

# EFFECT OF QUERCETIN AND *ALBIZZIA* SAPONINS ON RAT MAST CELL

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( Received on April 4, 1984 )

**Summary** : In the present work the effect of quercetin obtained from (*Allium cepa*). *Albizzia lebbek* (crude extract of seeds) and a pure saponin fraction of *Albizzia* has been studied on the mast cells in the mesentery and peritoneal fluid of rats subjected to anaphylaxis. The results show a mast cell membrane stabilizing effect of these test drugs.

**Key words** : quercetin      *Albizzia lebbek*      saponin      mast cell      anaphylaxis

## INTRODUCTION

Ayurvedic practitioners in South India have been prescribing a special variety of onions in management of bronchial asthma. It has been reported that this variety of onions (*Allium cepa* Linn.) is effective against respiratory disorders (2). Decoctions of *Albizzia lebbek* has also been recommended in Ayurveda as a possible cure for asthma (5). It is reported that *Allium cepa* contains large amounts of quercetin and that saponins are one of the major constituents of *Albizzia* sp. (J. Singh, personal communication).

In order to elicit the possible mode of action of quercetin and *Albizzia* saponins, the effect of these substances on rat mast cells have been studied, since mast cells have been considered to play an important role in the pathogenesis of asthma (6).

## MATERIAL AND METHODS

Quercetin (the principle constituent of *Allium cepa*), alcoholic extract of the seeds of *Albizzia* and a pure saponin fraction of *Albizzia* were obtained by procedures standardized in this Institute. Disodium cromoglycate (DSCG) was supplied by Unique Chemicals, Bombay, Horse serum was obtained from King's Institute, Madras.

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Study was conducted on albino rats (100-150 *gms*) maintained under uniform husbandry conditions (temperature,  $27 \pm 2^\circ\text{C}$ , 12 hr light and dark cycles). The animals were fed Hindustan Lever diet; water was given *ad libitum*.

*Active anaphylaxis* : Active anaphylaxis was induced by sensitizing the rats with horse serum by the method of Kabat and Mayer (4). Two sc injections of 0.5 *ml* horse serum (diluted in normal saline to contain 10 *mg* protein per *ml*) were given 4 days apart to each animal. On the sixth day of sensitization, animals were divided into groups of 6 animals each, for treatment with distilled water (vehicle) or quercetin (25 *mg/kg/day*, po), extract of *Albizia lebbek* (25 *mg/kg/day*), saponin fraction (25 *mg/kg/day*) and DSCG (25 *mg/kg/day*, ip). At the end of treatments the rats were sacrificed by cervical dislocation. Tyrode solution (10 *ml*) was injected ip and after 5 min the peritoneal fluid was taken out. The fluid was centrifuged at 250 g for 10 min at  $10^\circ\text{C}$  and the pellet containing mast cells was resuspended in Tyrode solution (2 *ml*). Intestinal mesentery was also removed, cut into small pieces and kept in Tyrode solution. The mesenteric bits and peritoneal mast cell suspensions were challenged with horse serum (5%). After 10 min of incubation at  $37^\circ\text{C}$  the mesenteric bits were removed, mounted on glass slides and dried overnight. The mast cells from mesenteric bits and peritoneal fluid were used for microscopic examination (see below).

*Passive anaphylaxis* : Passive anaphylaxis was induced by injections (ip) of 1.0 *ml* of undiluted serum (collected from a rat of sensitized control group) into individual fresh rats. The passively sensitized animals were divided into different experimental groups and treated with various test drugs as described above. At the end of drug treatment, the mast cells from peritoneal fluid and mesenteric bits were examined microscopically as mentioned below.

*Effect of drugs added in vitro* : Another group of sensitized rats was utilized for *in vitro* study. The animals were given 2 injections of horse serum 4 days apart. On the sixth day, mesenteric bits and peritoneal mast cell suspensions were obtained. The samples of mesenteric bits and peritoneal fluids were incubated separately for 30 min at  $37^\circ\text{C}$  with quercetin, crude extract of *Albizia* seeds, the pure saponin fraction (all at a dose of 0.5 *mg/ml* of Tyrode solution, and DSCG (25  $\mu\text{g/ml}$  of Tyrode solution) At the end of incubation the mesenteric bits and peritoneal fluids were challenged with horse serum (5%) for 10 min, after which the mast cells were examined microscopically.

*Microscopic study of mast cell* : Mast cells were stained first with 0.1% toluidine for 5 min and then with 0.1% light green for 2 min. Microscopic examination was carried out by randomly selecting 5 fields to count the intact/ruptured cells. Significance of difference was calculated by student's t-test.

## RESULTS

It is observed that the mean count of ruptured mast cells in sensitized untreated animals (control group) was  $86 \pm 5$  in mesenteric bits and  $80 \pm 7$  in peritoneal fluid. All the test compounds significantly reduced the number of ruptured mast cells, in both mesenteric bits and peritoneal fluid, obtained from sensitized animals, in comparison to the control group (Table I and II). This effect was found to be identical in both types of systemic anaphylaxis (active and passive). These results are further reinforced by *in vitro* experiments. Further, it can be observed from Table I and II that among the test drug studied, quercetin was most effective followed by *Albizzia* and saponin fraction.

TABLE I : Effect of test substances on mesenteric mast cells in rat.

Groups	Drug treatment			
	Quercetin	<i>Albizzia</i> (crude extract)	Saponin fraction	DSCG
A. Active anaphylaxis	$38 \pm 4$ (55%)	$44 \pm 5$ (40%)	$63 \pm 3$ (27%)	$23 \pm 6$ (74%)
B. Passive anaphylaxis	$35 \pm 6$ (59%)	$44 \pm 4$ (48%)	$45 \pm 7$ (47%)	$22 \pm$ (75%)
C. <i>In vitro</i>	$42 \pm 3$ (50%)	$52 \pm 7$ (40%)	$62 \pm 5$ (28%)	$29 \pm 3$ (66%)

Mean count of ruptured mast cells  $\pm$  S.D. Figures in parenthesis refer to the percentage protection after drug treatment. Average count of ruptured mast cells in sensitized (control) group was  $86 \pm 5$  (N=12). All values differ significantly from control ( $P < 0.01$ ).

TABLE II : Effect of test substances on peritoneal mast cells in rats.

Groups	Treatment			
	Quercetin	<i>Albizzia</i> (crude extract)	Saponin fraction	DSCG
A. Active anaphylaxis	$32 \pm 2$ (60%)	$43 \pm 7$ (47%)	$59 \pm 3$ (27%)	$23 \pm 5$ (72%)
B. Passive anaphylaxis	$25 \pm 4$ (69%)	$24 \pm 3$ (70%)	$25 \pm 5$ (69%)	$19 \pm 4$ (76%)
C. <i>In vitro</i>	$43 \pm 4$ (47%)	$48 \pm 6$ (40%)	$52 \pm 5$ (35%)	$29 \pm 3$ (64%)

Mean count of ruptured mast cells  $\pm$  S.D. Figures in parentheses refer to the percentage protection after drug treatment. Average count of ruptured mast cells in sensitized (control) group was  $80 \pm 7$  (N=12). All values differ significantly from control ( $P < 0.01$ ).

## DISCUSSION

The results presented here show that quercetin, *Albizzia lebbek* (crude extract of seeds) and pure saponin fraction offer significant protection to mast cells, both in mesenteric bits and peritoneal fluid, from degranulation due to antigen shock. The effect produced by these compounds was found to be qualitatively identical with that of DSCG, which is a well known mast cell membrane stabilizing agent, reducing release of mediators from mast cell in response to antigen and other challenges (6). It appears that the test compounds used in this report may possibly act by inhibiting the phenomenon of sensitization and may possess potent mast cell membrane stabilizing activity. Thus after treatment with these test compounds, a reduction in the release of mediators from mast cells may be expected thereby reducing the severity of anaphylactic shock.

In this context, it has been demonstrated earlier that extracts of *Allium cepa*, possessed well marked bronchodilatory activity and provided effective relief in human volunteers with acute and chronic asthma (1). The antihistaminic action of quercetin is also established (3). The observed anti-anaphylactic activity of quercetin, *Albizzia* and saponin may be of value for developing indigenous drug against asthma.

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