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Original Article

Vasodilatory effect of *asafoetida* essential oil on rat aorta rings: The role of nitric oxide, prostacyclin, and calcium channels

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ABSTRACT

Background: *Asafoetida* is an oleo-gum resin mainly obtained from *Ferula assa-foetida L.* species in the apiaceae family. Previous studies have shown that it has antispasmodic effects on rat's and pig's ileums.
Purpose: The main goals of this study were to assess the vasodilatory effect of *asafoetida* essential oil (AEO) on the contractile response of rat's aorta rings and to find the role of nitric oxide, cyclooxygenase, and calcium channels. Thoracic aorta rings were stretched under a steady-state tension of 1 g in an organ bath apparatus for 1 h and then precontracted by KCl (80 mM) in the presence and absence of *AEO*. L-NAME (blocker of nitric oxide synthase) and indomethacin (blocker of cyclooxygenase) were used to assess the role of nitric oxide (NO) and prostacyclin in the vasodilatory effect of *AEO*. Also, the effect of *AEO* on the influx of calcium through the cell membrane calcium channels was determined.
Results: Data showed that *AEO* had vasodilatory effects on aorta rings with both intact (IC_{50} = 1.6 µl/l) or denuded endothelium (IC_{50} = 19.2 µl/l) with a significantly higher potency in intact endothelium rings. The vasodilatory effects of AEO were reduced, but not completely inhibited, in the presence of L-NAME or indomethacin. Adding AEO to the free-calcium medium also significantly reduced the $CaCl_2$-induced contractions.
Conclusion: The results indicated that AEO has a potent vasodilatory effect that is endothelium-dependent and endothelium-independent. Also, it reduced the influx of calcium into the cell through plasma membrane calcium channels.

Introduction

Asafoetida is an oleo-gum resin mainly obtained from the exudates of the roots and the stems of a medicinal plant named *Ferula assa-foetida L.* (Alves-Santos et al., 2016; Ramadan et al., 2004). This plant, which belongs to the apiaceae family, is an aromatic plant abundant in north-east of Iran, where it is known as "*Anghouzeh*" (Mahendra and Bisht, 2012; Ross, 2003). It is used as a flavoring spice in various kinds of food, and is also traditionally introduced as a valuable remedy to treat various diseases in different countries, especially respiratory and gastrointestinal diseases (Iranshahy and Iranshahi, 2011; Mahendra and Bisht, 2012; Ross, 2003).

Recent pharmacological and biological studies indicated that *asafoetida* has antioxidant, anti-diabetic, antimicrobial, hypotensive, anticancer, hepato-protective, antispasmolytic activities, etc. (Abu-Zaiton, 2010; Saleem et al., 2001; Ramadan and Al Khadrawy, 2003; Safari et al., 2016; Kavoosi et al., 2013; Ramadan et al., 2004). *Asafoetida* is composed of three fractions including resin (which consists of ferulic acid and its esters, coumarins, sesquiterpene coumarins, and other terpenoids by 40–64%), gum (which contains glucose, galactose, l-arabinose, rhamnose, and glucuronic acid by 25%) and essential oil (which contains sulfur-containing compounds, monoterpenes and other volatile terpenoids by 10–17%) (Mahendra and Bisht, 2012). It seems that the essential oil is partly responsible for the pharmacological action of *asafoetida* (Skalli et al., 2007).

The *asafoetida* essential oil is volatile, has an unpleasant odor and a bitter taste, and is extracted by distillation (Kavoosi and Rowshan, 2013). It has complex compositions. The major components of *asafoetida* essential oil (AEO) have been reported differently in various studies (Ahmadvand et al., 2013; Bahrami et al., 2013; Bamoniri

Abbreviations: AEO, *Asafoetida* essential oil; KCl, potassium chloride; NO, nitric oxide; COX, cyclooxygenase; L-NAME, L-NG-Nitroarginine methyl ester; $CaCl_2$, calcium chloride; IC_{50}, concentration required to relax the induced tone by 50%; cGMP, cyclic guanosine monophosphate

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and Mazoochi, 2009; Dehpour et al., 2009; Kavoosi et al., 2013; Kavoosi and Rowshan, 2013; Moghaddam et al., 2013; Sadraei et al., 2003; Sefidkon et al., 1998). Some reported that disulfides are the major components (Skalli et al., 2007) while others reported the pinene and cymene as the most frequent ones (Kavoosi et al., 2013; Bamoniri and Mazoochi, 2009). The main reasons for these differences are attributed to the climatological factors.

It has been reported that *Ferula asafoetida* gum extract reduced the spontaneous contraction of the isolated guinea-pig ileum, possibly through interference with membrane calcium channels activity (Fatehi et al., 2004). It also reduced the ileum contractions induced by acetylcholine, histamine, and KCl (Fatehi et al., 2004). In addition, intravenous infusion of *Ferula asafoetida* gum extract showed antihypertensive properties in anesthetized rats in a dose–dependent manner (Fatehi et al., 2004). Sadraei and co-workers reported that AEO has spasmolytic activity against potassium chloride (KCL), acetylcholine, and 5-hydroxytryptamine-induced ileum contraction in rats (Skalli et al., 2007). Also, it did show the relaxatory effect in pig's trachea rings (Kiyanmehr et al., 2016; Bayrami et al., 2013).

To the extent of our knowledge, there is no study on the effect of AEO on the vascular tone. In the present study, we determined the effect of AEO on the contractile responses of isolated rat's aorta induced by KCl. Finally, the role of nitric oxide (NO), cyclooxygenase (COX), and plasma membrane calcium channels in the vasodilatory effect of AEO were investigated.

Materials and methods

Animals

To perform this study, male Wistar rats weighing 250–350 g (4–6 months old) were used. They were kept under standard conditions with free access to rat chow and water *ad libitum*. All of the animal procedures were performed in accordance with the international guidelines for the care and use of animal laboratories. All of the experiments were approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Ferula asafoetida preparation

Ferula asafoetida oleo-gum (*asafetida*) was collected from Dorbid area, Yazd, Iran in spring. A voucher specimen was kept in record (A2343) at the Herbarium of the Herbal Medicine Research Center of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The Specimen was identified at the Botany Department, Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The plant name was confirmed with www.theplantlist.org on January 20, 2017.

Ferula asafoetida essential oil preparation

In brief, 100 g *Ferula asafoetida* was powdered and its essential oil was extracted and isolated using Clevenger apparatus for 6 h. Finally, 4 ml of yellowish essential oil was obtained (4% v/w) and its water removed by sodium sulfate. The essential oil was kept in a dark container in a refrigerator (4 °C).

Identification of AEO compounds using GC–MS

An Agilent Technologies GC–MS (Santa Clara, CA, USA) consisting of a 6890 GC system coupled with a 5973 network mass selective detector and equipped with a BP-10 capillary fused silica column (length, 30 m; internal diameter, 0.32 mm; 0.25 μm film thicknesses) was used for separation and identification of chemical compounds of AEO. The temperature program was initiated at 40 °C for 1 min, then was increased by 5 °C to 250 °C every one min and finally was held constant for 20 min. The injector temperature was 250 °C with the split ratio of 1:20. The carrier gas was helium with the purity of 99.999% and the flow rate of 1 ml per min. Mass spectra was taken at 70 eV and scanned mass range was set at 50–500 m/z (mass to charge ratio).

Preparation of aorta rings

Rats were anesthetized by intra-peritoneal injection of sodium pentobarbital (50 mg/kg) and sacrificed by cutting their abdominal aorta. Then, their thoracic aorta were removed and placed in a Petri dish containing Krebs solution. Following the removing of fat and connective tissues, the cleaned aorta was cut into 3–5 mm sections and mounted on a steel hook in an organ bath apparatus (Bioscience, UK) containing perfusion medium continuously bubbled with 95% O_2 and 5% CO_2 at 37 °C. The rings were passively stretched by a passive tension of 1 g for 60 min for stabilization. The tension was recorded using an isometric transducer connected to a Narco Physiograph (NARCO Bio-System, USA). All data were saved on the computer using a power lab data acquisition system (AD Instrument, Australia). After 60 min of equilibration, the stretched rings were contracted with KCl (80 mM). When the contraction reached to the steady maximal response, the essential oil and inhibitors were added to the medium.

To confirm the existence of endothelium, acetylcholine chloride (10^{-6} M) was added to the medium. If the rings were exhibited 15–20% relaxation against KCl–induced maximum contraction, the endothelium was considered as intact.

For relaxation experiments, each aortic contracted ring was cumulatively exposed to 25 μl/l AEO in eight steps. Relaxation was expressed as a percentage of the decrease in maximal tension obtained by KCl-induced contraction.

To investigate the role of NO and prostacyclin in the vasodilatory effect of AEO, aorta rings with intact-endothelium were exposed to 10^{-4} M L-NAME (Sigma, USA), an inhibitor of NO synthase, and 10^{-5} M indomethacin (Sigma, USA) as an inhibitor of COX for 60 min before adding KCl.

To determine the effect of AEO on the influx of calcium through the plasma membrane calcium channels, the aorta rings were exposed to free-calcium solution, AEO, KCl, and calcium chloride ($CaCl_2$), respectively.

Experimental grouping

To perform this study, four experiments were done as follows:

Experiment 1: Aortic rings were first contracted by adding KCl (80 mM) to the medium till the maximum contraction was achieved. Then, AEO was cumulatively added (25 μl).
Experiment 2: Aortic rings were first exposed to AEO (IC_{50} = 1.6 μl/l, for intact-endothelium and 19.2 μl/l for denuded-endothelium rings); then KCl was added cumulatively.
Experiment 3: Aortic rings were first exposed to L-NAME (10^{-4} M) or indomethacin (10^{-5} M) and then to AEO and KCl, respectively.
Experiment 4: Aortic rings were placed in a free-calcium medium and then KCl, AEO and $CaCl_2$ were added to the medium, respectively.

Statistical analysis

The data were expressed as Mean ± SEM. The IC_{50} value (the concentration to reduce the maximal contractile response to KCl in intact-endothelium and denuded-endothelium rings) was determined from the nonlinear regression (log [inhibitor] vs. normalized response-variable slope). The other data were analyzed by nonlinear regression (plateau followed by one phase decay). The statistical significance was determined by two-way ANOVA. The data were analyzed by Graphpad Prism version 6.00 for Windows (Graphpad Software, La Jolla

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