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ORIGINAL ARTICLE **OPEN ACCESS**

Anti-Asthmatic Activity of Leaves of *Calotropis gigantea*

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ABSTRACT
Calotropis gigantea Linn, a widely grown plant in the Asclepiadaceae family, is said to have several therapeutic qualities. It is said to have a long history of being used as a folk remedy for many ailments. Research has been conducted on the anti-diabetic characteristics of calotropis leaves. Extracts of C. gigantea leaves were prepared using petroleum ether, chloroform, ethanol, and water, and then evaluated for their anti-asthmatic properties. The current study assesses the impact of Calotropis gigantea leaf extract using in vivo models. Chlorpheniramine (CPM) and Dexamethasone were utilized as the standard medications. The study shows that the leaves of Calotropis gigantea has anti-asthmatic effects. The study revealed that the pet ether extract of Calotropis gigantea is beneficial for those with asthma.
Keywords: Calotropis gigantea, anti-asthmatic action.

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INTRODUCTION

The term "asthma" in modern English is derived from the Greek word meaning "breathless" [1]. Asthma is a chronic inflammatory condition that can be triggered by antigen sensitization or a hereditary tendency towards airway inflammation. Asthma is defined by increased sensitivity of the bronchial tubes, increased mucus production, and structural changes in the airways leading to congestion. The symptoms are a result of the immune system's invasion of the lungs, leading to inflammation [2]. Neurons, T lymphocytes, mast cells, eosinophils, and neutrophils' dendrites play a crucial role in the inflammatory process that leads to asthma. Mortality data from wealthy nations indicate that asthma prevalence in school-aged children has risen by 75%, with an estimated 15–20 million asthmatics in India alone. The rate fluctuates between 0.1 and 0.8 per 10,000 individuals aged 5-34. The main focus in managing an asthma episode is providing respite from symptoms. India has several traditional medical systems including Ayurveda, Unani, and Siddha that recommend multiple medicines for treating asthma. Calotropis is a genus of laticiferous shrubs native to Asia and Africa. There are two species of this plant: Calotropis gigantea R. with purple or red flowers, also known as "arka," and Calotropis pricera R. with white flowers, also known as "alarka." Located in the Punjab region of India, Ceylon, Singapore, the Malay Peninsula, and southern China, as well as locations south of the Himalayas. The Asclepiadaceae family, which encompasses the milky shrub Calotropis gigantea (crown flower), known as madar in Hindi, is prevalent in India. It is considered a vital herb for treating asthma in both Ayurvedic and Unani medicine. Ayurvedic medicine use the whole dried plant as a tonic, expectorant, depurative, and antihelminthic. The leaves have medicinal properties that can be utilized for treating conditions such as intermittent fever, arthralgia, paralysis, and edema. The leaves possess bitter, astringent, stomachic, anthelmintic, and tonic properties, among other medicinal virtues. Calotropis gigantea contains proteases, 3-methyl butanoates of amyrin, flavonol glycosides, calotropis stigmasterol, sitosterol, cardenolides, pregnanone, and other compounds. Calotropis gigantea, a plant belonging to the Asclepiadaceae family, is distributed across India up to an elevation of 900 meters, including the Andaman Islands. Another common location is known as mudar. The young leaves are rectangular and wide, with a leathery base and a pointed or sometimes rounded tip. Their color is a glaucous green and they range in size from 6 to 20 centimeters in length and 3 to 8 centimeters in breadth. The length ranges from 0.5 to 2 cm [7,8]. The fruits are solitary or in pairs, measuring between 7 and 10 centimeters in length. They are also swollen and regained. The seeds are broadly ovate, brown, flattened, and measure from 2.5 to 3.2 centimeters in length. They have a white tuft of silky hair at the top and are

coated in brown pigment. The axillary nodes have pedunculate corymbs with lilac, pale pink, or purple flowers, sometimes featuring light greenish-yellow o r white blooms [9-13]. The odorless flowers feature corolla lobes that are reflexed and spreading. Flowers are seen year-round, with the peak blooming period in central India occurring from November to March.

MATERIAL AND METHODS

Plant material:

The leaves of *Calotropis gigantea* were taken from the dry parts of Nashik districts in Maharashtra, India. They were then authenticated at the Department of Botany, HPT (Arts) & RYK (Science) College Road, Nashik, for taxonomic identification. The leaves from the plant were taken off, dried, and ground into a coarse powder.

PREPARATION OF PLANT EXTRACT:

The gathered *Calotropis gigantea* leaves were procured and brought to the laboratory and washed thoroughly with running water to remove dirt and other extraneous matter. They were then rinsed with distilled water and shed dried prior to being ground into powder to yield between 500g to 1 kg of powder for each sample. Leaves were reduced to small pieces and dried in the shade. The dried leaves were further reduced to a powdered state through grinding. By passing powdered plant components through a screen (sieve), fine powder was separated from coarse powder, and coarse powder was stored in airtight, well-labeled containers until use. Approximately 1 kilogramme of powdered material was extracted with petroleum ether (60-800), chloroform, ethanol & distilled water.

EXPERIMENTAL ANIMALS:

Albino mice (25–30 g), Wistar rats (150–170 g) & guinea pigs (350–400 g) were preserved in polypropylene cages at a temp. of 22±20 °C & under a 12-hr day/night cycle. The animals received a healthy diet & access to fresh water throughout the experiment, the animals received a healthy diet and access to fresh water. The Sir Dr. M. S. Gosavi College of Pharmaceutical Education & Research, Gokhale Education Society, Nashik, Maharashtra, institutional animal ethical committee accepted the study's methods.

ACUTE TOXICITY STUDY

According to OECD guideline 425, the acute toxicity of test extracts was assessed employing the up-and-down method & a body weight limit of 2,000 mg/kg.

ANTIASTHMATIC ACTIVITY

Clonidine-induced catalepsy in mice

The bar test was empoyed to examine the anti-histaminic properties of *Calotropis gigantea* R. Br. (Linn) leaf extracts on clonidine-induced cataplexy. In this investigation, five sets of six mice each were employed. The control group got intraperitoneal injections of 1% v/v tween-80 solution (5 ml/kg body weight) while the test groups got test extracts at 100 and 150 mg/kg body weight. CPM (10 mg/kg body weight) was intraperitoneally injected into the placebo group. All groups received subcutaneous clonidine (1 mg/kg) 30 minutes after the last dosage. The time required for mice to eleminated their forepaws from a horizontal bar with a diameter and height of 1 cm was timed. After giving a dosage of clonidine, catalepsy was timed at 30, 60, 90, 120, 150 & 180 minutes [14, 15].

Effect of Chlorpheniramine Maleate on Haloperidol-Induced Cataplesy

The same bar test with haloperidol was conducted on the leaves of CG. In total, there were six mice, which were divided into two groups. For the first and second groups, intraperitoneal administration of 1% tween-80 (5 ml/kg body weight) & CPM (10 mg/kg body weight) were utilized, respectively. Haloperidol was administered intraperitoneally in all groups 30 minutes after treatment at a rate of one milligram per kilogram of body weight. We measured the removal of mice's forepaws from a horizontal bar with a diameter of 1 cm & a height of 3 cm. After 30, 60, 90, 120, 150, & 180 minutes of treatment with haloperidol, the duration of catalepsy was recorded [14].

Effect of Test Extracts on Milk Induced Leukocytosis & Eosinophilia

Twenty groups of six mice each were used to investigate the effectiveness of *Calotropis gigantea* leaves. The active groups received 100 & 150 mg of extracts intraperitoneally per kilogram of body weight, whereas the placebo group received 5 ml of a 1% v/v tween-80 solution. The "positive control" group received 50 mg/kg of dexamethasone intravenously. 30 minutes after treatment, 4 ml/kg of cold and boiling milk was subcutaneously given to all groups. Blood was drawn from the retro-orbital space. Both before & after the milk injection, measurements of the eosinophil count and total leukocyte count were made. Counts of total leukocytes and eosinophils were compared [16, 17].

RESULT AND DISCUSSION

The goal of this investigation was to examine the anti-asthmatic activity of various *Calotropis gigantea* leaf extracts.

Clonidine Induced Catalepsy

Table 1: Effect of PECGL, CCGL, EECGL & AECGL on clonidine induced catalepsy

Group	Dosage mg/kg (i.p.)	Duration of catalepsy (sec) Mean ± SEM at					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	94.33±4.80 **	97.50±3.81**	103.33±12.38**	93.00±5.19**	98.80± 7.29**	101.4± 6.57**
PECGL	100	56.50±3.22**	62.75±3.01**	66.32±5.03**	61.98±4.23**	63.30±4.76**	64.83±5.54**
	150	58.20±3.02 **	64.22± 4.02**	67.34± 6.21**	62.15±3.54**	65.34± 5.23**	66.63±6.02**
CCGL	100	92.5 ± 5.40	95.9 ± 2.01	98.6 ± 20.50	90.0 ± 5.09	93.7 ± 4.37	99.8 ± 2.20
	150	93.43±5.03	96.35±1.02	102.65±1.03	92.76±2.78	95.46±3.42	100.45±4.68
EECGL	100	89.8 ± 1.80	92.8 ± 2.49	98.27±10.02	88.7 ± 2.79	96.8 ± 3.40	97.9 ± 2.67
	150	91.32±2.82	96.46±3.67	100.52±7.20	91.54±2.42	97.56±4.37	99.37±6.31
AECGL	100	91.1 ± 3.09	96.3 ± 1.80	101.9 ± 5.34	90.7 ± 4.86	93.8 ± 4.54	96.7 ± 2.47
	150	92.69±3.62	97.57±0.25	55.38±2.04	92.56±3.27	95.85±7.80	100.32±4.01
CPM	10	50.01 ± 3.15**	57.91 ± 2.85**	60.14 ± 4.47**	54.9 ± 2.38**	59.5 ± 3.79**	58.2 ± 1.89**

n = six in each group. **P<0.05 significant compared with control group

This extract significantly inhibited clonidine-induced cataplesy compared to control group [Table 1].

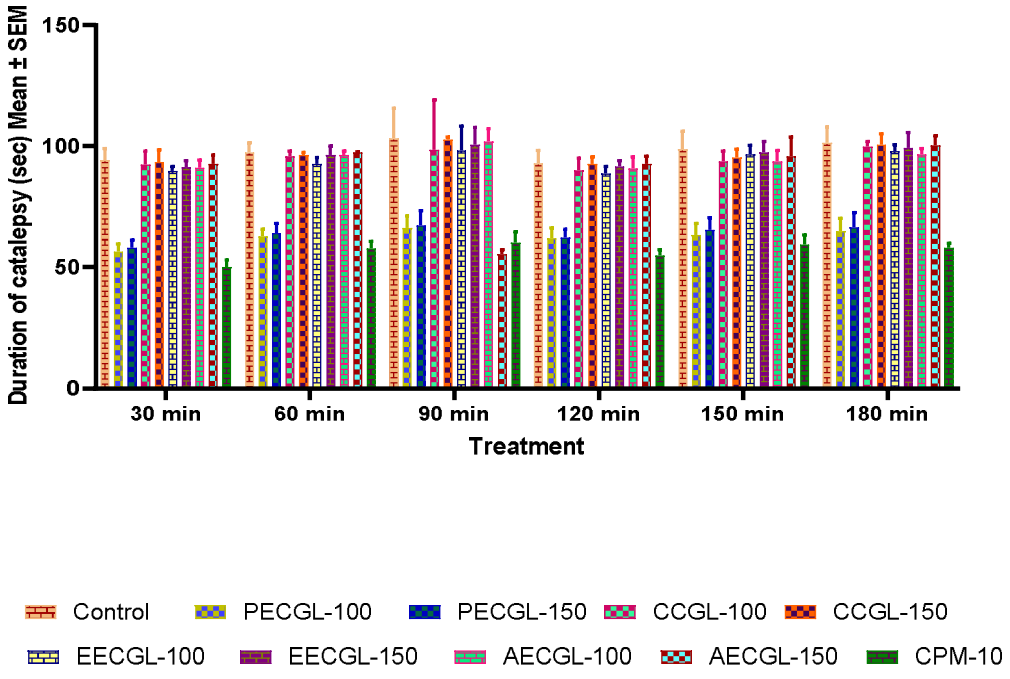

Fig. 1: Effect of PECGL, CCGL, EECGL and AECGL on clonidine induced catalepsy

Animals suffering from catalepsy will remain in a forced position for a long time before resuming their natural posture. A pharmaceutical reaction is known as cataleptic symptoms occur. The mice, however, abruptly go into catatonia when given clonidine by subcutaneous injection. The release of histamine is brought on by clonidine, a 2-receptor agonist.

Antihistamines are effective at treating the catalepsy brought on by clonidine [28]. By utilizing plant extracts with antihistaminic or mast cell stabilizing properties, Dhanalakshmi et al. [29] discovered a way to prevent clonidine-induced catalepsy.

Mice had cataplesy for three hours after receiving a 1 mg/kg subcutaneous injection of clonidine. In the experimental study, PECGL demonstrates the maximum duration of cataplesy to be 130.32±5.03** at 90 minutes after subcutaneous clonidine administration, as shown in Table 1 and Figure 1. PECGL was found to decrease clonidine-induced catalepsy counts more than EECGL, CCGL, and AECGL, respectively. The adaptogenic or antiallergic properties of plant extracts thus justify the employ of particular plants in the medication of asthma.

Table 2: Effect chlorpheniramine maleate on haloperidol induced catalepsy

Treatments	Dosage (i.p.)	Duration of Catalepsy (Sec.) Mean±SEM					
		30 min min	60 min	90 min	120 min	*150 min*	180 min
Control 1% Tween-80	5 ml/kg	32.4± 2.30**	46.6±2.39**	56.0± 5.64**	45.4± 4.95**	30.8 ± 4.96**	27.6 ± 6.45**
CPM	10 mg/kg	28.0± 6.45**	40.6± 0.07**	49.2± 4.98**	26.4± 7.01**	21.4 ± 9.02**	22.2 ± 6.21**

n = six in each group. **P<0.05 significant compared with control group

This extract not inhibited haloperidol induced catalepsy compared to control group. Thus, Cpm has no effect on haloperidol induced catalepsy [Table 2].

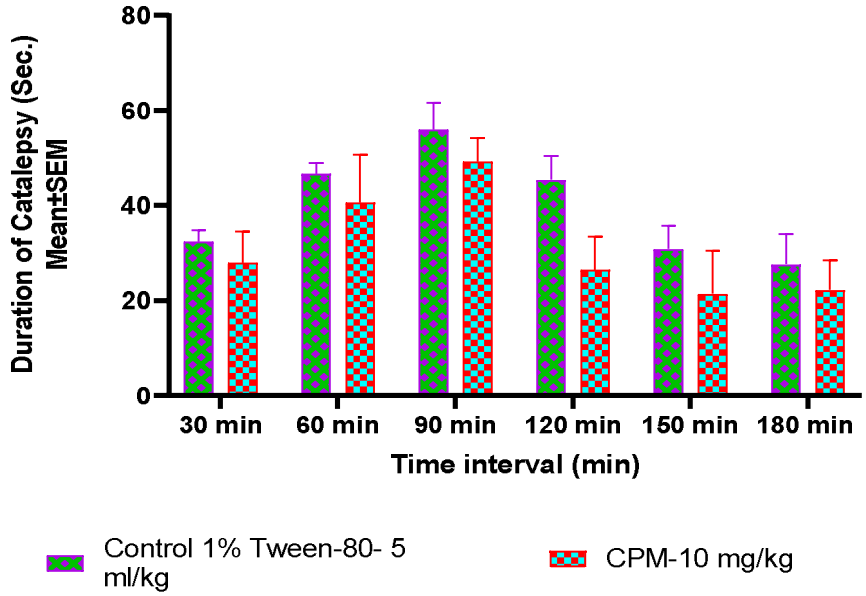

Fig. 2: Effect chlorpheniramine maleate on haloperidol induced catalepsy

Antihistamine chlorpheniramine maleate was examined to determine if it may lessen the cataplesy brought on by haloperidol in mice. 90 minutes after the haloperidol administration, the greatest amount of catalepsy in the placebo group was measured at 56.0 ± 5.64 minutes. Table 2 and Figure 2 show that chlorpheniramine maleate (10 mg/kg b.w.) did not shield mice from cataplesy brought on by haloperidol. There is evidence to support the idea that haloperidol can cause cataplesy by preventing the release of dopamine. Our results indicate that chlorpheniramine maleate had no effect on haloperidol-induced cataplesy, whereas it significantly inhibited clonidine-induced cataplesy.

Milk Induced Leucocytosis & Eosinophilia

24 hours after receiving milk subcutaneously, mice had more leukocytes and eosinophils. Patients with asthma had an increase in both leukocytes and eosinophils.

Table 3: Effect of leaves test extracts on milk induced leucocytosis

Treatments	Dosage (i.p.) mg/kg	Difference in no. of Leucocytes (Before and after treatment) (Mean ± SEM)
Control (1% Tween-80)	5 ml/kg	4550.33±297.95**
PECGL	100	2641.67±213.470**
	150	2792.67±203.886**
CCGL	100	3775±164.72
	150	3542±114.75
EECGL	100	3980±8.04
	150	3842±10.01
AECGL	100	3536.00±487.13
	150	3664±126.21
Dexamethasone	50	2550.00±110.40**

n = six in each group. **P<0.05 significant compared with control group

This extract significantly inhibited milk induced leucocytosis compared to control group [Table 3].

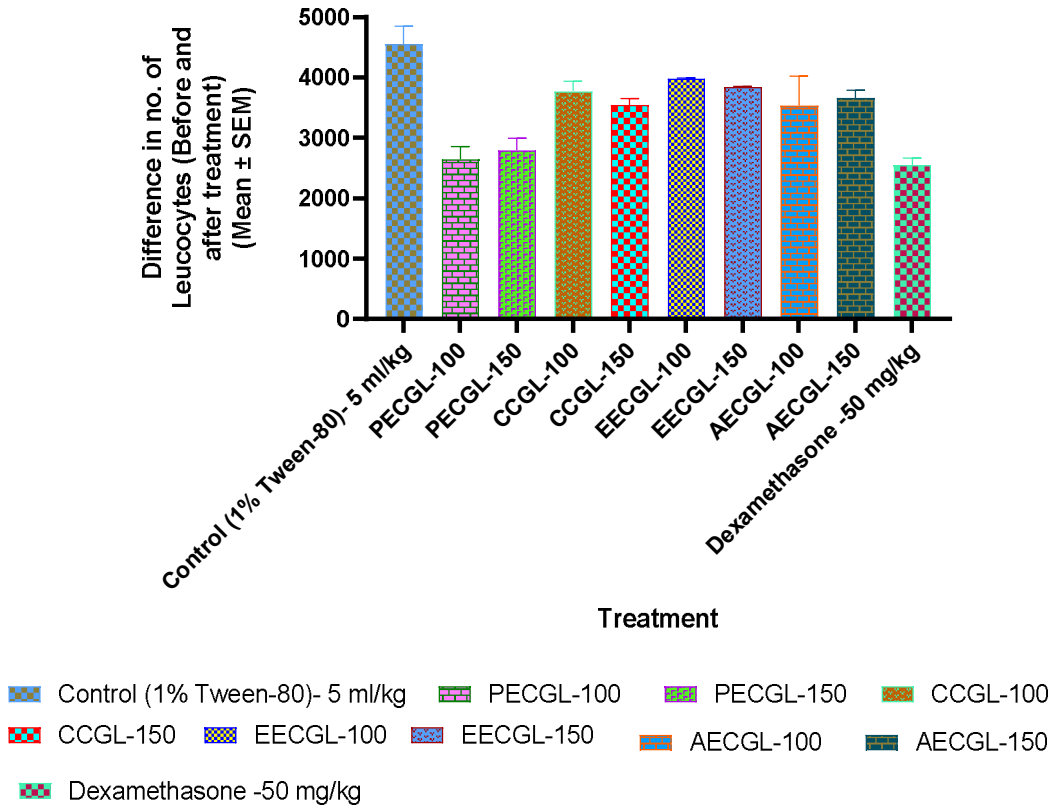

Fig. 3: Effect of leaves test extracts on milk induced leucocytosis

24 hours after subcutaneous milk injection in mice, leukocyte and eosinophil levels increase [30, 31]. Patients with asthma were found to have higher leukocyte and eosinophil levels.

Due to the additional stress they cause, boosts in total leucocyte count may make asthma symptoms worse [32]. In the face of internal and external pressures, adaptogenic or anti-stress drugs have the power to strengthen the body's defenses [33].

After receiving subcutaneous milk at a dosage of 5 ml/kg body weight for 24 hours, the leucocyte count in the control group had enhanced the most. In contrast, the leucocyte count in the dexamethasone-treated mice had reduced significantly (P<0.05) in comparison to the control group. PECGL > EECGL > CCGL > AECGL resulted in a greater reduction in leucocytes than the other test extracts, as depicted in Table 3 and Figure 3. Hence, test extracts of selected plants reveal adaptogenic or antiallergic properties, validating their application in the treatment of asthma.

Table 4: Effect of leaves test extracts on milk induced Eosinophilia

Treatments	Dosage (i.p.) mg/kg	Difference in no. of Eosinophilia (Before and after treatment) (Mean ± SEM)
Control (1% Tween-80)	5 ml/kg	120.21±2.42**
PECGL	100	93±149.98**
	150	97±157.54**
CCGL	100	102±142.53
	150	108±162.30
EECGL	100	105±139.86
	150	107±136.75
AECGL	100	102.00±38.64
	150	101.43± 42.87
Dexamethasone	50	90.00±22.00**

n = six in each group. **P<0.05 significant compared with control group

This extract significantly inhibited milk induced eosinophilia compared to control group [Table 4].

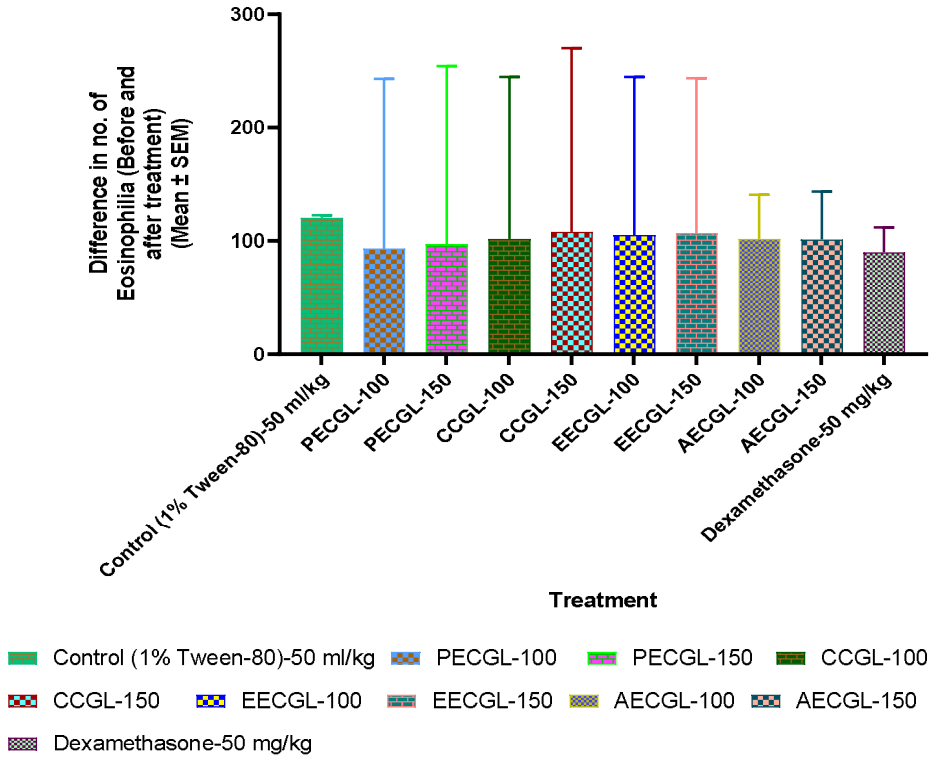

Fig 4: Effect of leaves test extracts on milk induced eosinophilia

Eosinophil degranulation is a crucial immunologic process that contributes to allergic inflammation [34]. Eosinophil counts were shown to be higher in asthma [35, 36]. Eosinophilia, which releases cytotoxic proteins, broncho-active mediators, cytokines (IL-4, IL-5 & IL-13) & mucus, is related with asthmatic airway hyperresponsiveness [37, 38].

The eosinophil count in the control group grew to a maximum of 120.21±2.42 after 24 hours of subcutaneous milk delivery at a dosage of 5 ml/kg body weight, but the eosinophil count in the dexamethasone-treated group reduced to a significant ($P<0.05$) 102.00±22.00. As demonstrated in table 4 and figure 4, the relative decrease in leucocyte counts by test extracts was PECGL > EECGL > AECGL > CCGL. Hence, the adaptogenic or antiallergic properties of plant extracts validate the utilized of selected plants in the cure of asthma.

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