WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.632
Volume 11, Issue 6, 2236-2244 Research Article ISSN 2278 – 4357

ANTI-HISTAMINIC AND BRONCHODILATOR SCREENING OF ADATHODA VASICA LEAF IN RODENNT MODEL

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Article Received on
19 April 2022,

Revised on 09 May 2022,
Accepted on 30 May 2022

DOI: 10.20959/wjpps20226-22439

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ABSTRACT

The present study is intended toinvestigate the Study of anti-asthmatic activity of methanolic extract of *Adhatoda vasica*. The leaves of medicinal plant *Adhatoda Vasica* (500 gm) was collected locally from M.P. The results of the present study revealed anti histaminic actions of different extracts of plant *Adhathoda vasica*. In this study control group of animals showed convulsion during the first 3 min of the experiment. Prior treatment of *Adhathoda vasica* extract (100 and 200 mg/kg body weight) protected animals to significant extent from the development of asphyxia produced by histamine aerosol confirming that it has an antihistaminic activity. The role of histamine in asthma is well established. Methanolic extract of *Adhathoda vasica* significantly and dose dependently increased the time of PCT following exposure to histamine aerosols induced bronchospasm in guinea pigs as shown in table. Increased in the time of PCT was more against histamine aerosol following administration of *Adhathoda vasica* extract. To conclude, our study indicate that the extract of *Adhathoda vasica* exert significant antiastmatic effect in experimentally induced asthma in animal comparable to standard drug and provides pharmacological evidence for the traditional use of extract as anti asthmatic agents. In conclusion, the results of the present investigation suggest that, higher dose of extract has significant broncho dilator activity against histamine.

KEYWORD: *Adhathoda vasica*, bronchospasm, antihistaminic, bronchodilator.

INTRODUCTION

Asthma is a common long-term inflammatory disease of the airways of the lungs that involves a complex interaction of airflow obstruction, bronchial hyper responsiveness and an

underlying inflammation.[1] This interaction can be highly variable among patients and within patients over time.[2] It is characterized by variable and recurring symptoms, reversible airflow obstruction, and easily triggered bronchospasm.[3] Symptoms include episodes of wheezing, coughing, chest tightness, and shortness of breath.[4] These may occur a few times a day or a few times per week. Depending on the person, they may become worse at night or with exercise. Asthma is thought to be caused by a combination of genetic and environmental factors.[5] A number of long-term prospective studies of children admitted to hospital with documented RSV have shown that approximately 40 percent of these infants willcontinue to wheeze or have asthma in later childhood.[6]

Adhatoda vasica is a shrub with lance-shaped leaves 10 to 15 centimeters in length by four wide.[7] Theyare oppositely arranged, smooth-edged, and borne on short petioles.[8] When dry they are of a dull brownish-green color. The vasicine yield of the herbage has been measured as 0.541 to 1.1% by dry weight.[9]

The present study is intended toinvestigate the Study of anti-asthmatic activity of methanolic extract of *Adhatoda vasica*. The main objective of the study was to evaluate the *In-vivo* Pharmacological Studies on fruits of *Adhatoda vasica* in validated experimental animal models.

MATERIAL AND METHODS

Plant collection: The leaves of medicinal plant *Adhatoda Vasica* (500 gm) was collected locally from M.P. After cleaning, plant parts were dried under shade at room temperature for 3 days and then in oven at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant: The leaves of medicinal plant *Adhatoda Vasica* were authenticated by a plant taxonomist in order to confirm its identity and purity.

Extraction of Plant: The air dried medicinal leaves of plant of *Adhatoda Vasica* (500 gm) was coarsely powdered and extracted by continuous hot extraction method by using Soxhlet apparatus with methanol at 60°C for 8 hours, individually. The extract was filtered through Whatman filter paper no.1, and the filtrate was dried using rotary evaporator under reduced pressure (335 mbar) at 40°C and stored in refrigerator (2-4°C) for further activity. The obtained crude extract was stored in a refrigerator at 4°C until time of use. The percentage

yield of the extract was calculated using the formula below.

% yield = weight of the extract/weight of plant material × 100

Phytochemical investigation: Qualitative phytochemical investigation was carried out by using standard procedure.

Experimental animals

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No.1824/PO/c/09/CPCSEA). For the experiment *Albino wister* rat were used animal weight was taken between 150 to 200 gm. Three animals were used in a group and animals were acclimatized for one week prior to dosing. Identification done by cage number and marking on animal and feed standard pellets supplied by Golden Feeds, New Delhi, Purified water *ad libitum* and drug was give via P.O. route.

Acute oral toxicity (OECD 423)

The acute toxic class method set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or themoribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher orthe next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight.

***In vivo* study**

(A) Antihistaminic activity: Bar test will be used to study effect of extracts on clonidine-induced catalepsy, todetermine indirect antihistaminic activity.

Experimental design: Mice will be divided into five groups, five animals in each group. Normal group willreceive vehicle.

Group I : Control group treated with distilled water (5 ml/ kg, p.o.)

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 s.c., 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 removethe 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.

(B) Evaluation of Bronchodilator activity: Overnight fasting of 24 hrs guinea pigs were divided into four groups each containing 6 animals. The drugs were dissolved in distilled water and administered orally through intubation canula. Guinea pigs exposed to histamine aerosol showed progressive sign of difficulty in breathing leading to convulsions and death. The time until the signs of convulsion appeared is called pre-convulsion dyspnoea (PCD).

The following schedule of treatment was administered:
Group 1 was treated as control p.o.
Group 2 received standard drug chlorpheniramine maleate (2 mg/kg) orally 30 minprior to exposure.
Groups 3 received *Adhatoda Vasica* 200 mg/kg body weight p.o.
Group 4 received *Adhatoda Vasica* 400 mg/kg body weight p.o.

Prior to drug treatment each animal was placed in the histamine chamber and exposedto 0.2 % histamine aerosol. The pre convulsive dyspnoea (PCD) was determined from the time of exposure to onset of convulsions.

As soon as the PCD commenced, the animal were removed from the chamber and placed in fresh air. This time for PCD was taken as day 0 value. Those animals which developed typical histamine asthma within 3 min were selected out three days prior to the experiment and were given habituation practice to restrain them in histamine chamber. Animals which did not develop typical asthma within 6 minute were taken as protected.

The % increase in time of PCD was calculated using the following formula: Theprotection offered by treatment was calculated by using the formula.

Percentage Protection = (1 - T1/T2) X 100 Where, T1 = the mean of PCD before administration of test drugs. T2 = the mean of PCD after administration of test drugs.

RESULTS AND DISCUSSION

Table 1: Phytochemical investigation.

Test for carbohydrates	
Test	Methanolic extract
Molish	+Ve
Fehling's	+Ve
Benedict's	+Ve
Test for protein and amino acid	
Biuret	- Ve
Ninhydrin	- Ve
Test for glycosides	
Borntrager's	+ Ve
Keller-killani	+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
Libermann-burchard's	+ Ve
Test for Tanin and phenolic compounds	
Ferric chloride	+Ve
Lead acetate	+ Ve
Gelatin	-Ve

Table 2: Acute Oral Toxicity.

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

(A) Antihistaminic activity

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. Control group received Normal saline (5 ml/ kg, i.p.); test group received AV at doses (100, 200 mg/kg, i.p) and standard group treated with Chlorpheniramine maleate (10 mg/kg,

i.p.). Clonidine 1 mg/kg, subcutaneously.

Bar test was used to study the effect of extract on clonidine induced catalepsy to determine anti asthmatic activity. The maximum catalepsy was observed at 90 min of clonidine administration in control group (117.16± 6.229). The result showed that ethanol extract of *Adhatoda Vasica* significantly inhibited clonidine-induced catalepsy at dose 100 and 200 mg/kg in dose dependent manner. The results are compared with chloropheniramine maleate (10 mg/kg, i.p.).

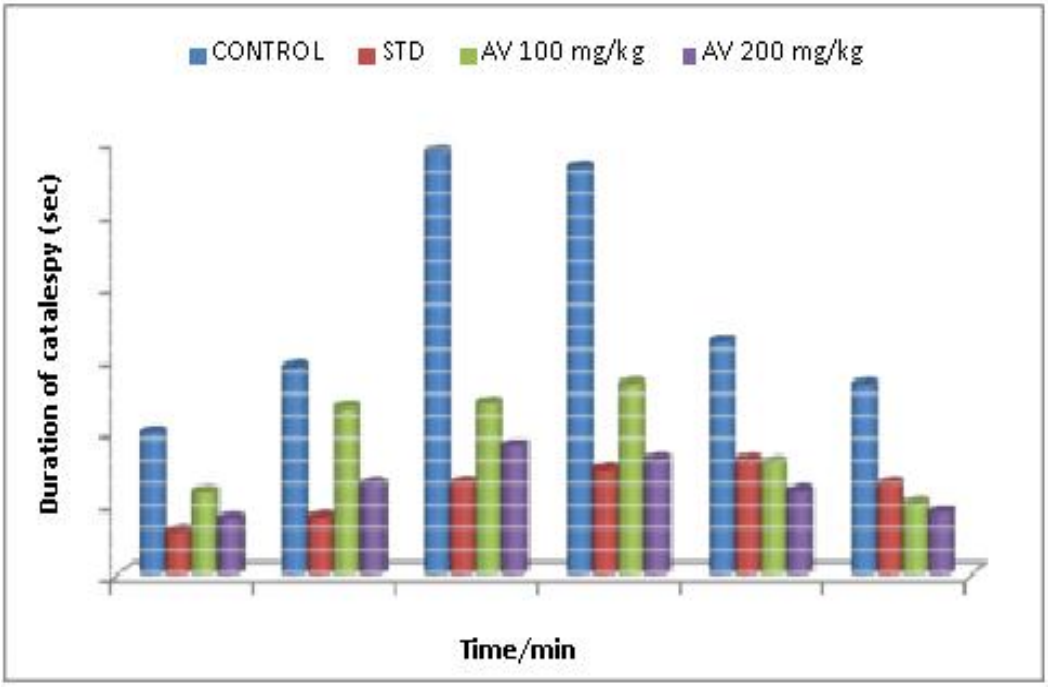

Figure 1: Effect of extract *Adhatoda Vasica* on clonidine iduced catalepsy in rats.

(B) Evaluation of Bronchodilator activity.

Table 3: Effect of extract on Histamine induced broncho constriction in animal.

S. No.	Groups	Dose (mg/kg)	PCD in sec	% Protection
1.	Normal control	10 mg/kg	36.5± 5.357	-
2.	Std Drug Chlorpheniramine Maleate	2 mg/kg	178.66±5.537	79.6
3.	*Adhatoda Vasica* 100 mg/kg	100 mg/kg	75.83 ± 4.792**	51.9
4.	*Adhatoda Vasica* 200 mg/kg	200 mg/kg	153.5 ± 5.822**	76.3

Values were Mean ± SD for (n=6) expressed as 6 animals in each group.

Data analysis was performedusing ANOVA. ** P < 0.05 vs. control

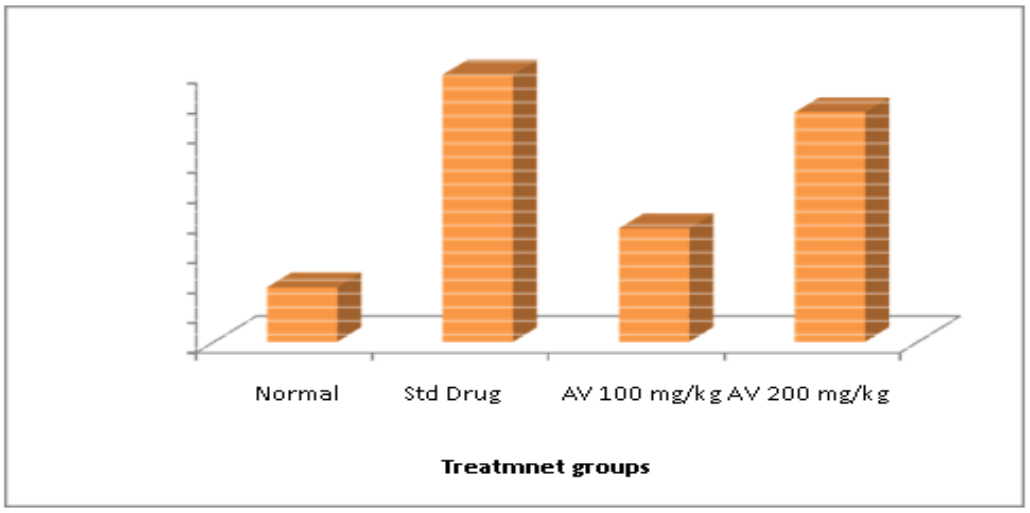

Figure 2: Effect of extract on Histamine induced broncho-constriction in animal.

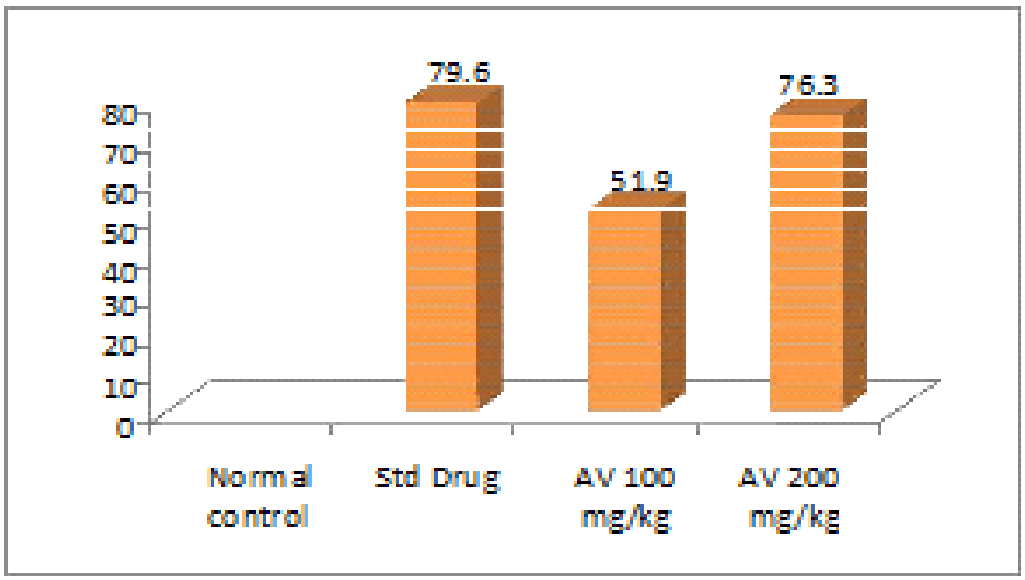

Figure 3: Percentage protection of *Adhathoda vasica* extract.

The results of the present study revealed anti histaminic actions of different extracts of plant *Adhathoda vasica*. In this study control group of animals showed convulsion during the first 3 min of the experiment. Prior treatment of *Adhathoda vasica* extract (100 and 200 mg/kg body weight) protected animals to significant extent from the development of asphyxia produced by histamine aerosol confirming that it has an antihistaminic activity. The role of histamine in asthma is well established. 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, animals 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, animal airways react to histamine, acetylcholine, leukotrienes, and other bronco-constrictors in a manner similar to that seen in humans. Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to adrenergic antagonists. 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.

CONCLUSION

Phytochemical screening of *Adhathoda vasica* revealed the presence of saponins, sterols/ triterpenes, alkaloids, flavonoids, polyphenolic compounds and carbohydrates.In this study, an attempt has been made to evaluate antiasthmatic activity of *Adhathoda vasica* in the experimental animals. The acute toxicity was studied. Based on these results of toxicity, doses of 50, 300, 500 and 2000 mg/kg of *Adhathoda vasica* extract were selected for various animal models. No mortality and the sign of toxicity were observed at the dose of 2000 mg/kg. Dose selected for pharmacological evaluation were 100 and 200 mg/kg. Methanolic extract of *Adhathoda vasica* significantly and dose dependently increased the time of PCT following exposure to histamine aerosols induced bronchospasm in guinea pigs as shown in table. Increased in the time of PCT was more against histamine aerosol following administration of *Adhathoda vasica* extract. To conclude, our study indicate that the extract of *Adhathoda vasica* exert significant antiastmatic effect in experimentally induced asthma in animal comparable to standard drug and provides pharmacological evidence for the traditional use of extract as anti asthmatic agents. In conclusion, the results of the present investigation suggest that, higher dose of extract has significant broncho dilator activity against histamine. However, further studies are suggested to establish molecular mechanism and also to isolate and characterize the active principle responsible for the action.

CONFLICT OF INTEREST

There is no conflict of interest.

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