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Phytochemical Screening and Assessment of *Adhatoda Vasica* (Leaf) For Antihistaminic Activity

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ABSTRACT

Biologically active compounds from natural sources are of interest as possible new drugs for different diseases. Over many centuries humans have been mining the bounties of nature for discovering natural products that have been used for the treatment of all human diseases. *Adhatoda vasica* Nees (Acanthaceae) is widely used in the Indian system of medicine as an Asthma, bleeding, bronchitis, cough, diarrhea, dysentery, fever, flu, hysteria, neuralgia, rheumatic pain, swelling, TB, urinary disorders, vomiting etc. The plant was also reported for its diabetes, epilepsy, skin disorders and many more such activities. Clonidine, a α_2 adrenoreceptor agonist induces dose dependent catalepsy in mice, which was inhibited by histamine H_1 receptor antagonists but not by H_2 receptor antagonist. Clonidine releases histamine from mast cells which is responsible for different asthmatic conditions. In present study methanol extract of *Adhatoda vasica* leave at doses 100and 200 mg/kg i.p were evaluated for antihistaminic activity using clonidine induced catalepsy in mice. Acute toxicity of the extract (2000 mg/kg) was examined in mice for 7 days. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. Phytochemical analysis revealed the presence of carbohydrates, glycosides, alkaloids, saponins, flavonoids, tannin and phenolic compounds. Methanolic extract up to 2000 mg/kg did not produce any toxic effects. The methanolic extract of leave of *Adhatoda vasica* (100, 200 mg/kg, p.o.) and chlorpheniramine maleate (10 mg/kg, i.p.) significantly inhibited (**P< 0.05) clonidine-induced catalepsy in mice at 180 min after the administration of clonidine. The methanolic extract of leave of *Adhatoda vasica* possesses significant antihistaminic activity (H_1-antagonist) and can be attributed to bronchodilating, anti-inflammatory, adaptogenic activity etc. Hence detailed study needs to be conducted to evaluate the phytoconstituent responsible for the above mentioned results and their clinical efficacy in the treatment of related diseases.

Keywords: *Adhatoda vasica,* Clonidine, Antihistaminic activity, Chlorpheniramine maleate, Qualitative analysis.

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INTRODUCTION

Adhatoda vasica Nees (Acanthaceae), also known as *Justicia adhatoda* L is a small evergreen, perennial shrub distributed throughout India, especially in the lower Himalayas. The shrub is the source of Vasaka, a drug in the Indian System of Medicine which is well known for its beneficial effects, particularly in bronchitis[1]. Vasicine and vasicinone alkaloids are the major chemical constituents of the plant and are responsible for its strong respiratory stimulant activity[2]. The drug mainly comprises fresh or dried leaves but the flowers, fruit and roots are also extensively used for various ailments. Various dosage forms of leaf-like powder, fresh juice, decoction, infusion, alcoholic and aqueous extract, etc., are described to be used for various ailments[3]. In different forms of extracts, the leaves are reported to show anti-inflammatory, antimicrobial, antioxidant and many other biological activities[4-6]. Herbal medicines are being increasingly utilized to treat a wide variety of diseases, though the knowledge about their mode of action is relatively scanty. There is a growing interest regarding the pharmacological evaluation of various plants used in traditional system of medicine[7]. Allergies occur when a hypersensitive immune system reacts to a common or unusual substance. The number of individuals suffering with allergic illnesses is increasing in the industrialized, as well as in large cities of developing countries. Allergies also have reached high prevalence and incidence in all over the world[8,9]. Most of the allergic diseases are due to allergens like airborne pollens (grass, trees, and weeds), house-dust, mites, animal dander, cockroaches, fungal spores, etc. Overproduction of histamine in body triggers the allergic and inflammatory responses. Drugs always exist in the nature to

prevent the effect of histamine. Hence, in the present study, the antihistaminic activity of methanolic extract of leaves of *Adhatoda vasica* was studied using clonidine-induced catalepsy in mice models.

MATERIALS AND METHODS

Plant material

The leaves of *Adhatoda vasica* were collected from local area of Bhopal (M.P.) in the month of June, 2019.The sample was identified by senior Botanist Dr. Zia-Ul-Hassan, Professor and head of department of Botany, Safia College of Arts and Science, peer gate Bhopal. A herbarium of plants was submitted to the specimen library of Safia College of Arts and Science, peer gate Bhopal and The specimen voucher no. of *Adhatoda vasica* is 119/Bot/Saf/345.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). Clonidine (Unichem, Ltd.); Chlorpheniramine maleate (Alkem, Mumbai), All the chemicals used in this study were of analytical grade.

Extraction

Dried pulverized leaves of *Adhatoda Vasica* were placed in thimble of soxhlet apparatus. Soxhlation was performed at 40-60ºC using petroleum ether as non-polar solvent at first. Exhausted plant material (marc) was dried and then extracted with methanol. For each solvent, soxhlation was continued till no colour was observed in siphon tube. For confirmation of exhausted plant marc (i.e. completion of extraction), colorless solvent was collected from siphon tube and completion of extraction was confirmed by absence of any residual solvent, The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[10].

Phytochemical screening

The crude methanolic extract of *Adhatoda Vasica* was qualitatively tested for the detection of alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, reducing sugars, proteins, glucosides, terpenoids, and steroids following standard procedures[11].

Animals

In the present investigation the Swiss albino mice of either sex weighing 25-30gm were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity studies

Mice were selected for this study. They were divided into four groups with six animals in each group. Methanolic extract of leave of *Adhatoda Vasica* was administered orally in varying doses (5, 50, 300 and 2000 mg/kg body weight) to these animals. They were continuously observed for 2 h to detect changes in the autonomic or behavioral responses like alertness, spontaneous activity, irritability, urination, etc. Any mortality during experimentation in the following 7 days was also recorded. A group of animals treated with vehicle (distilled water) was served as control. Based on the results of preliminary toxicity testing, the doses of 100 and 200 mg/kg p.o were chosen for further experiments.

Antihistaminic activity

Clonidine-induced catalepsy in mice

Bar test was used to study effect of extracts on clonidine-induced catalepsy, to determine indirect antihistaminic activity. Mice were divided into four groups, six animals in each group.

Group I: Control group treated with normal saline (5 ml/ kg, i.p.)

Group II: Standard group treated with chlorpheniramine maleate (10 mg/kg, i.p.)

Group III treated with *Adhatoda Vasica* at a dose of 100mg/kg

Group IV treated with *Adhatoda Vasica* at dose of 200mg/kg.

Clonidine 1 mg/kg subcutaneously, will be administered to all groups except normal group 30 min after treatment. The forepaws of mice will be placed on a horizontal bar (1 cm in diameter, 3 cm above the table) and the time required to remove the paws from bar will be noted for each animal. The duration of catalepsy will be measured at 30, 60, 90, 120, 150 and 180 min interval after administration of clonidine[12,13].

Statistical analysis

The results were reported as mean ± SEM and analyzed for statistical significance using One way ANOVA followed by Dunnett' test $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The crude extracts so obtained after the soxhlation process, extract were further concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield calculated by the formula was found to be 2.41 % (by petroleum ether) and 8.43 % (by methanol). Phytochemical analysis of methanolic extract of *Adhatoda Vasica* leaves showed the presence of carbohydrates, glycosides, alkaloids, saponins, flavonoids, tannin and phenolic compounds Table 1.

Table1 Phytochemical analysis of methanolic leaves extract of *Adhatoda Vasica*

Test for carbohydrates	
Test	**Methanolic extract**
Moloch's	+Ve
Fehling's	+Ve
Benedict's	+Ve
Test for protein and amino acid	
Biuret	- Ve
Ninhydrin	- Ve
Test for glycosides	
Borntrager's	+ Ve
Keller–Kiliani	+Ve
Test for alkaloids	
Mayer's	+Ve
Hager's	+ Ve
Wagner's	+ Ve
Test for saponins	
Froth Test	+ Ve
Test for flavonoids	
Lead acetate	+ Ve
Alkaline reagent	+ Ve
Test for triterpenoids and steroids	
Salkowski's	+ Ve
Liebermann–Burchard	+ Ve
Test for Tannin and phenolic compounds	
Ferric chloride	+Ve
Lead acetate	+ Ve
Gelatin	-Ve

Acute oral toxicity was calculated at four different concentrations 5mg/kg, 50mg/kg, 300 mg/kg and 2000 mg/kg. Observations were performed in groups of three and no mortality was observed Table 2.

Table 2 Acute oral toxicity

S. No.	**Groups**	**Observations/ Mortality**
1.	5 mg/kg Bodyweight	0/3
2.	50 mg/kg Bodyweight	0/3
3.	300 mg/kg Bodyweight	0/3
4.	2000 mg/kg Bodyweight	0/3

The acute toxicity results showed that methanolic extracts of *Adhatoda Vasica* was safe up to a dose of 2000 mg/kg body weight. Based on acute toxicity data, two different dosages 100 and 200 mg/kg (p.o.) were selected for in vivo Antihistaminic activity.Clonidine releases histamine from mast cells which is responsible for different asthmatic conditions. Catalepsy produced by clonidine is mediated by histamine via H_1 receptors. The maximum catalepsy was observed after 90 minute of clonidine administration (1 mg/kg, i.p.) in vehicle treated (control) group (117.16± 6.229). Prior treatment with *Adhatoda Vasica* at doses (100, 200 mg/kg i.p) and Chlorpheniramine maleate (10 mg/kg, i.p.) showed significant ($P<0.05$) inhibition of clonidine induced catalepsy in dose dependent as shown in (Figure 1).

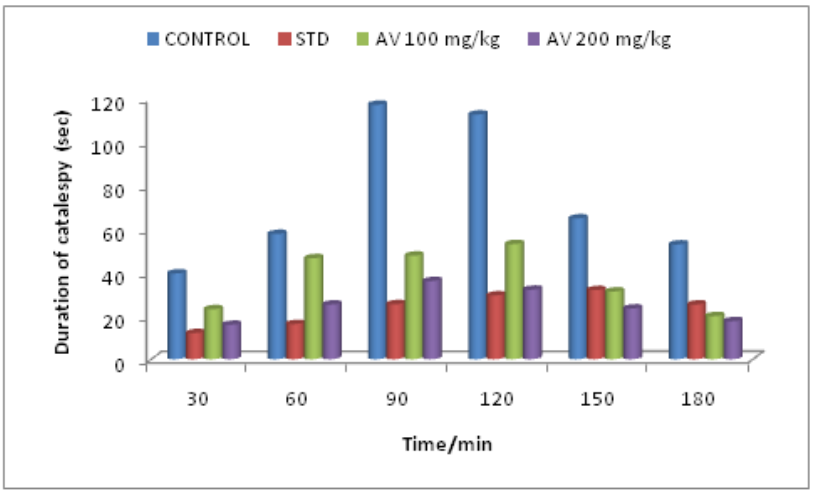

Figure 1 Effect of *Adhatoda Vasica* on clonidine induced catalepsy in mice

CONCLUSION

Present study concluded that the drugs having antihistaminic potential inhibit clonidine induced catalepsy, so methanol extract of *Adhatoda Vasica* leaves possesses antihistaminic activity. Future scope of present investigation is isolate active phytoconstituents which is responsible for antihistaminic activity.

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