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Possible mechanism(s) for relaxant effects of *Foeniculum vulgare* on guinea pig tracheal chains

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In a previous study the relaxant (bronchodilatory) effect of *Foeniculum vulgare* on isolated guinea pig tracheal chains was demonstrated. To study mechanisms responsible for this effect the present study evaluated the inhibitory effect of this plant on contracted tracheal chains of guinea pig. The relaxant effects of aqueous and ethanol extracts and an essential oil from *Foeniculum vulgare* were compared to negative controls (saline for aqueous extract and essential oil and ethanol for ethanol extract) and a positive control (diltiazem) using isolated tracheal chains of the guinea pig precontracted by 10 μM methacholine (group 1) and 60 mM KCl (group 2, $n = 7$ for each group). In the group 1, experiments diltiazem, ethanol extract, and essential oil from *Foeniculum vulgare* showed a significant relaxant effect on methacholine induced contraction of tracheal chains compared to those of negative controls ($p < 0.05$ to $p < 0.001$). In addition the effect of the ethanol extract was significantly greater than that of diltiazem ($p < 0.001$). However, the aqueous extract did not show any relaxant effect in group 1. In the group 2 experiments, only diltiazem showed a significant relaxant effect on KCl induced contraction of tracheal chains ($p < 0.001$). The relaxant effects of ethanol extracts and essential oil obtained in the group 2 experiments were significantly lower than those in group 1 ($p < 0.05$ to $p < 0.001$). These results confirm the bronchodilatory effects of ethanol extract and essential oil from *Foeniculum vulgare*. However with regard to the effect of KCl on calcium channels, the results indicated that the inhibitory effect of ethanol extracts and essential oil from *Foeniculum vulgare* on calcium channels is not contributing to their relaxant (bronchodilatory) effects on guinea pig tracheal chains. However the results suggest a potassium channel opening effect for this plant, which may contribute on its relaxant effect on guinea pig tracheal chains.

1. Introduction

Foeniculum vulgare Mill. is a grassy plant with yellow flowers and small green to yellow seeds which is from the Labiatea family and grows in Iran and Mediterranean countries. The seeds of *Foeniculum vulgare* contain mainly *trans*-anethole, stragol, fenchyl acetate, limonene (Stahl 1982), (−) endo-fenchol (Croteau et al. 1995), and phonyl carbonic acid (Trankle et al. 1971).

Several therapeutic effects including reduction in blood pressure (Abdul-Ghani and Amin, 1986), antichronic colitis (Chakurski et al. 1981), increased milk secretion, promotion of menstruation, facilitating birth, alleviating the symptoms of the male climacteric, and increasing libido (Albert-Puleo 1980) have been observed for the seeds of *Foeniculum vulgare*. In addition, anti-asthma and dyspnea effects have been described for this plant in ancient Iranian medical books (Avesina 1990). Shah et al. (1991) also listed different therapeutic effects of this plant and showed that there is no significant toxic effect on mice of the ethanol extract from *Foeniculum vulgare*.

There is evidence of a relaxant effect of a volatile oil from this plant on isolated tracheal muscles of guinea pigs (Forster et al. 1980). However, a contractile effect of anethole, the major constituent of this plant on ileal smooth muscle of guinea pigs has also been demonstrated (Reiter and Brondet 1985). In contrast it has been shown that anethole has a relaxant effect on skeletal muscles (Albuquerque et al. 1995).

In a previous study, we have demonstrated the relaxant effects of different extracts and essential oil from *Foeniculum vulgare* on guinea pig tracheal chains (Boskabady and Khatami 2003).

In the present study the other possible mechanisms for the relaxant effect of essential oil, aqueous and ethanol extracts of this plant on guinea pig tracheal chains were investigated.

2. Investigations, results and discussion

The present study investigated the relaxant effect of essential oil, and aqueous and ethanol extracts from *Foeniculum*

Table: Relaxant effect of extracts and essential oil from *Foeniculum vulgare* on contracted tracheal chains of guinea pig in comparison with negative control (ethanol for ethanol extract, saline for aqueous extract and essential oil) and positive control (diltiazem).

Experimental designs	Group 1	Group 2
Saline	0.17 ± 0.12	-5.18 ± 3.42
Ethanol	23.71 ± 5.19	-8.7 ± 4.95
Aqueous extract	-21.52 ± 11.70 NS +	-22.52 ± 4.29** + nS
Ethanol extract	71.78 ± 6.7*** ++	-5.35 ± 3.78 NS + ⋆⋆
Essential oil	30.98 ± 4.79* ns	-3.15 ± 11.87 NS + ⋆
Diltiazem	19.66 ± 5.07*	90.71 ± 6.21 *** ⋆⋆

Group 1 (tissues contracted by 10 μM methacholine); Group 2 experiments (tissues contracted by 60 mM Kcl), (for each group n = 7). Values are presented as mean ± SEM. Statistical differences between the effects of extracts, essential oil and diltiazem with those of negative control; NS: non significant difference, *: $p < 0.05$, **: $p < 0.01$,***: $p < 0.001$. Statistical differences between the effects of extracts, essential oil with those of positive control ns: non significant difference, +: $p < 0.01$, ++: $p < 0.001$. Statistical differences between the result of two groups; nS: non significant difference, ⋆: $p < 0.05$, ⋆⋆: $p < 0.001$.

vulgare on guinea pig tracheal chains contracted by methacholine and KCl.

In group 1 experiments, diltiazem, and ethanol extract, and essential oil from *Foeniculum vulgare* showed a significant relaxant effect on methacholine induced contraction of guinea pig tracheal chains ($p < 0.05$ for diltiazem and essential oil and $p < 0.001$ for ethanol extract). However, the aqueous extract showed no significant relaxant effect compared to that of saline. The effect of aqueous extract was significantly lower ($p < 0.05$) and the effect of ethanol extract was greater ($p < 0.001$) than that of diltiazem (Table, Fig.).

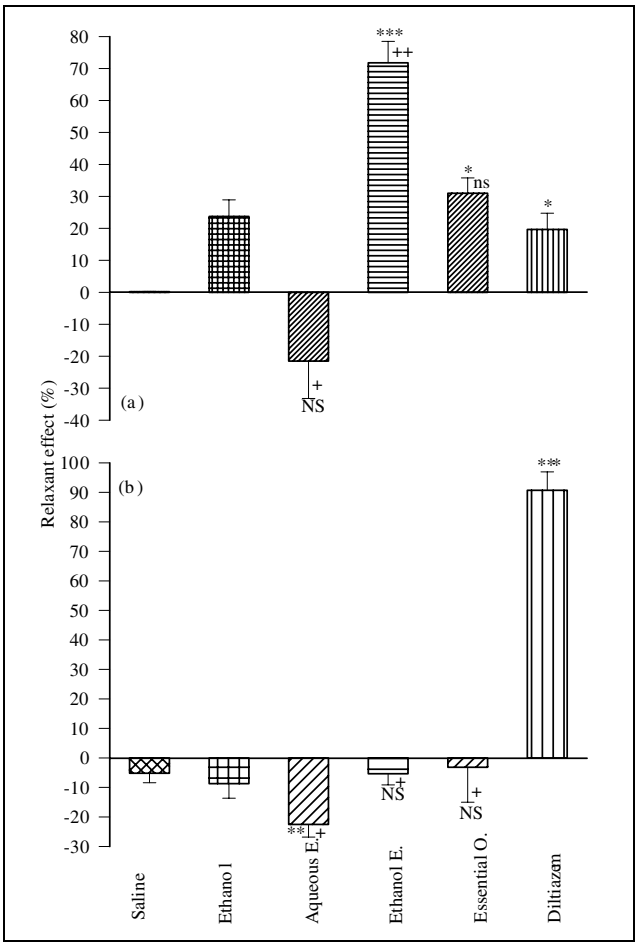

Fig.: Relaxant effect of different extracts and essential oil from *Foeniculum vulgare* and saline, ethanol and diltiazem on contracted tracheal chains of guinea pigs in group 1 (tissues contracted by 10 μM methacholine, a) and group 2 experiments (tissues contracted by 60 mM KCl, b), (for each group, n = 7). Differences between relaxant effect of negative controls (ethanol for ethanol extract and saline for aqueous extract and essential oil) with the effect of plant solutions; NS: non significant difference, *; $p < 0.05$, **; $p < 0.01$, ***: $p < 0.001$. Statistical differences between the effects of extracts, essential oil with those of positive control ns: non significant difference, +: $p < 0.01$, ++: $p < 0.001$.

In group 2 experiments, extracts and essential oil from *Foeniculum vulgare* showed no significant relaxant effect on KCl induced contraction of guinea pig tracheal chains. In fact aqueous extract showed a significant contractile effect compared to that of saline ($p < 0.01$, Table, Fig b). The effects of the extracts and essential oil were significantly different from that of diltiazem ($p < 0.05$ for all cases, Table, Fig b).

The effects of ethanol extract and essential oil in the group 2 experiments were significantly lower than those in group 1 ($p < 0.05$ for essential oil and $p < 0.001$ for ethanol extract, Table 1). However the effect of diltiazem in group 2 was significantly greater than that in group 1 ($p < 0.001$, Table 1).

In the present study examined other possible mechanisms for the relaxant effects of essential oil and extracts (aqueous and ethanol) of *Foeniculum vulgare* on guinea pig tracheal chains. In the group 1 experiments, essential oil, ethanol extract and diltiazem showed significant relaxant effects on methacholin induced contraction of tracheal chains. The results of this group support the findings of previous studies demonstrating a relaxant effect of volatile oil from *Foeniculum vulgare* on isolated tracheal and ileal smooth muscle of the guinea pig and a relaxant effect of essential oil and ethanol extract from this plant on tracheal chains. The relaxant effect of ethanol extract was significantly and the effect of essential oil non-significantly greater than that of diltiazem in this group of studies.

The absence of an observed relaxant effect for aqueous extract in group 1 could be due to a lower level of effective substance(s) or to the presence of a greater amount of anethole in aqueous extract than in essential oil and ethanol extract, because anethole has a contractile effect on smooth muscle (Reiter and Brandt 1985). This is presumably due to the difference in methods of extraction between extracts and essential oil. The relaxant effect of ethanol might indicate that the effect of ethanol extract is due to its ethanol content. However, the relaxant effect of ethanol extract was significantly higher than that of the same volume of ethanol in group 1 experiments.

In group 2, extracts and essential oil from *Foeniculum vulgare* did not show any relaxant effect on KCl induced contraction of tracheal chains. The relaxant effects of both extracts and essential oil were significantly lower than that of diltiazem in group 2 experiments.

Given that KCl affects calcium channels (Perez-Guerrero et al. 1997) and having regard to the bronchodilatory ef-

fect of calcium channel blockers (Miyahara et al. 1993; McCaig and DeJonckeere 1993), these findings showed the absence of a blocking effect of the two extracts and essential oil from *Foeniculum vulgare* on calcium channels. The absence of an obvious relaxant effect of ethanol extract and essential oil from this plant in group 2 and the relatively potent relaxant effect of extracts and essential oil in group 1 experiments may also indicate an opening effect of these solutions on potassium channels, because the bronchodilatory effect of potassium channel opening has been demonstrated previously (Buckle et al. 1993). If the ethanol extract and essential oil from *Foeniculum vulgare* had a potassium channel opening effect, they would not have a relaxant effect on tracheal chains contracted by KCl, whereas they could show a relaxant effect when the tracheal chain was contracted by metacholine. However aqueous extract showed a contractile effect in group 2. The reason of this effect is not clear to us and needs to be clarified in further studies.

Our previous study also showed that muscarinic and histamine (H_1) receptor inhibitory and ß-adrenoceptor stimulatory effects of this plant do not contribute to the relaxant property of the plant.

The other possible mechanisms causing a bronchodilatory effect include: stimulation of inhibitory non-adrenergic, non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC (Linden et al. 1993), methyl xanthin activity (Meini et al. 1993), and inhibition of phosphodiesterase (Van Amsterdam et al. 1993). The contributions of these mechanisms to the bronchodilatory effect of *Foeniculum vulgare* remain to be clarified in further studies.

In conclusion the results of the present study confirmed the relaxant effect of essential oil and ethanol extract from *Foeniculum vulgare*. However the relaxant effect of the extracts and essential oil from this plant is not due to an inhibitory effect of the plant on calcium channels. Indeed, the results suggest a potassium channel opening effect for this plant, which may contribute to its relaxant effect on guinea pig tracheal chains.

3. Experimental

3.1. Plant and extracts

Foeniculum vulgare was identified by Mr M. H. Joharchi in the herbarium of Ferdowsi University, Mashhad and the specimen number of the plant is 7702. The plant extracts were prepared as follows:

Aqueous extract: Fifty grams of the chopped, dried plant were extracted with 300 ml distilled water by Soxhlet apparatus.

Ethanol extract was prepared in the same way, as aqueous extract, except that the solvent was ethanol instead of distilled water. The solvents of both extracts were then removed under reduced pressure until the extract volume reached 20 ml. Plant ingredient concentrations in the final extracts were 33.3% W/V for both aqueous and ethanol extracts.

Essential oil: One millilitre of essential oil was extracted from 100 g of the chopped, dried plant with 1000 ml distilled water by steam distillation apparatus. The concentration of plant ingredients in the essential oil was 10% V/V.

3.2. Tissue preparations

Male guinea pigs (400–700 g) were killed by a blow on the neck and trachea were removed. Each trachea was cut into 10 rings (each containing 2–3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain (Holroyde 1986). Tissue was then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent U. K.) containing Krebs-Henseleit solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11.

The Krebs solution was maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

3.3. Protocols

The relaxant effects on calcium channels of isolated guinea pig tracheal chains of 0.02 ml of essential oil, 0.6 ml of aqueous extract and 0.1 ml of ethanol extract from *Foeniculum vulgare* and 5 μM diltiazem (Sigma Chemical Ltd, UK) as a positive control were examined. Saline (0.6 ml) was used as a negative control for essential oil and aqueous extract, and ethanol (0.1 ml) for ethanol extract.

In each experiment the effect of one of the solutions on contracted tracheal smooth muscle was measured after exposing the tissue to the solution for 10 min. A decrease in tone was considered as a relaxant effect and expressed as positive percentage change and an increase in tone was considered as a contractile effect and expressed as a negative percentage change in proportion to the maximum contraction obtained due to contractile agents.

The relaxant effect of different solutions was tested with two different experimental designs as follows:

1. On tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK), (group 1 experiments).
2. On tracheal chains contracted by 60 mM KCl (group 2 experiments).

The relaxant effects in the two groups of experiments were examined in two different series of tracheal chains (for all groups, $n = 7$). All of the experiments were performed randomly with a 1 h resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and were measured after fixation.

3.4. Statistical analysis

The data on the relaxant effects of the different solutions were expressed as mean ± SEM. The relaxant effects of the different concentrations of extracts and diltiazeml were compared using the ANOVA test. The relaxant effects of the two experimental groups were compared using the unpaired "t" test. Significance was taken as $p < 0.05$.

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