



Antianaphylactic Activity of Alcoholic Extract of *Eclipta alba*

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ABSTRACT

The antianaphylactic activity of alcoholic extract of *Eclipta alba* with two different doses of 250 and 500 mg/kg was studied by using different animal models such as effect on mast cell degranulation using rat mesentery, passive cutaneous anaphylaxis using rat, by measuring leakage of Evans blue dye in skin, passive paw anaphylaxis using rat by measuring the paw volume by plethysmometer. Bronchoalveolar lavage (BaL) fluid study in guinea pig trachea, measurement of different blood cells, and level of histamine in lung tissues was performed. Treatment with alcoholic extract of *Eclipta alba* showed a dose dependent beneficial effect on degranulation of mast cells in rats when challenged with Compound 48/80. Further, a dose-dependent beneficial effect was also observed on leakage of Evans blue dye in skin challenged with antigen. *Eclipta alba* showed beneficial effect on paw anaphylaxis induced by antiserum and also on infiltration of various inflammatory cells as well as on histamine release from lungs. Antianaphylactic activity of alcoholic extract of *Eclipta alba* may be possibly due to its membrane stabilizing potential, inhibition of antigen induced histamine release, and inhibition of release of various inflammatory mediators.

Key words: Allergy, compound 48/80, ovalbumin

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INTRODUCTION

Allergy is an interaction between the immune system and substance foreign to the body. Allergy producing substances are called as “allergens”. A disorder caused by an allergic reaction or allergy is called allergic disorders. Allergic disorders are in rise every year and are stated as an endemic disease of the 21st century. Some of the allergic disorders, which may be caused by an allergen originating from immune system, environment, and by genes^[1] are, asthma, eczema, hay fever, anaphylaxis, autoimmune disorders, etc. Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances. A number of plants are described in Ayurveda for use in the treatment of allergic disorders, namely psoriasis, eczema,

bronchial asthma, etc. Only a few have been studied for their antiallergic activity, which was not studied earlier. On activation, mast cells released immediately the preformed and the *de novo* synthesized mediators such as histamine, proteases, leukotrienes, prostaglandins, and cytokines.^[2] As a consequence, the acute reactions such as vasodilation, increased vascular permeability, and bronchoconstriction were induced. In addition, allergic responses also trigger the influx and activation of a variety of inflammatory cells including eosinophils and lymphocytes. Rapidly released mediators and numerous cytokines produced by mast cells are strongly believed to induce and sustain these responses, which may contribute to chronic inflammation.^[3] The plant *Eclipta alba* (Linn.) Hassk [Synonym—*Eclipta prostrata* (Linn.); (family—Asteraceae)] has been mentioned in ancient texts to be a nervine tonic,^[4,5] in addition to

possessing hepatoprotective, hair growth promoting and antiaging properties. The plant is reported to contain the phytoconstituents eclalbatin, alpha-amyrin, ursolic acid, oleanolic acid^[6] ecliptasaponin, daucosterol, stigmasterol-3-*O*-glucoside,^[7] and coumestans like wedelolactone and demethylwedelolactone as main active principles.^[8] Wedelolactone and demethylwedelolactone have been reported for their 5-lipoxygenase enzyme inhibitory activity, a major enzyme responsible for synthesis of inflammatory mediators such as PGs.^[9] The plant has been extensively studied for its hepatoprotective activity and a number of herbal preparations comprising *Eclipta alba* are available for treatment of jaundice and viral hepatitis.^[10-13] The aqueous and alcoholic extracts of the plant are proved to confer protection against the myotoxic effects of snake venom.^[14] Additionally, it is also reported to possess antinociceptive, antiinflammatory activities. However, no investigative reports exist pertaining to its antianaphylactic activity; hence, present investigation was carried out to study the antianaphylactic activity of the plant.

MATERIALS AND METHODS

Animals

Male Wistar rats (200–250g) were obtained from the Central Animals House Facility of the institute. All the experiments were performed before obtaining prior approval from IAEC. The animals were housed in suitable environmental conditions with suitable nutrition and filtered tap water *ad libitum*.

Preparation of plant extract

The plant material was collected from LVG (Herbal Drug Supplier), Ahmedabad, Gujarat, India. The powder was passed through 40 # sieve. The extract was prepared using Soxhlet extractor using 50% ethanol as a solvent. The extract was concentrated under controlled temperature below 50°C in porcelain dish to get the syrupy mass.

Acute toxicity study

Acute Toxicity Study was carried out as per OECD guidelines.

Preliminary phytochemical screening

Standard phytochemical tests were used in screening the extract for different constituents. Briefly, FeCl₃ test was used to characterize for tannins, Dragendroff's reaction and Mayer's test was used for alkaloids, and Fehling's test

was used for reducing sugars. Similarly, Shinoda, HCL-Magnesium test was used for flavonoids while frothing test was deployed for saponins, for coumarin extract made alkaline test was used, baljet and borntreger's test for presence of glycosides.

Study on rat mesenteric mast cell degranulation by compound 48/80^[15,16]

The animal was sacrificed by ethically acceptable method and the pieces of mesentery were collected in petridish containing Ringer Locke solution and then subjected to the following treatment schedules:

- Petri Dish No: 1 Ringer Lock solution (vehicle control)
- Petri Dish No: 2 Ringer Lock solution (positive control)
- Petri Dish No: 3 Dexamethasone (10 µg/ml)
- Petri Dish No: 4 Alcoholic extract of *Eclipta alba* (100 µg/ml)
- Petri Dish No: 5 Alcoholic extract of *Eclipta alba* (250 µg/ml)
- Petri Dish No: 6 Alcoholic extract of *Eclipta alba* (500 µg/ml)

Each petri dishes were incubated for 10 min at 37°C and then to each petri dish 0.1 ml of compound 48/80, a mast cell degranulator used to induce mast cell degranulation, having concentration of 10 µg/ml was added and again incubated for 10 min at 37°C. Thereafter, all pieces were transferred to 4% formaldehyde solution containing 0.1% toluidine blue and kept aside for 20 to 25 mins. After staining and fixation, mesentery pieces were transferred through acetone and xylene two times and mounted on slides. All the pieces were examined under light microscope with 450x magnification. Minimum of 100 cells were counted and percentage of intact and disrupted mast cells were determined. Disrupted mast cells were stained with toluidine blue and undisrupted mast cells remain as such almost round shaped. Percentage protection from degranulation of mast cells by the drug was determined.

Study on passive cutaneous anaphylaxis model^[17]

Preparation of antiserum from rats

The Wistar rats of either sex were injected intraperitoneally with 0.2 ml, 10% egg albumin, and 0.2 ml of Bordetella pertussis vaccine on day 1, 3, and 5. After 21 days of first immunization, blood was collected from orbital plexus under light ether anesthesia. The collected blood was allowed to clot and serum was separated by centrifugation at 1500 rpm. The separated serum was stored at -20°C until it was used for the experiment.

Then animals were divided into the following groups:

1. Model Control
2. Standard (5 mg/kg)
3. AEEA (250 mg/kg)
4. AEEA (500 mg/kg)

The antiovalbumin serum was injected intradermally on the clipped dorsal skin of the animal. Drug/extracts were administered to animal according to their group for three consecutive days from the day of sensitization. After treatment, inject 1 ml of 0.5% Evans blue solution containing 20 mg of egg albumin was injected intravenously through tail vein. Because of antigen-antibody reaction there was increased vascular permeability and dye will penetrate in that tissue area. This area of skin was removed after sacrificed. The skin portion was transferred to the solution of 70% acetone for 24 hrs. The dye was extract out in the acetone and Evans blue dye was measured calorimetrically at 620 nm. The amount of dye penetrate in the skin area reflect the severity of hypersensitivity reaction.

Study on passive paw anaphylaxis model

Preparation of antiserum from rats

The Wistar rats of either sex were injected intraperitoneally with 0.2 ml, 10% egg albumin and 0.2 ml of Bordetella pertussis vaccine on day 1, 3, and 5. After 21 days of the first immunization, blood was collected from orbital plexus under light ether anesthesia. The collected blood was allowed to clot and serum was separated by centrifugation at 1500 rpm. The separated serum was stored at -20°C until it was used for the experiment.

Passive paw anaphylaxis

Wistar rats of either sex weighing 200–275 g were selected and randomly divided into four groups each containing six animals. The drugs were administered orally in distilled water. The animals were dosed once daily for seven days. The following schedule of treatment was administered:

Group I	Control
Group II	Standard
Group III	AEEA (250 mg/kg)
Group IV	AEEA (500 mg/kg)

Two hours after the last dose of drug administration (on seventh day), rats were passively sensitized into the left hind paw with 0.1 ml of the undiluted serum. The contralateral paw received an equal volume of saline. 24 hours after sensitization, the rats were challenged in the left hind paw with 10 mg of Egg albumin in 0.1 ml saline. The hind paw volume was measured after 30 minutes

by volume displacement method using mercury column plethysmometer.

Effect on Histamine release^[18]

Guinea pigs of either sex weighing 350–500g were selected and randomly divided into five groups each containing six animals. The drugs were administered orally in distilled water. The following schedule of treatment was administered:

Group I	Distilled water (Control)
Group II	Egg Albumin (Sensitized)
Group III	Standard
Group III	Egg Albumin + AEEA (250 mg/kg)
Group IV	Egg Albumin + AEEA (500 mg/kg)

The guinea pigs of all the groups except group I were sensitized with egg albumin (1 ml, 10% w/v, i.p.). The animals of group III, IV, and V were dosed once daily for 15 days with Standard, AEEA (250 mg/kg), and AEEA (500 mg/kg). Two hours after the last dose of drug administration (on 15th day), all the animals except group I animals were challenged with egg albumin (0.5 ml, 2% w/v) through saphenous vein. After three hours of the challenge of the egg albumin or just prior to death of animals, the lungs of the guinea pigs were chopped into fragments. The chopped lung tissues were placed in tubes with 2 ml of ice-cold Ca²⁺ free Tyrode's solution and kept on ice till further use. 200 mg (wet weight) of lung tissues were taken in test tube. The test tubes were then supplemented with 1.8 mM CaCl₂ and incubated for 10 mins at 37°C. After that the lung tissues were incubated with 2 mg/ml egg albumin for 15 mins at 37°C. After 15 mins, the reaction was stopped by filtration of the medium through nylon mesh. Histamine in the medium was determined fluorometrically.

Histamine standard curve

Known amount of standard histamine hydrochloride were added to prepare standard solutions of 20, 40, 60, 80 and 100 ng/ml concentrations. 2ml of pure triple distilled water was then added to each solution. To each test tube 0.4 ml of 1.0 N NaOH was added followed by 0.1 ml of o-phthalaldehyde reagent (1% in absolute methanol, AR grade). After four minutes, 0.2 ml of 2.0 M citric acid was added. The contents of tubes were mixed thoroughly after each addition. The fluorescence was then measured at emission wavelength of 440 nm resulting from excitation at 350 nm.

Estimation of histamine in the test medium

From the filtered test medium, 0.2 ml of test solution was taken to which 1.8 ml of absolute methanol was added. Then histamine was assayed by repeating the same

procedure as described for the standard solution at 440 nm emission and 350 nm excitation wavelength.

Effect on bronchoalveolar lavage (bal) fluid in guinea pigs^[19]

Guinea pigs were selected and divided into following groups:

Group I	Distilled water (Control)
Group II	Egg Albumin (Sensitized)
Group III	Standard
Group III	Egg Albumin + AEEA (250 mg/kg)
Group IV	Egg Albumin + AEEA (500 mg/kg)

The guinea pigs of all the groups except group I were sensitized with egg albumin (1 ml, 10% w/v, i.p.). The animals of group III, IV and group V were dosed once daily for fifteen days with Standard drug, AEEA (250 mg/kg, p.o.) and AEEA (500 mg/kg, p.o.).

Two hours after the last dose of drug administration (on fifteenth day), all the animals except group I animals were challenged with egg albumin (0.5 ml, 2% w/v) through saphenous vein. After three hours of the challenge of the egg albumin or just prior to death of animals, which ever was earlier, the tracheobronchial tree was lavaged with 10 ml of saline and the fluid so collected was centrifuged at 2000 rpm for 5 min. and the pellet was resuspended in 0.5 ml saline. 0.2 ml of geimsa stain in buffered saline (pH 6.8) was added to it. After five minutes, the number of each type of leukocyte in 0.5 ml fluid was determined under the microscope 450x magnifications. The results obtained were compared with unsensitized and untreated egg albumin sensitized guinea pigs.

Statistical analysis

All the values were expressed as mean \pm SEM of six observations. The statistical analysis was performed using student's unpaired t-test. Value of p less than 5% ($p < 0.05$) was considered statistically significant.

RESULTS

Acute toxicity study

Alcoholic extract of *Ediпта alba* was found to be safe upto 5000 mg/kg p.o. given to mice. No any sign for behavioural as well as any physical changes were found.

Preliminary phytochemical screening

The alcoholic extract of *Ediпта alba* was found to be rich in glycoside, saponin, flavonoid, tannin, alkaloid and coumarins.

Effect of PF on rat mesentery mast cell

C48/80, a known mast cell defragmentation or degranulation agent produced significant increase in degranulation of rat mesentery mast cell when compared to the mesentery exposed to Ringer Locke solution alone. AEEA produced a significant dose dependent increase in the number of intact cells. Standard drug also produces significant reduction in degranulated mast cell compared to control [Figure 1].

Study on passive cutaneous anaphylaxis model

AEEA produced a significant dose dependent decrease in the amount of Evans blue dye leaked at site. Standard drug also produces significant decrease in the amount of Evans blue dye leaked at site [Figure 2].

Study on passive paw anaphylaxis model

AEEA produced a significant dose dependent decrease in the paw volume induced by antiserum. Standard drug also produces significant decrease in the paw volume induced by antiserum. [Figure 3].

Effect on histamine release

AEEA produced a significant dose dependent decrease in the histamine level in lung tissue of G.pigs. Standard drug also produces significant decrease in the histamine level in lung tissue of G.pigs. [Figure 4].

Effect on bronchoalveolar lavage (Bal) fluid in guinea pigs

AEEA produced a significant dose dependent decrease in the number of Eosinophil in Bronchoalveolar Lavage (Bal) Fluid In Guinea Pigs. Standard drug also produces significant decrease in the number of Eosinophil Bronchoalveolar Lavage (Bal) Fluid In Guinea Pigs [Figure 5].

DISCUSSION

The present study was undertaken for the evaluation of anti anaphylactic activity of alcoholic extract of *Ediпта alba*. It is also reported to possess anti inflammatory, analgesic, hepatoprotective and anti ageing property. Mast cell disruption is mediated by activation of IgE antibodies. Sodium cromoglycate and Nedocromil are important agents for the prevention of mast cell defragmentation and stabilization of mast cell membrane is the major mechanism responsible for their effectiveness. They act by reducing the synthesis and release of several proinflammatory cytokines. Anaphylactic allergic reaction

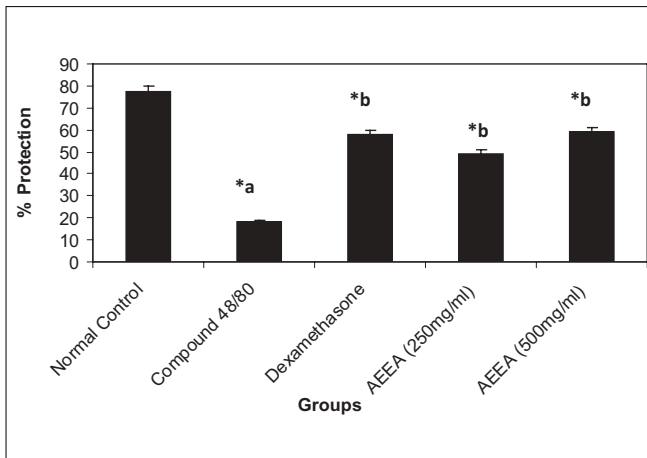


Figure 1: Effect of Alcoholic extract of *Eclipta alba* on rat mesenteric mast cell degranulation. * $P < 0.05$ when compared with control, $n = 6$ in each group. a - Model group compared with normal control group. b - Model group compared with treatment group

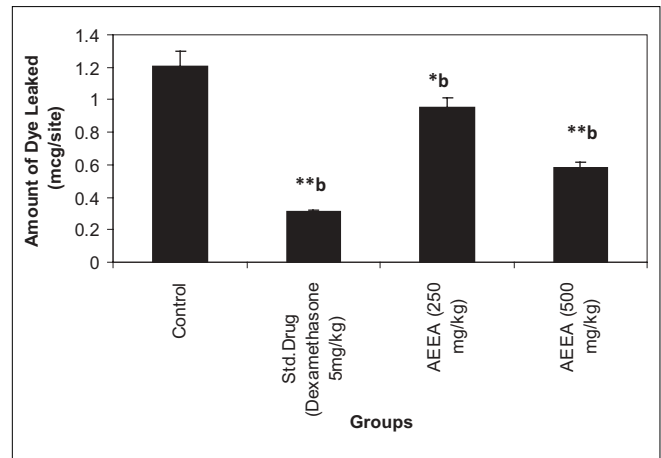


Figure 2: Effect of Alcoholic extract of *Eclipta alba* on Passive Cutaneous Anaphylaxis. * $P < 0.05$ when compared with control, $n = 6$ in each group. ** $P < 0.001$ when compared with control, $n = 6$ in each group, b - Model group compared with treatment group.

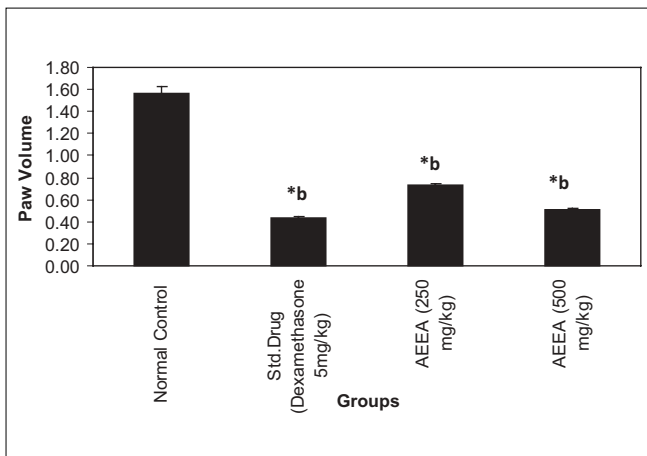


Figure 3: Effect of Alcoholic extract of *Eclipta alba* on Passive Paw Anaphylaxis. * $P < 0.001$ when compared with control, $n = 6$ in each group. b - Model group compared with treatment group.

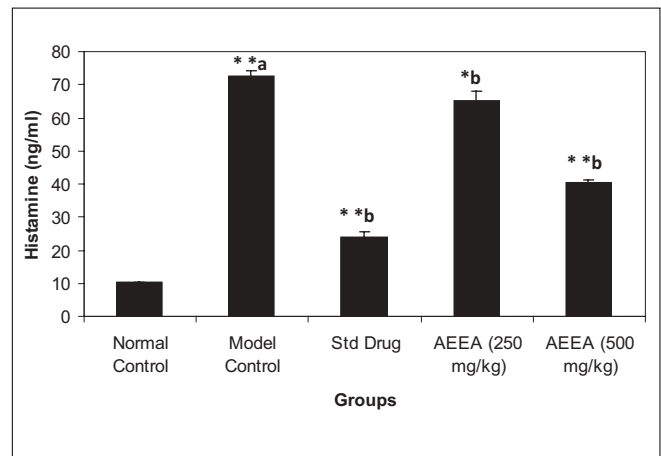


Figure 4: Effect of Alcoholic extract of *Eclipta alba* on Histamine Level In G.pig Lungs. * $P < 0.05$ when compared with control, $n = 6$ in each group. ** $P < 0.001$ when compared with control, $n = 6$ in each group, a - Model group compared with Normal group, b - Model group compared with treatment group

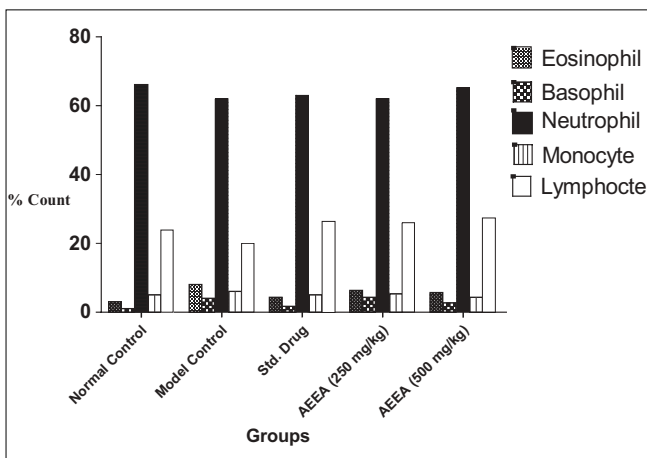


Figure 5: Effect of Alcoholic extract of *Eclipta alba* on Bronchoalveolar Lavage Fluid Study. * $P < 0.05$ when compared with control, $n = 6$ in each group, a - Model group compared with the normal group, b - Model group compared with the treatment group

is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators such as histamine and pro-inflammatory cytokines and can be elicited by various stimulators including compound 48/80 and anti-IgE.^[20] Results of the present investigation showed that AEEA possessed significant anti-anaphylactic activity in preventing compound 48/80-induced of histamine release from compound 48/80 activated mast cells. Tasaka *et al.*^[21] reported that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This result indicates that the membrane permeability increase may be an essential trigger for the release of the mediator from the mast cells. AEEA might act on the lipid bilayer membrane preventing

the perturbation induced by compound 48/80. On the other hand, compound 48/80 induces signal transduction pathways, leading to exocytosis and degranulation of mast cells.^[22]

PCA is an anti-IgE antibody induced *in vivo* model of anaphylaxis in local allergic reactions in which mast cells play an important role. The secretion process of mediators in PCA differs from that in compound 48/80-induced anaphylaxis: it results from aggregation of specific IgE receptors on the surface of mast cells by the corresponding antigen.^[23] There are two types of PCA models: Homologous PCA and Heterologous PCA. The present study was done using Homologous PCA model i.e. from rat to rat for evaluation of alcoholic extract of *Eclipta alba* on immediate hypersensitivity reaction. Mediators like leukotriene, prostaglandins, PAF and cytokines are reported to be responsible for the immediate hypersensitivity reaction, but it is observed that enhanced vascular permeability and leukocyte infiltration at the sites of allergen challenge. In our model antiovalbumin serum obtained from sensitized rats was injected to the rats. The enhanced vascular permeability was estimated by Evans blue dye. The leakage of dye was significantly less in the rats treated with alcoholic extract of *Eclipta alba* than the control animals. This activity can partly be due to inhibition of leukotriene synthesis. Passive paw anaphylaxis is another *in vivo* model for IgE-mediated immediate hypersensitivity reactions.^[24] In both these models a prominent inhibitory effect of AEEA is suggestive of its antianaphylactic activity. The presence of eosinophil in the airways of asthma patient was recognized over years ago in histological preparation of lung tissue. Exposures of actively sensitized guinea pigs to allergens results in selective pulmonary eosinophilia, a response that has been associated with hyperreactivity. The evidence of eosinophil involved in inflammatory reaction within the asthmatic lung has been provided, by demonstration of an increased bronchial eosinophilia in patients of asthma. Eosinophils cause epithelial damage by releasing major basic protein. In our study we have used sensitized guinea pigs to demonstrate the effect of alcoholic extract of *Eclipta alba* leaves on eosinophil accumulation following antigen challenge. As compared to unsensitized animals, significant increase in eosinophil was observed in sensitized animals. Alcoholic extract of *Eclipta alba* had produced significant reduction in the eosinophil at the concentration 500mg/kg, but not in concentration at 250mg/kg dose. The other types of WBC were not significantly affected by the extract. So the result of study shows that the alcoholic extract of *Eclipta alba* is found to be effective in various experimental models of

anaphylaxis. It also shows significant reduction in paw volume induced by various inflammatory mediators in a dose dependent manner. Histamine is an important inflammatory mediator for various allergic reactions. In present investigation alcoholic extract of *Eclipta alba* shows significant decrease in histamine level as compared to control group. The activity like stabilization of mast cell degranulation and inhibition of release of various inflammatory mediators involved in the anaphylaxis, the reduction of dye infiltration in inflamed areas show reduction in inflammation and lastly the reduction in eosinophil infiltration in lung in egg albumin sensitized guinea pigs shows antianaphylactic activity by the treatment of alcoholic extract of *Eclipta alba*.

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