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Evaluation of Mast Cell Stabilization Potential of Euphorbia Wallichaii Hook

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Abstract

The present research article find out the potential of Euphorbia wallichaii root extract on stabilization of mast cells or antihistaminic activities in albino rats. The aqueous root extract of the Euphorbia wallichaii when screened for the presence of different phytochemical, revealed the presence of steroids, triterpenoides and flavonoides. The rats were divided into four groups. The group I which was control sensitized showed 88.20 ± 2% disrupted mast cells and 15.50 ± 2% of intact mast cells, the group II was orally treated with aqueous root extract at the dose of 50 mg/kg b.w showed the disruption of mast cells 29.80 ± 2% and intact mast cells were 71.20 ± 2% but at the dose of 100 mg/kg b.w weight in group III 24.70 ± 2% of mast cells were disrupted and 81.10 ± 2% of mast cells were intact. The group IV was treated with Prednisolone at 10 mg/kg b.w showed the 84.50 ± 2% of intact mast cells and 20.40 ± 2% of disrupted mast cells. This mast cell stabilization property of euphorbia wallichaii root seems to be due to the presence of high content of steroids, triterpenoides and flavonoides in the plant extract.

Keywords: Euphorbia wallichaii; Mast cells; Asthma; Phytochemical; Histamine

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Introduction

Medicinal herbs make an effective source for the traditional and modern medicine. Euphorbia wallichaii locally in Kashmir (J&K) known as Kaali Heerbi and is used as a folk medicine for the treatment of various dreadful diseases. Asthma is a disease of the human respiratory system in which the airways constrict and become narrow, causing difficulty in breathing with sounds of whistle [1]. Asthma is caused by a large number of reasons in which allergy is one of them. When someone has the capability to become allergic and produce IgE antibodies, the antibody attaches itself to certain cells, called mast cells, in his body. Millions of these mast cells line the wall of a person's skin, nose and bronchial tubes. Mast cells are immune systems (Watchman) spread across the body and have been used to test for agents against allergic disorders and chronic bronchial asthma [2]. Each mast cell contains about a thousand tiny granules these granules are loaded with potent chemicals or mediators, the most powerful of which are histamine and leukotrienes [3]. Histamine secreted by the mast cells play important role in the inflammatory reaction in the body. There is a category of medicine that competes with histamine released after allergic response to nullify its destructive effects on the tissues [4]. Herbal approaches have regained their popularity, with their efficacy and safety aspects being supported by controlled clinical studies. Herbal alternatives employed against the asthma have proven to provide symptomatic relief and assist in the inhibition of this disease.

Materials and Methods

The On the basis of traditional Knowledge of the area the whole plant of Euphorbia wallichaii was collected from Danwas area of Gulmarg Kashmir, India **(Figure 1)**. The plant was identified and authenticated from the Centre of Biodiversity and Plant Taxonomy University of Kashmir, India. The herbarium of the plant has been deposited in the Herbarium Record of the Centre of Biodiversity and Plant Taxonomy University of Kashmir, India The root part of the plant was dried in shade and subjected to Extraction [5]. Soxhlet apparatus was used for the extraction of root by using different solvents in increasing order of polarity. They are n-hexane, petroleum ether, chloroform, methanol and distilled water. The Extraction was performed for 48 hours at 42°C-45°C or 8 cycles. The Aqueous crude extract of root was used for Preliminary photochemical investigation and analysis of mast cell stabilization in Albino rats. Preliminary photochemical investigation was carried out by using methodology adopted **(Table 1)** [6].

Table 1: Preliminary phytochemical screening of root aqueous extract.

Presence of components		Name of the test performed	Euphorbia wallichaii
Alkaloids		Dragendroff reaction	-
		Mayer's reaction	-
Flavonoides		Alkaline reagent test	+ +
Glycosides	Cardiac glycosides	Keller-Killiani test	+
	Sponin glycosides	Froth formation test	+
	Steroids and triterpenoides	Salkowski test	++
Note: Positive (+), Strong positive (++), Negative (-)			

Figure 1 Euphorbia wallichaii.

Experimental animal

The present study was conducted on male albino rats (175 g-200 g) of Wistar strain (Rattus norvegicus) **(Figure 2)**. The experimental work was carried as per the guidelines of CPCSEA with the approval No. 804/03/CA/CPCSEA maintained under controlled conditions at temperature of 22 ± 2°C, humidity 60 ± 10% and a 12 h light/dark cycle. They had free access to standard rodent pellet diet and water ad-libitum. Twenty 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 [5].The sensitized rats were divided into four groups having five rats in each group. Group I received water and served as control. By intra-gastric catheter the rats of group II and III were orally administered with aqueous root extract of 50 and 100 mg/kg body weight respectively for the 14 days. The rats of group IV received 10 mg/kg of Prednisolone (reference drug) orally by intra-gastric catheter for 4 days [6]. On the 14th day 25 hours after the last dose of treatment rats were sacrificed and intestinal mesenteries 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, $NaHCO_3$ 0.15 and glucose 1.0 gm/l of distilled water) at 37°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, thin sections were made and staining was done with 1.0% toluidine blue solution. The mesenteries were finally 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 [7]. Mast cells were readily identified by their metachromatic cytoplasmic granules under the light microscope. The results of various studies were expressed as mean ± SEM and analyzed statistically using one-way ANOVA, Chi-square test or unpaired Student's t-test to find out the level of significance. $P<0.05$ was considered statistically significant. The analysis was performed using Graphpad Prism software package.

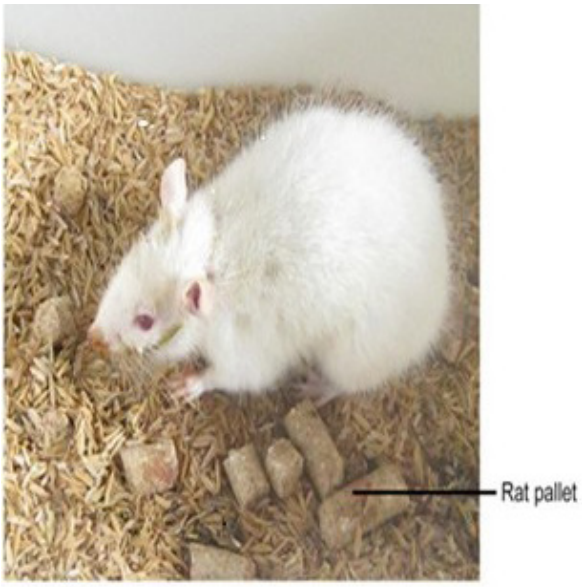


Figure 2 Albino rat.

Results

During preliminary phytochemical screening tests aqueous root extract of Euphorbia wallichaii was found to be strong positive for flavonoides, steroids and triterpenoides which when tested on albino rats showed their impact on mast cell stabilization. In the present study, anti-histaminic or mast cell stabilizing activity was evaluated by the aqueous root extract of Euphorbia wallichaii in anaphylactic Wister albino rats. Group I was served as control and have received water with ad-libitum but not treated and was sacrificed for the observation of mast cells which were found 15.50 ± 2% intact and 88.20 ± 2% disrupted **(Figure 3, Table 2, Graph 1)**.

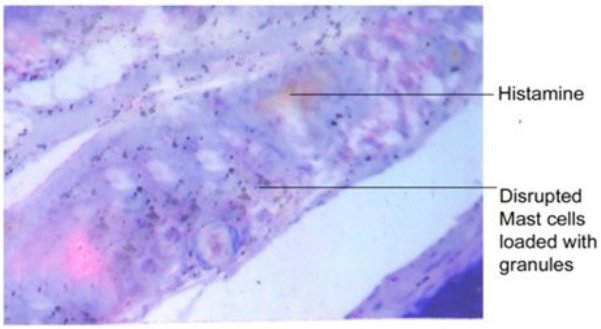


Figure 3 Histopathology of Mesenteries in sensitized control group of albino rats.

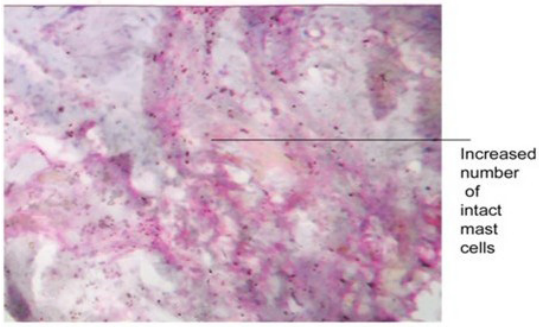
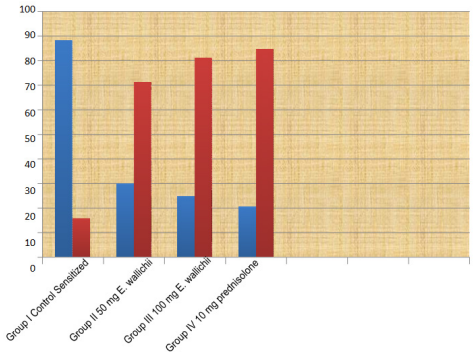


Figure 4 Histopathology of Mesenteries of albino rats treated with extract of Euphorbia wallichaii 50 mg/kg b.w.



Graph 1 Results of experimental bioassay. Note: ■ Disrupted mast cells; ■ Intact mast cells.

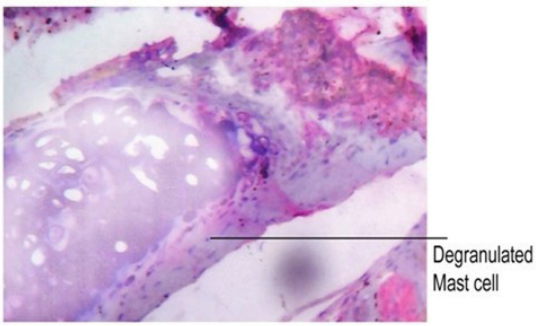


Figure 5 Histopathology of Mesenteries of albino rats treated with Euphorbia wallichaii extract 100 mg/kg b.w.

The results of the present study coincides with the findings made by Patel [8] and Chaudhary [9].Thus from the above findings it can be concluded that the mast cell stabilization in albino rats seems to be due to the presence of Steroids and flavonoides in the aqueous root extract of Euphorbia wallichaii [10-12] **(Figures 4-6, Graph 1 and Table 2)**.

Table 2: Effect of root extract on mast cells of albino rats.

Group	Treatment	Dose (Mg/kg B. w.)	Route of administration	Mast cells degranulation	
				Disrupted %	Intact %
I	Control sensitized	---	Orally	88.20 ± 2%	15.50 ± 2%
II	Treated with Euphorbia wallichaii root extract	50	Orally	29.80 ± 2%	71.20 ± 2%
III	Treated with Euphorbia wallichaii root extract	10	Orally	24.70 ± 2%	81.10 ± 2%
IV	Standard drug Prednisolone	10	Orally	20.40 ± 2%	84.50 ± 2%

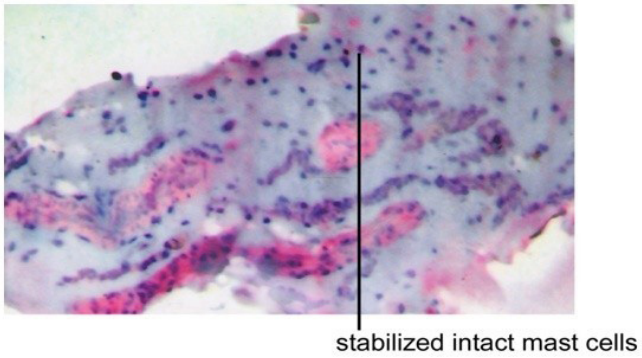


Figure 6 Histopathology of Mesenteries of albino rats treated with Prednisolone 10 mg/kg b.w.

Conclusion

The group II when treated with aqueous Euphorbia wallichaii root extract, it was noticed that when the dose of crude extract 50 mg/kg body weight given orally, the disruption of mast cells were found 29.80 ± 2% and intact mast cells were found 71.20 ± 2%. But at the dose of 100 mg/kg body weight in Group III 24.70 ± 2% of mast cells disrupted and 81.10 ± 2% of intact mast cells were observed. However in the group IV the standard drug Prednisolone of 10 mg/kg body weight, the percentage of disrupted mast cells was 20.40 ± 2% and the percentage of intact mast cells was 84.50

± 2%, which was quite similar to the maximum 100 mg/kg/b. w. of Euphorbia wallichaii root extract. The above results when compared to the control seem to be quite significant at p<0.05% when student "t" test was applied. All the values obtained after the treatment by plant extract were highly significant. However when both the treated groups were compared with the group IV of Prednisolone the results of the root extract was quite significant with excellent stabilization of mast cells. It was also observed that mast cells after stabilization show trans-granulation, which occurs between mast cell apparently transferring their granules to the cytoplasm of fibroblast.

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