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Original Article

Antitussive Effect of *Plantago lanceolata* in Guinea Pigs

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Abstract

Background: Several therapeutic effects including anti-asthma and dyspnea have been described for *Plantago lanceolata*. In the present study the antitussive effect of this plant was evaluated.

Methods: The antitussive effects of aerosols of two different concentrations of Soxhlet and macerated ethanolic extracts, codeine and saline were tested by counting the number of coughs produced due to aerosol of citric acid 10 min after exposing the animal to aerosols of different solutions (n=8 for each solution).

Results: The results showed significant reductions of cough number observed in the presence of both concentrations of Soxhlet and macerated extracts of *Plantago lanceolata* as compared to saline treated group ($p<0.001$). The reduced cough number observed in the presence of higher concentrations of extracts was not significantly different from that of lower concentrations of LP. Furthermore, there was no significant difference between the cough numbers observed in the presence of both concentrations of the extracts with that of codeine.

Conclusion: The ethanolic extracts of *Plantago lanceolata* have antitussive effects comparable with that of codeine.
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Keywords ● Plantago lanceolata ● antitussive effect ● guinea pig ● citric acid ● codeine

Introduction

Plantago lanceolata L. (PL; *Plantaginaceae*), a perennial plant species with a worldwide distribution and large ecological amplitude. IGs (Iridoid Glycosides) are a group of monoterpene-derived compounds that have been recorded in over 50 plant families.[1] The main IGs found in *P. lanceolata* are catalpol and its precursor aucubin.[2] Several therapeutic effects including: therapeutic effect on gastrointestinal, blood and respiratory (asthma and dyspnea) disorders have been described for the *Plantago lanceolata* in Iranian ancient medical books.[3]

Different pharmacological effects have been reported for the *Plantago lanceolata* including growth hormone like effect,[4] anti-pollen,[5] anti-oxidant,[6] anti-inflammatory,[7] as well as therapeutic effect on upper airway inflammation,[8] and therapeutic effect on asthma.[9] Therefore, in the present study the antitussive effects of different extracts from this plant were evaluated.

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Materials and Methods

Plant and extract

Plantago lanceolata was collected from Sangsefid region in Khorasan Province, North-east of Iran. The identity of the plant was confirmed and for future reference a voucher specimen (Herbarium No: 220-1612-02) was preserved in the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences. Mashhad, Iran.

The plant extracts were prepared as follows: For macerated ethanolic extract: 50 g of the chopped, dried plant was macerated with 300 ml 96% ethanol and shaken (on a shaker) for 48 hours. For Soxhlet ethanolic extract the same amount of plant was extracted with 500 ml 96% ethanol by Soxhlet apparatus. The solvent of both extracts were then removed under vacuum and then distilled water was added to the dried residue to have a final plant ingredient concentration of 10 g/100ml in both extracts.

Protocols

All experiments were performed randomly with two hour resting period between each two experiments. The study was approved by the ethical committee of Mashhad University of Medical Sciences. Dunkin-Hartley guinea pigs of both sexes were used in the study (body weight 500-600g). The animals were divided into six groups in random order (n=8 for each group). The method used has been described previously.[10]

Unanesthetized unrestrained animals were placed individually in a transparent Perspex chamber, dimensions 30x20x20 cm and exposed to a nebulized aqueous solution of 0.1 g/ml citric acid for seven min. The aerosol was produced by an air flow of 8 l/min through a Wright nebulizer. The aerosol particles had a mass median aerodynamic diameter of 0.9 µm as determined by laser light scattering (Malvern Instruments 2600 HSD analyzer; Malvern, U.K). The output of nebulizer was 0.6±0.04 ml solution/min. The same nebulizer was used throughout the experiments. During the last five min of the exposure, a trained observer continuously watched the animal and counted the numbers of coughs as described earlier.[10] Coughs could easily be distinguished from sneeze, since there is a clear difference in sound as well as in behavior of the animals.[10]

The above protocol was performed 10 min after exposing the animals to aerosols of the following solutions for a period of 7 min in 6 groups of animals:

i Normal saline (baseline)
ii Codeine (0.03 g/ml, positive control)
iii Macerated extract (2.5 g%)
iv Macerated extract (5 g%)
v Soxhlet extract (2.5 g%)
iv Soxhlet extract (5 g%)

Statistical analysis

Data were expressed as mean±SEM. Comparison of baseline data with the number of coughs obtained in the presence of plant extracts and codeine were made using ANOVA. Comparison of data obtained in the presence of two different concentrations of aqueous and macerated extracts were made using unpaired Students t test and statistical significance was accepted at $p<0.05$.

Results

Both concentrations of Soxhlet and macerated extracts, and codeine caused significant reductions in cough numbers compared to that of saline group as baseline value ($p<0.01$ to $p<0.001$), (Table 1). The antitussive effects of both concentrations of aqueous and macerated extracts were not significantly different with that of codeine (Table 1).

There was no significant difference between the effects of two extracts of macerated and Soxhlet. In addition the antitussive effects of higher concentrations of Soxhlet and macerated extracts were not significantly different than those of lower concentrations.

Discussion

In the present study the antitussive effects of extracts from *PL* were evaluated using a standard method used previously by several investigators.[10,11] The result of the present study demonstrated a relatively potent antitussive effect for both extracts from *Plantago lanceolata*.

Table 1: Comparison of the number of coughs observed in the presence of two extracts (Soxhlet and macerated) from *Plantago lanceolata* with those obtained in the presence of saline (baseline) and codeine

Groups (n=8)	n of coughs	St dif *vs* Baseline	St dif *vs* Codeine
Baseline	16.13±1.34		
Codeine 0.03 g/ml	9.75±1.08	p<0.001	
Soxhlet 2.5 g%	11.63±0.94	p<0.01	NS
Soxhlet 5.0 g%	9.50±0.88	p<0.001	NS
Macerated 2.5 g%	11.63±1.51	p<0.01	NS
Macerated 5.0 g%	9.50±1.72	p<0.001	NS

Values are presented as mean±SEM. St dif= statistical difference; NS: not significant

However, the effect of the higher concentration of each extract was not significantly different from those of the lower concentrations. The antitussive effects of both extracts from *PL* were comparable with that the effect of codeine at concentration used.

Although, the antitussive effects of different extracts from *PL* were similar to that of codeine, the mechanism(s) of antitussive effect of this plant cannot be concluded from the results of the present study. Opioids, such as morphine and codeine, are generally considered to be the most potent and effective antitussive drugs available and are believed to inhibit coughs through suppression of a cough center in the central nervous system.[12,13] Morphine is shown to reduce vagally mediated bronchoconstriction produced by inhaled distilled water in asthmatics and in healthy human subjects.[14]

The bronchoconstriction to inhaled capsaicin was attenuated by nebulized codeine and morphine.[15] The mechanism behind this inhibitory effect is unknown, but suppression of neurotransmitter release has been suggested. Inhibitory opioid receptors have been demonstrated on peripheral nerves,[16] inducing vagal sensory neurons.[17,18] Some experimental data indicate that opioids may interact with the peripheral nervous system of the tracheobronchial tree. A partial antagonism of a noncholinergic neurogenic bronchoconstriction in the guinea pig by opioid agonists has been reported.[19-21] Karlsson *et al.* also showed that nebulized codeine and morphine could inhibit bronchoconstriction and coughs induced by citric acid using a method similar to that of the present study.[22] Therefore, the similarity of the effects of *PL* extracts with that of codeine indicate that the antitussive effect of this plant might possibly be due to its bronchodilator property.

In addition, coughs can be induced by irritation of sensory receptors located in the vicinity or below the epithelial lining of tracheobronchial tree. The sites of airway branching may be particularly sensitive to tussive stimuli.[23] Sensory receptors mediating reflex bronchoconstriction seem, however, to be distributed all along the tracheobronchial tree.[24] Advenier *et al.* showed the tachykinin receptor antagonists have also antitussive effect.[25] Therefore, the antitussive effect of *PL* might be due to its possible tachykinin inhibitor substance(s) mediating both bronchodilatory and antitussive effect.

With regard to inflammatory effect of tachykinin substance(s) the antitussive effect of this plant may be due to its anti-inflammatory effect because it has anti-inflammatory effect on the respiratory system.[7,8] However, this inflammatory effect does not seem to occur during in a short period of time and is not effective in time period used in the present study. Therefore, the mechanism(s) of antitussive effect of *PL* should be investigated in further studies.

Although codeine is a central antitussive drug, it is possible that both codeine and plant extracts are absorbed through muco and induce their antitussive effects centrally. This may happen for just codeine or both extracts of the plant and codeine can affect their antitussive effect peripherally.

Misawa and colleagues also showed the antitussive effect of several volatile oils by inhalation and IP injection.[11] The antitussive effect of volatile oils in their study was smaller than that of codeine. Although, the antitussive effect of *PL* was smaller than *Caurium copticom*,[26] *Nigella sativa*,[27] but it was greater than of *Rosa damascene*.[28] Therefore, further studies are needed to evaluate the potency of the antitussive effect of *PL* extracts.

The similar antitussive effect of two extract may suggest that the effective antitussive substance(s) of two extracts are similar. The non significant difference in antitussive effect between two concentrations of extract may indicate that in lower concentration of extracts (2.5 g%) the maximum effect is achieved.

Conclusion

The antitussive effect of *Plantago lanceolata* is comparable with that of codeine at concentrations used but the exact mechanism of this effect should be clarified in further studies.

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