**Bronchodilatory Activity of Vitis vinifera Leaf Hydroalcoholic Extract in Rat**

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**ABSTRACT**

Several reports have shown the various effects of grape (Vitis vinifera) seed extract such as antioxidant, hypotensive, hypolipidemic and vasodilatory effects. The aim of present study was to investigate the effect of grape leaf hydroalcoholic extract on isolated rat tracheal contractions induced by KCl and acetylcholine. The trachea was removed from male adult Sprague-Dawley rat and placed in an organ bath containing Krebs-Henseleit solution and contractions were recorded isometrically. The results demonstrate that the grape leaf extract at 0.5, 1, 2, 4 and 8 mg/ml significantly reduces the tracheal contractions induced by KCl (60 mM) dose-dependently (**P**<0.0001). Acetylcholine (55 μM)-induced tracheal contractions were also attenuated at the same concentration of the extract (**P**<0.0001). The grape leaf extract induced relaxation in the KCl-induced contraction in trachea was unaffected neither by nitric oxide (NO) synthase inhibitor (L-NAME, 100 μM) nor by beta-adrenoceptor antagonist (propranolol 1 μM). Our results suggest that the bronchodilatory effect of grape leaf extract is mediated via the voltage dependent calcium channels. Furthermore, the beta-adrenergic and NO are not involved. Iran. Biomed. J. 10 (2): 79-83, 2006

**Keywords:** Grape leaf, Bronchodilatation, Rat

**INTRODUCTION**

Grape (Vitis vinifera) from Vitaceae is a perennial woody vine native to Asia Minor and then introduced in Europe [1]. Its leaves are consumed in some traditional foods (Dolmathes) and used for diarrhea, vomiting and varicose treatment [2]. The pharmacological properties of grape seeds are well investigated and it is believed that flavonoids (from polyphenols) are its most potent constituents. Grape seed extract reduces blood lipids in rabbit [3] with no side effects after long term consumption [4]. The procyanidins in grape seed induces endothelial-dependent vasorelaxation in human artery [5]. This effect is carried out through nitric oxide (NO) and enhancement of cGMP [6,7]. Procyanidins of grape seed extract show several effects, for instance, protective effect against oxidative stress [8], cataract [9], colon cancer [10] and the increase in plasma antioxidant activity [11]. Recently, the vasorelaxatory effect of grape leaf extract on isolated rat aorta was shown to be dependent on endothelium integrity, and production of NO and cGMP [12]. The aim of the present study was to investigate the effect of grape leaf hydroalcoholic extract (GLHE) on rat isolated trachea.

**MATERIALS AND METHODS**

Acetylcholine, propranolol and L-NAME were purchased from Sigma (USA) and other chemicals were from Merck (Germany). All chemicals were dissolved in the Krebs-Henseleit solution. Grape leaves were collected from the Jondi Shapour Ahwaz University of Medical Sciences Campus (Ahwaz, Iran) in April 2004. The collected leaves were identified by Dr. Siahpoosh from Department of Pharmacogenosy, Faculty of Pharmacy, Jondi Shapour Ahwaz University of Medical Sciences. A voucher specimen was deposited in the herbarium of the same department under number A06390001M. Grape leaves were dried under...
shade and powdered by electrical grinder. The powder (50 g) was mixed with 230 ml of alcohol (70%) for 72 h at room temperature and stirred four times daily. The mixture was filtered with Whatman filter paper (No.1) and then the solvent was evaporated at room temperature. The obtained extract powder was 9.5 g (19% extraction ratio) which stored at 4ºC until being used.

Animals and tissue preparation. Male Sprague-Dawley rats (200-245 g) were housed in cages with ambient temperature at 20-24ºC, 12-h light/dark cycle and free access to water and food. All animal experiments were approved by the Local Animal Ethics Committee of Ahwaz Jondi Shapour University of Medical Sciences. The rats were anaesthetized by ketamine hydrochloride (50 mg/kg, ip) and chest and neck were opened. The trachea (2 cm) was dissected out and its connective tissue was removed in a cold oxygenated Krebs-Henseleit solution. A piece of trachea (5 mm) with 5-6 cartilage rings was cut and mounted between two stainless steel hooks horizontally. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isometric transducer (UF1 Harvard transducer, UK) connected to an ink-writing curvilinear recorder (Harvard Universal Oscillograph, UK). The organ bath contained Krebs-Henseleit (10 ml, pH 7.4 and at 37ºC) with following composition (mM): NaCl, 118; KCl, 4.7; CaCl$_2$, 2.52; MgSO$_4$, 1.64; KH$_2$PO$_4$, 1.18; NaHCO$_3$, 7 and glucose, 5.5 bubbled with oxygen. The initial tension was 1.5 g throughout the experiment and equilibrium period was 60 min in which, the bath solution was refreshed every 15 min. After equilibrium period, the trachea was contracted by 60 mM of KCl [13] or by 55 μM [14] of acetylcholine and when the plateau was achieved, the extract (0.5, 1, 2, 4 and 8 mg/ml) was added non-cumulatively to the organ bath and left to achieve a new plateau. Then, during 10 min, the bath solution was exchanged three times with fresh Krebs-Henseleit solution. The same protocol was repeated but with the higher extract concentration. To investigate the involvement of NO and β-adrenoceptors, first, the inhibitory effect of extract (3 mg/ml) was recorded on KCl-induced contraction. After 15 min and several exchanging the bath solution, the same protocol was repeated in the presence of L-NAME (100 μM, 10 min) or propranolol (1 μM, 10 min). At the end of the experiments, the tissue water was absorbed by filter paper and tissue weighed.

Statistical analysis. Data are expressed as mean ± SEM of g force/100 mg tissue or in percentage of force changes. Statistical comparisons were made by Student’s t-test and one-way ANOVA. P<0.05 was considered significant.

RESULTS

Effect of GLHE on KCl and acetylcholine-induced tracheal contractions (Ach). Figure 1 shows that non-cumulative concentrations of GLHE (0.5, 1, 2 and 4 mg/ml) have reduced the KCl and ACh-induced tracheal contractions in a dose-dependent manner and significantly (n = 8, P<0.0001, tested by ANOVA). Each piece of trachea was used only for one of these two spasmogens.

![Fig. 1. Effect of non-cumulative concentrations of the grape leaf hydroalcoholic extract on rat tracheal contractions induced by KCl (60 mM) or ACh (55 μM). Spasmogens-induced contractions in the absence of extract were considered as 100% (n = 8). The P values of dose-response for the both curves (ANOVA) are <0.0001.](image-url)
Fig. 2. The comparison of spasmyolytic effect of grape leaf hydroalcoholic extract (Ext. 3 mg/ml) on contraction induced by KCl (60 mM) in absence and in the presence (10 min) of the nitric oxide synthase inhibitor (L-NAME, 100 µM) in rat trachea (n = 8; *, P<0.05; **, P<0.0001 and NS, non-significant).

Inhibitory effect of extract on trachea KCl-induced contraction in the presence of propranolol. As Figure 3 shows, the inhibitory effects of GLHE (3 mg/ml) on the KCl-induced contraction are not different in the absence and in the presence of propranolol [16] as a β-adrenoceptor antagonist (1 µM, for 10 min, n = 8).

Study of the cholinergic antagonistic effect of extract. The extract inhibited the contraction induced by acetylcholine to study whether this inhibitory effect is due to antagonistic effect on acetylcholine receptors or is just due to inhibiting the contracting effect of acetycholine, the following protocol was carried out. The trachea was contracted by KCl (30 mM) and after achievement of the plateau, ACh (27 µM) was added to organ bath. The ACh-induced contraction was super-imposed on KCl-induced contraction. The tissue was washed several times and was left for 20 min. In the next stage, atropine was first added to bath for 10 min and then the previous protocol was repeated. Figure 4 shows that only KCl induces contraction but not ACh. Meanwhile, applying the extract (4 mg/ml) abolished this contraction.

Fig. 3. Spasmyolytic effect of grape leaf hydroalcoholic extract (Ext. = 3 mg/ml) on contraction induced by KCl (60 mM) in absence and in the presence (10 min) of β-adrenoceptor antagonist, propranolol, (Prop. = 1 µM) in rat trachea (n = 8, *, P<0.05; **, P<0.0001 and NS = non significant).

Fig. 4. Effect of atropine (Atro. = 30 µM) on rat trachea superimposed contractions induced by KCl (30 mM) and acetylcholine (ACh = 27 µM). Atropine has only prevented the contractile effect of acetylcholine but not the KCl effect. Extract (4 mg/ml) has abolished the contractile effect of KCl (n = 7).

DISCUSSION

The present study showed that GLHE reduces the tracheal contractions induced by KCl and acetylcholine. In this bronchodilating effect, neither NO nor β-adrenoceptors were involved. This spasmyolytic effect was reversible due to the fact that, this effect was abolished when the bath solution was exchanged with fresh solution. Therefore, it seems that extract effects occur on the smooth muscle cell membrane. The depolarization of smooth muscle cells by KCl causes the activation of voltage dependent calcium channels (VDCC), which ultimately induces the contraction [16-18]. The existence of L-type calcium channels in the rat trachea has been reported [18] and supported by the inhibitory effect of verapamil on rat trachea contraction induced by KCl [19]. Furthermore, it is reported that the same extract reduces the rat aorta contracted by KCl [12] which support that, the

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VDCC are involved in the spasmolytic effect of GLHE. In the vasorelaxatory effect of the extract, NO and cGMP was involved but in trachea the involvement of NO was not the case, since the spasmyloytic effect of extract was unaffected by L-NAME. However, It has been reported that, the rat trachea produces NO and cGMP without NO synthase activation [20]. Therefore, it was possible that grape leaf flavonoids, for instance, quercetin [21] promotes NO production and induces the tracheal relaxation but without NO synthase activation. The inhibitory effect of quercetin on uterus and intestine contractility has been also reported [22, 23]. The β-adrenoceptors activation by isoproterenol causes tracheal relaxation [24] but the ineffectiveness of propranolol to reduce the extract spasmyloytic activity indicates that, these receptors are not involved. Acetylcholine depolarizes the tracheal smooth muscle via increasing intracellular calcium concentration [25]. Inhibitory effect of extract on ACh-induced tracheal contraction, could be an evidence for anticholinergic property of extract. In the presence of atropine, KCl induced contraction, however, ACh was unable to induce more contraction. Meanwhile, adding extract abolished the contraction induced by KCl. This result supports that, extract reduces tracheal contractility, possibly, through VDCC and the cholinergic receptors are not involved. Acetylcholine depolarizes the tracheal smooth muscle via increasing intracellular calcium concentration [25]. Inhibitory effect of extract on ACh-induced tracheal contraction, could be an evidence for anticholinergic property of extract. On the other hand, it has been reported that atropine reverses the ACh-induced relaxation in rat aorta precontracted by phenylephrine but the vasorelaxatory effect of the same extract was unaffected by atropine [12]. This report supports that GLHE reduces the ACh-induced tracheal contraction via blocking the events occurred by coupling ACh to its receptors rather than blocking cholinergic receptors.

In conclusion, these data suggest that the GLHE inhibits the rat tracheal contractions induced by KCl and ACh via blocking the VDCC on the smooth muscle cells membrane.

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REFERENCES


