

RELAXANT EFFECTS OF *ACHILLEA WILHELMSII* ON GUINEA-PIG TRACHEAL CHAINS

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Summary

Achillea wilhelmsii have been used in folk remedies and its different pharmacological effects including relaxant effect on some smooth muscle was demonstrated. Therefore in the present study, the relaxant effects of the extract of *Achillea wilhelmsii* on tracheal chains of guinea pigs were examined. The relaxant effects of four cumulative concentrations of the extract (2, 4, 6 and 8 mg/ml) in comparison with saline as negative control and four cumulative concentrations of theophylline (0.2, 0.4, 0.6 and 0.8 mM) were examined by their relaxant effects on precontracted tracheal chains of guinea pig by 10 μ M methacholine (n = 11). All concentrations of theophylline and three last concentrations of the extract (4, 6, and 8 mg/ml) showed significant relaxant effects compared to that of saline (p<0.001 for all cases). However, the relaxant effects of three lower concentrations of the extract (2, 4, and 6 mg/ml) were significantly less than those of theophylline (p<0.01 to p<0.001). There were significant positive correlations between the relaxant effects and concentrations for both theophylline and the extract (p<0.001 for both cases). These results showed a relatively potent relaxant effect for the extract of *Achillea wilhelmsii* on tracheal chains of guinea pigs.

Key words: *Achillea wilhelmsii*, Extract, Relaxant effects, Guinea pig, Trachea

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Relaxant effect of *Achillea wilhelmsii*

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Introduction

The herb *Achillea* (Asteraceae Compositae), is a genus with more than 100 species all around the world [1]. These plants are medicinal perennial rhizomous herbs, are also in Australia, New Zealand and North America and specially in Europe and Western Asia [2]. *A. millefolium* is the best known species, and was used as traditional medicine for treating swollen tissues and wounds [3]. The aerial parts of different species of the genus are widely used in folk medicine due to numerous medicinal properties, such as anti-inflammatory, antispasmodic, antihemorrhoidal, stomachic, antiseptic and emmenagogue [4–6].

The main components of the oil of *A. wilhelmsii* were carvacrol (25.1%), linalool (11.0%), 1,8-cineol (10.3%), E-nerolidol (9.0%) and borneol (6.4%), [7].

The analgesic [8] and anticonvulsant [9] of *A. wilhelmsii* has been demonstrated. Several studies [10, 11] showed different effects from the extracts of *A. wilhelmsii* or *A. talagonica* grown in Iran as being antilipidemic, antihypertensive or immunosuppressive for humans or laboratory animals respectively. The relative radical scavenging activity of the plant also documented (12).

The antispasmodic activity of total extract of *A. nobilis* subsp. *sipylea* on rat duodenum demonstrate was observed [13]. Some flavonoids can act as spasmolytic agents by relaxing various smooth muscles [14, 15]. They have also reduced the tone of guinea-pig isolated trachea, main pulmonary artery, rat uterus and rat vas deferens [16-18]. It has been also suggested that the inhibitory effects of cirsiol on smooth muscle are attributed to inhibition of transmembrane Ca^{2+} influx [19]. In addition the relaxant effect of carvacrol on tracheal smooth muscle is observed in our previous study [20].

Therefore in the present study, the relaxant effects of hydro alcoholic extract from *A. wilhelmsii* on tracheal chains of guinea pigs were examined.

Materials and Methods

Experimental Plant and fractions

Achillea wilhelmsii was collected from north east region of Iran and identified by MR Joharghi. A voucher specimen was preserved in the Herbarium of the School of Agriculture, Ferdowsi University (Herbarium No: 40377, FUMH). The aqueous-ethanolic extract of the plant was prepared as follows: fifty grams of *Achillea millefolium* were grinded, added to 700 ml of ethanol 50% (350 ml distilled water and 350 ml ethanol) using the Soxhlet apparatus. The solvent was then removed under reduced pressure. The plant ingredient concentration in the final extract was adjusted to 0.1 g/ml by adding distilled water to the dried extract.

Tissue preparation

Guinea pigs (400-700 g, both sexes) were killed by a blow on the neck and tracheas 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 (21). Tissue was then suspended in a 10 ml organ bath (organ bath 61300, Bio Science Palmer-Washington, Sheerness, Kent U.K.) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, NaHCO₃ 25, MgSO₄ 0.5, KH₂PO₄ 1.2, KCl 4.72, CaCl₂ 2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95% O₂ and 5% CO₂. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

Protocols

The relaxant effects of four cumulative concentrations (2, 4, 6 and 8 mg/ml) of the extract of *A. wilhelmsii* and theophylline anhydrous (Sigma Chemical Ltd UK) (0.2, 0.4, 0.6 and 0.8 mM) as positive control, and saline (1 ml) as negative control were examined. To produce the first concentration of the extract, 0.2 ml of 0.1 g/ml was added to a 10 ml organ bath and for other three concentrations; 0.2 ml of 10 g% was added to organ bath three times respectively. For theophylline, 0.2 ml of 20 mM concentrated solution was added to organ bath 4 times. The consecutive volumes were added to organ bath at five minutes intervals.

In each experiment, the effect of saline, four cumulative concentrations from the extract and theophylline on contracted tracheal smooth muscle induced by 10 µM methacholine hydrochloride (Sigma Chemical Ltd UK) was measured after exposing tissue to each concentration of the solution for 5 min (n = 11). A decrease in tone was considered to be a relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to the maximum contraction. An increase in tone was considered as a contractile (bronchoconstrictory) effect which was expressed as negative percentage change (32).

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 contractions were measured using an isotonic transducer (Harvard APP LTD, 50-6360 SINO . 0210) and measured by using a software by a computer (Acer model NO.: G781) recording.

Statistical analysis

All data were expressed as mean±SEM. Data of relaxant effects of different concentrations of the extract were compared with the results of negative and positive control using paired t test. The relaxant effect of the extract and theophylline were related to the concentrations using least square regression. Significance was accepted at p<0.05.

Results

Relaxant (bronchodilatory) effect

All concentrations of theophylline and three last concentrations of the extract (4, 6 and 8 mg/ml) showed significant relaxant effects compared to that of saline (p<0.001 for all cases), (Table 1).

Table 1. Relaxant effect of four concentrations of the extract of *A. wilhelmsii* in comparison with negative control (saline) and positive control (theophylline), (n=11)

Concentrations	Saline	Theophylline	St. Dif. vs Saline	Extract	St. Dif. vs Saline
1	-	28.9±2.80	p< 0.001	0±2.70	NS
2	-	45.75±4.33	p< 0.001	19.59±2.89	P<0.001
3	-	65.29±4.50	p< 0.001	43.88±6.03	P<0.001
4	0.56±0.2 1	90.43±4.87	p< 0.001	81.32±8.61	P<0.001

Values are presented as mean±SEM. St. Dif.: Statistical deference. The four different concentrations for the extract were 2, 4, 6 and 8 mg/ml, and for theophylline, 0.2, 0.4. 0.6 and 0.8 mM.

Comparison of the relaxant effect of theophylline with different fractions

The relaxant effects of three lower concentrations of the extract (2, 4 and 6 mg/ml) were significantly less than those of theophylline (p<0.05 to p<0.001), (Fig. 1).

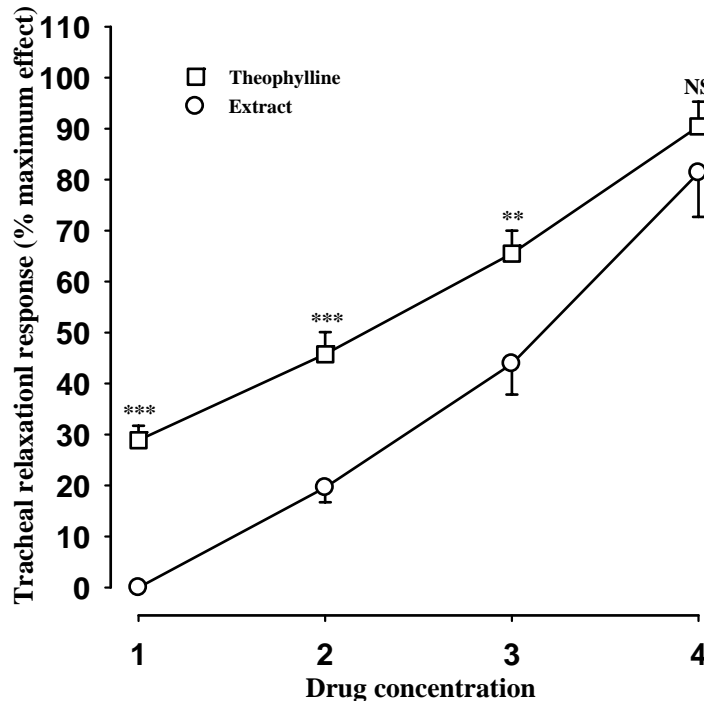


Fig. 1 Concentration response curves of the relaxant effect of theophylline and the extract of *A. wilhelmsii* in methacholine induced contraction of tracheal chains (n=11). Statistical differences in the relaxant effect different concentrations of the extract with those of theophylline; NS: non-significant difference, **; $p < 0.01$, ***; $p < 0.001$. The concentrations of the extract were 2, 4, 6 and 8 mg/ml and for theophylline, 0.2, 0.4, 0.6 and 0.8 mM.

Correlation between concentrations of solutions and their relaxant effect

There were significant positive correlations between the relaxant effects and concentrations for both theophylline and the extract ($p < 0.001$ for both cases).

Discussion

In this study, the relaxant effects of the extract from *A. wilhelmsii* in comparison with saline as negative control and theophylline as positive control were studied. The extract and theophylline showed relaxant effect on tracheal smooth muscle. The relaxant effects of the extract was significantly less than that of theophylline. There were positive correlations between concentrations and the relaxant effects of both theophylline and the extract.

The results of this study confirmed those of the study of Asgari *et al.* [11] indicating the antihypertensive effect of the plant which is perhaps due to its relaxant effect on vascular smooth muscle. The reduction of the tone of guinea-pig isolated trachea, main pulmonary artery, rat uterus and rat vas deferens observed for the plant in different studies [16-18] also support the findings of the present studies.

The results of the present study showed a relative potent relaxant effect on tracheal chains contracted by methacholine. These results may suggest a muscarinic inhibitory effect for the plant. However the exact effect of the plant on muscarinic receptors should be evaluated by performing concentration-response curves to a muscarinic receptor and examining the rightward shift of the curve obtained in the presence of the plant extract. It has been also suggested that the inhibitory effects of cirsiol on smooth muscle are attributed to inhibition of transmembrane Ca^{2+} influx [19]. Therefore, the inhibitory of the plant on calcium channels could be contributed in observed relaxant effect of the plant extract in the present study. The contribution of the other possible mechanism(s) for the observed relaxant effect of the plant on tracheal smooth muscle including the stimulatory effect of the plant on β -adrenoceptors should be examined in further studies. While the relaxant effect of carvacrol, the main constituent of the plant is demonstrated in our previous study [20], the relaxant effect observed in the present study could be due to this component of the plant which should be clarified in further studies.

In conclusion These results of the present study showed a relatively potent relaxant effect for the extract of *A. wilhelmsii* on tracheal chains of guinea pigs which was almost comparable to that of theophylline. The possible mechanism(s) for observed relaxant effect on tracheal muscle should be examined in further studies.

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