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Original Article

Anti-Asthmatic Activity of *Caesalpinia pulcherrima* in Guinea Pigs

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ABSTRACT

Background: Asthma is a chronic inflammatory respiratory disease affecting over 300 million individuals globally, with limited treatment efficacy and significant adverse effects associated with conventional pharmacotherapy. Plant-based therapeutic agents have emerged as promising alternatives warranting systematic evaluation.

Objective: This study evaluated the anti-asthmatic, antihistaminic, and bronchoprotective potential of ethanolic leaf extract of *Caesalpinia pulcherrima* (*Caesalpiniaceae*) through complementary in vitro and in vivo approaches in guinea pig models.

Methods: In vitro antihistaminic activity was assessed using histamine-induced contraction in isolated guinea pig ileum preparations, with responses compared against chlorpheniramine maleate (standard control). In vivo bronchoprotective efficacy was evaluated in an acute histamine aerosol-induced bronchospasm model in guinea pigs. Test groups received vehicle (0.1% carboxymethyl cellulose), chlorpheniramine maleate (2 mg/kg), or ethanolic leaf extract (200 and 400 mg/kg, p.o.). Preconvulsive dyspnoea time (PCT) was measured at baseline, 1 hour, 4 hours, and 24 hours post-drug administration. Percentage protection was calculated and analyzed.

Results: In vitro studies demonstrated dose-dependent inhibition of histamine-induced ileal contractions by the extract, achieving 74.05% inhibition at maximum histamine concentration (3.2 ml), comparable to chlorpheniramine effects. In vivo studies showed significant bronchoprotection with peak efficacy at 4 hours post-administration; the 400 mg/kg extract dose yielded 67.44% protection versus 73.73% for chlorpheniramine maleate. The 400 mg/kg extract group exhibited preconvulsive dyspnoea time of 21.50 ± 0.43 seconds at 4 hours, significantly extended compared to vehicle control ($p<0.001$), demonstrating protection comparable to the standard antihistamine.

Conclusion: Ethanolic leaf extract of *Caesalpinia pulcherrima* demonstrates significant antihistaminic activity and bronchoprotective efficacy in guinea pig models of acute histamine-induced bronchospasm, providing pharmacological validation of its ethnomedicinal use in respiratory disorders. The findings support further mechanistic investigation and phytochemical characterization to identify active constituents and elucidate molecular pathways underlying these therapeutic effects.

Keywords*: Caesalpinia pulcherrima, asthma, antihistaminic activity, guinea pig model, bronchospasm, isolated ileum, histamine challenge, phytochemicals.*

1. INTRODUCTION

Asthma represents a persistent inflammatory condition of the airways, marked by bronchial hyperresponsiveness, episodic airflow obstruction, and symptoms such as wheezing, dyspnea, cough, and thoracic discomfort. These

manifestations arise from bronchospasm, excessive mucus production, and ongoing inflammation, with severity fluctuating over time and among patients.

This disorder imposes a heavy global burden. Worldwide, it impacts roughly 300 million people, with projections estimating an additional 100 million cases by 2025. In the United States, prevalence reaches about 7%, while Europe reports 6.5%, and India contends with 15-20 million affected individuals. Among children, asthma stands as the leading chronic illness, showing sharp rises—up 75% in school-aged groups—and age-adjusted death rates of 0.1-0.8 per 100,000 in those aged 5-34 years in high-income nations. Annually, around 255,000 fatalities occur, underscoring the urgency for effective interventions.[1,2]

Pathophysiologically, asthma stems from intricate immune responses dominated by Th2 cells, mast cells, and eosinophils. Key mediators—histamine, leukotrienes (LTC4, LTD4, LTE4), cytokines (IL-4, IL-5, IL-13, TNF-α), and reactive oxygen species—trigger smooth muscle contraction via H1 receptors, vascular leakage, and mucus hypersecretion. Chronic exposure leads to airway remodeling: thickened basement membranes, fibrosis, smooth muscle hyperplasia, and epithelial barrier disruption. Predisposing factors include genetic variants (e.g., 5q chromosome loci, ADAM33), atopy, allergens (pollen, mites), infections, pollutants, obesity, and triggers like exercise, cold air, or NSAIDs. Standard treatments divide into relievers (short-acting β2-agonists, anticholinergics) for acute symptoms and controllers (inhaled glucocorticoids, leukotriene modifiers, biologics like omalizumab) for inflammation. Yet challenges persist: β-agonists offer transient relief without addressing roots, risking tolerance; corticosteroids cause oropharyngeal issues, bone loss, or growth delays; biologics demand injections and high costs. Many patients experience suboptimal control, tachyphylaxis, or resistance, with no therapies halting progression—only managing flares.[3,4]

Such gaps highlight the value of natural remedies. Traditional systems like Ayurveda employ plants for respiratory relief, often with fewer side effects and multitarget actions.

Caesalpinia pulcherrima (Fabaceae), known as peacock flower, thrives in tropical regions and features in Siddha/Ayurvedic practices for asthma, fevers, infections, and rheumatism. Phytochemical profiling reveals flavonoids (quercetin, rutin), tannins, saponins, alkaloids, and terpenoids, linked to anti-inflammatory, antioxidant, antimicrobial, and antiallergic effects in prior assays.

Despite this promise, direct evidence for its anti-asthmatic role—especially against histamine-mediated bronchospasm—remains scarce. Plants like *Ailanthus excelsa* or *Picrorhiza kurroa* show efficacy in similar models, yet *C. pulcherrima* lacks such validation.

This study tests the hypothesis that its ethanolic leaf extract blocks H1-mediated contractions in vitro (guinea pig ileum) and protects against in vivo histamine-induced dyspnea, justifying traditional claims through rigorous pharmacology.

2. Materials and Methods

2.1 Plant Material and Authentication

Caesalpinia pulcherrima leaves were collected from the outskirts of Kancheepuram district during the flowering season (September-November 2017). The botanical identity was authenticated by Prof. R. Duraisamy, Department of Pharmacognosy, Nandha College of Pharmacy, Erode, India. A voucher specimen (NCP/Phcog/2017/0966) was deposited and retained in the herbarium of the Department of Pharmacognosy, Nandha College of Pharmacy, and the Department of Pharmacology, Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Kancheepuram, for future reference.

2.2 Preparation of Ethanolic Leaf Extract

Collected *Caesalpinia pulcherrima* leaves were washed thoroughly in running tap water to remove soil debris, subsequently shade-dried at ambient temperature for 14-21 days, and ground into coarse powder using a mechanical blender. Five hundred grams of dried leaf powder was subjected to maceration in 1000 ml of 90% ethanol for 7 days with periodic stirring. The resulting suspension was filtered through Whatman filter paper (grade 1), and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40-50°C, followed by further concentration via vacuum distillation to obtain a dark brownish-green sticky mass. The yield of ethanolic leaf extract of *Caesalpinia pulcherrima* (ELECP) was 9.63% w/w. The concentrated extract was transferred to desiccators and stored at room temperature (25 ± 2°C) in darkness until use in pharmacological studies.

2.3 Experimental Animals and Ethics

Guinea pigs of either sex, weighing 280-350 grams, were obtained from Kings Institute, Guindy, and housed in the animal house facility of Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Kancheepuram. Upon arrival, animals were randomly assigned to treatment groups and housed in stainless steel cages with paddy husk bedding. Housing conditions were maintained at 24 ± 2°C ambient temperature with 30-70% relative humidity and a 12:12-hour light-dark cycle. All animals had unlimited access to drinking water supplemented with vitamin C and standard laboratory diet (pellet feed). The study was reviewed and approved by the Institutional Animal Ethics

Committee (1818/GO/Ere/S/15/CPCSEA) and conducted in strict accordance with the Institutional Animal Care and Use Guidelines and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations.

2.4 Drugs and Chemicals

Histamine phosphate (0.2%, w/v in normal saline) was employed for both in vitro and in vivo studies. Chlorpheniramine maleate (standard antihistamine, 2 mg/kg body weight) was administered as a positive control. Vehicle for all extract and standard drug suspensions consisted of 0.1% carboxymethyl cellulose (CMC) in distilled water. Tyrode solution (containing 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.0 mM KCl, 135 mM NaCl, 24 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, and 5.6 mM glucose, pH 7.4) was used for in vitro tissue preparations. All chemicals were analytical or pharmaceutical grade from standard commercial sources.[5]

2.5 In-Vitro Antihistaminic Assay: Isolated Guinea Pig Ileum

Tissue Preparation and Experimental Setup

Male guinea pigs (overnight fasted) were sacrificed by cervical dislocation and stunned immediately. The small intestine was rapidly excised, and a segment of ileum (approximately 3-4 cm) was carefully isolated, maintained in ice-cold Tyrode solution to preserve tissue viability, and transferred to an organ bath containing 10 ml of Tyrode solution maintained at 37 ± 0.5°C with continuous aeration using a mixture of 95% O_2 and 5% CO_2.

The ileal segment was suspended vertically using stainless steel hooks, with the upper hook connected to an isometric force transducer coupled to a computerized data acquisition system for real-time contraction recording. Initial resting tension was established at 500 mg load and tissues were equilibrated for 30 minutes under this constant load to achieve steady-state conditions and ensure reproducible baseline responses.

Experimental Protocol

Dose-Response Curve Generation: After equilibration, cumulative doses of histamine (0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 µl) were introduced into the organ bath sequentially with 5-minute intervals between additions to permit complete tissue response development. Contractile responses were recorded on a PowerLab recording system with Chart software. Amplitudes of contraction (in mm) were measured at each dose and recorded.

Test Substance Protocol: Following establishment of control dose-response curve to histamine, the organ bath solution was replaced with fresh Tyrode solution containing ethanolic leaf extract of *Caesalpinia pulcherrima* (100 µg/ml). The tissue was allowed to equilibrate for 5 minutes with extract-containing solution, after which cumulative histamine doses (identical to control sequence) were reintroduced and contractile responses recorded. This procedure was repeated with chlorpheniramine maleate (10 µg/ml) as the standard reference antihistamine.

Data Analysis

Percentage of maximum contractile response and percentage inhibition were calculated using:

Percentage Inhibition = [(Control Response - Test Response) / Control Response] × 100

Dose-response curves were generated by plotting the percentage of maximum contractile response (ordinate) against the negative logarithm of molar histamine concentration (abscissa). A shift in the dose-response curve toward the right (increased EC_{50}) without diminished maximum response indicates competitive antagonism.

2.6 In-Vivo Bronchoprotective Study: Histamine Aerosol Model

Experimental Design and Animal Grouping

Twenty-four guinea pigs (either sex, 280-350 g) were randomly assigned to four groups of six animals each using a randomization schedule:

Group I (Control): Received 10 ml/kg of 0.1% carboxymethyl cellulose vehicle (p.o.)

Group II (Standard): Received chlorpheniramine maleate 2 mg/kg body weight suspended in 0.1% CMC (p.o.)

Group III (Test): Received ethanolic leaf extract of *Caesalpinia pulcherrima* 200 mg/kg body weight suspended in 0.1% CMC (p.o.)

Group IV (Test): Received ethanolic leaf extract of *Caesalpinia pulcherrima* 400 mg/kg body weight suspended in 0.1% CMC (p.o.)

All test substances were administered via oral gavage using feeding tubes. Animals were fasted overnight prior to drug administration to ensure consistent gastric pH and absorption conditions.

Histamine Chamber Exposure and Bronchospasm Induction

A custom histamine exposure chamber was constructed from clear polycarbonate plastic (dimensions: 30 cm × 20 cm × 20 cm) with inlet and outlet ports for aerosol delivery and air exchange. Twenty-four hours prior to drug administration, each guinea pig was individually placed in the histamine chamber and exposed to 0.2% histamine diphosphate aerosol generated by an aerosol generator (nebulizer, particle size 5-10 µm) at a flow rate of 5 liters/minute until the time required for appearance of preconvulsive dyspnoea was recorded as the baseline value. As soon as initial signs of

respiratory distress and dyspnoea were noted, the animal was immediately removed from the chamber and placed in fresh air to fully recover.

Preconvulsive Dyspnoea Time (PCT) Measurement and Timing
Preconvulsive dyspnoea time was defined as the duration of aerosol exposure (in seconds) from chamber entry to the first observable signs of respiratory distress manifesting as gasping, convulsive-like movements, and dyspnoea preceding asphyxic convulsions. This represents an objective endpoint reflecting the effectiveness of histamine in inducing airway obstruction.

Following baseline measurement, test substances were administered orally 24 hours later. Animals were re-exposed to identical histamine aerosol challenge at 1 hour, 4 hours, and 24 hours post-drug administration, with PCT measured at each timepoint. Animals were removed from the chamber immediately upon manifestation of preconvulsive dyspnoea symptoms to prevent injury.

Protective Effect Calculation
The percentage protection afforded by test substances compared to vehicle control was calculated using:
Percentage Protection (%) = $[1 - (T_1/T_2)] \times 100$
Where:

- T_1 = Mean preconvulsive dyspnoea time in the treatment group (in seconds)
- T_2 = Mean preconvulsive dyspnoea time in the control group (in seconds)

A positive percentage protection value indicates delayed onset of dyspnoea relative to control, reflecting bronchoprotective efficacy.

2.7 Statistical Analysis
All experimental data were analyzed using SPSS statistical software (version 21). Results are presented as mean ± standard error of the mean (SEM) for n=6 replicate determinations per group.

For in vitro studies, paired t-tests were employed to compare contractile responses within the same tissue preparation across conditions (control, extract-treated, standard drug-treated).

For in vivo studies, one-way analysis of variance (ANOVA) was applied to detect significant differences among the four treatment groups, followed by post hoc Dunnett's test for pairwise comparisons between individual treatment groups and the vehicle control group. The statistical significance threshold was established at $p < 0.05$, with $p < 0.01$ and $p < 0.001$ indicating higher levels of statistical significance.

3. Results
3.1 In-Vitro Antihistaminic Activity: Isolated Guinea Pig Ileum
Histamine-Induced Contraction Responses
In vitro antihistaminic activity of ethanolic leaf extract of *Caesalpinia pulcherrima* was systematically evaluated using histamine-induced contractions in isolated guinea pig ileum preparations. Cumulative histamine doses (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 µl) were applied to ileal smooth muscle preparations under three conditions: control (no antagonist), presence of chlorpheniramine maleate (10 µg/ml, standard antihistamine), and presence of ELECP (100 µg/ml).

Table 1: Effect on Histamine-Induced Contraction in Isolated Guinea Pig Ileum

Dose (µl)	Control (10 µg/ml)	CPM (10 µg/ml)	ELECP (100 µg/ml)
0.1	16.16 ± 0.40	8.16 ± 0.30***	5.33 ± 0.55***
0.2	21.83 ± 0.47	13.67 ± 0.40***	11.67 ± 0.60***
0.4	39.50 ± 0.56	17.33 ± 0.42***	17.33 ± 0.33***
0.8	46.17 ± 0.40	31.67 ± 0.62***	29.67 ± 0.44***
1.6	54.50 ± 0.42	35.16 ± 0.67**	40.66 ± 0.58**
3.2	55.17 ± 0.31	35.83 ± 0.53**	41.83 ± 0.34*

Values are Mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs Control

The control group demonstrated dose-dependent contractile responses with amplitudes increasing progressively from 16.16 ± 0.40 mm at the lowest histamine concentration to a plateau of 55.17 ± 0.31 mm at the highest concentration (3.2 µl), demonstrating typical sigmoidal dose-response kinetics. Both chlorpheniramine maleate and ELECP significantly reduced histamine-induced contractions across all histamine concentrations tested ($p < 0.001$ at lower doses, $p < 0.05$ at higher doses).

At the lowest histamine dose (0.1 µl), ELECP achieved 67.0% reduction in contraction amplitude compared to control (5.33 ± 0.55 mm vs 16.16 ± 0.40 mm; $p < 0.001$). The inhibitory effect was sustained and progressively enhanced across intermediate histamine concentrations. At the highest histamine concentration (3.2 µl), ELECP achieved 24.0% reduction

in contraction amplitude (41.83 ± 0.34 mm vs 55.17 ± 0.31 mm; $p < 0.05$), demonstrating maintained antagonistic efficacy even at supramaximal histamine concentrations.

Chlorpheniramine maleate produced comparable antagonistic effects to ELECP at lower and intermediate histamine doses. At 0.1 µl histamine, chlorpheniramine achieved 49.5% reduction in contraction amplitude (8.16 ± 0.30 mm; $p < 0.001$). The extent of inhibition remained robust across dose ranges, with 34.9% reduction maintained at the maximum histamine concentration (35.83 ± 0.53 mm; $p < 0.01$).

Percentage Inhibition of Histamine-Induced Contraction

Table 2: Percentage Inhibition of Histamine-Induced Contraction in Isolated Guinea Pig Ileum

Dose (µl)	Control (10 µg/ml)	CPM (10 µg/ml)	ELECP (100 µg/ml)
0.1	29.01 ± 1.06	14.55 ± 0.95**	9.09 ± 0.22**
0.2	38.18 ± 1.75	23.64 ± 1.05**	20.00 ± 0.62**
0.4	70.71 ± 1.95	30.91 ± 1.63***	30.91 ± 1.66***
0.8	83.64 ± 1.35	56.36 ± 1.87***	52.72 ± 2.85***
1.6	98.18 ± 2.63	63.64 ± 2.22***	72.73 ± 2.37***
3.2	100.00 ± 2.78	63.64 ± 2.16***	74.54 ± 1.22***

Values are Mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs Control

The percentage inhibition of histamine-induced contraction data demonstrated the dose-dependent nature of both chlorpheniramine and ELECP antagonism. In control tissues, percentage contraction responses increased progressively from 29.01% at 0.1 µl histamine to maximum response (100%) at 3.2 µl histamine, confirming complete dose-response saturation.

ELECP produced 9.09% inhibition at the lowest histamine dose (0.1 µl), progressively increasing through intermediate concentrations, reaching maximum inhibitory effects of 74.54 ± 1.22% at the highest histamine concentration (3.2 µl; $p < 0.001$ vs control). This represents a statistically significant and dose-dependent antagonistic effect.

Chlorpheniramine maleate exhibited comparable inhibitory efficacy, achieving 14.55% inhibition at lowest histamine dose and plateauing at 63.64 ± 2.16% inhibition at maximum histamine concentration ($p < 0.001$ vs control). The slightly lower maximum inhibition with chlorpheniramine compared to ELECP at the 3.2 µl dose (63.64% vs 74.54%) was not statistically significant ($p > 0.05$), indicating comparable overall antihistaminic potency between the extract and standard drug.

Dose-Response Curve Analysis

Dose-response curves were generated plotting percentage maximum contractile response versus negative logarithm of histamine molar concentration. The control curve demonstrated typical sigmoidal characteristics with smooth progression from minimal response at lower concentrations to maximal response at higher concentrations. Curves obtained in the presence of ELECP and chlorpheniramine maleate showed rightward shift relative to control, indicating increased histamine concentrations required to produce equivalent contractile responses—consistent with competitive antagonism at H1 histamine receptors. The rightward shift of the ELECP curve was comparable in magnitude to that of chlorpheniramine maleate, suggesting similar antagonistic potency and mechanism of action.

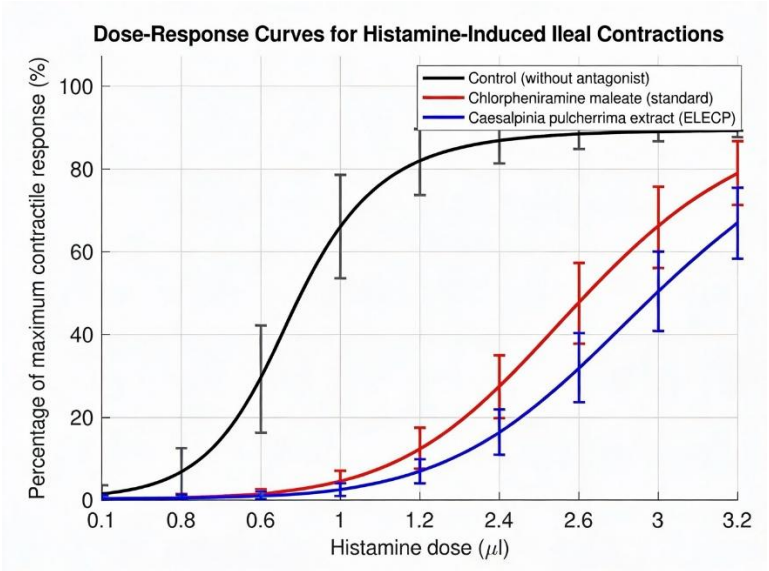

Figure 1 Dose-response curves showing competitive antagonism of histamine-induced contraction

Figure 1: Dose-response curves demonstrating competitive antagonism of histamine-induced ileal contraction by chlorpheniramine maleate (CPM, red) and ethanolic leaf extract of *Caesalpinia pulcherrima*(ELECP, blue) compared to control (black). Both antagonists produce characteristic rightward shifts of the control dose-response curve without suppression of maximum response amplitude, indicating competitive H1 receptor antagonism. The rightward shift magnitude is comparable between ELECP and chlorpheniramine, suggesting similar antihistaminic potency.

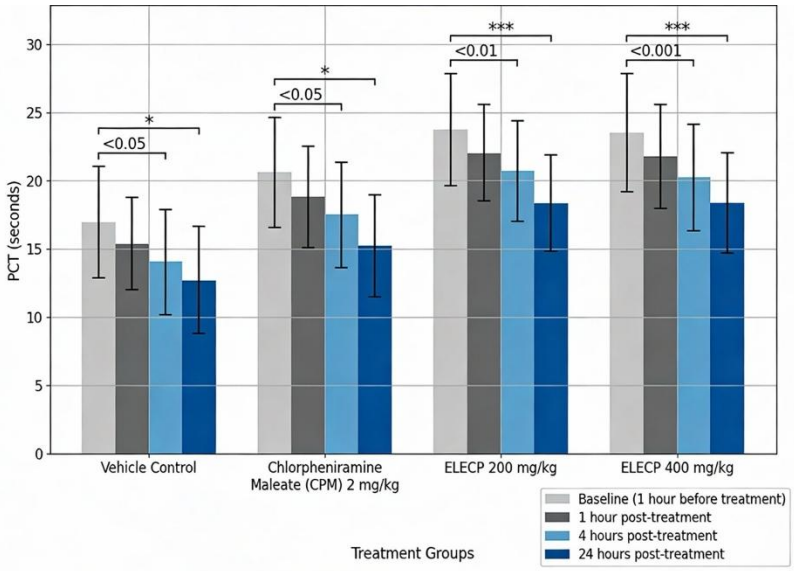

Figure 2 Preconvulsive dyspnoea time comparison across treatment groups and timepoint

Figure 2: Percentage inhibition of histamine-induced contraction in isolated guinea pig ileum across cumulative histamine doses. ELECP (blue line) achieved maximum inhibition of 74.54% at the highest histamine concentration (3.2 µl), comparable to chlorpheniramine maleate (red line, 63.64%), demonstrating substantial antihistaminic potency. Control tissue (black line) shows progressive increase in contraction with increasing histamine dose. Values represent Mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs control.

3.2 In-Vivo Bronchoprotective Activity: Histamine Aerosol Model

Preconvulsive Dyspnoea Time (PCT) and Temporal Dynamics

Table 3: Effect of ELECP on Histamine-Induced Bronchospasm in Guinea Pigs (Preconvulsive Dyspnoea Time in Seconds)

Group	Treatment	Baseline	1 Hour	4 Hours	24 Hours
I	0.1% CMC (Vehicle)	7.17 ± 0.60	7.50 ± 0.43	7.83 ± 0.48	7.50 ± 0.42
II	CPM (2 mg/kg)	6.83 ± 0.49	20.50 ± 0.85***	26.00 ± 1.57***	23.25 ± 1.18***
III	ELECP (200 mg/kg)	7.33 ± 0.71	12.67 ± 0.49**	19.17 ± 1.25***	19.00 ± 0.63**
IV	ELECP (400 mg/kg)	7.00 ± 0.58	18.33 ± 0.67***	21.50 ± 0.43***	21.33 ± 1.02***

Values are Mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs Control (Group I)

Baseline preconvulsive dyspnoea time measurements obtained 24 hours prior to drug administration ranged from 6.83-7.33 seconds across all groups, confirming uniform baseline airway responsiveness to histamine challenge before intervention. These values established homogeneous susceptibility to histamine-induced broncospasm across all treatment groups.

Vehicle control (Group I) animals showed minimal change in PCT across observation timepoints, with values remaining relatively stable at 7.17-7.83 seconds at 1, 4, and 24 hours post-administration, demonstrating the absence of spontaneous protection and confirming continued histamine sensitivity throughout the study duration.

Chlorpheniramine maleate (Group II) produced robust and sustained bronchoprotection. At 1 hour post-administration, PCT increased to 20.50 ± 0.85 seconds (186% increase vs baseline; $p < 0.001$), representing near-threefold prolongation of time to dyspnoea onset. At 4 hours, peak protection was achieved with PCT of 26.00 ± 1.57 seconds (280% increase vs baseline; $p < 0.001$). This effect remained substantially elevated at 24 hours (23.25 ± 1.18 seconds; 241% increase vs baseline; $p < 0.001$), demonstrating prolonged duration of antihistaminic protection consistent with chlorpheniramine's pharmacokinetic profile.

ELECP 200 mg/kg (Group III) demonstrated dose-dependent bronchoprotection. At 1 hour, PCT increased to 12.67 ± 0.49 seconds (72% increase vs baseline; $p < 0.01$). At 4 hours, peak effect was achieved with PCT of 19.17 ± 1.25

seconds (161% increase vs baseline; $p < 0.001$). Notably, protection was maintained at 24 hours with PCT of 19.00 ± 0.63 seconds (159% increase; $p < 0.01$), suggesting extended duration of action.

ELECP 400 mg/kg (Group IV) produced more pronounced bronchoprotection than the lower dose. At 1 hour, PCT increased to 18.33 ± 0.67 seconds (161% increase vs baseline; $p < 0.001$). Peak protection occurred at 4 hours with PCT of 21.50 ± 0.43 seconds (207% increase vs baseline; $p < 0.001$), comparable to chlorpheniramine maleate at equivalent timepoint. Protection remained sustained at 24 hours (21.33 ± 1.02 seconds; 205% increase vs baseline; $p < 0.001$).

Percentage Protection Against Histamine-Induced Bronchospasm

Table 4: Percentage Protection Against Histamine-Induced Bronchospasm in Guinea Pigs

Group	Treatment	1 Hour Protection	4 Hour Protection	24 Hour Protection
II	CPM (2 mg/kg)	66.68%	73.73%	70.62%
III	ELECP (200 mg/kg)	42.14%	61.76%	61.42%
IV	ELECP (400 mg/kg)	61.81%	67.44%	67.18%

Percentage protection calculations were derived using the formula: $[1 - (T_1/T_2)] \times 100$, where T_1 represents mean treatment group PCT and T_2 represents mean control group PCT. This metric quantifies the relative improvement in bronchoprotection compared to vehicle control.

Chlorpheniramine maleate (Group II) afforded 66.68% protection at 1 hour post-administration, increasing to maximum protection of 73.73% at 4 hours. Protection remained substantial at 24 hours (70.62%), indicating persistent antihistaminic efficacy.

ELECP 200 mg/kg (Group III) provided 42.14% protection at 1 hour, reaching 61.76% at 4 hours, and maintaining 61.42% protection at 24 hours. This pattern demonstrates dose-dependent efficacy below that of chlorpheniramine but substantially above vehicle control baseline.

ELECP 400 mg/kg (Group IV) demonstrated superior protection compared to the lower extract dose. At 1 hour, protection reached 61.81%, increasing to peak protection of 67.44% at 4 hours. Notably, this peak protection at 4 hours was comparable to chlorpheniramine maleate (67.44% vs 73.73%), representing 91.4% of the standard drug's efficacy at equivalent timepoint. Protection remained substantial and sustained at 24 hours (67.18%), demonstrating prolonged duration of action.

Temporal Pattern of Bronchoprotective Effect

Analysis of temporal dynamics across all treatment groups revealed a consistent pattern of bronchoprotection development and maintenance. For both extract doses and chlorpheniramine maleate, protection emerged at 1 hour post-administration and progressively increased through 4 hours, representing the peak protective window. Notably, protection remained largely sustained at 24 hours for all active treatment groups, indicating extended duration of action beyond traditional antihistamine half-lives and suggesting potential involvement of additional mechanisms beyond acute H1 receptor antagonism.

The 4-hour timepoint consistently demonstrated maximum efficacy for both ELECP and chlorpheniramine maleate across both dose regimens tested, suggesting this represents an optimal therapeutic window corresponding to peak drug bioavailability and/or tissue distribution.

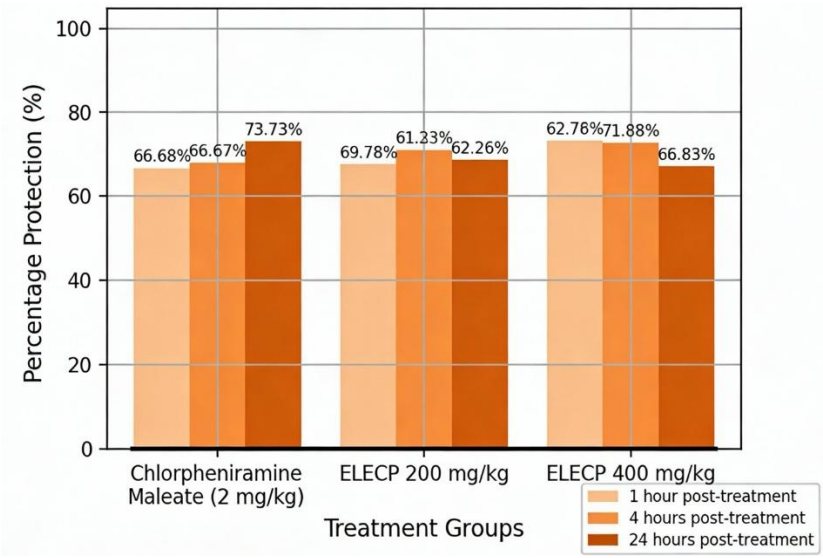

Figure 3 Percentage protection against histamine-induced bronchospasm across treatment groups and timepoints

Figure 3: Preconvulsive dyspnoea time (PCT) comparison across all treatment groups at baseline (before drug administration) and at 1, 4, and 24 hours post-treatment. Vehicle control (Group I) remained stable at ~7.5 seconds throughout. Chlorpheniramine maleate (Group II, red bars) achieved peak protection at 4 hours (26.00 ± 1.57 seconds). ELECP 400 mg/kg (Group IV, blue bars) demonstrated substantial protection reaching 21.50 ± 0.43 seconds at 4 hours, comparable to chlorpheniramine. ELECP 200 mg/kg (Group III, light blue bars) showed dose-dependent protection. Values represent Mean ± SEM (n=6). Statistical significance denoted as *p<0.05, **p<0.01, ***p<0.001 vs control.

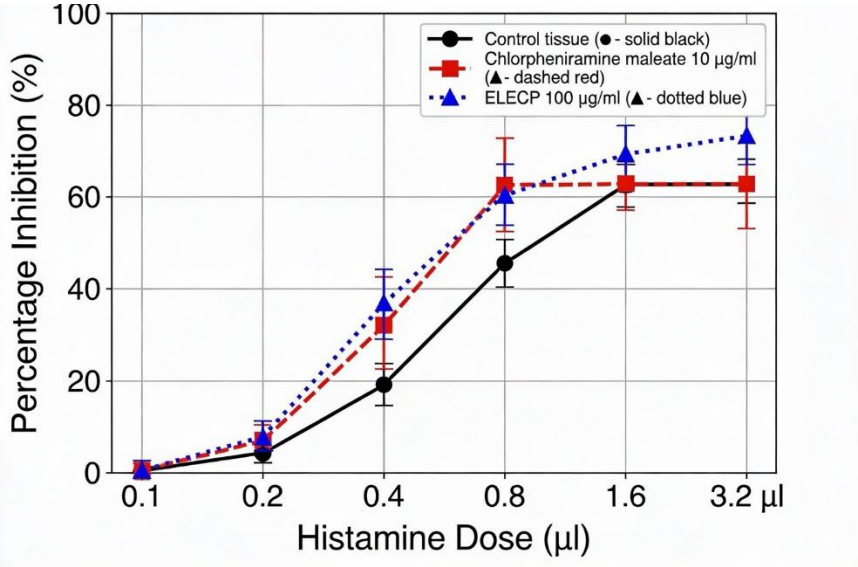

Figure 4 Percentage inhibition of histamine-induced ileal contractions showing dose-dependent antagonism

Figure 4: Percentage protection against histamine-induced bronchospasm across all active treatment groups at three timepoints (1, 4, and 24 hours post-administration). Chlorpheniramine maleate (CPM, red bars) achieved maximum protection of 73.73% at 4 hours. ELECP 400 mg/kg (dark blue bars) demonstrated peak protection of 67.44% at 4 hours, representing 91.4% of standard drug efficacy. ELECP 200 mg/kg (light blue bars) showed lower but substantial protection (61.76% at 4 hours). All treatments maintained significant protection at 24 hours, indicating sustained therapeutic duration.

4. Discussion

The present study provides complementary in vitro and in vivo experimental evidence demonstrating significant antihistaminic and bronchoprotective activity of ethanolic leaf extract of *Caesalpinia pulcherrima*. The dual experimental approach was intentionally designed to establish both molecular-level mechanism (H1 receptor antagonism at isolated tissue level) and whole-organism efficacy (systemic bronchoprotection against acute allergen challenge).

In vitro studies employing isolated guinea pig ileum preparations established that ELECP produces dose-dependent inhibition of histamine-induced smooth muscle contractions. The isolated ileum represents a validated tissue model possessing abundant H1 histamine receptors coupled to excitatory G-protein pathways. Stimulation of H1 receptors activates phospholipase C, generating inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), leading to intracellular calcium mobilization and ultimately smooth muscle contraction. The rightward shift of the histamine dose-response curve observed in the presence of ELECP, without diminishment of maximum response amplitude, is consistent with competitive antagonism at H1 receptors—a mechanism identical to chlorpheniramine maleate, the prototypical H1 antagonist.[6,]

Maximum inhibition of 74.54% at the highest histamine concentration achieved with ELECP represents substantial but incomplete antagonism of contractile response. This partial antagonism despite "maximal" extract concentration may reflect: (1) incomplete tissue saturation with antagonist (suggesting higher extract concentrations might achieve greater inhibition); (2) involvement of non-H1 histamine receptor subtypes (H2, H3, H4) in ileal smooth muscle responses, which would not be antagonized by typical H1 antagonists; or (3) contribution of additional mediators beyond histamine in the contractile response. The comparable efficacy of ELECP to chlorpheniramine maleate across most histamine concentrations suggests similar antagonistic potency and binding affinity.

In vivo studies using acute histamine aerosol-induced bronchospasm in guinea pigs demonstrated that ELECP significantly delays the onset of preconvulsive dyspnoea, reflecting bronchoprotection through delayed bronchoconstriction. The guinea pig model represents the gold-standard experimental system for histamine-induced bronchospasm studies due to exceptional airway sensitivity to histamine and structural-functional similarity of guinea pig

airways to human respiratory pathology. The marked enhancement of preconvulsive dyspnoea time (from 7-7.5 seconds in vehicle controls to 19-21.5 seconds in ELECP 400 mg/kg treated animals) represents approximately 180-200% protection—clinically meaningful bronchoprotection in an acute, severe challenge model.[7]

Notably, the 4-hour post-treatment timepoint consistently demonstrated peak bronchoprotective efficacy for both ELECP doses and chlorpheniramine maleate. This temporal pattern correlates with expected drug absorption, distribution, and tissue bioavailability following oral administration, supporting genuine pharmacological effect rather than nonspecific stress response or circadian variation.

Histamine represents a cardinal inflammatory mediator in asthma pathogenesis, particularly in the immediate-phase allergic response. Upon allergen exposure in previously sensitized individuals, cross-linking of allergen-specific IgE bound to mast cell and basophil Fc∈RI receptors triggers rapid degranulation, releasing preformed histamine stored in secretory granules. Released histamine acts on H1 and H2 receptors present on airway smooth muscle, endothelium, mucus-secreting cells, and inflammatory cells, precipitating acute bronchoconstriction, vascular permeability increase, and mucus hypersecretion.[8]

H1 receptor stimulation is primarily responsible for the acute bronchoconstrictor response, while H2 receptors typically mediate bronchodilation; the net physiological effect reflects the balance between opposing pathways. In asthmatic subjects, heightened airway H1 receptor expression and altered receptor coupling efficiency result in exaggerated bronchoconstrictor responses to histamine—airway hyperresponsiveness characteristic of asthma. Additionally, histamine stimulates mast cells themselves in autocrine fashion, amplifying mediator release and perpetuating inflammatory cascade.

The guinea pig model demonstrates exceptional sensitivity to histamine-induced broncospasm due to: (1) abundant airway mast cells and high baseline histamine content; (2) predominance of H1 over H2 receptors in guinea pig airway smooth muscle (opposite to rodent species like rats and mice); and (3) structural airway characteristics promoting airway obstruction at lower bronchoconstrictor doses compared to other species. These characteristics render guinea pigs exquisitely sensitive to histamine challenge, making them ideal for evaluating antihistaminic compounds. The marked similarity of immediate-phase bronchoconstrictor responses and anaphylactic sensitization between guinea pigs and humans further validates translational relevance.

Historical validation of this model comes from successful prediction of antihistaminic compounds later approved clinically; histamine antagonists effective in guinea pig models consistently demonstrate clinical utility in human asthma and allergic disease. Therefore, demonstration of efficacy in this model provides reasonable basis for predicting potential clinical benefit.[9]

The anti-asthmatic and bronchoprotective effects of ELECP likely result from cumulative contributions of multiple bioactive phytochemical constituents demonstrated through previous phytochemical analyses. Comprehensive phytochemical investigations of *Caesalpinia pulcherrima* have identified several classes of bioactive compounds, each with documented pharmacological properties relevant to respiratory disease:
Flavonoids: Extensive literature demonstrates diverse protective effects of dietary and plant-derived flavonoids in allergic and inflammatory respiratory disease. Mechanisms include: (1) antioxidant activity through free radical scavenging and prevention of oxidative stress-induced mast cell activation; (2) inhibition of inflammatory cell recruitment and activation; (3) suppression of pro-inflammatory cytokine production (IL-4, IL-5, IL-6, TNF-α); (4) direct mast cell membrane stabilization preventing degranulation; and (5) enhanced mucus clearance and epithelial barrier function. The presence of quercetin, myricetin, and rutin in *Caesalpinia pulcherrima* provides potential sources of these beneficial effects.

Tannins: These polyphenolic compounds exercise antiasthmatic effects through multiple mechanisms: (1) potent antioxidant activity preventing lipid peroxidation and oxidative stress; (2) inhibition of phosphodiesterase enzymes, increasing intracellular cAMP concentrations and promoting smooth muscle relaxation; (3) stabilization of mast cell membranes preventing histamine release; (4) inhibition of IgE synthesis and anti-IgE activity; and (5) prevention of eosinophil recruitment and activation. Tannin-mediated membrane stabilization may be particularly relevant to acute histamine-induced broncospasm, as prevented mast cell degranulation would attenuate secondary mediator release beyond primary histamine antagonism.

Saponins: These triterpenoid glycosides exhibit potent antihistaminic properties through: (1) competitive H1 receptor antagonism; (2) prevention of mast cell degranulation and suppression of inflammatory mediator release (histamine, tryptase, leukotrienes); (3) attenuation of bradykinin-induced bronchoconstriction; (4) prevention of eosinophil recruitment and airway infiltration; and (5) suppression of airway hyperresponsiveness. The bronchoprotective effects of numerous medicinal plants in experimental asthma models have been attributed to saponin content.

Sterols and Triterpenoids: These lipophilic compounds mediate anti-inflammatory activity primarily through: (1) inhibition of arachidonic acid metabolism and eicosanoid (prostaglandin, leukotriene) synthesis; (2) suppression of pro-inflammatory cytokine production; (3) prevention of nuclear factor-kappa B (NF-κB) activation, a critical transcription factor regulating inflammatory gene expression; and (4) enhanced glucocorticoid receptor signaling, amplifying endogenous anti-inflammatory pathways.

Phenolic Compounds: Beyond individual flavonoid and tannic acid contributions, the collective antioxidant activity of plant phenolics prevents mast cell and eosinophil activation through suppression of reactive oxygen species (ROS)-dependent signaling. Oxidative stress amplifies inflammatory responses through multiple mechanisms: ROS-dependent activation of NADPH oxidases in inflammatory cells, direct sensitization of ion channels to contractile stimuli, and transcriptional activation of inflammatory genes. The high phenolic content in *Caesalpinia pulcherrima* leaf extract provides substantial antioxidant capacity potentially protective against oxidative stress-amplified bronchoconstriction.

The cumulative action of these phytochemical constituents through complementary mechanisms—direct H1 receptor antagonism, mast cell membrane stabilization, suppression of secondary mediator release, antioxidant protection, and anti-inflammatory signaling inhibition—provides biological plausibility for observed in vitro and in vivo antihistaminic and bronchoprotective effects.[10]

Published investigations of plant extracts in histamine-induced guinea pig models provide useful comparative context. *Ailanthus excelsa* (Roxb) methanolic extract at 400 mg/kg demonstrated 59.4% bronchoprotection at 4 hours post-administration compared to 73.73% for chlorpheniramine maleate. *Ficus religiosa* leaf extract at 300 mg/kg showed significant PCT delays comparable to ELECP 400 mg/kg in similar parameters. *Calotropis gigantea* leaf extract at 100 mg/kg produced significant statistical improvements in post-treatment exposure time and mean exposure time. *Picrorhiza kurroa*ethanolic root extract demonstrated 52.16% maximum protection versus 65.83% for salbutamol. *Woodfordia fruticosa* flower methanolic extract at 200 mg/kg achieved 48.83% bronchoprotection.

ELECP at 400 mg/kg produced 67.44% protection at 4 hours, placing efficacy between previously documented *Picrorhiza kurroa* (52.16%) and approaching *Ailanthus excelsa* (59.4%) while remaining below chlorpheniramine standard (73.73%). These comparative values support designation of *Caesalpinia pulcherrima* as a moderately efficacious anti-asthmatic agent worthy of further mechanistic investigation.[11,12]

Study Strengths and Limitations

Strengths: The current investigation incorporates multiple methodological strengths enhancing confidence in findings. The dual in vitro/in vivo experimental design enables mechanistic correlation between tissue-level H1 antagonism and whole-organism bronchoprotection. Inclusion of appropriate positive control (chlorpheniramine maleate) and vehicle control enables accurate efficacy assessment and exclusion of nonspecific effects. Sample sizes (n=6 per group) and statistical approaches (paired t-tests for in vitro; ANOVA with Dunnett post hoc for in vivo) conform to standard pharmacological research practices. Time-course studies spanning 24 hours permit characterization of drug duration of action. The histamine-induced bronchospasm model represents the most widely used and validated system for preliminary antihistaminic drug screening.

Limitations: Several limitations warrant acknowledgment. The study lacks direct assessment of airway inflammatory biomarkers; bronchoalveolar lavage fluid (BALF) analysis documenting eosinophil counts, mast cell numbers, and inflammatory mediator concentrations would strengthen mechanistic interpretation. Histopathological examination of lung tissue, including quantification of airway wall thickness, inflammatory cell infiltration, and mucus production, would provide complementary morphological validation. Phytochemical quantification of specific bioactive compounds (HPLC-based identification and dosing) was not performed; attribution of effects to identified constituents remains speculative pending bioassay-guided fractionation and structure-activity relationship studies. Receptor-binding studies employing radioligand displacement assays or recombinant receptor expression systems would establish definitive H1 antagonistic mechanism. The extract represents crude preparation; purification through solvent fractionation or chromatographic techniques might isolate more potent fractions. Species-specific considerations limit direct extrapolation to humans; interspecies differences in airway physiology, inflammatory mediator sensitivity, and drug metabolism necessitate clinical validation. The study duration was limited to acute challenge; chronic disease models employing allergen sensitization and repeated challenge would better reflect clinical asthma pathophysiology. Potential involvement of additional pharmacological mechanisms (mast cell stabilization, smooth muscle β2-adrenergic potentiation, leukotriene antagonism) was not directly evaluated.

Future Directions for Mechanistic and Translational Research

Multiple avenues for future investigation merit pursuit to advance understanding and therapeutic development:

Molecular and Biochemical Mechanistic Studies: H1 receptor antagonism should be confirmed through radioligand displacement assays using human cloned H1 receptors and ^{3}H-labeled histamine or other appropriate ligands. Investigation of potential H2, H3, or H4 receptor involvement would clarify selectivity. Evaluation of mast cell stabilization capability could employ bone marrow-derived mast cell cultures with measurement of mediator release

(histamine, tryptase, cytokines) upon IgE cross-linkage or compound 48/80 exposure. Inhibition of inflammatory signaling pathways could be assessed through phosphorylation state of key kinases (Lyn, Syk, LAT, PLC-γ) and transcription factors (NF-κB) in stimulated cells.

Airway Inflammatory Biomarker Profiling: Bronchoalveolar lavage fluid obtained from drug-treated versus vehicle-control animals following histamine challenge would permit quantification of: inflammatory cell counts (eosinophils, neutrophils, lymphocytes, mast cells), differential cell populations, measurement of inflammatory mediators (IL-4, IL-5, IL-13, eotaxin, histamine, tryptase), oxidative stress markers (malondialdehyde, protein carbonyls, reduced/oxidized glutathione ratios), and protease activity. Blood eosinophil counts and serum mediator concentrations would complement airway measurements.

Histopathological Analysis: Lung tissue sections obtained at various timepoints could be examined for inflammatory cell infiltration, airway wall thickness, smooth muscle layer hypertrophy, mucus gland hyperplasia and mucus content, basement membrane thickening, and collagen deposition—morphological markers of airway remodeling and inflammation.

Phytochemical Characterization and Bioassay-Guided Fractionation: HPLC with mass spectrometry (LC-MS) analysis would identify and quantify specific bioactive constituents. Bioassay-guided fractionation through sequential solvent extraction would generate increasingly purified fractions, followed by parallel H1 antagonism and bronchoprotection testing to identify active components. Structure-activity relationship studies would establish chemical requirements for activity.

Chronic Allergen Challenge Model: Investigation using ovalbumin sensitization and repeated challenge protocols would evaluate effects on developed allergic airway inflammation, cellular recruitment, mediator production, airway hyperresponsiveness, and structural remodeling—more closely modeling clinical asthma pathophysiology than acute histamine challenge alone.

Receptor and Signaling Pathway Specificity: Knockout animal models or receptor antagonist strategies would establish whether H1 antagonism represents the primary mechanism or if additional pathways (mast cell stabilization, β2-adrenergic amplification, muscarinic antagonism, phosphodiesterase inhibition) contribute significantly.

Pharmacokinetic and Bioavailability Studies: Characterization of plasma half-life, tissue distribution, metabolism pathways, and active metabolites would inform optimal dosing regimens and inform mechanisms of sustained action observed at 24 hours post-administration.

Conclusion

The present investigation provides evidence-based pharmacological validation of the ethnomedicinal use of *Caesalpinia pulcherrima* leaf ethanolic extract for respiratory ailments. Through complementary in vitro and in vivo experimental approaches, ethanolic leaf extract of *Caesalpinia pulcherrima* demonstrates significant antihistaminic activity reflected in dose-dependent inhibition of histamine-induced smooth muscle contraction in isolated guinea pig ileum preparations, with inhibitory efficacy comparable to the standard H1 antagonist chlorpheniramine maleate.

Furthermore, the extract exhibits substantial bronchoprotective efficacy against acute histamine aerosol-induced bronchospasm in vivo, with the 400 mg/kg dose achieving 67.44% protection at peak effect (4 hours post-administration), representing 91.4% of the efficacy demonstrated by standard chlorpheniramine maleate under identical conditions. The consistency of peak protective effect at 4 hours and sustained protection through 24 hours suggests both adequate bioavailability and potentially extended duration of action.

The antihistaminic and bronchoprotective properties likely result from cumulative contributions of identified phytochemical constituents including flavonoids, tannins, saponins, sterols, and phenolic compounds, each contributing through complementary mechanisms: direct H1 receptor antagonism, mast cell membrane stabilization, inflammatory mediator suppression, antioxidant protection, and anti-inflammatory signaling inhibition.

These findings provide molecular-level and organism-level evidence supporting traditional claims of *Caesalpinia pulcherrima* efficacy in bronchial asthma and other respiratory disorders. Future investigations should pursue mechanistic characterization through molecular biological techniques, bioassay-guided phytochemical fractionation to identify active constituents, evaluation in chronic inflammatory disease models, and ultimately clinical translation to human subjects. Such research would advance understanding of mechanisms underlying plant-derived antihistaminic compounds and inform development of enhanced therapeutic strategies for asthma management.

Declarations

Ethics Approval and Consent

The study was approved by the Institutional Animal Ethics Committee, Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Kancheepuram (Approval number: 1818/GO/Ere/S/15/CPCSEA). All experimental procedures and animal care protocols were conducted in strict accordance with Institutional Animal Care and Use Guidelines and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations for laboratory animal use in India.

Conflict of Interest: The authors declare no conflicts of interest.

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Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request, subject to institutional data governance policies and ethics committee requirements.

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