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# Corilagin Attenuates Allergy and Anaphylactic Reaction by Inhibiting Degranulation of Mast Cells

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**Background:** This study evaluated the anti-allergic activity of corilagin and also postulates the possible mechanism of its action.





**Material/Methods:** Corilagin was given orally at dose of 10, 20, and 40 mg/kg/day. All the animals (guinea pigs, rats, and mice) were sensitized for allergy such as eosinophilia and leukocytosis induced by milk; degranulation of mast cell by compound 48/80; and passive and active anaphylaxis. Moreover, the antagonistic effect was determined by estimating the effect of corilagin on contraction of guinea pig tracheal chain and ileum induced by Ach and histamine, respectively.

**Result:** There was a significant decrease in the leukocyte and eosinophil counts in the corilagin-treated group compared to the negative control group. Treatment with corilagin significantly protects the degranulation of mast cells, and it also has significant anti-muscarinic and antihistaminic activity by reducing the muscle contraction induced by Acetylcholine (Ach) and histamine in guinea pig tracheal chain and ileum.

**Conclusions:** Corilagin possess anti-anaphylactic and anti-allergic activity by inhibiting the release of mediators from mast cells and by decreasing the serum concentration of immunoglobulin E (IgE).

**MeSH Keywords:** **Allergy and Immunology • Anaphylaxis • Medicine, Chinese Traditional**

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## Background

Anaphylactic reaction is a type I hypersensitivity reaction that occurs in an antigen-sensitized individual in which antigen antibody reaction activates the mast cells [1]. Activated mast cells degranulate and release histamine, which contributes to the anaphylactic reaction [2]. However, degranulation of mast cells also occurs by non-immunologic reactions such as activation with certain drugs, complement components, basic compounds, and neuropeptides [3]. In anaphylactic reaction, IgE bind with the receptor located on the cell membrane and thereby initiates the release of prostaglandin, histamine, and interleukin in the systemic circulation [4,5]. These chemicals alter the physiological function and contribute to the anaphylactic reaction.

Reports in the literature suggest that several natural products show promising effects in the management of anaphylactic reactions. Corilagin is isolated from the herb *Caesalpinia coriaria* and is chemically known as ellagitannin [6]. Previous reports suggested that corilagin has potent antioxidant, hepatoprotective, anti-inflammatory, analgesic, antihypertensive, anti-tumor, and carbonic anhydrase inhibitor properties [7–10]. It was reported that corilagin possesses analgesic activity by altering the glutamergic system and anti-inflammatory activity by decreasing the production of pro-inflammatory cytokines and mediates tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$ , IL-6, NO (iNOS), and cyclooxygenase-2 (COX-2) at both the protein and gene level by blocking NF- $\kappa$ B nuclear translocation [11,12]. Thus, the present investigation evaluated the effect of corilagin against anaphylactic reaction.

## Material and Methods

### Chemicals

Histamine Hcl and Compound 48/80 were procured from Sigma Aldrich, USA. Horse serum and egg albumin were purchased from Loba Chemicals, India. Ketotifen and dexamethasone was procured from Pfizer Ltd., USA.

### Animals

Dunkin Hartley guinea pigs (320–400 g), Swiss albino mice (20–25 g), and Sprague Dawley rats (180–200 g) were procured from Shanghai Changzheng Hospital, China. All the animals were kept in a well-maintained and controlled environment according to experimental animal care guidelines. The protocols used in this study were approved by Institutional Animal Ethics Committee of the Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine (SUTCM/2016/20) and we followed the guidelines of the Association for the

Assessment and Accreditation of Laboratory Animal Care International (AAALAC) for experimentation and animal use.

### Estimation of anti-allergic effect of corilagin

#### *Estimation of effect of corilagin on eosinophilia and leukocytosis*

All the mice were separated into 6 groups (n=6): the control group received solvent, the negative control group received subcutaneous injection of milk (4 ml/kg), the standard (STD) group received intraperitoneal injection of dexamethasone (0.27 mg/kg), and the corilagin group received corilagin 10, 20, and 40 mg/kg orally. Leukocyte and eosinophil counts in the blood were estimated before and after administration of milk [14].

#### *Passive cutaneous anaphylaxis*

Subcutaneous injection of egg albumin with aluminum hydroxide was used to sensitize the rats on days 1, 3, and 5 of the protocol. Serum was separated from the blood drawn on day 11. All the animals were separated into 6 groups: the control group, the negative control group, the STD group, and the corilagin 10, 20, and 40 mg/kg groups. We shaved all the rats and subcutaneously injected 100  $\mu$ l of homologous anti-serum. A mixture of EA (100  $\mu$ g) and Evan's blue (0.5% w/v) at a ratio of 1: 1 was injected intravenously to each group of rats and used a Vernier caliper was used to measure the area of dye leakage [15].

#### *Estimation of serum IgE*

Following the procedure described earlier, we sensitized the rats and separated them into 4 different groups with 6 rats in each group: control, STD, and corilagin 20 and 40 mg/kg treated groups. Treatment was given between day 4 and day 10 of the protocol. Blood was drawn on days 6, 8, and 11 of the protocol and the concentration of IgE in the serum was estimated using anti-rat IgE antibody by ELISA method. The concentration of standard IgE used was 0.15–20 ng/ml.

#### *Estimation of anaphylaxis reaction*

Horse serum (0.5 ml) was subcutaneously injected to sensitize the rats and we separated them into 6 different groups with 6 rats in each group: control, negative control, STD, and corilagin 10, 20, and 40 mg/kg treated groups for the duration of 10 days. After the treatment, horse serum (0.25 ml) was injected intravenously and we observed symptoms such as cyanosis, dyspnea, enhanced respiratory rate, and respiratory distress of anaphylactic reactions. Anaphylactic reaction was scaled from 0 to 20 on the basis of its severity [16].

**Table 1.** Effect of corilagin on milk induced leukocytosis and eosinophilia.

Sr. No.	Group	Eosinophilis per cu. mm ( $\times 10^6$ c/ $\mu$ l) <sup>a</sup>	Differential Leukocytes ( $\times 10^6$ c/ $\mu$ l) <sup>b</sup>		Total Leukocytes per cu. mmc
			Monocytes	Neutrophils	
1	Control	62.83 $\pm$ 4.72	34.22 $\pm$ 2.16	3.35 $\pm$ 0.21	6310 $\pm$ 29.52
2	Negative control	151.26 $\pm$ 11.29 <sup>###</sup>	48.92 $\pm$ 1.94 <sup>###</sup>	10.48 $\pm$ 0.87 <sup>###</sup>	9880 $\pm$ 31.82 <sup>###</sup>
3	Corilagin 10 mg/kg	109.32 $\pm$ 8.64 <sup>**</sup>	42.71 $\pm$ 1.37 <sup>*</sup>	7.93 $\pm$ 0.63 <sup>**</sup>	9138 $\pm$ 18.74 <sup>*</sup>
4	Corilagin 20 mg/kg	91.78 $\pm$ 7.92 <sup>**</sup>	38.89 $\pm$ 1.53 <sup>**</sup>	6.11 $\pm$ 0.71 <sup>**</sup>	7640 $\pm$ 20.15 <sup>**</sup>
5	Corilagin 40 mg/kg	82.51 $\pm$ 3.84 <sup>**</sup>	35.72 $\pm$ 1.27 <sup>**</sup>	4.82 $\pm$ 0.26 <sup>**</sup>	6844 $\pm$ 12.62 <sup>**</sup>
6	STD	79.13 $\pm$ 6.32 <sup>**</sup>	36.69 $\pm$ 1.46 <sup>**</sup>	4.29 $\pm$ 0.32 <sup>**</sup>	6519 $\pm$ 14.79 <sup>**</sup>

Means  $\pm$ SD (n=6), <sup>###</sup> p<0.01 than control, <sup>\*</sup> p<0.05, <sup>\*\*</sup> p<0.01 than negative control.

### Assessment of paw edema

All the rats were separated into 6 different groups with 6 rats in each group: control, negative control, STD (ibuprofen 50 mg/kg, p.o.), and corilagin 10, 20, and 40 mg/kg. Histamine 1% w/v was injected into the planter region of the right paw and the drug was administered 30 min later. A plethysmometer was used to assess the paw volume at 2 and 3 h after the injection [17].

### Estimation of mast cell degranulation

Subcutaneous injection of compound 48/80 (1 mg/kg) was used to sensitized the rats and we separated them into 6 different groups with 6 rats in each group: control, negative control, STD (ketotifen fumarate, 1 mg/kg, i.p.), and corilagin 10, 20, and 40 mg/kg treated groups for 7 days. Phosphate buffer saline was injected intraperitoneally on day 7 of the protocol and we massaged the abdomen gently. Thereafter, all the rats were sacrificed and peritoneal fluid was collected in a tube containing phosphate buffer. Percoll density centrifugation method was used for the purification of mast cells. Mast cells were incubated for 10 min at 37°C with 5  $\mu$ g/ml concentration of compound 48/80 after resuspending it into phosphate buffer solution. Later, cells were separated out by centrifugation and stained with 0.1% Toluidine blue. A high-power microscope was used for the estimation of degranulated cells [18].

### Effect of corilagin on the contraction of guinea pig ileum

Guinea pigs were fasted overnight and the ileum was isolated after sacrifice. Tissue was mounted in Tyrode solution containing the organ bath maintained at 37°C. IC50 value was calculated by taking the concentration response curve of histamine on ileum in presence or absence of corilagin.

### Effect of corilagin on guinea pig tracheal chain

The trachea was isolated from the guinea pigs and we tied them together to prepare the chain from it. Krebs solution was used to mount the chain and CRC of Ach was carried out in the presence and absence of corilagin and atropine to calculate IC50 value [18].

### Statistical analysis

Values of the given data are represented as mean  $\pm$ SD (n=6) and the data were statistically analyzed by one-way ANOVA (Dunnett post hoc test). Level of significance was considered as p<0.05.

## Results

### Determination of the effect of corilagin on leukocytosis and eosinophilia

The effect of corilagin on milk-induced leukocytosis and eosinophilia is shown in Table 1. Differential leucocytes, WBCs, and eosinophil counts in the blood were significantly enhanced in the negative control group compared to the control group due to administration of milk. Treatment with corilagin significantly (p<0.01) decreased the count of differential leucocytes, WBCs, and eosinophils compared to the negative control group. We observed that corilagin attenuates the leukocytosis and eosinophilia in a dose-dependent manner.

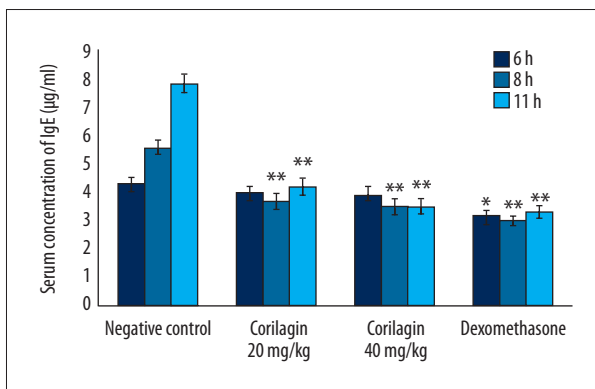
### Determination of effect of corilagin on anaphylactic reaction

Table 2 shows the effect of corilagin on passive cutaneous and active anaphylaxis and degranulation of mast cells. There was significant reduction in the anaphylactic reactions (passive cutaneous and active) in the corilagin-treated group compared

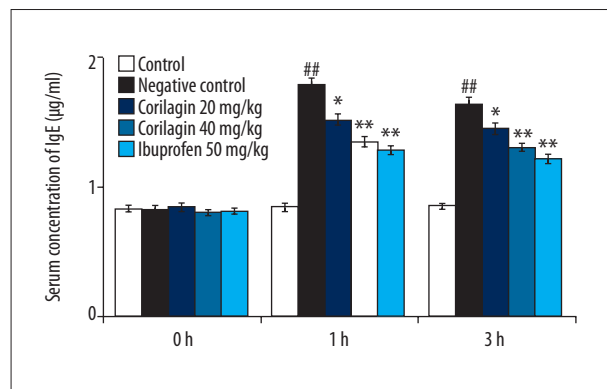
**Table 2.** Effect of corilagin on passive cutaneous anaphylaxis, active anaphylaxis and degranulation of mast cell.

Sr. No.	Group	Passive cutaneous anaphylaxis		Active anaphylaxis	Mast cell degranulation	
		Blueing reaction mm <sup>3</sup>	Behavioural symptoms (% of mortality)	Respiratory score	Intact	Degranulated
1	Control	00	Normal	00 (00)	100	00
2	Negative control	78.39±3.15	Respiratory distress/collapse 3–5 min (70%)	8.80±5.2 (65%)	22.62±2.94##	79.32±4.72##
3	Corilagin 10 mg/kg	69.17±3.09	Dyspnoea 5–8 min (40%)	6.25±3.15 (40%)	31.72±2.45*	67.37±4.31**
4	Corilagin 20 mg/kg	54.92±2.90	Dyspnoea 15–20 min (25%)	4.10±1.28 (25%)	43.89±3.56**	56.18±3.78**
5	Corilagin 40 mg/kg	47.36±1.97	Normal	2.74±1.45 (15%)	55.73±4.71**	44.27±3.26**
6	STD Dexomethasone	43.18±2.69	Normal	2.36±0.75 (15%)	–	–
	Ketotifen	–	–	–	62.39±4.26**	38.29±2.46**

Means ±SD (n=6), ## p<0.01 than control, \* p<0.05, \*\* p<0.01 than negative control.



**Figure 1.** Effect of corilagin on the serum concentration of IgE in sensitized rats. Means ±SD (n=6), \* p<0.05, \*\* p<0.01 compared to negative control.



**Figure 2.** Effect of corilagin on histamine-induced paw edema. Means ±SD (n=6), ## p<0.01 compared to control, \* p<0.05, \*\* p<0.01 compared to negative control.

**Table 3.** Effect of corilagin on IC<sub>50</sub> value of Ach and histamine in guinea pig tracheal chain and ileum.

Sr. No.	Antagonists	IC <sub>50</sub> values	
		Response of Histamine in presence of antagonist	Response of Ach in presence of antagonist
1	Nil	16.5 (14.15–17.46) µg/ml	162 (142–176) µg/ml
2	Chlorpheniramine maleate	2.75 (2.21–2.89) µg/ml	
3	Atropine maleate		2.79 (2.55–3.10) µg/ml
4	Corilagin	415 (382–429) µg/ml	261 (239–290) µg/ml

to the negative control group. We observed that corilagin attenuated the anaphylactic reactions such as cyanosis, dyspnea, respiratory rate, and score in a dose-dependent manner. Moreover, the corilagin-treated group showed a significant reduction in the compound 48/80-induced degranulation of mast cells compared to the negative control group.

#### Determination of effect of corilagin on the serum concentration of IgE

Figure 1 shows the effect of corilagin on the serum concentration of IgE in sensitized rats. The serum concentration of IgE was significantly decreased ( $p < 0.01$ ) in the corilagin-treated group compared to the negative control group at 8 and 11 h after treatment.

#### Determination of effect of corilagin on $IC_{50}$ value of Ach and histamine

Table 3 shows the effect of corilagin on Ach- and histamine-induced contraction of guinea pig tracheal chain and ileum, respectively. Treatment with corilagin significantly inhibited the Ach- and histamine-induced contraction of guinea pig tracheal chain and ileum, respectively. This inhibition of contraction was found to be in a dose-dependent manner. The  $IC_{50}$  value of corilagin for histamine was 415 (382–429)  $\mu\text{g/ml}$  and Ach was 261 (239–290)  $\mu\text{g/ml}$ .

#### Determination of effect of corilagin on paw edema

The effect of corilagin on the histamine-induced paw edema in rats is shown in Figure 2. There was significantly increased paw edema volume in the negative control group of rats compared to the control group at 1 and 3 h after drug administration. However, treatment with corilagin significantly ( $p < 0.05$ ,  $p < 0.01$ ) decreased the paw edema compared to the negative control group, in a dose-dependent manner.

## Discussion

The present study investigated the effect of corilagin against anaphylactic and allergic reaction. Eosinophilia and leukocytosis were induced by injecting milk into mice and anaphylactic reaction was induced by sensitizing rats with albumin.

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Moreover, degranulation of mast cells was induced by 48/80 compound in rats. We estimated the concentration of IgE in the serum and  $IC_{50}$  value of corilagin was estimated against histamine- and Ach-induced contraction of tracheal chain and ileum, respectively.

The literature reveals that degranulation of eosinophils results into immunological reactions, and in various allergic conditions, the eosinophil count and leukocytosis significantly increases in allergy/asthma [19]. Allergic reaction involves the release of several inflammatory mediators such as cytokines, D4, C4, leukotrienes, and histamine, and the result of this study suggest that treatment with corilagin significantly reduces the count of leucocytes and eosinophils compared to the negative control group [20–22].

Reports reveal that immunoglobulin (IgE) is mainly responsible for the anaphylactic reaction and to the degranulation of mast cells [23]. Our results suggest that corilagin significantly attenuates the anaphylactic reaction and degranulation of mast cells. Moreover, it also decreases the concentration of IgE in the blood. Several studies suggest that lipid layer permeability is significantly enhanced by compound 40/80 and results in the release of mediators [24]. This compound enhances the level of cAMP in the cells that stimulates Ca influx and thereby activates the mast cells for degranulation. Corilagin attenuates the anaphylaxis and allergic reaction, probably by activating G-protein, membrane stabilization, and releasing the intracellular  $Ca^{+}$ .

Treatment with corilagin significantly decreased the Ach- and histamine-induced contraction in guinea pig tracheal chain and ileum, suggesting the antagonistic activity of corilagin on histamine and muscarinic receptors.

## Conclusions

Our study shows that corilagin has anti-anaphylactic and anti-allergic activity by inhibiting the release of mediators from mast cells and by decreasing the IgE serum concentration.

#### Conflicts of interest

None.

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