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Evaluation of antiasthmatic activity of *Stephania japonica*m Thunb. Miers.,

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ABSTRACT

Antiasthmatic activity of Ethanolic extract of *Stephania japonica* Thumb, Miers., in various experimental models**.** The present study deals with the effect of aqueous and ethanolic extract of leaves of S*tephania japonica* by using in vivo animal models. The study shows that extract is effective against histamine induced contraction. The study in guinea pigs by the aerosol testing method and histamine induced broncho contraction in guinea pig ileum. The studies showed significant protection at lower doses while further increase in dose level showed reduced activity. The result of test extracts of *stephania japonica* suggested that is effective in reducing the symptoms of bronchial asthma and also improve the lung function parameters of asthmatic subjects.

Keywords: Antiasthmatic activity, Histamine induced bronchoconstriction, bronchodilator, Histamine aerosol, *Stephania japonica*.

1. INTRODUCTION

Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world. The currently used drugs for the treatment of this disease in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. Asthma is a chronic condition involving the respiratory system in which the airways occasionally constrict, become inflamed, and are lined with excessive amount of mucous, often in response to one or more triggers. These episodes may be triggered by exposure to environmental stimulants such as an allergen, tobacco smoke, cold or warm air, perfume, pet, dander, moist air, exercise or exertion or emotional stress. [1]

More than 400 medicinal plant species have been used ethno pharmacologically and traditionally to treat the symptoms of asthmatic and allergic disorders worldwide. The World Health Organization (WHO) has recognized herbal medicine as an essential building block for primary health care of vast countries like India and China. [2]

Asthma is characterized by:

- *Airway inflammation:* The airway lining becomes red, swollen and narrow.
- *Airway obstruction:* The muscles encircling the airway tighten causing the airway to narrow making it difficult to get air in and out of the lungs.
- *Airway hyper-responsiveness:* The muscles encircling the airway respond more quickly and vigorously to small amounts of allergens and irritants.

1.1. Medicinal Plants Used in the treatment of Asthma

Large number of medicinal plants are claimed to be useful in asthma in all traditional systems of medicine and folklore. While these plant remedies (both herbal and multi herbal formulations) are being used orally and by local application since ancient times, the mechanisms where by such effects are elicited have not been looked into. These effects may be brought by their inherent Antibacterial, Antifungal, Antiviral, Antiinflammatory, Antiallergic, Antihistaminic properties [3,4].

Asthma is a chronic condition involving the respiratory system in which the airways occasionally constrict, become inflamed and are lined with excessive amount of mucous often in response to one are more triggers. Histamine is one of the important mediators of allergy, inflammation and bronchoconstriction, which were released after degranulation of mast cell by

an antigen exposure. Targeting histamine, either prevention of its release from mast cell or use of histaminergic receptor. [5]

2. MATERIALS AND METHODS

2.1. Plant collection and authentication

The plant leaves (Figure 1) of *Stephania japonica* was collected from surrounding areas of Komarapalayam and Sankagiri, Namakkal District, Tamilnadu, India.

The fresh plant material was collected from Namakkal District in the month of June 2014. The plant material was taxonomically authentified by Dr. Jayaraman, Botanist, Chennai, Tamilnadu. (CARC/2014/3069).

Figure - 1: Leaves of *Stephania japonica* Thunb. Miers.

2.2. Plant drying and size reduction

The shade dried leaves of *Stephania japonica* were cut into small pieces of 2 cm and dried in shade 20-25°C at room temperature. The plant material stored in PVC bag and stored in dark & clean place in airtight container. [6]

2.3. Method

About 1kg of the air dried powdered plant material was extracted successively with solvents of increasing polarity using soxhlet extractor.

2.3.1. Petroleum ether extract of leaves of *Stephania japonica*

The dried coarse powder of *Stephania japonica* was extracted with 2.0 litres of petroleum ether (60 - 80°C) by continuous hot percolation method using soxhlet apparatus. After 24 hours the extraction was completed then petroleum ether extract was taken and the solvent was redistilled. A dark green coloured extract was obtained and stored in a desiccator. [7]

2.3.2. Ethanolic extract of the leaves of *Stephania japonica*

The plant leaves was dried and subsequently extracted with 5 litres of ethanol by continuous hot percolation method. After the completion of the extraction it was filtered and solvent was removed by distillation under reduced pressure, and the green coloured extract was stored in a desiccator. The marc was dried for aqueous extraction. [7,8]

2.3.3. Aqueous extract of the leaves of *Stephania japonica*

The marc left after ethanolic extraction was dried and macerated with 2-3 litres of chloroform water (0.25%) in a narrow mouthed bottle for three days. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extract was then stored in desiccator. The results are shown in table 1.

Above extracts were used for identification of constituents by phytochemical tests, separation and isolation of plant constituents by chromatographic, spectral methods and for the pharmacological studies.

Table - 1: Successive Solvent Extraction of *Stephania japonica*

Extracts	Colour and Consistency	Percentage yield of extracts of *Stephania japonica* w/w
Petroleum ether	Green & viscous mass	1.48
Ethanol	Green & semisolid in nature	9.86
Aqueous	Brown semi solid in nature	13.5

2.3.4. Anti asthmatic activity

2.3.4.1. Histamine-induced bronchospasm in guinea pigs -Aerosol testing method. [9,10]

Animals used:

Guinea pigs of pir Bright white strain (400-500 g).

Drugs Used:

Standard: Promethazine hydrochloride (300µg/kg); Test: Ethanol, Aqueous extracts of *Stephania japonica* (100mg/kg) respectively.

Animals were divided into 4 groups of 6 animals each.

The groups were as follows:

Group 1: Control (Saline 1.0ml/kg).

Group 2: Standard (Promethazine hydrochloride 300µg/kg) p.o.

Group 3: Ethanolic extract of *Stephania japonica* (100mg/kg) p.o.

Group 4: Aqueous extract of *Stephania japonica* (100mg/kg) p.o.

Animals belonging to each group were subjected to histamine aerosol (0.2% histamine diphosphate in saline) using a nebulizer for 2 seconds in an airtight Perspex Histamine chamber. Aerosolization of the solution was achieved via a compressed air line operating at a pressure of 8psi and flow rate of 5mL/min. After exposure to the histamine aerosol, the animal showed a sign of immediate immobilization, bouts of coughing.

This was followed by shallow breathing symptoms, after which the animals collapsed fell on its back and convulsed. The time taken at the animal to fall on its back after exposure to the aerosol was designated as the exposition time. The exposition time for each animal in all the groups were noted. Once the animal fell on its back, it was after exposure to the aerosol was designated as the exposition time. The exposition time for each animal in all three groups were noted. Once the animal fell on its back, it was immediately taken out of the chamber and exposed to fresh air for the recovery of treated animals.

After one hour, the animals in the test groups were administered orally 100mg/kg with the ethanolic and aqueous extracts of *Stephania japonica* respectively, (Figure 3) while reference group of animals received 300µg/kg Promethazine hydrochloride by oral route. One hour later, the animals were exposed on the aerosol and exposition time before and after extract administration was taken as a measure of the protective effort of the drugs and percent protection afforded by the samples was calculated by the formula

$$\text{Percentage protection (\%)} = \frac{\text{Eta-Etb}}{\text{Etb}} \times 100$$

Eta – mean exposition time after treatment with the extracts.

Etb – mean exposition time before treatment with the extracts.

2.3.4.2. Effect of *Stephania japonica* extracts on histamine induced contraction in guinea pig ileum

Hı antagonist inhibits most responses of smooth muscle to histamine, Hı receptor antagonist act on vestibular afferents and within the brain storm. In guinea pig, for example death by anaphylaxis follows quite small doses of histamine.

Procedure

The antihistamine effect was studied in isolated guinea pig ileum. The guinea pig was sacrificed by a blow on the head and carotid bleeding was done. The abdomen was cut and the caecum was opened to trace the ileo-caecal junction. A few cm length of the ileal position was cut out and removed. It was immediately placed in watch glass containing Tyrode solution. The ileum was cut into pieces of 2-3 cm long [11].

One piece of the ileum was taken and tied with a thread in the top and bottom ends without closing the lumen. The tissue was mounted in the organ bath containing Tyrode solution maintained at 37 ^{0}C and bubbled with air. A tension of 0.5 gm was applied on the tissue. The tissue was allowed to stabilize for 30 minutes after placing in the organ bath. The contraction dependent responses of histamine (using Standard stock solution 1µg/ml) using frontal writing lever were recorded. A contact time of 30 seconds and 5 minutes time cycle were followed for recording the responses. Atleast four concentration responses of histamine (using standard solution 1µg/ml) were recorded.[12]

Table - 2: Effect of *Stephania japonica* extracts on Histamine induced Bronchospasm in guinea pig

Treatment	Pre-treatment Exposition time in Seconds ± SEM	Post-treatment exposition time in Seconds±SEM	Percentage Protection
Control	108±1.12	108±1.56	0
Promethazine Hydrochloride (300µg/kg/p.o)	110±1.29	387±1.59*	71.57
Ethanolic extract (100mg/kg/p.o)	108±1.26	310±1.30	65.2
Aqueous extract (100mg/kg/p.o)	112±1.53	228±1.46*	45.5

n=6;*P<0.001 Vs Control; P value was calculated by comparing with Control by Student "t" Test

Table - 3: Effect of *Stephania japonica* extracts on histamine induced contractions in isolated guinea pig ileum

Method	Amount
Concentration of Standard stock solution of Histamine	1µg/mL
Ethanolic extract of *Stephania japonica*	250mg/mL
Aqueous extract of *Stephania japonica*	250mg/mL
Contact time of *Stephania japonica*	30 Seconds
Time cycle	3 Minutes
Bath volume	30 mL
Temperature of bath	37 ^{0}C
Physiological solution	Tyrode solution

Tyrode solution containing 250mg/ml of *Stephania japonica* charged in the reservoir and the tissue was allowed to stabilize in it for 30 minutes, after that the sub- maximal dose of histamine was repeated. The inhibitions of the response were observed by comparing it with the maximal response of histamine (Figure 7 and 8). Responses to the test extracts of *Stephania japonica* were expressed as percentages of the maximum relaxation of contracted tissues induced by histamine [13].

Histamine induced a substantial contraction of the ileum of guinea pig and the tissue returned to the baseline after washout with Tyrode solution. The alcohol and aqueous extracts of *Stephania japonica* relaxed the contractions induced by histamine (Figure 9 and 10). [14,15]

Figure - 2: Housing of animals.

Figure - 3: Oral Administration of drug.

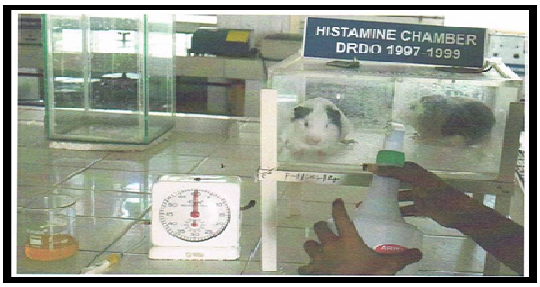

Figure - 4: Chamber Saturated with Histamine Aerosol.

Figure - 5: Noted the recovery time.

Figure - 6: Recovery of animals after breathing difficulty.

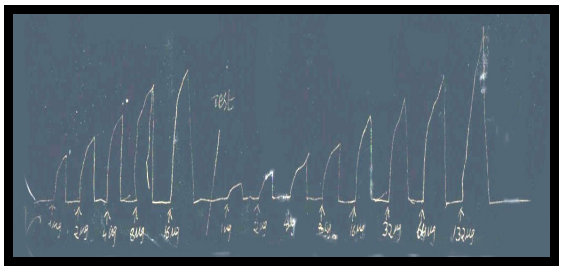

Figure - 7: Effect of ethanolic extract on *Stephania japonica* on Isolated guinea pig ileum.

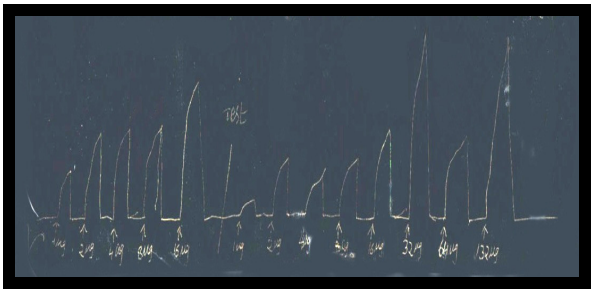

Figure - 8: Effect of Aqueous extract on *Stephania japonica* on isolated guinea pig ileum.

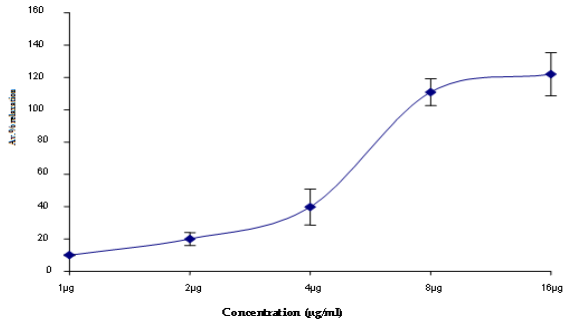

Figure - 9: Effect of the alcoholic extract on histamine induced contraction of the isolated guinepilleum.

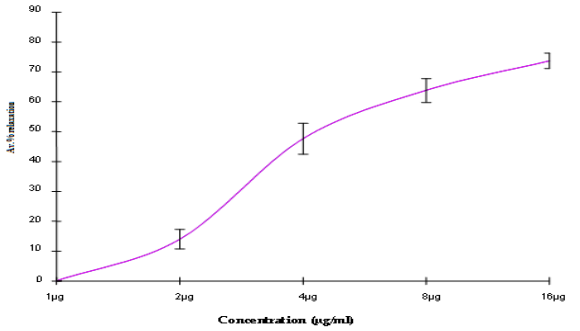

Figure - 10: Effect of the aqueous extract on histamine induced contraction of the isolated guinea pig illeum.

3. RESULTS AND DISCUSSION

The leaves of *Stephania japonica* Thunb, Miers., belonging to familyMenispermaceae has been investigated in a sytematic way covering pharmacological studies**.**

Litrerature survey revealed that not much work is done on this plant. Therefore, it was thought worthwhile to cary out the pharmacological studies on this plant. The ethanolic and aqueous extracts of leaves of *Stephania japonica*were selected for its Pharmacological activity as they were found to contain more phytoconstituents like phytosteroids and steroids, alkaloids, glycosides, flavonoids, etc.,

Antiasthmatic activity for the ethanol and aqueous extracts of *Stephania japonica* were studied in guinea pigs by the Aerosol testing and Histamine induced contraction in guinea pig ileum method.

In the histamine aerosol study, the control animals showed convulsion during the first 3 minutes of the experiment. Prior treatment of ethanolic and aqueous extracts of *Stephania japonica*(100mg/kg, p.o) protected the animals (Table-2) to a significant extent ($P < 0.001$) from the evelopment of asphyxia produced by histamine aerosol confirming that it has antihistaminic activity.

The role of histamine in asthma is well established. [16] The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins. Although there are various model of asthma, guinea pig airways react to histamine, acetylcholine, leukotrienes, and other bronchoconstrictors in a manner similar to that seen in humans [17].

Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to β-adrenergic antagonists. [18] Thus, the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release. Histamine antagonists can be conveniently recognized and assayed by their ability to protect guinea pigs against lethal effects of histamine-induced bronchospasm [19].

In vivo study of ethanolic & aqueous extracts of*Stephania japonica*toguinea pigs had shown the significant increase in preconvulsion time when the guinea pigs were exposed to histamine. The results of present investigation suggest that, ethanolic and aqueous extracts of *Stephania japonica*have significant bronchodilatory activity against histamine. [20,21]

3.1. Histamine induced contraction in guinea pig ileum

Histamine is one of the important mediators of allergy, inflammation and bronchoconstriction, which were released after degranulation of mast cell by an antigen exposure. Targeting Histamine, either prevention of its release from mast cell or use of histaminergic receptor antagonist becomes part of antihistaminic therapy in allergic disease. [22]

The alcohol and aqueous extracts of *Stephania japonica* inhibited contractions induced by histamine -the agent which implicate in various ways in the pathogenesis of asthma (Figure 9 and 10).

The results of this study indicated that the alcoholic and aqueous extracts of *Stephania japonica* relaxed significantly ileumpre-contracted by histamine, the extracts probably might have acted by blocking the muscarinic receptors. The ability of the alcoholic and aqueous extracts of *Stephania japonica*to inhibit the contractions

induced by the histamine suggests a possible role in the treatment of asthma. The relaxation of histamine pre-contracted ileum by the extracts of *Stephania japonica* indicates their potency in ameliorating established asthma.

The results of test extracts of *Stephania japonica* suggested that it is effective in reducing the symptoms of bronchial asthma and also improve the lung function parameters of asthmatic subjects.

Results of the experimental studies of ethanolic and aqueous extract of leaves of *Stephania japonica* suggested that anti-asthmatic activity could be due to its bronchodilator, mast cell stabilizing. [23-25]

4. CONCLUSION

Inconclusion, thepresentstudy showed that the phytosteroids as one of the major phytoconstituent. The ethanol extract was screened for anti-asthmatic activities. The resultshows that the extract produced significant anti-asthmatic activity when compared with the standard drug. The present study shows that the phytosteroids present in the extract may be responsible for the anti-asthmatic activity.

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