ANTITUSSIVE EFFECT OF *ASTRAGALUS GUMMIFER* IN GUINEA PIGS

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Running head: Antitussive effect of *Astragalus gummifer*

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Summary

Several therapeutic effects including anti-asthma and dyspnoea have been described for *Astragalus gummifer*. In the present study the antitussive effect of this plant was evaluated. The antitussive effects of aerosols of two different concentrations of macerated aqueous and ethanolic extracts, codeine, and saline were tested by counting the number of coughs produced due to aerosol of citric acid 10 min after exposing animal to aerosols of different solutions (n=8 for each solution). The results showed significant reduction of cough number observed in the presence of both concentrations of extracts and codeine ($p<0.01$ to $p<0.001$). The cough number observed in the presence of higher concentration of macerated aqueous extract was significantly and that of ethanolic extracts non-significantly less than those of lower concentrations. In addition the effects of both concentrations of ethanolic extract were significantly greater than cough numbers observed in the presence of codeine. The antitussive effects of both concentrations of ethanolic extract were significant greater than cough numbers observed in the presence aqueous extract ($p<0.05$ for both concentrations). These results indicated potent antitussive effect of aqueous and ethanolic extract from *Astragalus gummifer* which was greater than that of codeine for ethanolic extract.

Introduction

Astragalus gummifer is a shrub that grows wild in the mountains of the Middle East, principally Iran and Turkey [1] Gum tragacanth is a complex heterogeneous acidic polysaccharide (containing galactose, arabinose, xylose, fucose, rhamnose, and galacturonic acid). Gum tragacanth also contains proteins. Analysis of amino acid content reveals that it is highest in hydroxyproline, but also contains most other amino acids, with the exception of cystine and methionine [1].

Several therapeutic effects including: therapeutic effect on respiratory diseases have been described for *Astragalus gummifer* in Iranian ancient medical books [2].

Different pharmacological effects have been reported for the *Astragalus gummifer* including ant-inflammatory effect on airway inflammation [3], protective effect on pulmonary epithelial damage [4], the effect on immune process [5], including the effect on non-specific immunity [6] and immune regulatory [7] effects. However, the effect of this plant on atopic prevalence also has been shown [8]. In addition, anti bacterial [7] and anti-carcinogenic [9] effects of *Astragalus gummifer*. Is effects on bone loss [10] hepatic fibrosis [11] and virus myocarditis [12] have been also demonstrated.

Therefore, in the present study the antitussive effects of different extracts from this plant were evaluated.

Materials and Methods

Plant and extract

Astragalus gummifer was purcharged from Glodaru Company, Esfahan, Iran. The plant extracts were prepared as follows: For macerated aqueous extract: 50 g of the gums of the plant was macerated with 300 ml distilled water and shaken (on a shaker) for 48 h. For macerated ethanolic extract the same amount of the gums of the plant was extracted with 300 ml ethanol using the same method as macerated aqueous extract. The solvent of both extracts were then removed under reduced pressure and distilled water was added so that the plant ingredient concentration in the final extracts was 10 g% in both extracts.

Protocols

Dunkin-Hartley guinea pigs of both sexes were used in the study (body weight 500-600g).The method used has been described previously [13].

Unanaesthetized unrestrained animals were placed individually in a transparent perspex chamber, dimensions 30 x 20 x 20 cm and exposed to a nebulized aqueous solution of 0.1 g/ml citric acid for 7 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 0.65±0.04 ml solution per minute. The same nebulizer was used throughout the experiments. During the last 5 min of the exposure, a trained observer continuously watched the animals, and the numbers of coughs were determined. Coughs could easily be distinguished from sneeze, since there is a clear difference in sound as well as in behaviour of the animals [13].

The above protocol was performed 10 min after exposing animals to aerosols of the following solutions for a period of 7 min (n=8 for each solution):

i. Normal saline (baseline measurements)
ii. Codeine solution (0.03 g/ml, positive control)
iii. Macerated aqueous extract (0.25 g%)
iv. Macerated aqueous extract (0.5% g%)
v. Macerated ethanolic extract (0.25% g%)
vi. Macerated ethanolic extract (0.5% g%)

All of the experiments were performed randomly with 2 h resting period between each two experiments. The study was approved by the ethical committee of Mashhad University of Medical Sciences.

Statistical analysis

Data were expressed as mean ± SEM. Comparison of baseline data with 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 paired “t” test. Significance was accepted at $p<0.05$.

Results

Both concentrations of aqueous and ethanolic extracts, and codeine caused significant reduction in cough numbers compared to baseline value ($p<0.05$ to $p<0.001$), (Table 1, Figure 1). The antitussive effect of aqueous extract was not significantly different with that of codeine. However, there were significant differences in antitussive effects of both concentrations of ethanolic extract and codeine ($p<0.05$ for both concentrations, Table 1, Figure 1).

The antitussive effects of higher concentrations of aqueous extract of the plant was significantly greater than those of lower concentrations ($p<0.05$) However, there was no significant difference between the antitussive effect of two concentrations of ethanolic extract. (Figure 1).

The effect of both concentrations of ethanolic extract were significantly greater than those of aqueous extract ($p<0.05$ for both cases, Figure 1)

Table 1

Comparison of number of coughs observed in the presence of two extracts (aqueous and macerated) from *Astragalus gummifer* with those obtained in the presence of saline (baseline) and codeine (for each experimental design, n=8)

Experimental design		Number of coughs	St. Dif. *vs* Baseline	St. Dif. *vs* Codeine
Baseline		16.13±1.34		
Macerated aqueous extract	0.25 g%	12.25±2.07	$p<0.05$	NS
Macerated aqueous extract	0.5 g%	8.50±0.86	$p<0.001$	NS
Macerated ethanolic extract	0.25 g%	6.13±0.30	$p<0.001$	$p<0.05$
Macerated ethanolic extract	0.5 g%	5.62±0.70	$p<0.001$	$p<0.05$
Codeine	0.03 g/ml	9.75±1.08	$p<0.001$	

Values are presented as mean±SEM. St. Dif.: statistical difference; NS: nonsignificant difference.

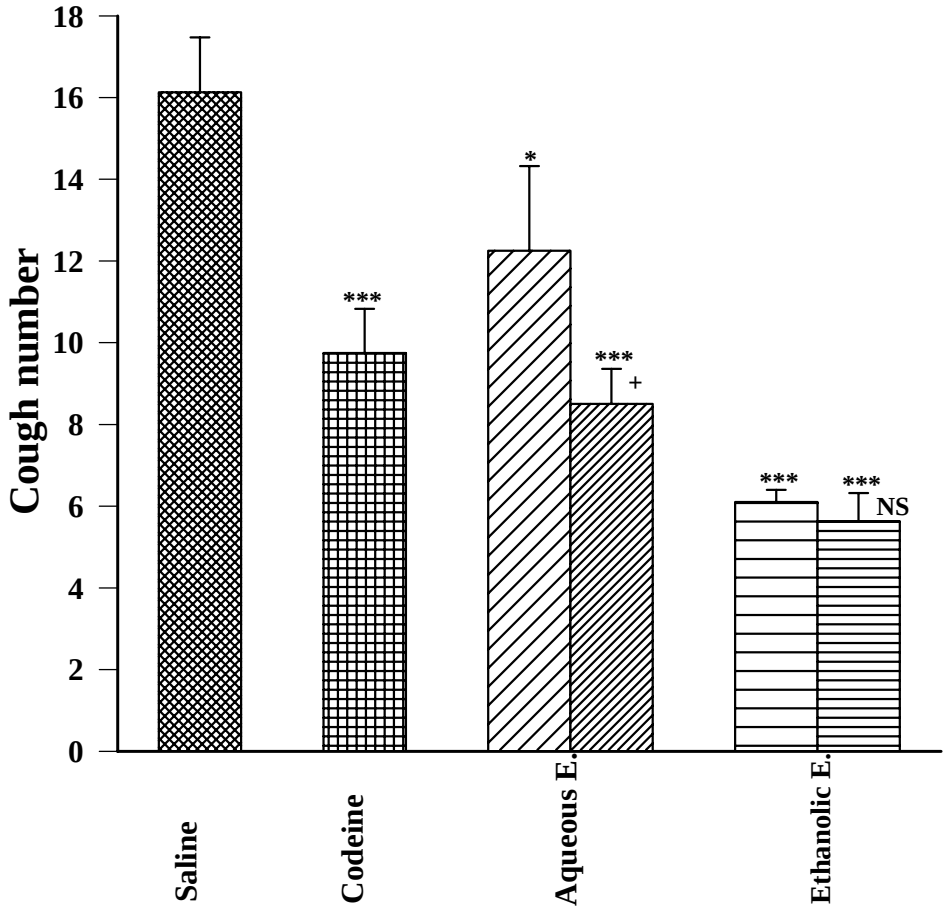

Figure 1 Cough numbers observed in the presence of lower concentration (2.5W/W, medium filled bars) and higher concentration (5.0 W/W, fine filled bars) of aqueous and ethanolic, extracts from *Astragalus gummifer* and those obtained in the presence of saline (baseline) and codeine. Statistical differences between the effects of plant extracts and baseline value; *: $p<0.05$,**: $p<0.01$, ***: $p<0.001$. Statistical differences in cough numbers between two concentrations of aqueous and macerated extracts; +: $p<0.05$, NS: non significant difference.

Discussion

In the present study the antitussive effects of extracts from *Astragalus gummifer* were evaluated using a standard method used previously by several investigators [13-14]. The result of the present study demonstrated a potent antitussive effect for both extracts from *Astragalus gummifer*. The effect of the higher concentration of aqueous extract was significantly greater than those of the lower concentrations. The antitussive effects of ethanolic extract from *Astragalus gummifer* was also greater than the effect codeine at minute concentration used of the extract.

Although the antitussive effects of different extracts from *Astragalus gummifer* were similar or even greater than 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 [15-16]. Morphine was recently shown to reduce a vagally mediated bronchoconstriction produced by inhaled distilled water in asthmatics and in healthy human subjects [17]. The bronchoconstriction to inhaled capsaicin was attenuated by nebulized codeine and morphine [18]. 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 [19], inducing vagal sensory neurons [20-21]. 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 [22-24]. Karlsson *et al* [25] also showed that nbulized codeine and morphine could inhibit bronchoconstriction and coughs induced by citric acid using a method similar to that of the present study. Therefore, the similar antitussive effect of extracts from *Astragalus gummifer* and codeine may indicate that the antitussive effect of this plant is due to its possible bronchodilator property.

In addition, coughs can be induced by irritation of sensory receptors located within and immediately below the epithelial lining. Sites of airway branching may be particulary sensitive to tussive stimuli [26]. Sensory receptors mediating reflex bronchoconstriction seem, however, to be distributed all along the tracheobronchial tree [27]. Advenier *et al* [28] showed the tachykinin receptor antagonists have also antitussive effect. Therefore, the antitusive effect of *Astragalus gummifer* might be due to its possible tachykinin inhibitor substance(s) content mediating both

bronchodilatory and antitussive effect.

With regard to inflammatory effect of tachykinin and because *Astragalus gummifer* has anti-inflammatory effect [3], the antitussive effect of this plant may be due to its anti-inflammatory effect. However, the inflammatory effect of *Astragalus gummifer* does not seems to occurs 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 *Astragalus gummifer* should be investigated in further studies.

Misawa and Kizawa [14] also showed the antitussive effect of several volatile oils by inhalation and i.p. injection. The antitussive effect of volatile oils in their study was smaller than that of codeine. Therefore the *Astragalus gummifer* has a potent antitussive effect that required further studies**.**

The higher antitussive effect of ethanolic extract compared to aqueous extract may suggest that the effective antitussive substance(s) of two extracts are different. This is due to variation in method of extraction in two different extracts. The non sgnificant difference in antitussiveeffect between two concentrations of ethanolic extract may indicate that in lower concentration of this extract (2.5 g%) the maximum effect is achieved.

In conclusion the results of the present study indicated a potent antitussive effect of *Astragalus gummifer* especially for ethanolic extract which was greater than that of codeine at concentration used but the exact mechanism of this effect, should be clarified in further studies.

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