomethacin (10^{-5} M) or L-NAME (10^{-6} M) before vasoconstriction induction by phenylephrine (10^{-6} M) (Figs. 7 and 8). In presence of indomethacin and L-NAME, maximum relaxation was obtained after using cumulative doses of hexanic and hydroalcoholic extracts of celery seed. Fig. 7 indicated that indomethacin

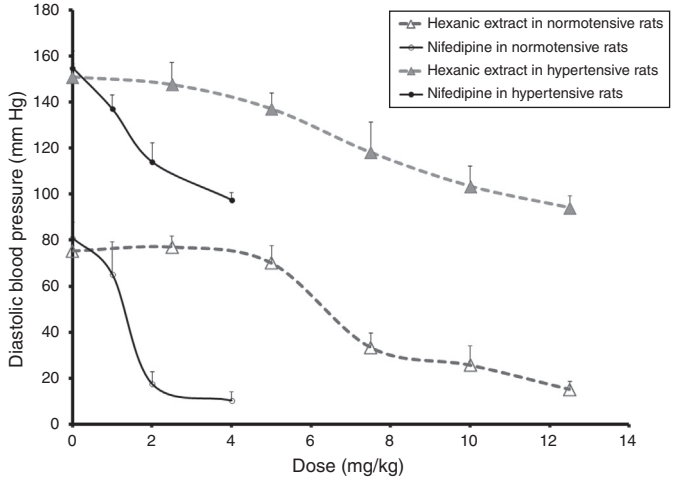

Fig. 3. Diastolic blood pressure (DBP) after intra jugular administration of different doses of hexanic celery seed extract and nifedipine in an invasive model of normotensive and hypertensive rats. Each point is mean ± SEM ($n = 6$).

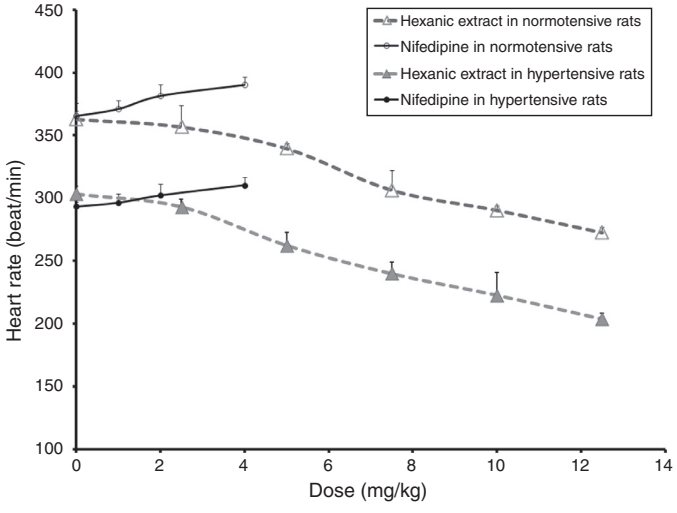

Fig. 5. Heart rate (HR) after intra jugular administration of different doses of hexanic celery seed extract and nifedipine in an invasive model of normotensive and hypertensive rats. Each point is mean ± SEM ($n = 6$).

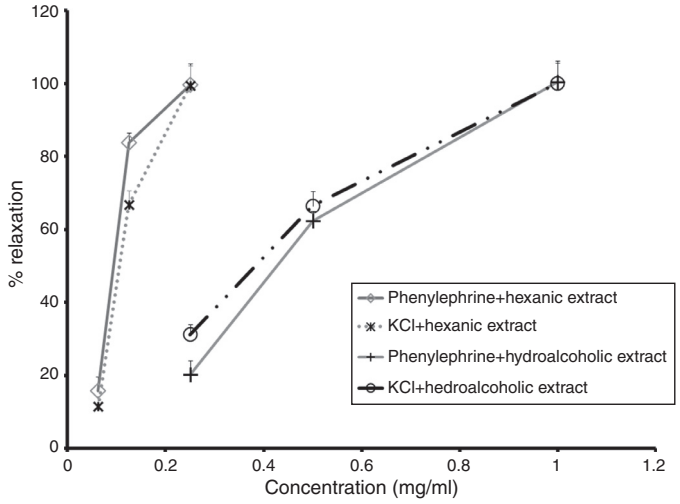

Fig. 6. Vasodilatory effect of cumulative doses of hexanic and hydroalcoholic extracts of celery seed on previously constricted aorta ring tissue by phenylephrine (10^{-6} M) and KCl (80 mM). Each point is mean ± SEM ($n = 6$).

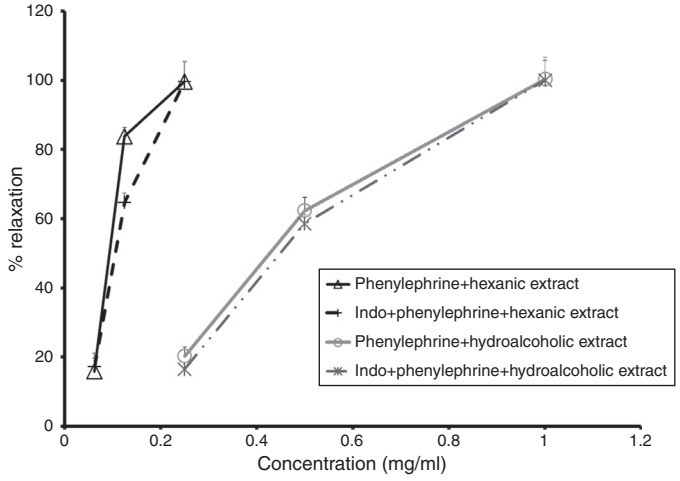

Fig. 7. Vasodilatory effect of cumulative doses of hexanic and hydroalcoholic extracts of celery seed on previously constricted aorta ring tissue by phenylephrin (10^{-6} M) and indomethacin (10^{5} M). Each point is mean ± SEM ($n = 6$).

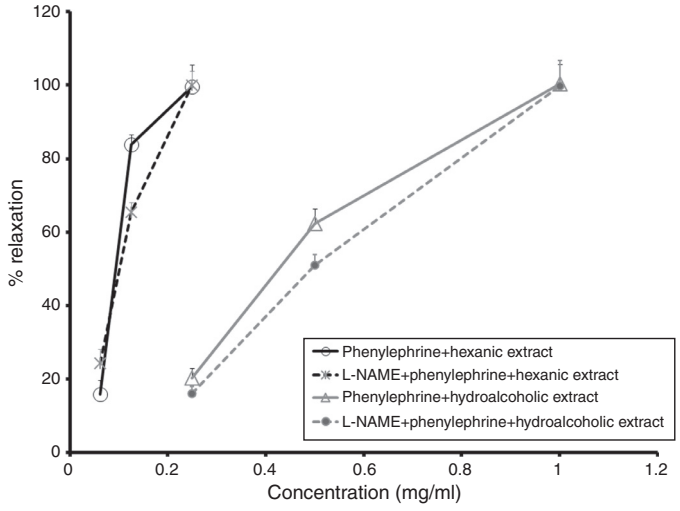

Fig. 8. Vasodilatory effect of cumulative doses of hexanic and hydroalcoholic extracts of celery seed on previously constricted aorta ring tissue by phenylephrin (10^{-6} M) and L-NAME (10^{-5} M). Each point is mean ± SEM ($n = 6$).

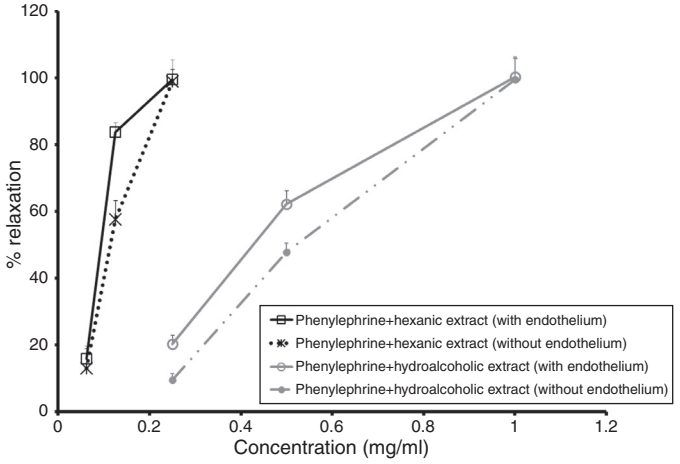

Fig. 9. Vasodilatory effect of cumulative doses of hexanic and hydroalcoholic extracts of celery seed on previously constricted aorta ring tissue by phenylephrin (10^{-6} M) in the absence of endothelium. Each point is mean ± SEM ($n = 6$).

could decrease the relaxation response only at 0.125 mg/kg of hexanic extract ($p < 0.01$), whereas the relaxation changes at other concentrations of hexanic and hydroalcoholic extracts were not significant ($p > 0.05$). According to our data, L-NAME (10^{-6} M) caused 8.5 and 18.5% reduction in relaxation at 0.0625 and 0.125 mg/kg hexanic extract, respectively, whereas the relaxation reduction values by L-NAME at 0.25 and 0.5 mg/kg hydroalcoholic extract were 4.5 and 11%, respectively ($p < 0.05$) (Fig. 8). Figs. 7 and 8 also show that the potency of hexanic extract, as a vasodilator agent, was significantly higher than that of hydroalcoholic extract. Fig. 9 represents the effect of endothelial destruction in reducing the relaxation response of the tissue after primary contraction by phenylephrine. The data revealed that maximum response was observed after adding cumulative doses of hexanic and hydroalcoholic extracts, even in the absence of endothelial pathways. Except at the maximum doses of both extracts, removing the endothelium could slightly decrease the relaxation response of the tissue made by other doses of the celery seed extracts (Fig. 9). This finding indicated the role of nonendothelial pathways in vasorelaxation effect of celery seed extract. As can be seen in Fig. 10, the potency and efficacy of hexanic and hydroalcoholic extracts of celery seed were

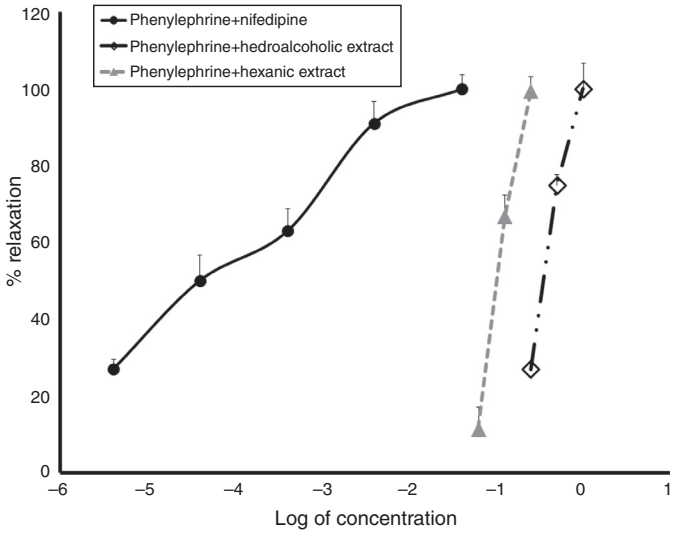

Fig. 10. Vasodilatory effect of cumulative doses of hexanic and hydroalcoholic extracts of celery seed and nifedipine on previously constricted aorta ring tissue by phenylephrin (10^{-6} M). Each point is mean ± SEM ($n = 6$).

compared to nifedipine as a positive control. Aorta tissue was firstly contracted by 10^{-6} M phenylephrine and then cumulative doses of extracts or nifedipine were administered. According to the data the efficacy of extracts was similar to nifedipine while the potency of nifedipine was significantly higher than that of celery seed extracts.

Discussion

HPLC analysis of celery seed extracts

In our previous study, we reported the antihypertensive effects of chronic administration of hexanic, methanolic, and aqueous-ethanolic extracts on rat BP (Moghadam et al., 2013). We also reported the higher content of *n*-butylphthalide (NBP), as an active agent in hexanic extract compared to methanolic and aqueous-ethanolic extracts. According to our former study, hexanic extract showed significantly stronger effect in lowering BP compared to methanolic and aqueous-ethanolic extracts (Moghadam et al., 2013).

Moreover, in the present study, HPLC analysis demonstrated that NBP levels in hexane and aqueous-ethanolic extracts (20/80 v/v) were 0.42 and 0.09%, respectively (Fig. 1). Consequently, it seems that NBP present in celery seed may be the main phytochemical responsible for reducing MABP, SBP, and DBP in normotensive and hypertensive rats; nevertheless, the presence of other non-polar compounds such as apigenin, sedanenolide, and sedanolide may also contribute to these observations. These compounds were studied by other researchers, as well (Mencherini et al., 2007; Tang et al., 1990).

Antihypertensive effects of celery seed extracts: in vivo *evaluation*

Various mechanisms including vasodilatory, negative chronotropic and ionotropic, and diuretic effects may be involved in management of hypertension. In the present study, we aimed to investigate the antihypertensive effects and mechanisms of celery seed extract in rats. To this end, the antihypertensive effects of hexanic extract of celery seed in an invasive model of normotensive and hypertensive rats were studied. Thereafter, the possible vasodilatory effects and mechanisms of the extract were evaluated in isolated rat aorta with or without endothelium.

Our results demonstrated that the hexanic extract of celery seed could dramatically diminish SBP, DBP, MABP, and HR following intrajugular administration. Hexanic and nifedipine extracts had similar efficacy in lowering SBP, DBP, and MABP in normotensive and hypertensive rats, while they had disparate effects on heart rate (HR). In other words, hexanic celery seed extract induced bradycardia, whereas nifedipine had a tachycardia effect on HR.

According to our results, potency of nifedipine was significantly higher than that of celery seed extract. With respect to HR, reflex tachycardia following nifedipine administration was reported in previous studies (Mohajeri et al., 2011; Valdivielso et al., 1997). However, our results indicated that the HR-lowering effect of hexanic extract (negative chronotropic effect) overcomes the reflex tachycardia after intrajugular injection (Fig. 5).

The effects of some calcium channel blockers on BP and HR are similar to those of celery seed extract. Verapamil is an L-type calcium channel blocker, which is used in management of hypertension, cardiac arrhythmia, and angina pectoris. Although verapamil produces vasodilatory effects through blocking voltage-dependent calcium channels in smooth muscles, it causes bradycardia as a side effect or in overdoses (Cheng et al., 2010). Thus, its direct bradycardic effect overcomes the reflex tachycardia after vasodilation. On the other hand, administration of dihydropyridine calcium channels blockers (*e.g.*, nifedipine) lowers BP and leads to reflex tachycardia (Brown et al., 2000; Pushparaj et al., 2001).

According to our data, the hexanic extract of celery seed exerts its hypotensive effects via its bradycardic and vasodilatory properties (Tang et al., 2007). A number of studies have been performed on antihypertensive effects of celery seed and its compounds; however, there is a paucity of data on the compounds that may play the main role in lowering BP. Branković et al. showed that both aqueous and ethanolic extracts of celery had hypotensive and negative chronotropic and inotropic effects (Branković et al., 2010). They reported that aqueous and ethanolic extracts of celery (0.5–15 mg/kg) immediately diminished SBP, DBP, and MABP in anesthetized rabbits. According to their data, 15 mg/kg of aqueous extract caused a 14.35% decrease, whereas 15 mg/kg of ethanolic extract of celery seed induced 45.8% fall in MABP. Our data revealed that hexanic extract of celery seed reduced SBP, DBP, and MABP in normotensive rats by 70–80%.

These results indicate that celery seed has higher levels of active components than aerate parts of the plant. Given the presence of lipophilic compounds, organic solvents with lower dielectric content (*e.g.*, hexane) are suitable for extraction of active agents from the celery seed. Branković et al. also investigated the effect of celery extract on isolated rat atria. According to their outcomes, ethanolic extract (0.75 mg/ml) of celery could effectively attenuate atrial rate and force by 34% and 45%, respectively. Thus, *in vivo* studies revealed that active ingredients of celery extract could reduce BP through direct induction of bradycardia and negative ionotropic effects.

These findings can explain the bradycardic effect after administration of hexanic extract of celery seed in our *in vivo* experiments (Fig. 5). In other words, direct bradycardic effect of celery seed extract overcomes the reflex tachycardia after vasodilation and BP reduction. The active ingredients of celery seed may cause its negative ionotropic and chronotropic effects through blocking the Ca^{2+} channels in cardiac muscle cells.

Mechanisms of antihypertensive effect of celery seed extract: ex vivo *evaluation*

According to our *ex vivo* outcomes, both hexanic and aqueous-ethanolic extracts of celery seed have vasodilatory effect in a dose-dependent manner. Compared to nifedipine, as a calcium channel blocker, both extracts had significantly less potency with similar efficacy. Fig. 6 indicates that celery seed extract could inhibit both phenylephrine-induced and KCl-induced vasoconstriction in the rat aorta through endothelial and non-endothelial pathways. Production of prostaglandins such as prostacyclin causes vasodilation, and thereby reduces BP (Alimohammadi et al., 2013). Incubation of aortic rings with indomethacin (a potent cyclooxygenase inhibitor) did not significantly alter the relaxation response induced by celery seed extract after vasoconstriction by phenylephrine (Fig. 7). These findings suggest that active ingredients in both extracts may not exert their effects through cyclooxygenase pathway and prostacyclin production in the aortic tissue.

Endothelial nitric oxide synthase (eNOS) is responsible for production of nitric oxide (NO), which plays an important role as a vasodilator and anti-inflammatory agent (Ramakrishna and Jailkhani, 2007). Nω-nitro-L-arginine methyl ester (L-NAME), as a non-selective NOS inhibitor, was applied to inhibit NO production in our experiments (van Bremen et al., 2013). Fig. 8 demonstrates that incubation of rat aorta with L-NAME reduced the vasodilatory effects of both extracts, especially in lower concentrations after phenylephrine-induced vasoconstriction. However, 100% vasorelaxation was obtained in higher concentrations of both extracts. Although other mechanisms may be involved, our data showed that NOS plays a role in vasodiatory effect of active compounds

of celery seed. Fig. 9 exhibits that endothelial disruption could effectively reduce the relaxation response, especially in lower concentrations, induced by celery extract. This finding confirms that non-endothelial vasodilatory pathways, such as inhibition of Ca^{2+} influx into smooth muscles, are effective in relaxation of the aortic rings.

Vasodilatory effect seems to be dependent on inhibition of Ca^{2+} influx into cells or the release of Ca^{2+} from sarcoplasmic network in the smooth muscles. Moreover, celery seed extracts were less potent than positive control (nifedipine), but they could inhibit phenylephrine-induced vasoconstriction in a dose-dependent manner (Fig. 10). In a study by Tsi et al., hypotensive and vasorelaxant effects of NBP (an important active compound in celery seed) were studied (Tsi and Tan, 1997). They indicated that vasorelaxant effect of NBP could be attributed to the blockade of Ca^{2+} entry, possibly through voltage-activated and receptor-operated Ca^{2+} channels.

In another study by Ko et al., the vasodilatory effect of apigenin (an active compound in celery seed extract) was investigated. They concluded that apigenin inhibits the Ca^{2+} influx through voltage- and ligand-gated calcium channels (Ko et al., 1991). In a study by Anjos et al., cardiovascular effects of linalool (an active substance in celery seed) in normotensive and hypertensive rats were investigated, and its vasodilatory effect was studied on isolated rat mesenteric artery. Their results indicated that linalool reduced BP through its direct effect on the vascular smooth muscles. Linalool is a calcium channel blocker, which causes reflex tachycardia (Anjos et al., 2013).

In a study by Santiago et al., it was noted that the D-limonene (a compound in celery seed) improved the status of circulatory lipids and antioxidants and decreased BP. Thus, it can reduce the pathological changes, improve physiological functions, and lower BP (Tan et al., 2005). In addition, Tang et al. studied the vasodilatory mechanism of celery juice and suggested that it might inhibit the receptor-operated channel (ROC) in the smooth muscles (Tang et al., 2007). Peripheral vascular resistance has a major impact on DBP; therefore, celery seed extract produces vasorelaxant effects, which could be due to its high levels of NBP, apigenin, linalool, D-limonene, or other similar compounds.

Conclusion

The present study indicated the hypotensive effect of the hexane extract of celery seed in an invasive rat model. This effect could be attributed to the presence of some hydrophobic constituents such as NBP in celery seed. Our data revealed that SBP, DBP, MABP and HR significantly decreased after administration of hexanic extract. Therefore, it exerts its hypotensive effects through its bradycardic and vasodilatory properties. Possible vasodelatory mechanisms of celery seed extract were also studied. The results indicated that active agents in celery seed extract could exert their vasodilatory properties through their Ca^{2+} channel blocking activity in endothelial and non-endothelial pathways. The efficacy of hexanic extract, as a vasorelaxant, was similar to that of nifedipine as a positive control but with lower potency. It can be concluded that the celery seed hexanic extract displays its vasodilatory effect mainly via endothelial and non-endothelial dependent pathways and particularly by interference with the extra or intracellular calcium.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contribution

FTS contributed in extracting the celery seeds, HPLC analysis, running the *in vivo* experiments, analysis of the data, writing the manuscript, BMR supervised the *ex vivo* experiments, MI supervised the *in vivo* experiments, MD and HF contributed in *ex vivo* studies, YE contributed *in vivo* experiments and SAM designed the study, supervised the laboratory work, contributed to critical reading and editing of the manuscript as corresponding author. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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