Research Article

In vitro evaluation of Antiasthmatic activity of *Achyranthes aspera* aerial parts

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Abstract

Objective: Asthma in chronic condition is very tedious to cure and which is very common disease. The aim of study was to evaluate anti-asthmatic activity the ethanol extract of aerial part of *A. aspera*. **Material and methods:** Collection of Aerial parts of *Achyranthes aspera* is done from Bhopal. For authentication of aerial parts of *A. aspera* Herbarium of plant was made and sent to botany department of Safia College, Bhopal. The collected material was proceeded for air dry at 35-40º C and then it was pulverized in electric grinder. Extraction of obtained powder was completed in ethanol by using Soxhlet Apparatus. 8.45% w/w yield of substance was found. The presence of flavonides, phenolic compound, glycosides, tannins, saponins, alkaloids and carbohydrates were revealed by phytochemical screening. Ethanol extract of aerial part of *A. aspera* was performed for the screening of anti-asthmatic activity. **Results and conclusion:** The current study of the ethanol extract of aerial part of *A. aspera* was performed for the screening of anti-asthmatic activity of drug. The ethanol extract of aerial part of *A. aspera* was result the right side shift of dose response curve in isolated goat chain and isolated guinea pig so it is indicating antiasthmatic action of drug extract.

Keywords: Asthma, *Achyranthes aspera*, extract, phytochemical screening

Introduction

Asthma in chronic condition is very tedious to cure and which is very common disease. 17-18 million people in United States are being affected by Asthma and in the last 20 years it is found to be increased about 75 %. As per the current status of asthma patients about 1 out of 13 children and 1 out of 20 adults are suffering from Asthma. It has been found that since 1980 number of cases of Asthma was increased for the children under the age of 5 years this alarming fact cannot be ignored. Children in school age 75% has captured by Asthma. 15-20 million asthmatic patients are estimated only in India. Data of death because of Asthma from developed countries reveals that the rate varies from 0.2-0.8 per 100,000 persons aged 6-35 (Nichols and Longworth, 1995). Symptomatic relief is pointing requirement for curing the attack of asthma by ayurveda, unani and traditional system. One of the most elite plants is mentioned in Ayurveda and Unani system for the treatment of Asthma (Shukla et al., 2011; Charde et al., 2012). Even in Chinese system of medicine *Achyranthes aspera* is one of most essential plant. *A. aspera* is pungent, bitter, laxative, heating, carminative, stomachic and also beneficial in the treatment of bronchitis, vomiting, piles, heart disease, ascites, itching abdominal pains, dysentery, blood diseases, dyspepsia etc (Shukla et al., 2011; Shukla et al.,2011; Shukla et al., 2014). Till now there is not more scientific evidence or proof for antiasthmatic activity of plant extract of *A. aspera*, so the objective of present study of *A. aspera* to evaluate for antiasthmatic activity.

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Materials and methods

Phytochemical studies

Collection of aerial parts of *A. aspera* is done from Bhopal. For authentication of *A. aspera* Herbarium of plant was made and sent to botany department of Safia College, Bhopal. The plant botanical identification was confirmed by Dr. Zeaul Hasan with no. 257/Bot/saifia/11. The collected

material proceeded for air dry at 35-40º C and then it was pulverized in electric grinder. Extraction of obtained powder was completed in ethanol by using Soxhlet Apparatus. 8.45% w/w yield of substance was found. The presence of flavonides, phenolic compound, glycosides, tannins, saponins, alkaloids and carbohydrates were revealed by Phytochemical screening (Shukla et al., 2011; Shukla et al., 2013; Gupta et al., 2015). For the further use extract was stored in a refrigerator.

Animals

Guinea pigs was brought of either sex of 350-400 gm from the local market of Jabalpur MP and Wistar rats of 150-250 gm and rats of either sex were breaded at the Pinnacal Research Lab Bhopal MP, and housed at (22±1º) temperature and 12/12 h light/dark cycle to maintain standard condition. Standard pellet diet was given and also they were free for water intake. (IAEC) Institutional Animal Ethical Committee was selected to grant permission to conduct this experiment. As per internationally accepted protocol toxicity study was conducted under OECD guidelines 420 at a dose level of extracts up to 10 g/kg in rats.

Goat tracheal chain and Guinea pig ileum preparation

Isolated adult goat tracheal tissue was taken from the slaughter abode. Incision was made into individual rings and rings were tied jointly in series to form a chain. Trachea was in floating condition in the Kreb's solution bath and solution was aerated at 37±0.5º. Kreb's solution and kreb's solution containing 100 µg/ml *A. aspera* extract were taken for performing dose response curve of Histamine. And to record dose response curve of Histamine in availability and absence of plant extract and percent of maximum concentration responses were plotted **(**Chaudhari and Lahiri, 1974).

Overnight fasted guinea pigs were taken and sacrified. An organ bath containing Tyrode solution that was aerated continuously at 37±0.5ºC was used to mout ileum. Tyrode solution and Tyrode solution containing 25 µg/ml aerial parts of *A. aspera* were taken for performing dose response curve of Histamine. And to generate dose response curve of Histamine in availability and absence of plant extract and percent of highest concentration responses were plotted (Vogel 1998).

Histamine induced bronchoconstriction

Eight groups (n=6) of Guinea pigs were made, saline was given to controlled groups and single dose of extract (75, 150, 200, 300, 600, 1200 mg/kg p.o.) respectively was given to other groups. Chlorpheniramine maleate, 2 mg/kg was taken as positive control. Histamine chamber was used to place each animal prior to and after drug treatment and 0.2% of aerosol of Histamine was used to expose. Determination of (PCT) pre-convulsive time was done from contact time to onset of dyspnoea pointing for looking preconvulsive dyspnoea in a min. Protection percentage of PCT was calculated which was offered by drugs for each dose and positive control (Gokhale and Saraf et al., 1996).

Passive paw anaphylaxis

Three doses of 100µg of egg albumin were given to Wistar rats on days 1, 3 and 5. For the separation of serum blood of rats were collected and centrifused on day 10 of sensitization. Eight groups (n=6) of Wistar rats were made, saline was given to controlled groups and single dose of extract (85, 175, 250, 350, 700, 1400 mg/kg p.o.) respectively was given to other groups. Dexamethasone 0.27 mg/kg was taken as standard. Sterilization was performed with serum prior to drug administration.10µg of egg albumin was again administered after the treatment with the drug and the measurement of edema inhibition was performed (Sanberg et al., 1980).

Milk-Induced leucocytosis

Eight groups (n=6) of rats were made, saline was given to

Table 1. The effect of ethanol extract of aerial part of *A. aspera* on histamine induced contractions on the isolated goat chain and isolated guinea pig ileum

(2.5 µg/ml)	Maximum percent contractions on the isolated goat chain		Maximum percent contractions on isolated guinea pig ileum	
	Control group	Test group	Control group	Test group
0.1	23.52±1.52	13.26±0.85	19.27±0.25	11.34±0.85
0.2	45.28±2.51	31.42±1.30	28.30±0.12	21.67±0.94
0.4	65.34±2.04	49.24±1.34	64.27±2.62	41.39±2.31
0.8	81.27±3.42	58.02±1.52	85.36±3.67	61.28±3.52
1.6	85.31±4.01	67.20±3.56	87.16±3.52	59.23±2.6

All values are expressed as mean ± SEM of a sample size of n=6, *p<0.05. All treated groups were compared with control group.

controlled groups and single dose of extract (125, 250, 375, 500, 1000, 2000 mg/kg p.o.) respectively was given to other groups. Only the group that was given milk served as intoxicant. Boiled and cooled milk injection (4 ml/kg s.c.) were given to all the groups except controlled group after 1 hour of drug treatment (Bhargava and Singh et al., 1981; Horn and Robin et al., 1975).

Table 2. The effect of ethanol extract of *Achyranthes aspera* (AS) on histamine-induced bronchoconstriction

Groups with Dose in mg/kg p.o.	% Protection
Control	12.36
75 AS	48.23
150 AS	64.28
200 AS	85.62
300 AS	72.28
600 AS	64.28
1200 AS	8.39
AA (2 mg/kg)	89.27

All values are expressed as mean ± SEM of a sample size of n=6; *p<0.05. All treated groups were compared with control group.

Table 3. The effect of ethanol extract of *A. aspera* (AS) on passive paw anaphylasis

Groups	Paw Edema Volume (ml) Mean ± SEM			
	1h	2h	3h	4h
Control	0.81±0.02	0.70±0.05	0.61±0.06	0.58±0.05
85 AS	0.47±0.04	0.42±0.06	0.35±0.07	0.31±0.02
175 AS	0.68±0.03	0.54±0.07	0.46±0.02	0.40±0.07
250 AS	0.51±0.06	0.36±0.02	0.30±0.07	0.24±0.03
350 AS	0.57±0.03	0.47±0.05	0.42±0.06	0.39±0.04
700 AS	0.59±0.07	0.42±0.07	0.40±0.15	0.40±0.06
1400 AS	0.65±0.08	0.41±0.06	0.38±0.02	0.36±0.07
Dexamethasone (0.27 mg/kg)	0.44±0.07	0.31±0.04	0.30±0.07	0.29±0.02

All values are expressed as mean ± SEM, n=6; *p<0.05. All treated groups were compared with control group.

Result and discussion

The current study of the ethanol extract of aerial part of *A. aspera* was performed for the screening of anti-asthmatic activity of drug. Bronchial asthma is one of the chronic inflammatory diseases which cause bronchoconstriction and inflammation in airway pathway that also responsible for the hyper bronchial responsiveness to most of the stimuli likes mast cell, T-lymphocytes and eosinophils. For the contractile responses various agonists like histamine, acetylcholine, bradykinin and 5-hydroxyltrytamine are responsible. The ethanol extract of aerial part of *A. aspera* was result the right side shift of dose response curve in isolated goat chain and isolated guinea pig so it is indicating antiasthmatic action of drug extract (Table 1).

Table 4. The effect of ethanol extract of aerial parts of *Achyranthes aspera* (as) on total leukocyte count

Group treatment	Difference in Number of leukocytes (Cu. mm.)
Control (10 ml/kg saline)	427±24.62
AS 125 + Milk (4 ml/kg sc)	1127±151.62
AS 250 + Milk (4 ml/kg sc)	1453±147.62
AS 375 + Milk (4 ml/kg sc)	2854±185.26
AS 500 + Milk (4 ml/kg sc)	351±25.18
AS 1000 + Milk (4 ml/kg sc)	1762±143.85
AS 2000 + Milk (4 ml/kg sc)	1638±114.62
Saline (10ml/kg) + Milk (4 ml/kg sc)	6248±438.21 ##

All values are expressed as mean ± SEM of a sample size of n=6; *p<0.05. All treated groups were compared with control group.

Histamine is one of the simple inflammatory mediator which causes bronchoconstriction and inflammation in airway pathway that also responsible for the hyper bronchial responsiveness even in immediate phase of Asthma. There was a prominent involvement of H1 receptor in comparison to H2 receptor in Asthma which was estimated by the experiment in guinea pig using respiratory smooth muscle (Gosh et al., 1984). For brochorelaxant study at 200 mg/kg of dose given and 85.62% of protection was observed which is the greatest protection percentage in comparison with that of 89.27% of standard chlorpheniramine maleate (Table 2). 200 mg/kg was found to be effective ($p< 0.01$) dose because this dose had statistical significance in post treated exposition and mean exposition time. Decease in activity was found as the amount of dose increased.

The activation of T-lymphocytes with subsequent release of inflammatory mediators is a result of exposure of allergen which causes allergic asthma that can also be called a chronic inflammatory disease. Asthma treatment by the inhibition of antigen-antibody reaction and also by inhibition of release of inflammatory mediators can be done so for that Immuno-modulating agents are valuable. 250 mg/kg was found to be effective ($p< 0.01$) dose for paw edema because this dose had statistical significance in protection against edema in comparison with

dexamethasone. Further decease in activity was found as the amount of dose increased (Table 3).

For the treatment of asthma some herbal formulation can also be used. Adaptogen normalization outcome may be experimented into milk induced leukocytosis after administration of milk parenterally. Milk induced leukocytes count was found to be deceased leukocyte count at 375 mg/kg. Exactly opposite result was found that is the dose 375mg/kg there was max increase in leukocyte count (Table 4).

It is found the improved leukocyte calculation in entire leucocytes count form, because of improved lymphocyte (B and T cells) count. Drugs which are steroidal in nature are more effective in asthma treatment. Extract of aerial parts of *A. aspera* contains steroidal nucleus in the form of triterpenoides and many various sapogenins and saponin glycosides. So antiasthmatic activity showed by *A. aspera* may be because of these chemical moieties.

Declaration of interest

The authors report no conflicts of interest.

References

Bhargava KP, Singh N.1981. Anti-stress Activity of *Ocimum sanctum* (linn). Indian Journal of Medical Research, 73:443–51.

Charde M, Shukla A, Bukhariya V, Mehta J, Chakole R.2011.Herbal Remedies as Antioxidants: An Overview. International Journal of Pharmacological Research, 1(2):25-34.

Charde M, Shukla A, Bukhariya V, Mehta J., Chakole R. 2012. A Review On: A Significance of Microwave Assist Technique. In green Chemistry International Journal of Phytopharmacy, 2 (2):39-50.

Chaudhari KN, Lahiri C.1974.Role of Goat Trachea for an Isolated Tracheal Chain Preparation. Indian Journal of Pharmacology, 6:149–51.

Gokhale AB, Saraf MN.1996. Bronchoprotective Effect of Ethanolic Extract of *Tephosia purpurea* in-vivo. Indian Drugs, 37:346–7.

Gosh MN. Fundamental of Experimental Pharmacology. 2nd ed. Calcutta: Scientific Book Agency; 1984.

Gupta M, Lodhi S, Shukla A. 2015. Preliminary phytochemical analysis and *in vitro* anti-helminthic activity of *Martynia annua* Linn and *Permotrema reticulatum.* Asian Journal of Biomaterial Research, 1(2):72-74

Horn BR, Robin ED.1975. Total Eosinophils Count in the Management of Bronchial Asthma. New England Journal of Medicine, 292:1152–5.

Mitra SK.1999.Antiasthmatic and Anti-anaphylactic Effect of E-721B: A Herbal Formulation. Indian Journal of Pharmacology, 31:133–7.

Nichols DJ, Longsworth FG.1995. Prevalence of exercise-induced asthma in schoolchildren in Kingston, St. Andrew and St. Catherine, Jamaica. West Indian Medicinal Journal, 44:16–9.

Sanberg PR.1980. Haloperidol-induced Catalepsy is Mediated by Postsynaptic Dopamine Receptors. Nature, 284:472–3.

Shukla A, Bukhariya V, Mehta J, Bajaj J, Charde R, Charde M, Gandhare B.2011. Herbal Remedies for Diabetes: An Overview. International Journal of Biomedical and Advance Research, 02 (01):57-68.

Shukla A, Bukhariya V, Mehta J, Bajaj J, Charde R. 2011. *Achyranthes aspera* linn. (chirchira): a magic herb in folk medicine. International Journal of Biomedical and Advance Research, 02(06):228-240.

Shukla A, Bukhariya V, Mehta J, Nema S.2011. A Review on "Anti Asthmatics - Herbal Folk Drugs: An Overview. Inventi Plant Active, 4:1-5

Shukla A, Gupta R, Sharma P, Jain AP. 2013. Comparative Study of Microwave Assisted with Conventional Extract of Calcium Sennasoids from Senna Leaf. Research Journal of Pharmaceutical Biological and Chemical Science, 04 (03):103-109.

Shukla A, Shukla R, Pandey V, Golhani D, Jain C. P. 2014. In-Vitro Antioxidant Activity of *Garcinia cambojia* Fruits. Journal of Medical Pharmaceutical and Allied Sciences, 3 (01):67-73.

Tripathi KD. Essentials of Medical Pharmacology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers; 2003.

Vogel GH, Vogel WH. Drug Discovery and Evaluation. Berlin: Spinger Verlag; 1998.