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Vasorelaxant and Hypotensive Effects of *Allium cepa* Peel Hydroalcoholic Extract in Rat

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Abstract: The aim of present study was to investigate the effect of onion (*Allium cepa*) peel hydroalcoholic extract (OPE) on rat hypertension induced by high-fructose diet and aorta contractility. The OPE was prepared by maceration method using 70% ethanol. The thoracic aorta from male adult rat (Wistar) was dissected and suspended in Krebs-Henseleit solution under 1 g resting tension. Tissue preparation was contracted by KCl (80 mM) or phenylephrine (Phe, 1 μM) and then the extract was applied cumulatively (0.0625-2 mg mL^{-1}). Hypertension was induced in negative control and three groups of rats by adding fructose (10% W/V) in drinking water for 6 weeks but control group received tap water. Hypertensive groups received saline or OPE at 200, 400 and 800 mg kg^{-1} daily for last 3 weeks by gavage. Results showed that OPE reduces aorta contractions induced by KCl or Phe in a concentration-dependent manner ($p<0.001$). Removing aorta endothelium did not attenuate the OPE activity. Inhibition of nitric oxide, cGMP and prostaglandin synthesis by L-NAME (100 μM), methylene blue (10 μM) and indomethacin (10 μM), respectively, did not attenuate OPE activity. Atropine abolished ACh-induced relaxation in Phe precontracted aorta but not the OPE-induced relaxation. Although the extract did not change heart rate but after 3 weeks reduced the hypertension induced by fructose ($p<0.001$). Present results indicated that OPE reduces aortic contractions possibly via inhibition of calcium influx but without involving NO, cGMP, endothelium and prostaglandins. The OPE hypotensive effect could be due to extract quercetin content, antioxidant activity and inhibiting vascular smooth muscle cells Ca^{2+} influx.

Key words: *Allium cepa*, rat, hypotensive, vasorelaxant

INTRODUCTION

Recently, increases in life-style related diseases like hypertension, arteriosclerosis, heart disease, obesity, diabetes mellitus and cancers have become a great social problem (Fujita *et al*., 2000). A good proportion of the world population particularly those living in developing countries depend mostly on herbal medicines for their health needs. Medicinal herbs are indispensable part of the traditional medicine practiced all over the world due to easy access, low cost and ancestral experience (Ayoka *et al*., 2005). Therefore, natural product scientists are now intensifying efforts towards scientific evaluation of medicinal plants used in traditional remedies (Amos *et al*., 2003).

Since antiquity *Allium* plants such as garlic and onion have been believed to be beneficial to human health. Anecdotal evidence supports the important roles of garlic and onion in the prevention and treatment of pathogenic infections, tumors and cardiovascular diseases (Jakubowski, 2003). At least 25 different anthocyanins have been reported from red onion (Slimestad *et al*., 2007) and S-methyl cysteine sulfoxide isolated from onion reduces rat serum cholesterol, triglyceride and phospholipids (Kumari and Augusti, 2007). Furthermore, the antithrombotic (Jung *et al*., 2002), antidiabetic (Kelkar *et al*., 2001; El-Demerdash *et al*., 2005) and antioxidant (Helen *et al*., 1999; Campos *et al*., 2003) effects of onion have also been shown. It is reported that boiling reduces the antihypertensive activity of onion (Kawamoto *et al*., 2004). Moreover, it has been demonstrated that onion peel with its antioxidant flavonoid quercetin and FRS 1000 (extracted from onion peel) has a strong phosphodiesterase 5A inhibitory activity (Lines and Ono, 2006) and onion peel also enhances antioxidant status in aged rats (Park *et al*., 2007). The effect of onion peel extract on the vasculature contractility and blood pressure has not been evaluated yet. Therefore, the aim of the present study was to investigate the effect of onion peel hydroalcoholic extract on rat aorta contraction and also on the hypertension induced by high-fructose diet.

MATERIALS AND METHODS

Plant extraction: Red onions were purchased from Ahwaz green-grocery in winter (2007) and dried skin was peeled.

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Red onion was identified by Dr. Aghel from Department of Pharmacognosy, Ahwaz Jundishapur University of Medical Sciences (AJUMS). After cleaning, the peels were powdered and then soaked in 70% ethanol at room temperature for 72 h and mixed occasionally daily. The mixture was then filtered (Whatman No. 1) and the filtrate was concentrated in rotary evaporator and dried at room temperature to obtain a reddish powder (yield: 21%). The Onion Peel Extract (OPE) powder was stored at 4°C until being used.

Animals and tissue preparation: Adult male Wistar rats (200-250 g) were purchased from AJUMS animal facility and the protocols were approved by the Committee of Ethics in Research of the university. Animals were kept at 20-24°C under 12 h light-dark cycle and were allowed free access to tap water and commercial chow. In *in vitro* study, animals were anaesthetized by ether and exsanguinated. Thoracic aorta was rapidly removed and cleaned of extraneous connective and fatty tissue in ice-cold Krebs-Henseleit solution and cut into rings 5 mm long. Aortic rings were suspended horizontally between two stainless steel hocks, one of the hocks was fixed to the chamber wall while the other was attached to an isometric force transducer (UF1 Harvard Transducer, UK) and to an ink-writing curvilinear polygraph (Universal Harvard Oscillograph, UK). The organ bath (10 mL) containing Krebs-Henseleit solution (37°C, pH 7.4) of the following composition (mM): NaCl (118), KCl (4.7), $CaCl_2$ (2.52), $MgSO_4$ (1.64), KH_2PO_4 (1.18), $NaHCO_3$ (25) and glucose (5.5) and gassed with oxygen. Tissue was then maintained under 1 g initial tension and allowed to equilibrate for 1 h during which bath solution was refreshed every 15 min. All dissecting procedures were done with extreme care to protect the endothelium from inadvertent damage.

Drugs: N^{ω}-nitro-L-arginine methyl ester (L-NAME), phenylephrine hydrochloride, atropine sulfate, methylene blue, indomethacin and acetylcholine chloride were purchased from Sigma (USA) and mineral solutes from Merck (Germany). OPE and all chemicals were dissolved in Krebs-Henseleit and volumes added to bath were less than 5% of the organ bath volume.

Experimental protocols

***In vitro* study:** In all *in vitro* experiments, after equilibration, the presence of a functional endothelium was assessed on the basis of relaxation evoked by the endothelium-dependent dilator acetylcholine (1 μM) in aorta rings precontracted with phenylephrine (1 μM) and if the relaxation was more than 70%, the endothelium was regarded as intact (Sung *et al*., 2002). To evaluate the effects of the OPE on the aorta contractile responses, aorta was precontracted by phenylephrine (1 μM) or KCl (80 mM) and once the plateau of the contraction was achieved, OPE was applied cumulatively (0.0625 to 2 mg mL^{-1}). In some aortic rings, the endothelium was denuded by gently rubbing the inner surface with a rough steel needle. The role of NO and cGMP was determined using nitric oxide synthase inhibitor (L-NAME, 100 μM for 30 min) and guanylyl cyclase inhibitor (methylene blue, 10 μM for 30 min) respectively. To study the role of prostaglandins, indomethacin as a prostaglandins synthesis inhibitor was dissolved in the tissue bath solution (10 μM). To evaluate the role of muscarinic receptors, a group of aorta (with intact endothelium) was contracted by phenylephrine (1 μM) and the acetylcholine (1 μM) or OPE (2 mg mL^{-1}) was applied to induce relaxation and then, atropine (1 μM) was added. All the mentioned concentrations are the final concentration in tissue bath.

***In vivo* study:** Five groups of rats (8 in each group) were treated for 6 weeks according to the method described by Dimo *et al*. (2002):

Group 1: As control group that received tap water freely.

Group 2: As negative control group that received fructose 10% (W/V) in drinking water for 6 weeks freely and from 4th week received 1 mL of normal saline by gavage daily.

Groups 3, 4 and 5: As treated groups which received fructose 10% (W/V) in drinking water for 6 weeks freely and from 4th week received 1 mL of different doses of OPE (200, 400 and 800 mg kg^{-1}) daily by gavage. Systolic arterial pressure in conscious and restrained rat was recorded weekly by using tail cuff (Powerlab, UK). Heart rate was also recorded by using the same instrument. Blood pressure and heart rate of all rats were recorded one week prior to beginning the experiment considered as basal levels (week 0).

Statistical analysis: Aorta contraction induced by phenylephrine (1 μM) or KCl (80 mM) were assumed as 100% and changes in the aorta contraction were calculated as the percent of relaxation and expressed as mean±SEM. The number of rings obtained from different rats was represented by n. The data was analyzed by one- and two-way ANOVA followed by Dunnet test and values of $p<0.05$ were considered to be significant.

RESULTS

Effect of OPE on rat aorta contraction: KCl and phenylephrine-induced contraction in aorta with intact endothelium was reduced by cumulative concentrations

Table 1: Percentage of relaxation induced by cumulative concentrations of onion peel hydroalcoholic extract in phenylephrine (1 μM) precontracted aorta with intact (+endo., n = 11) or denuded endothelium (-endo., n = 9)

	Onion peel extract (mg mL^{-1})					
Substances	0.0625	0.125	0.25	0.5	1	2
Phenylephrine (+ endo)	2.3±5.1	13.9±8.1	24.3±9.4	52.0±8.8	85.2±7.9	110.5±9.5
Phenylephrine (- endo)	5.6±3.3	13.8±4.5	26.8±6.7	48.5±7.5	81.2±8.7	100.8±7.6

Table 2: Percentage of relaxation induced by cumulative concentrations of onion peel hydroalcoholic extract in aorta, with intact endothelium, precontracted by phenylephrine (1 μM, n = 11) in the absence (control) and in the presence of L-NAME (100 μM, 30 min, n = 8), methylene blue (10 μM, 30 min, n = 10) or indomethacin (10 μM, n = 7)

	Onion peel extract (mg mL^{-1})					
Substances	0.0625	0.125	0.25	0.5	1	2
Phenylephrine (control)	2.3±5.1	13.9±8.1	24.3±9.4	52.0±8.8	85.2±7.9	110.5±9.5
(+) L-NAME	0.4±0.7	2.5±2.6	12.9±5.2	28.0±7.0*	70.0±15.2	104.2±14
(+) Methylene blue	8.8±3.3	18.7±4.1	40.3±3.4	64.6±5.1	83.1±4.2	99.0±4.1
(+) Indomethacin	-2.1±5.7	14.3±7.3	38.6±13.1	54.9±13	85.9±7.1	124.8±11.3

Statistical analysis were performed between phenylephrine as control group and other groups at the same extract concentration, *p<0.05

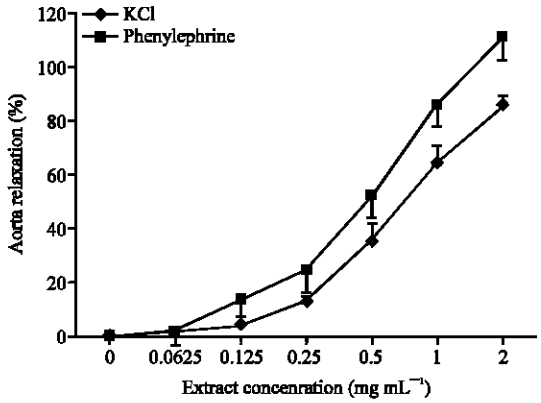

Fig. 1: Vasorelaxatory effect of cumulative concentrations of onion peel hydroalcoholic extract (OPE) on rat aorta precontracted with KCl (80 mM, n = 9) or phenylephrine (1 μM, n = 11). These two concentration-response curves are not significantly different (two-way ANOVA)

of OPE (0.0625-2 mg mL^{-1}) in a concentration-dependent manner (one-way ANOVA, p<0.001). Two-way ANOVA of OPE vasorelaxatory effects for these two spasmogens were not significantly different (Fig. 1).

The role of the endothelium in OPE vasorelaxatory effect: The OPE (0.0625-2 mg mL^{-1}) vasorelaxatory effects on phenylephrine precontracted aorta with or without endothelium (n = 11 and n = 9, respectively) were not significantly different (Table 1).

Effect of L-NAME and methylene blue on the OPE vasorelaxatory activity: Incubation (30 min) of the aorta with L-NAME (100 μM, n = 8) or with methylene blue (10 μM, n = 9) with intact endothelium did not attenuate the OPE vasorelaxatory activity (Table 2). Although the OPE vasorelaxatory effect (at 0.5 mg mL^{-1}) is augmented in the presence of L-NAME but statistical analysis (two-way ANOVA) indicated that L-NAME has not changed the overall vasorelaxatory of OPE activity.

Role of prostaglandins in the OPE vasorelaxatory activity: In the presence of indomethacin (10 μM, n = 7), the cumulative concentrations of OPE caused vasorelaxation in phenylephrine precontracted aorta (with intact endothelium) in a concentration-dependent manner (p<0.001). However, two-way ANOVA indicated that the OPE vasorelaxatory effect in the absence and presence of indomethacin are not different (Table 2).

The comparison of vasorelaxatory effect of atropine and OPE: A group of aortae with intact endothelium was contracted by phenylephrine (1 μM) and then, acetylcholine (1 μM) was added to the tissue bath which induced a significant relaxation (p<0.001, n = 8). Adding atropine restored the contraction in the aorta (p<0.001). After 30 min and several refreshing of the bath solution, the protocol was repeated but OPE extract (2 mg mL^{-1}) was used instead of acetylcholine. The OPE induced vasorelaxation (p<0.001, n = 8) was unaffected by atropine (Fig. 2).

Effect of OPE on heart rate and hypertension induced by fructose: Systolic Blood Pressure (SBP) was gradually increased (p<0.05) from 1st to 3rd week in groups receiving-fructose diet compared with week 0 (beginning of the experiment) and also compared with control group which receiving tap water (p<0.05). Animals in negative control group showed a sustained a high SBP from 4th to 6th weeks. However, the induced hypertension in treated groups was attenuated by OPE (200, 400 and 800 mg kg^{-1})

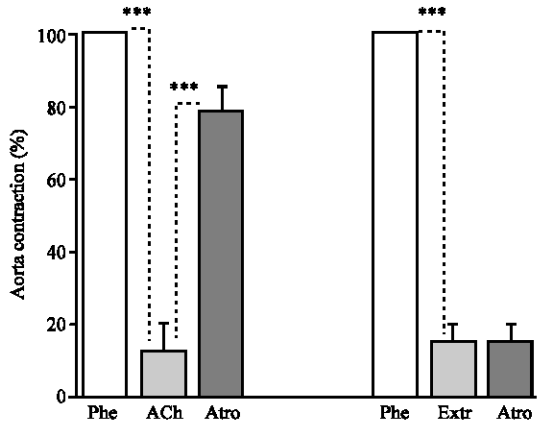

Fig. 2: Effect of atropine (Atro, 1 μM) on acetylcholine (ACh, 1 μM) or OPE (Extr, 2 mg mL^{-1}) induced relaxation in phenylephrine (Phe, 1 μM) precontracted aorta, with intact endothelium (n = 8, ***p<0.001). The extract vasorelaxant effect is unaffected by atropine

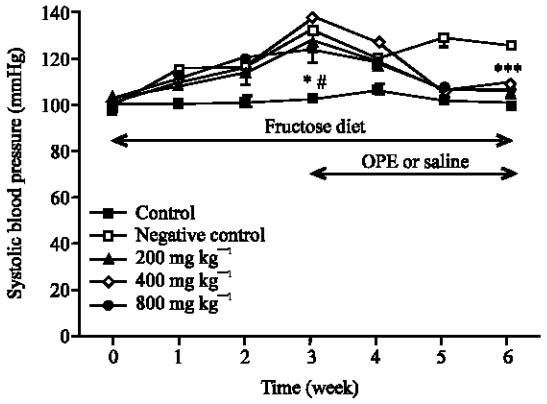

Fig. 3: Effect of OPE on hypertension induced by high-fructose diet in rats. Control group received tap water and other groups received high-fructose diet for 6 weeks. The negative control and treated groups received saline (1 mL) or different doses of OPE, respectively (n = 8) for last three weeks. *p<0.05 compared with week 0 and #p<0.05 in compare to control group at the same week, ***p<0.001 compared with negative control group

at 4th and 5th weeks and returned to a level which was not different from control group but different (p<0.001) from negative control group. There were no significant differences between the SBP in OPE treated groups at 5th and 6th weeks (Fig. 3). As mentioned above, although, the SBP had changed during the experiment but the heart rate of all groups did not alter during this period (Fig. 4).

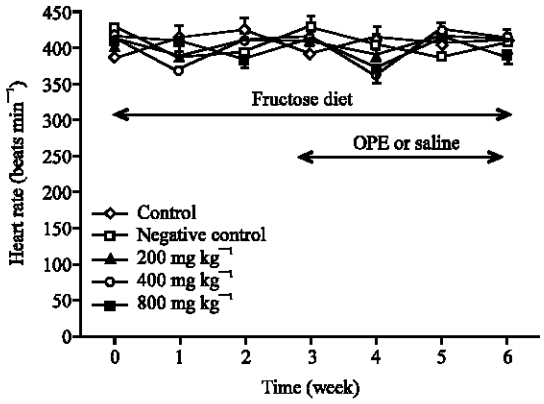

Fig. 4: Effect of high-fructose diet and oral administration of OPE on heart rate in rats. Control group received tap water and other groups received high-fructose diet for 6 weeks. The negative control and treated groups received saline (1 mL) and different doses of OPE, respectively (n = 8) for last three weeks. Heart rate has been affected neither by high-fructose diet nor by OPE treatment during experiment period

DISCUSSION

Present results showed the vasorelaxatory and hypotensive effects of the onion peel hydroalcoholic extract (OPE) in rat. Intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ is the main regulator of contraction in smooth muscle. A rise in $[Ca^{2+}]_i$ leads to the activation of myosin light chain kinase and phosphorylation of myosin light chain which results in muscle contraction (Ghisdal *et al*., 2005). Extracellular high K^+ levels induce membrane depolarization that in turn opens the voltage-dependent Ca^{2+} channels (Karaki *et al*., 1997). On the other hand, interaction of phenylephrine (Phe, α_1-adrenoceptor agonist) with its receptors induces the generation of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol that activates protein kinase C (Karaki *et al*., (1997). IP_3 binds to its receptor in the Sarcoplasmic Reticulum (SR). As a result Ca^{2+} is released from SR to induce contraction (Marin *et al*., 1999). Since, KCl and Phe cause Ca^{2+} channels activation and increment in $[Ca^{2+}]_i$ (Shin *et al*., 2005) and on the other hand, OPE vasorelaxatory effect on the contractions induced by both these spasmogens were identical, therefore, it seems that OPE vasorelaxation activity is mediated via Ca^{2+} influx.

Acetylcholine stimulates releasing Nitric Oxide (NO) from endothelium via muscarinic receptors (Dauphin and Hamel, 1990) and NO increases cGMP synthesis. Cyclic GMP causes vasorelaxation via membrane hyperpolarization by potassium channel activation and

inactivation of voltage-gated Ca^{2+} channels thus reducing intracellular Ca^{2+} and ultimately causing relaxation (Sung *et al*., 2002). The ineffectiveness of L-NAME, methylene blue, atropine and denuding endothelium to attenuate the OPE vasorelaxatory activity indicate that the OPE vasorelaxatory activity was not mediated through NO, cGMP, endothelium and muscarinic receptors. Onion contains flavonoids such as quercetin (Jalili *et al*., 2006; Lines and Ono, 2006). However, most of the flavonoids described to date exhibit a relaxant effect independent of the presence of a functional endothelium (Lemos *et al*., 1999) which is in agreement with our results in which removing of the endothelium had no effect on the OPE vasorelaxant effect. Prostaglandin E1 induces vasorelaxation by ATP-sensitive potassium channels (Eguchi *et al*., 2007). However, OPE activity was unaffected by indomethacin, as a cyclooxygenase inhibitor, which rules out possible involvement of prostaglandins in the OPE activity.

Present *in vivo* results showed that High-Fructose Diet (HFD) causes hypertension which was in agreement with other reports (Dimo *et al*., 2002; Zhao *et al*., 2003; Sanchez-Lozada *et al*., 2007). It has been demonstrated that the same HFD after 3 weeks was accompanied with hypertension and elevated plasma triglyceride and cholesterol concentrations (Suzuki *et al*., 1994; Dimo *et al*., 2002). We may assume, therefore, that same elevations in triglyceride and cholesterol levels have been occurred in our study and the hypotensive effect of OPE partly was due to a reduction in plasma triglyceride and cholesterol concentration. In this study the hypertension induced by HFD was unaccompanied by heart rate alteration as reported (Suzuki *et al*., 1997; Dimo *et al*., 2002) and furthermore, heart rate was unaffected by oral administration of OPE in hypertensive rats. Therefore, the hypotensive activity of OPE was not due to alteration in heart rate. Babu and Srinivasan (1999) reported that HFD induces hypertension by increasing uric acid production and Ostrowska *et al*. (2004) suggested that NO synthesis is inhibited by uric acid. Furthermore, Nakagawa *et al*. (2006) showed that uric acid inhibits endothelial function in response to acetylcholine. These evidences indicate that hypertension induced by HFD is caused by disturbances in vascular tissue. Moreover, it is reported hypertension induced by HFD was associated with increased cytosolic free Ca^{2+} and aortic Ca^{2+} uptake (Vasdev *et al*., 1994). It has also been shown that fructose causes oxidative stress (Jalili *et al*., 2006). As a matter of fact, onion has high amounts of quercetin (Murota *et al*., 2007) and both onion and onion peel elevate plasma concentration of quercetin (Park *et al*., 2007). The antidiabetic effect of quercetin (Vessal *et al*., 2003; Kanter *et al*., 2007) and antioxidant activity of onion peel (Park *et al*., 2007) have also been reported. It seems therefore that antidiabetic and antioxidant activities of onion peel lowers the oxidative stress and hypertension induced by HFD. OPE oral administration daily for two weeks returned the elevated SBP to control level (Fig. 3) which indicates the hypotensive potency of the extract and suggests necessity of further studies on the effect of OPE on plasma biochemical parameters related to hypertension such as insulin, glucose, cholesterol and triglycerides. The identical antihypertensive effects of different doses of the extract indicate that lower doses could be used to have a reasonable dose-response curve. Although *in vivo* results cannot explain the precise mechanism(s) of OPE action, however, *in vitro* results suggest that main action of extract was through inducing relaxation in the vasculature tissue. Since Vasdev *et al*. (1994) demonstrated that hypertension induced by fructose is associated with elevation in $[Ca^{2+}]_i$, therefore, it seems that Ca^{2+} influx inhibition had a role in the observed OPE activities.

CONCLUSION

The onion peel hydroalcoholic extract hypotensive effect could be due to extract quercetin content, antioxidant activity and inhibiting Ca^{2+} influx in the vascular smooth muscle cells.

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