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EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF *CITRULLUS COLOCYNTHIS* FRUIT

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ABSTRACT: The present investigation was under taken to evaluate the anti-asthmatic activity of ethanolic extract of *Citrullus colocynthis* Fruits in experimental animals. *Citrullus colocynthis* Fruits was evaluated for anti-histaminic activity using isolated goat tracheal chain preparation and histamine induced Bronchoconstriction in Guinea pig. *Citrullus colocynthis* Fruits significantly inhibited dose dependent contraction of goat tracheal chain produced by histamine and also showed significant protection by prolonging Preconvulsion dyspnea time (PCD) in guinea pigs. Thus, *Citrullus colocynthis* Fruits showed anti-allergic activity against histamine and hence possesses potential role in the treatment of asthma.

INTRODUCTION: Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. During an acute asthma episode, the airway lining in the lungs becomes inflamed and swollen and excess mucus production occurs in the airway¹ asthma prevalence increases globally by 50% every decade². With the projected increase in the proportion of the world's urban population from 45% to 59% in 2025, there is likely to be a marked increase in the number of asthmatics worldwide over the next two decades³.

Although the fundamental causes of asthma are not completely understood, the strongest risk factors for developing asthma are inhaled asthma triggers such as pollens and moulds, tobacco smoke; and Chemical irritants.

The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population. Approximately 300 million people worldwide currently have asthma, with estimates suggesting that in the workplace⁴. Large numbers of drugs are used for in the treatment of asthma. However none of them seems to be an ideal drug⁵.

The currently used drugs for the treatment of asthma in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use^{6,7}. The search for new drug is still the need of the day. *Citrullus colocynthis* (Fruits.) belonging to the family *Cucurbitaceae* a large number of such compounds as flavonoids, alkaloids, steroids, flavonoids are having anti-allergic and antioxidant activity⁸. Alkaloids are having anti-inflammatory activity. Traditionally, the Fruits juice of *Citrullus colocynthis* is used in treating asthma, scanty urine, and internal inflammation disorders⁹. Therefore, by considering by considering the traditional claim reported pharmacological activity and chemical constituents present in plant, the need was felt to

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investigate the anti-asthmatic activity of *Citrullus colocynthis* (Fruits.) in laboratory animals¹⁰.

MATERIALS AND METHODS:

Plant collection and Authentication: The collection of plant materials of *Citrullus colocynthis* Fruits was done in month of January 2015 from CAJRI (Central Arid Zone Research Institute) of Jodhpur, Rajasthan and the identified and authenticated by S.L. Menna, Scientist C & H.O.O Botanical survey of India, Jodhpur, Rajasthan (voucher specimen no: BSI/AZRC/I.Tech./2014-15/(PI.Id.)/714) and submitted at G.D. Memorial College of Pharmacy, Jodhpur, (Raj)

Preparation of plant extract: The whole plant parts of plant were washed and dried under shade for 7 days at temperature 20-25°C. Cleaned and grind with help of iron Morter pestle. The powders (40 size mesh) were used for the soxhlet extraction. Plant material 20 g was extracted with 250 ml of petroleum ether in soxhlet apparatus for 40hrs at temp. 60-80°C to the powder and then mark was extracted with 250 ml ethanol (70%) for 72 hrs at temp. 75-80°C. The extracts were collected and evaporated to dryness to give dry crude extract. The yield of ethanolic extract was 8.50% w/w. The obtained extracts were subjected to phytochemical investigation.

Experimental animals: Dunkin-Hartley Guinea pig weighing 350-400 gm of both sexes and Wistar albino rat (200-250g) either sex were procured from animal house of G. D. Memorial College of Pharmacy, Jodhpur (Raj.). They were housed in well ventilated cage under controlled condition of light (12 hr light-dark) and temp (20-22°C). The animals were allowed standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Reg. no. (1491/PO/a/11/CPCSEA).

Phytochemical Screening: Ethanolic extract of *Citrullus colocynthis* plant was screened for presence such as protien fiber carbohydrate, glycosides, flavanoids (Table 1). The screening was done using standard protocol described in Khandelwal and Kokate.^{11, 12}

Acute Toxicity Study¹³: Albino rats of either sex weighing 200-250 gm were used in the study.

Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. There was no behavioral abnormality and zero mortality was recorded till 48 h post treatment with the dose 2000mg/kg, Therefore 1/10th of the dose 2000mg/kg of *Citrullus Colocynthis* was selected i.e., 200mg/kg as middle dose. Different doses (100, 200 mg/kg, p.o) of *Citrullus colocynthis* were later chosen for this study based on the acute toxicity testing.

Antiasthmatic activity:

Isolated Goat tracheal chain preparation:¹⁴ The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Krebs's solution (Composition NaCl, 115; KCl, 4.7; CaCl₂, 2; NaHCO₃, 25; KH₂PO₄, 1.2; MgCl₂, 1.2; glucose, 11.5). Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Krebs's solution and maintained at 37 ± 1°C, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 400 mg. A dose response curve for histamine was recorded at variant molar concentrations by maintaining 15 mint time cycle.

After obtaining dose response curve of histamine (30ug/ml) on trachea, the *Citrullus colocynthis* (100µg/ml) was added to reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative log of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine.

In absence and in presence of *Citrullus colocynthis* and standard drug Chlorpheniramine maleate (1 µg/ml).

Histamine induced Bronchoconstriction in Guinea pig:¹⁵ Overnight fasted guinea pigs were randomly divided into five groups (n=5). Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol.

The Preconvulsive dyspnea time (PCD) was noted for each animal. The Pre convulsive dyspnea time is the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion. As soon as preconvulsive dyspnea commenced, animals were removed from the chamber and placed in fresh air to recover from dyspnea for 24 hours. This time for pre convulsive dyspnea was recorded as basal value. After 24 hours, animals belonging to group I served as control and were administered with phosphate buffer (1ml/kg, p.o.); Animals belonging to group II were administered with Chlorpheniramine maleate (2 mg/kg, i.p.) while group III to IV were received respective doses of *Citrullus colocynthis*. These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hr and 24 hr and to determine Pre convulsive dyspnea time (PCD). The protection offered by the treatment was calculated by using the following formula:

$$\% \text{ protection} = (1 - T1/T2) \times 100$$

T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs at 1 hr, 4 hr and 24 hrs.

Statistical Analysis: The results of various studies were expressed as mean \pm SEM and analyzed statistically using one way ANOVA followed by Student's t-Test to find out the level of significance. Data were considered statistically significant at minimum level of $p < 0.05$.

Milk induced Leucocytosis in mice: ¹⁶ Mice were divided into four groups, six animal in each group. Animal belonging to group-I received distilled water 10ml/kg (p.o). Animal belonging to group II, III, received boiled and cooled milk injection in dose of 4ml/kg (s.c). Animal belonging to group II, III, IV were compared with control, 1hr before milk injection blood sample was collected from each mice from the retro orbital plexus, under light either anaesthesia. Total leukocytes count was done in each group before drug administration in total leukocytes count before and 24hour after drug administration was calculated.

RESULT:

Phytochemical Screening : (Table 1)

Effect of ethanolic extracts of fruits of *Citrullus colocynthis* on histamine induced contraction of isolated goat tracheal chain preparation: In the present study, histamine (30 μ g/ml) produced dose dependent contraction of goat tracheal chain preparation maximum percentage of contractile response versus negative log molar concentration of histamine (Table 2). The modified physiological salt solution containing Chlorpheniramine maleate (1 μ g/ml) significantly inhibited ($p < 0.01$) the contractile effect of histamine. The modified physiological salt solution containing ethanolic extract of *Citrullus colocynthis* (100 μ g/ml) significantly inhibited ($p < 0.01$) the contractile effect of histamine.

Hence Chlorpheniramine maleate and ethanolic extract of *Citrullus colocynthis* (100 μ g/ml) shifted the DRC of Histamine towards the right side indicating that there was competitive antagonism between histamine and both the drugs for histaminergic receptors. (Fig. 2).

Effect of ethanolic extract of *Citrullus Colocynthis* on histamine induced bronchoconstriction in guinea pig: The guinea pigs when exposed to 0.2% w/v histamine aerosol showed signs of progressive Dyspnea leading to convulsions. Chlorpheniramine maleate (2mg/kg, i.p) significantly prolonged ($p < 0.01$) the preconvulsive dyspnea in 1st, 4th and 24th hr as compared to control and the percent % protection observed was respectively (Table3). The ethanolic extract of fruits of *Citrullus colocynthis* at doses of 100 mg/kg ($p < 0.05$) and at the dose of 200 p.o ($p < 0.01$) significantly prolonged the preconvulsive dyspnea at 1st, 4th hr and 24 hr as compared to control. Thus showed more protection against preconvulsive dyspnea as compared to control, following exposure to histamine aerosol.

The percent protection observed for *Citrullus colocynthis* at the dose of 100 mg/kg was 47.66, 56.49 & 31.49 in 1st, 4th and 24th hr respectively. The percent protection observed for at the dose of 200 mg/kg was 62.99, 70.10 & 34.29 in 1st, 4th and 24th hr respectively. (Fig. 3).

Effect of ethanolic extracts of *Citrullus colocynthis* on milk induced Leucocytosis in mice; Table 4 Subcutaneous administration of

boiled and cooled milk(4ml/kg) into the swiss albino mice as antigen and produced allergic response in mice. The total leucocytes count was increased 24 hour after milk injection (Table 4).

The total leucocytes count in control group was significantly higher as compared to ethanolic extract of *Citrullus colocynthis* group($p < 0.0001$) (Fig. 4)

TABLE 1: RESULT OF PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF CITRULLUS COLOCYNTHIS FRUITS

Extract	Glycosides	Protein	Flavonoid	Steroids	Alkaloids	Tanins	Amino acid
Ethanolic Extract of <i>Citrullus colocynthis</i> . Fruits	+	+	+	+	+	-	-

(+): Present; (-): Absent

TABLE 2: EFFECT OF ETHANOLIC EXTRACTS OF FRUITS OF CITRULLUS COLOCYNTHIS ON HISTAMINE INDUCED CONTRACTION OF ISOLATED GOAT TRACHEAL CHAIN PREPARATION

Sr. No.	Dose of Histamine (30µg/ml) (ml)	- ve Log molar concentration of Histamine	% Maximum Contraction		
			Control	Test	Standard
1	0.1	6.38	21.24 ± 0.89	16.52 ± 1.02	9.64 ± 0.69
2	0.2	5.91	48.14 ± 1.75	36.91±1.32**	20.80 ± 1.04**
3	0.4	5.54	61.34 ± 2.03	49.33±2.94**	33.78±1.12**
4	0.8	5.21	76.49 ± 1.24	65.66±1.58**	41.99±1.19**
5	1.6	4.89	89.69 ± 2.39	75.48±0.94**	54.25±1.33**
6	3.2	4.59	100 ± 0.00	79.26±2.10**	58.55±2.03**

Data are expressed as Mean± S.E.M. Where, n= 5,

Statistical analysis done by using Student's - test. Where, ** $p < 0.01$, significantly different from control

Control = D.R.C of histamine (30µg/ml) in absence of test drugs of *Citrullus colocynthis*.

Test = D.R.C histamine (30µg/ml) in presence of *Citrullus colocynthis*.

Standard = D.R.C of histamine (30µg/ml) in presence of Chlorpheniramine maleate

TABLE 3: EFFECT OF ETHANOLIC EXTRACTS OF FRUITS OF CITRULLUS COLOCYNTHIS ON PERCENT PROTECTION IN HISTAMINE INDUCED BRONCHOCONSTRICTION IN GUINEA PIGS

Group (n=4)	Preconvulsive dyspnea (in sec) (Mean±SEM)at						Percent protection (%)		
	Before tretment	After tretment			1 hr	3hr	24 hr		
		1 hr	3 hr	24 hr					
I	20.05±1.15	-	-	-	-	-	-	-	
II	22.8±1.19	68.5±1.17	88.06±1.23	31.17±1.43	66.71	74.10	26.85		
III	19.95±1.05	38.12±1.10	48.86±1.50	29.12±1.80	47.66	56.49	31.49		
IV	21.78±1.50	58.86±1.25	72.86±1.61	33.15±1.80	62.99	70.10	34.29		

Data are expressed as Mean± S.E.M. Where, n= 5,

Statistical analysis done by ANOVA followed by Dunnett's test, where * $p < 0.05$, ** $p < 0.01$ when group II, III, and IV were compared with group I.

Group- I (Control) = Aerosolized Histamine (0.2 % w/v)

Group-II (Std) = Aerosolized Histamine (0.2 % w/v) + Chlorpheniramine maleate (2 mg/kg, i.p.)

Group-III (Citrullus colocynthis.CC-100) = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of *Citrullus colocynthis* (100mg/kg, p.o.)

Group-IV(Citrullus colocynthis-CC-200) = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of *Citrullus colocynthis* (200mg/kg, p.o.)

TABLE 4: EFFECT OF ETHANOLIC EXTRACTS OF CITRULLUS COLOCYNTHIS ON MILK INDUCED LEUCOCYTOSIS IN MICE

Group (n=4)	Difference in number of Leucocytes (per cu mm) (Mean ± SEM)
I (Control)	6355.8±5.63
II (Std)	4805.66±5.36
III (CC-100)	5954±6.25
IV (CC-200)	5607±7.70

Data are expressed as Mean± S.E.M. Where, n= 4,

Statistical analysis done by ANOVA followed by Dunnett's test, where * $p < 0.05$, ** $p < 0.01$ when group II, III, IV were compared with group I.



FIG. 1: CITRULLUS COLOCYNTHIS PLANT AND FRUITS

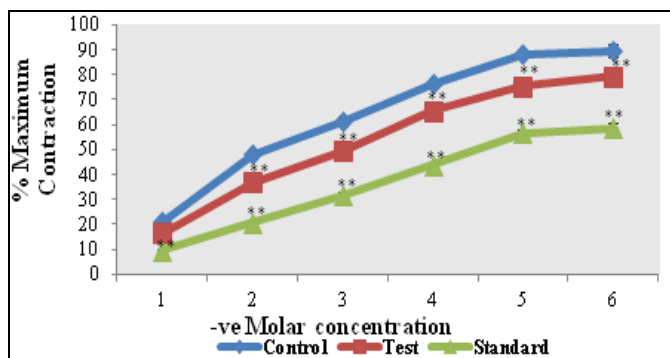


FIG. 2: HISTAMINE INDUCED CONTRACTION OF ISOLATED GOAT TRACHEAL CHAIN PREPARATION

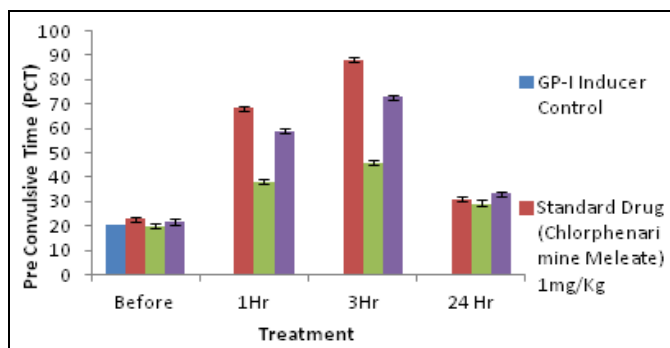


FIG. 3: HISTAMINE INDUCED BRONCHOCONSTRICTION IN GUINEA PIGS

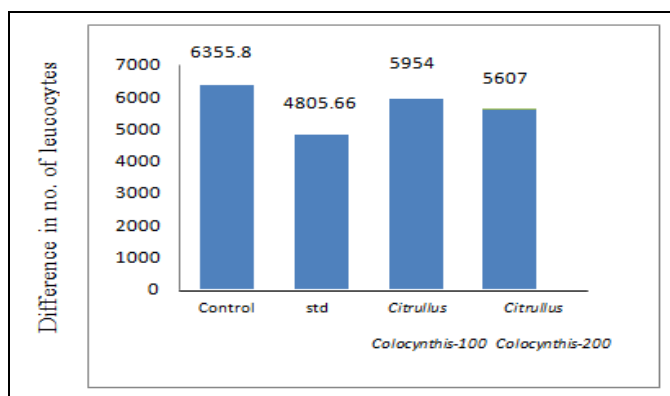


FIG. 4: MILK INDUCED LEUCOCYTOSIS IN MICE

DISCUSSION: Asthma is a chronic respiratory disease affecting a large proportion of population throughout the world. Bronchial provocation with allergen induce a prompt early phase immunoglobulin E (IgE)- mediated decrease in the brochial airflow (forced expiratory volume in one second) followed by a late phase IgE-mediated reaction with a decrease in the bronchial airflow for 4-8 hours. Initially asthma is characterized by the presence of increased number of various inflammatory mediators that are eosinophils, neutrophils, lymphocytes and plasma cells in the bronchial allergens tissues, bronchials secretion and mucus. The cross linkage of IgE molecules by allergens causes mast cells to degranulate, releasing histamine, leukotrienes, and other mediators that perpetuate the airway inflammation. Ultimately the mediators promote vascular permeability, smooth-muscle contraction and mucus production, which cause symptoms of asthma including airway constriction, coughing, shortness of breath and wheezing. The stimulation of H₁receptor causes contraction of bronchial smooth muscle. In the present study, the potential of ethanolic extract of *Citrullus colocynthis* has antagonized the histamine induced contractions on goat tracheal chain preparation which have shown a significant relaxation indicated by right shift of DRC of histamine.

CONCLUSION: The drug may be further explored for its phytochemical profile to identify the active coustituent. It can be concluded that ethanolic extracts of *Citrullus colocynthis* fruits may posseses antihistaminic activity which may be due to H₁-receptor blocking or anti-allergic activity. Thus *Citrullus colocynthis* fruits may be used in the management of asthma.

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