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## Antihistaminic Activity of Herbal Drug Isolated from *Cuscuta reflexa* Roxb. Growing in Kashmir Valley

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### ABSTRACT

The aim of present study was to find out the herbal drug which has potential antihistaminic properties. The ethanolic extract of *Cuscuta reflexa* Roxb was found quite useful in showing anti histaminic activities, when tested on experimental albino rats. The rats were divided into four groups of two animals each. Group I was served as control and received water with *ad-libitum* but not treated and sacrificed for the observation of mast cells which were found  $15.50 \pm 2\%$  intact and  $88.20 \pm 2\%$  disrupted. In the II group which was treated with ethanolic extract of *Cuscuta reflexa*, it was noticed that when the dose of 50 mg/kg body weight were given orally with water, the disruption of mast cells were found  $35.60 \pm 2\%$  disrupted and intact mast cells were found  $64.40 \pm 2\%$ . In the III group dose of 100 mg/kg body weight, the disruption of mast cells were found  $27.70 \pm 2\%$  and intact mast cells were found  $72.30 \pm 2\%$ . The rats of group IV received 10mg/kg of prednisolone (reference drug) orally for 4 days.

**Keywords:** Mast cell stabilization, *Cuscuta reflexa*, Phytochemical, Antihistaminic, Asthma.

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## INTRODUCTION

Mast cells are the group of connective tissues which show close relationship of histamine. Histamine secreted by the mast cells play important role in the inflammatory reaction in the body. Mast cells are immune systems (Watchman) spread across the body and have been used to test for newer agents against allergic disorders and chronic bronchial asthma. The influence of natural products derived from plants is broadly recognized for their great structural diversity as well as their wide range of pharmaceutical activities (Mukherjee 2001)<sup>1</sup>. Since time immemorial the Adivasi of Kashmir valleys have been used local flora for their elements. The *Cuscuta reflexa* Roxb is mostly utilized by the local herbalists (*Hakims*) for cough cold and asthma. *Cuscuta reflexa* belongs to family Cuscutaceae and is a parasitic plant commonly known as Kokli port. The medicinal uses and chemical constituents of *Cuscuta reflexa* have been widely studied by Sharma et al.(2009)<sup>2</sup>. The *Cuscuta reflexa* has been investigated for antispasmodic, antisteroidogenic(Gupta et al. 2003)<sup>3</sup> antihypertensive, muscle relaxant, cardio tonic(Singh et al 1973) and anticonvulsant activities(Gupta et al. 2004)<sup>6</sup>. Many chemical constituents have been isolated from the *Cuscuta reflexa* such as cuscutin, amarbelin, beta- sterol, sigmasterol, myrecetin, quercetin and olenolic acid. (Mohmmad Ali 2004)<sup>4</sup> Considering the available information and folklore use of the plant, the present study was designed to evaluate the antihistaminic /mast cell stabilizing activity of ethanolic extract of whole plant material of *Cuscuta reflexa*.

## MATERIALS AND METHOD

*Cuscuta reflexa* Roxb. was collected from the Baramulla district of Kashmir valley and was traditionally used for cough, cold and asthma by the tribal Gujjar and Bakerwals of that region. The whole plant was identified and authenticated from the Centre of Biodiversity and Plant Taxonomy University of Kashmir (J & K). The herbarium of the plant has been deposited and the voucher specimen is procured in the Herbarium Record of Pest control and Ayurvedic Drug Research Laboratory S.S. L. Jain College Vidisha at Serial Number 37.

### Preparation of Extract

The air dried whole plant material of *Cuscuta Reflexa* (500g) was reduced to coarse powder and subjected to extraction with ethanol in soxhlet extractor. The extract was concentrated to dryness on water bath to yield ethanol extract of *Cuscuta reflexa*.

### Animals

The study was conducted on male albino rats of Wistar strain (*Rattus-norvegicus*). The rats (150-200gms) were obtained from the animal house of Pest Control and Ayurvedic Drug Research

Laboratory, S.S.L. Jain College Vidisha (M.P.). The experimental work was carried out under the supervision of IAEC as per the guidelines of CPCSEA with the approval No. 804/03/CA/CPCSEA maintained under controlled conditions at temperature of  $22\pm 2^{\circ}\text{C}$ , humidity  $60\pm 10\%$  and a 12h light/dark cycle. They had free access to standard rodent pellet diet (Golden Feeds Private Ltd., Badodara) and water *ad-libitum*. Horse serum were procured from Himedia laborites, Mumbai, DPT antigen which contain Bordetella pertussis from Serum Institute of India, Pune, toludine blue from Loba chemie, Mumbai and prednisolone 10mg (I.P.), Wyeth limited, Goa. Other chemicals and reagents were procured from Merck Mumbai.

### **Preliminary Phytochemical Testing**

The standard phytochemical tests were used in screening the extract for different constituents.

#### **Test for Flavonoids**

##### **Alkaline Reagent test:**

In the test sample (ethnolic extract) a few drops of NaOH solution were added, immense yellow color was formed which turns colorless on addition of a few drops of acid. This indicates the presence of flavonoids in the extract.

#### **Test for glycosides**

##### **Cardiac glycosides Keller-Killiani Test (Test for Deoxy sugars)**

The sample was extracted with chloroform and evaporated with dryness and then 0.4ml of glacial acetic acid was added containing trace amount of ferric chloride. It was transferred to a small test tube and 0.5ml conc.  $\text{H}_2\text{SO}_4$  was carefully added by the side of the test tube. Acetic acid layer showed blue color.

##### **Saponin Glycosides (Presence of saponin)**

**Froth formation test** Two ml solution of crude ethanolic extract of *Cuscuta reflexa* was mixed in 7ml of distilled water in a test tube and shaken well, stable froth (foam) was formed, which showed the presence of saponin in the test sample.

#### **Test for steroids and tri-terpenoids**

##### **Salkowski test**

The ethanolic extract of *Cuscuta reflexa* was treated with a few drops of conc.  $\text{H}_2\text{SO}_4$ ; yellow color at lower layer indicated the presence of triterpenoids.

### **Evaluation of mast cell stabilizing activity**

#### **Active anaphylaxis**

Male albino rats were weighed and randomly selected. All rats were sensitized by injecting subcutaneously 0.5 ml of horse serum along with 0.5 ml of triple antigen containing 20,000 million

*Bordetella pertussis* organisms (Gupta et.al., 1973)<sup>3</sup>. The sensitized rats were divided into four groups. Group I received water and served as control. The rats of group II and III were orally administered with herbal extract of 50 and 100 mg/kg body weight respectively for the same duration. The rats of group IV received 10mg/kg of prednisolone (reference drug) orally for 4 days. On the 14<sup>th</sup> day 25 hours after the last dose of treatment rats were sacrificed and intestinal mesenteries and trachea were taken for the study of mast cells. Mesenteries of sacrificed rats were kept in Ringer-Locke solution (NaCl 9.0, KCl 0.42, CaCl 0.24, NaHCO<sub>3</sub> 0.15 and glucose 1.0gm/l of distilled water) at 37<sup>o</sup> C. The mesenteric pieces were then shifted to a beaker containing 5% horse serum distilled in Ringer-Locke Solution. After an incubation period of 10 minutes, the tissues were removed, trimmed and stained with 1.0% toluidine blue solution and examined microscopically for the number of intact and de-granulated mast cells. A mast cell was considered disrupted if four or five granules were observed around the mast cells as reported by Norton (1954)<sup>5</sup>.

### Mast cell staining

Pieces of intestinal mesenteries were mounted on slides. All slides were air dried, then stained with 1.0% Toluidine blue at room temperature for 5 minutes. Mast cells were readily identified by their metachromatic cytoplasmic granules under the light microscope.

### Statistical Analysis

The results were expressed as mean  $\pm$ SEM and analyzed statistically using student t-test to find out the level of significance.

## RESULTS AND DISCUSSION

### Preliminary Phytochemical Screening

Preliminary phyto-chemical study of ethanolic fraction revealed the presence of flavonoids, cardiac glycosides; saponin glycosides steroids and triterpenoids table 1.

**Table 1: Showing Preliminary Phytochemical Screening of Selected Plant Materials**

S. No.	Presence of Components	Name of the test performed	<i>Cuscuta reflexa</i>	
2	Flavonoids	Alkaline reagent test	++	
3	Glycosides	Cardiac Glycosides	Keller-Killiani test	-
		Saponin Glycosides	Froth formation test	++
		Steroids and triterpenoids	Salkowski test	++

Positive (+), Strong positive (++), Negative (-)

In the present study, anti-histaminic or mast cell stabilizing activity was evaluated using ethanolic fractions of *Cuscuta reflexa* in anaphylactic Wistar albino rats (Figure 1). In the present investigation group I was served as control and have received water with *ad-libitum* but not treated

and sacrificed for the observation of mast cells which were found  $15.50 \pm 2\%$  intact and  $88.20 \pm 2\%$  disrupted. Mast cells were observed carefully and percentage of intact and disrupted mast cells were calculated (Figure 2, Graph 1 and table 2). In the II group which was treated with ethanolic extract, it was noticed that when the dose of 50 mg/kg body weight were given orally with water by using oral feeding tube needle, the disruption of mast cells were found  $35.60 \pm 2\%$  disrupted and intact mast cells were found  $64.40 \pm 2\%$  (Figure 3, Graph 1 and table 2). In group III, the dose of 100 mg/kg body weight for the extract, the disruption of mast cells were found  $27.70 \pm 2\%$  and intact mast cells were found  $72.30 \pm 2\%$  (Figure 4, Graph 1, table 2). However in the group IV the standard drug Prednisolone of 10 mg/kg body weight, the percentage of intact mast cells showed was  $84.50 \pm 2\%$  and disrupted was  $20.40 \pm 2\%$  (Figure 5, Graph 1 and table 2).

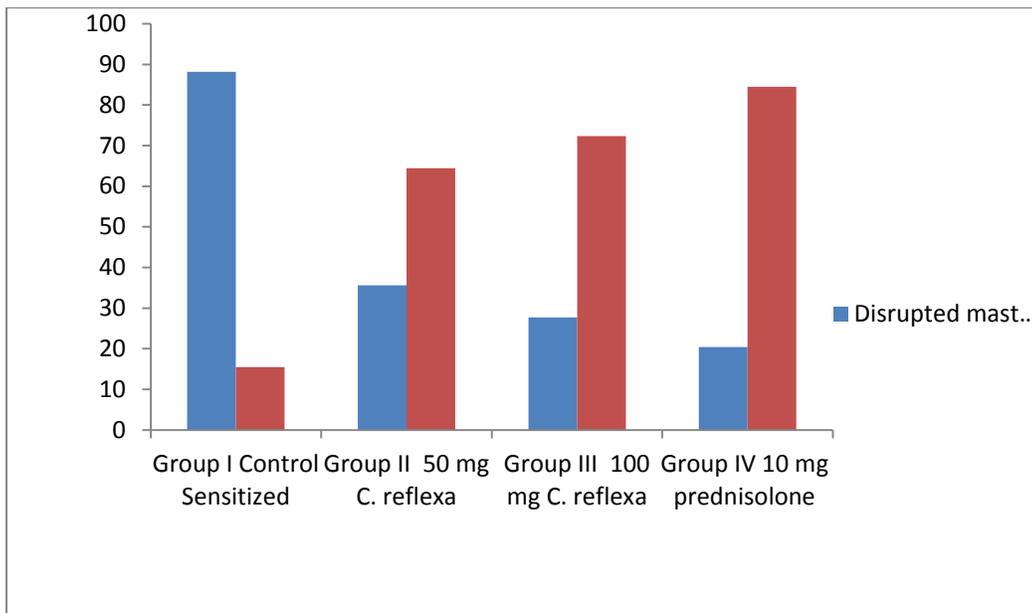
**Table 2: Effect of Active Fraction of Plant Extracts on Albino Rats**

Group	Treatment	Dose (mg/kg b. w.)	Route of administration	Mast cells de-granulation	
				Disrupted %	Intact %
I	Control Sensitized	--	Not given	$88.20 \pm 2\%$	$15.50 \pm 2\%$
II	Treated with <i>Cuscuta reflexa</i> extract	50	Orally	$35.60 \pm 2\%$	$64.40 \pm 2\%$
III	Treated with <i>Cuscuta reflexa</i> extract	100	Orally	$27.70 \pm 2\%$	$72.30 \pm 2\%$
IV	Standard drug Prednisolone	10	Intra muscular	$20.40 \pm 2\%$	$84.50 \pm 2\%$

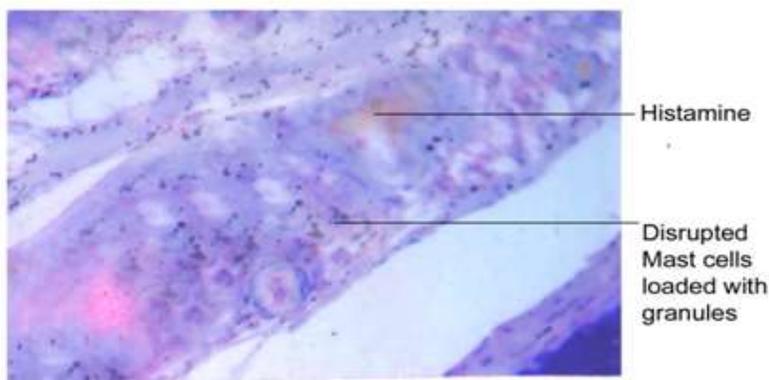
*P* value 0.05, \* SEM



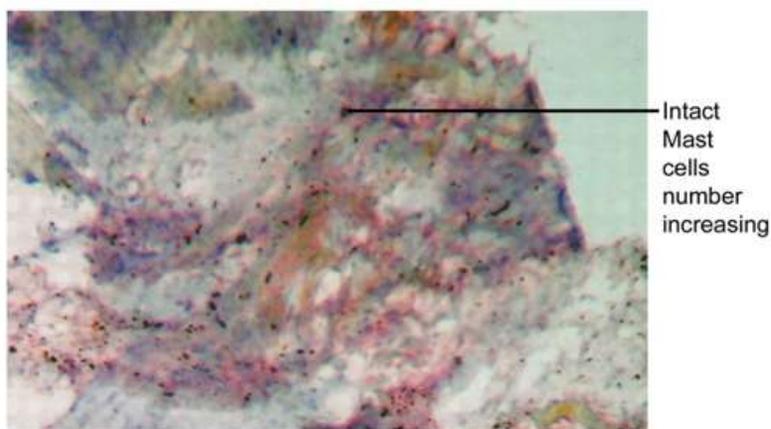
**Figure 1: Experimental Animal – Wistar Albino Rat**



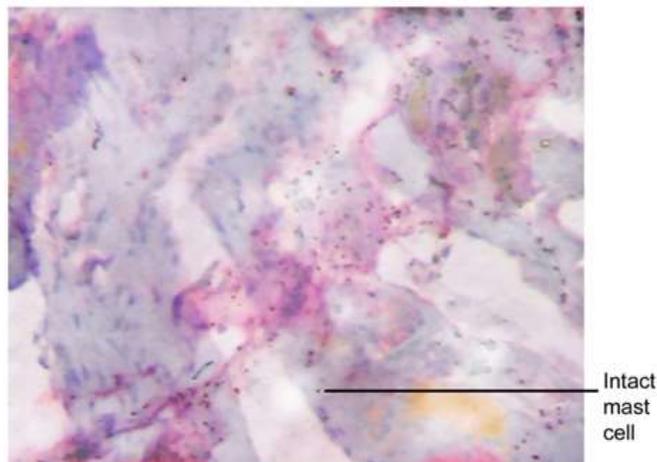
**Graph 1: Showing the Percentage of Intact and Disrupted Mast Cells**



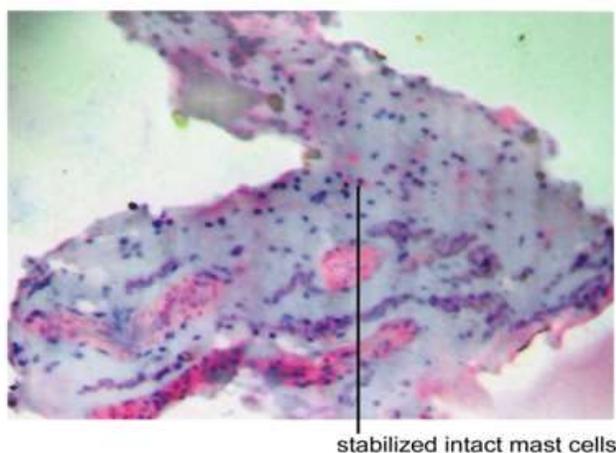
**Figure 2: Histopathology of Mesenteries in Sensitized Control Group of Albino Rats Stained with Toluidine Blue X 450**



**Figure 3: Histopathology of Mesenteries in Treated Group of Albino Rats when Given 50mg/kg b.w. Dose of Cuscuta Reflexa Extract Stained with Toluidine Blue X 450**



**Figure 4: Histopathology of Mesenteries in Treated Group of Albino Rats when Given 100mg/kg b.w. Dose of Cuscuta Reflexa Extract Stained with Toluidine Blue X 450**



**Figure 5: Histopathology of Mesenteries in Treated Group of Rats when given 10 mg/kg b.w. Dose of Standard Drug Prednisolone Stained with Toluidine Blue X 450**

Mast cells play a crucial role in the development of many physiological changes during allergic conditions of asthma. When the foreign particles come in to contact of mast cells, it trigger number of dramatic actions in the mast cells because they work as antigens. During dramatic actions mast cells release variety of chemicals like histamine that is a vasodilator which dilates blood vessels in the body. Another is serotonin which constricts blood vessels. Heparin is a anticoagulant but it doesn't play role in asthmatic conditions. After histamine, leukotrienes and other substances also play important role in allergic and asthmatic conditions. However, body always develops immunity against antigen through increasing the production of antibody. Immunoglobulin E (IgE) is an antibody which always binds histaminic receptors on the surface of mast cells during asthma and allergy. The present study observed the influence of saponins and flavonoids treatment on the stabilization of mast cells .Chaudhary (2010)<sup>7</sup> have reported such activity in *Ocimum sanctum* leaf

extract. Mast cells are evenly distributed in mesenteries in trachea. They have been found to display abundant granules which can be clearly seen by the present author by using toluidine blue staining which gave metachromatic cytoplasmic granules. Mast cells de-granulation was seen with numerous extruded granules both near and the distance from the cell body. A thorough microscopic examination of mast cells in the treated animals was observed in the present study. In intact cells, granulation was seen after the treatment of extracts of *Cuscuta reflexa*. These mast cells were more immune reactive and granules were found to be distributed outside the cell body. De-granulated cells contain less pre-nuclear granule. Near the de-granulated cells, numerous collagen fibers were found. Similar observations have been carried out earlier by Soni et al. (2004)<sup>8</sup>, where they have described the mast cells de-granulation activity in the methanolic extract of *Tephrosia purpurea*. In the present investigation, evaluation of antihistaminic activity was carried out using a traditionally important plant of Kashmir valley. The animal treated with *Cuscuta reflexa* ethanolic extract at 100 mg/kg body weight showed 72.30% intact mast cells, Which is quite comparable with the standard drug prednisolone (84.5%) as shown in (Graph 1 and Table). Thus, the results of the present study clearly suggest an antihistaminic activity in the formulation of *Cuscuta reflexa* ethanolic extract. Sunilson et al. (2010)<sup>9</sup> have also reported mast cells stabilizing activity of the cough syrup where they suggested that this may be attributed to the presence of plant phytoconstituents. Preliminary phytochemical screening of the selected plant extract was done by using various chemical tests and it was reported that alkaloids, flavonoids, saponin glycosides, steroids and triterpenoids were found to be strong positive in the *Cuscuta reflexa*. Similarly, Sharma et al. (2009)<sup>2</sup> have studied *Cuscuta reflexa* for preliminary phytochemical screening which revealed the presence of steroids, saponins, triterpenes and flavones in *Cuscuta reflexa*.

## CONCLUSION

The present study provides evidence for the mast cell stabilizing activity of ethanolic extract of *Cuscuta reflexa* in experimental animals, which may be do to presence of flavonoids, and saponins. The need of the hour is to identify and isolate the phyto-constituents responsible for the observed central effects in animals and to understand their molecular mechanism.

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