Research Article

Effect of *Orthosiphon aristatus* Extract as Histamine Antagonist on Tracheal Chain of Guinea Pig.*

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Abstract

The extract of *Orthosiphon aristatus* (Ya-nuadmaew or Cat's beard herb) was studied *in vitro* on isolated tracheal chain of guinea pig preparation. It was found that an aqueous extract in the dose of 8 mg/mL caused a relaxation of smooth muscle trachea and also could completely block histamine-contraction. This paper suggested that the active substance in this herb should be extracted for studying further on chemical structure, mechanism of action and side effects before being used as anti-asthma agent.

Key words : *Orthosiphon aristatus*, anti-asthma, histamine

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บทคัดย่อ : ผลของสารสกัดหญ้าหนวดแมว *Orthosiphon aristatus* ในการต้านฤทธิ์ฮิสตามีนในหลอดลมหนูตะเภา*

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สารสกัดจากหญ้าหนวดแมว *(Orthosiphon aristatus)* เมื่อนำมาศึกษาฤทธิ์ต่อหลอดลมหนูตะเภา พบว่า สารสกัดด้วยการต้มกับน้ำแล้วระเหยแห้งในปริมาณ 8 มก/มล สามารถทำให้กล้ามเนื้อเรียบของหลอดลมคลายตัว และสามารถต้านฤทธิ์ของฮิสตามีนในการทำให้หลอดลมหดตัวได้เท่าๆ กับ theophylline ปริมาณ 4 x 10^{-3} โมลาร์ รายงานนี้เสนอแนะว่า ควรมีการศึกษาเพิ่มเติมถึงผลของสารสกัดหญ้าหนวดแมวต่อสารอื่นๆ ที่ทำให้หลอดลมหดตัว และหาวิธีสกัดสารออกฤทธิ์ให้มีความเข้มข้นมากขึ้น เพื่อศึกษาชนิดของสารและกลไกการออกฤทธิ์ต่อหลอดลมและฤทธิ์ข้างเคียงอื่นๆ อันจะเป็นประโยชน์ในการนำมาใช้รักษาหรือบรรเทาอาการหอบหืดต่อไป

คำหัส : หญ้าหนวดแมว, *Orthosiphon aristatus*, สารต้านอาการหอบหืด, ฮิสตามีน

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Orthosiphon aristatus is a Southeast Asian plant which has been used for medicinal purposes. Its leaves is used for alternative treatment of renal and urinary tract diseases. It was welknown that the crude extract has potent diuretic activity in Thai traditional medicine.[1] The tea of *O. aristatus* leaf is also believed to have relief action on bronchial asthma but there were still have controversial conclusions.[2-3] The present report aims to study the action of *O. aristatus* hot-water extract as a bronchodilator using tracheal chain of guinea pig as a model and histamine as a mediator of allergic reaction.

Materials and Methods

O. aristatus was extracted by boiling 300 g of fresh herbs in 3 L of distilled water for

1 hours. After filtration to remove the residue, the extract was further boiled to have 100-200 mL solution for lyophilization. The powder obtained was weighed and dissolved in Kreb's solution[2] before experimentation.

Tracheal chain was prepared by cutting the trachea from 300-350 g guinea pig, remove the fat and other tissues before keeping in Kreb's solution. The trachea was cut at the junction of cartilages into 5-6 rings, then wrapped each ring together by fixing the part of smooth muscle of adjacent ring in the opposite side as shown in Figure 1 A.

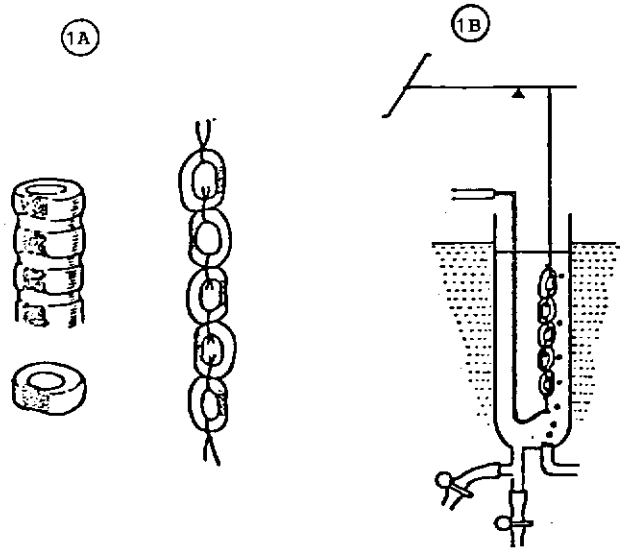


Figure 1 Guinea pig tracheal chain preparation

The tracheal chain was put into a tissue chamber containing 10 mL of Kreb's solution (Figure 1 B) in which oxygen gas (oxygen + 5% carbondioxide) was passed continuously and the temperature of the solution was stabilized at 37 °C. One end of the tracheal chain was fastened with the hook of tissue chamber and the other end with force-displacement transducer FT-03C. Tension of the tracheal chain was adjusted to be 1 g everytime before starting every experiment in the study.

The tracheal chain was equilibrated as indicated by stable graph on polygram using Grass Model 7D Polygraph. The test solution (*O. oristatus* 0, 2, 6, 8 mg/mL, histamine 2×10^{-3} M or theophylline 4×10^{-3} M) was then added one by one into the chamber and the changing of tracheal chain tension was recorded.

Results

The hot water extract of *O. aristatus* leaf (2, 4, 6 and 8 mg/mL) could decrease the muscle tension. The 8 mg/mL fraction was

repeated 5 times to get average bronchodilatation effect of 0.52 ± 0.0425 g decreased from the original 1 g tension as shown in Table 1.

Table 1 Tension decreased (in g) of bronchodilatation by *O. aristatus* extract (8 mg/mL).

No. of experiment	Bronchodilatation (tension decreased)
1	0.35
2	0.75
3	0.35
4	0.55
5	0.60
Mean ± SE	0.52 ± 0.0425

The inhibition effect of the extract was demonstrated by addition of the extract before and after histamine 2 x 10^{-3} M (Figure 2). This effect was confirmed by adding theophylline (4 x 10^{-3} M) as bronchodilator control after histamine addition. It was found that the extract had the same inhibition action on histamine as theophylline did (Figure 3).

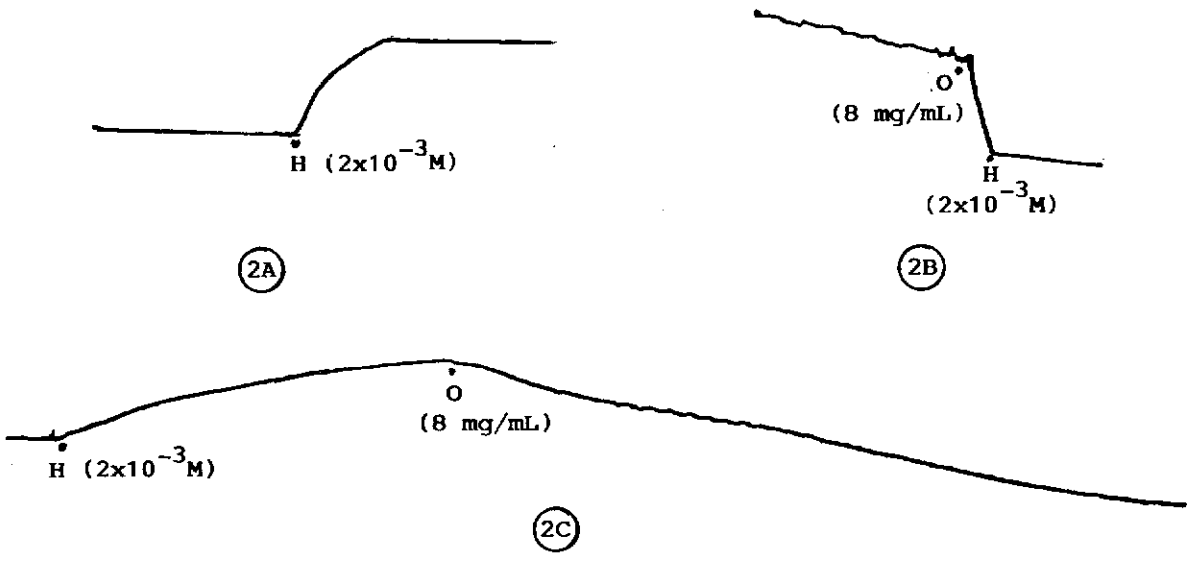


Figure 2 Effect of **2A** Histamine (H, 2x10^{-3} M) (paper feed 10 mm/min); **2B** *O. aristatus* extracts (O) on guinea pig bronchodilatation before histamine action (paper feed 5 mm/min) and **2C** *O. aristatus* extracts (paper feed 50 mm/min) after histamine action.

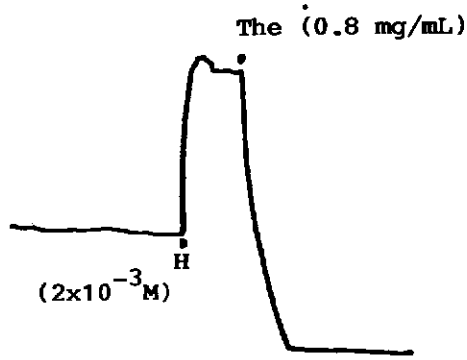


Figure 3 Effect of theophylline (The) after histamine (H) action on guinea pig trachea (paper feed 5 mm/min).

Discussion

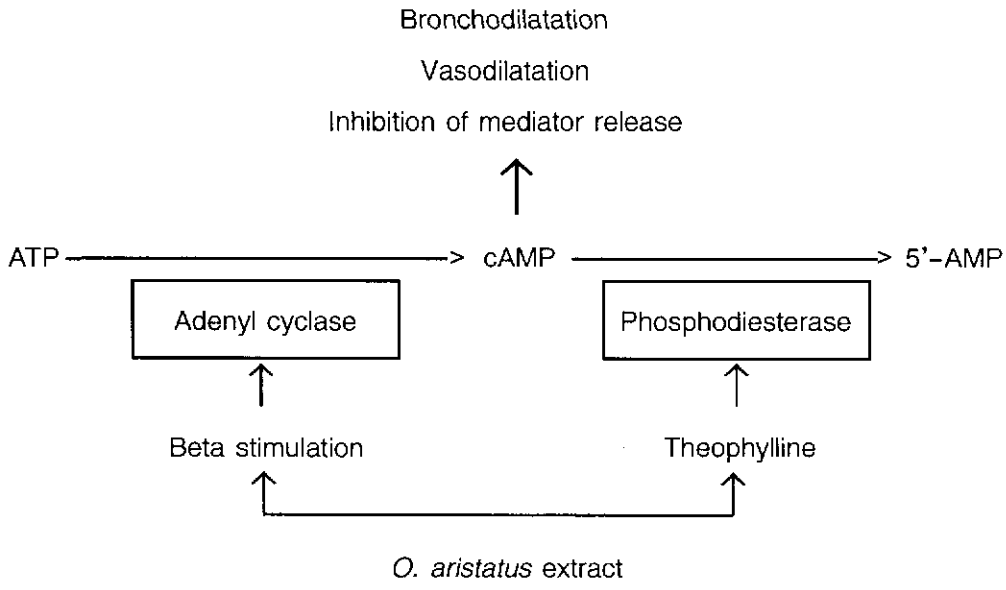
Asthma is an allergic response characterized by spasm of smooth muscle in the bronchioles, intermittent reversible obstruct of the airways.[4] Allergen may be histamine (β-imidazolylethylamine), platelet activating factor (PAF), prostaglandins, peptidoleukotrienes, *etc*, released from mast cells or tissues. PAF was recognized to be 1,000 times more potent than histamine.

Various drugs were investigated to be used for asthmatic therapy including β-agonist, corticosteroids, anticholinergic drugs, lipoxygenase inhibitor, *etc.*[4-6] Oral administration of these drugs usually made systemic side effects. Modification to lessen the side effects was tried, for example, aerosol form was used instead.[7]

The other attempt for seeking the new drugs that has as least side effect as possible are going on. Many herbs were studied for this purpose. One example was platelet activation factor antagonist (PAF-agonist) extracted from Chinese medicinal herbs which have a marked inhibitory effect on ability of antigen to produce late-onset airway obstruction.[4]

O. aristatus which was used in our preliminary study showed bronchodilator and antihistamine action as theophylline did. As our preliminary studies of *O. aristatus* extract with hot water showed that the extracts contained at least 3 fractions of acidic polysaccharide without sulfate group.[8] Another preliminary test was performed by further extraction of the hot-water extract with ethanol. It was found that the ethanol insoluble part could inhibit acetylcholine bronchoconstriction of guinea pig trachea (unpublished data). From our findings we could propose the possible mechanisms of *O. aristatus* as (Scheme 1): (1) there may be some chemical constituents in the extract that can stimulate adenyl cyclase for increase production of cAMP from ATP. The cAMP produced may inhibit mediator releasing from cells, then vasodilatation and bronchodilatation occurred; (2) the extract itself may have inhibitory effect against phosphodiesterase, making cAMP increased due to the cessation

of inactivation to 5'-AMP; (3) the extract itself is histamine antagonist; or (4) the extract have anticholinergic activity. Further studies should be performed by extracting the active substance in this herb for identification of chemical structure(s), mechanism of action on various bronchoconstrictors and side effects of the substances before being used as anti-asthma agent.



Scheme 1 Proposed mechanism of action of *O. aristatus* extract

References

1. Masuda T, Masuda K, Nakatani N. Orthosiphil A, a highly oxygenated diterpene from the leaves of *Orthosiphon stamineus*. Tetrahedron Letter 1992; 33: 945-6.
2. Malterud CE, Hanche-Olsen IM, Smith-Kielland I. Flavonoids from *Orthosiphon spicatus*. Planta Medica 1989; 55: 569-70.
3. Sumaryono W, Proksch P, Hartmann T, Nimiz M, Wray V. Induction of rosmarinic acid accumulation in cell suspension cultures of *Orthosiphon aristatus* after treatment with yeast extract. Phytochem 1991; 30: 3267-71.
4. Page C. Asthma as a chronic inflammatory disease and the implication for future therapy. Ann Allergy 1992; 62: 251-60.

5. Suziki F, Kuroda T, Nakasato Y, Manabe H, Ohmori K, Kitamura S, Ichikawa S, Ono T. New bronchodilators. 1. 1,5-substituted 1H-Imidazo[4,5-c]quinolin-4(5H)-ones. J Med Chem 1992; 35: 4045-53.
6. Lyckander IM, Malterud KE. Lipophilic flavonoids from *Orthosiphon spicatus* as inhibitors of 15-lipoxygenase. Acta Pharm Nord 1992; 4: 159-66.
7. Vichayanond P, Chokephaibulkit K, Kerdsomnuig S, Visitsuntorn N, Tuchinda M. Clinical evaluation of the 'Siriraj spacer' in asthmatic Thai children. Ann Allergy 1993; 69: 433-8.
8. Chanarat N, Amatrat A, Apisariyakul A, Michio F, Nagumo T. Effect of *Orthosiphon aristatus* extract as hypoglycemic agent. Bull Chiang Mai AMS 1997; 30 (Suppl 1): S32-9.