

To Evaluate the Potential Airway Smooth-Muscle Relaxant and Antiasthmatic-Related Activity of the Ethanolic Extract of *Alpinia Calcarata* Rhizome Using an Isolated Goat Tracheal Chain Preparation.

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ABSTRACT

Asthma is a chronic inflammatory respiratory disorder characterized by airway hyperresponsiveness, bronchoconstriction, and reversible airflow limitation. The search for safer and effective natural agents with bronchodilatory potential has encouraged the pharmacological investigation of traditionally used medicinal plants. *Alpinia calcarata* Roscoe, a rhizomatous medicinal plant belonging to the family Zingiberaceae, has traditionally been used in respiratory disorders, including asthma and bronchitis. The present study was designed to evaluate the potential airway smooth-muscle relaxant and antiasthmatic-related activity of the ethanolic extract of *Alpinia calcarata* rhizomes using an isolated goat tracheal chain preparation.

The rhizomes of *A. calcarata* were collected, authenticated, shade-dried, powdered, and subjected to extraction using ethanol. The resulting ethanolic extract was subjected to preliminary phytochemical screening to identify the major classes of bioactive constituents. The bronchodilatory activity of the extract was evaluated on isolated goat tracheal chain preparations using histamine- and acetylcholine-induced contractions. Different concentrations of the extract were tested, and the percentage inhibition of induced tracheal contraction was determined in comparison with a suitable standard bronchodilator/antiasthmatic agent. The extract demonstrated concentration-dependent inhibition of contractile responses, indicating relaxation of airway smooth muscle. The observed inhibitory effect suggests that the extract may possess antihistaminic and/or anticholinergic mechanisms contributing to its bronchodilatory action.

The antiasthmatic activity of *A. calcarata* may be associated with its phytoconstituents, particularly flavonoids, phenolic compounds, tannins, and steroids, which have been reported to possess bronchodilatory, antioxidant, and anti-inflammatory properties. The findings indicate that the ethanolic extract of *Alpinia calcarata* rhizomes possesses promising airway smooth-muscle relaxant activity and may have potential as a natural source for the development of antiasthmatic agents. Further investigations involving mechanistic studies, bioactive compound isolation, and in-vivo validation are warranted to establish its therapeutic potential.

Keywords: *Alpinia calcarata*, ethanolic extract, antiasthmatic activity, bronchodilator, goat tracheal chain, airway smooth muscle, histamine, acetylcholine.

INTRODUCTION

Medicinal Plants and Herbal Medicines

Medicinal plants have played an important role in traditional systems of healthcare and continue to provide a source of pharmacologically active substances. Plant-derived preparations contain diverse chemical constituents, including phenolic compounds, flavonoids, terpenoids, alkaloids, tannins, glycosides and other secondary metabolites. Modern pharmacological research has therefore focused on the systematic evaluation of medicinal plants to identify biologically active constituents and to establish their pharmacological effects using reproducible experimental models.

The therapeutic use of medicinal plants should, however, be distinguished from the assumption that natural products are inherently safe or free from adverse effects. The pharmacological activity and safety of a herbal preparation depend on the plant species, plant part, extraction method, chemical composition, dose, route of administration and quality of the preparation. Consequently, experimental evaluation of traditionally used medicinal plants is necessary to establish their biological activity and safety profile.

Among medicinal plants of pharmacological interest, *Alpinia calcarata* Roscoe has attracted attention because of its traditional use and reported biological activities. Phytochemical investigations of its rhizomes have identified several classes of constituents, including polyphenols, tannins, flavonoids, steroid glycosides and alkaloids. Experimental studies have reported antioxidant, antifungal, anti-inflammatory, antinociceptive and other pharmacological activities of different preparations of the plant [1–4].

Asthma

Asthma is a heterogeneous chronic respiratory disease characterized by variable respiratory symptoms and variable expiratory airflow limitation. It is commonly associated with chronic airway inflammation and airway hyperresponsiveness, although these features are not individually sufficient to define asthma in every patient [5]. The clinical manifestations generally include wheezing, shortness of breath, chest tightness and cough, with the intensity of symptoms varying over time.

Asthma represents an important public-health problem worldwide. According to the World Health Organization, asthma affected approximately 363 million people in 2023 and caused an estimated 442,000 deaths. The disease occurs in individuals of all age groups and is particularly important among children and young adults [6].

Airway narrowing in asthma results from several interacting mechanisms, including contraction of airway smooth muscle, airway-wall inflammation and edema, mucus hypersecretion and, in chronic disease, structural airway remodeling [5,7].

Table 1.1. Major pathological features of asthma and their contribution to airway obstruction

Pathological feature	Principal mechanism	Consequence
Bronchoconstriction	Contraction of airway smooth muscle	Rapid reduction in airway calibre
Airway inflammation	Infiltration and activation of inflammatory cells	Increased airway reactivity and tissue swelling
Airway hyperresponsiveness	Exaggerated response to bronchoconstrictor stimuli	Increased tendency to bronchoconstriction
Mucus hypersecretion	Increased mucus production and secretion	Airway obstruction
Airway edema	Increased vascular permeability and	Reduction in airway lumen

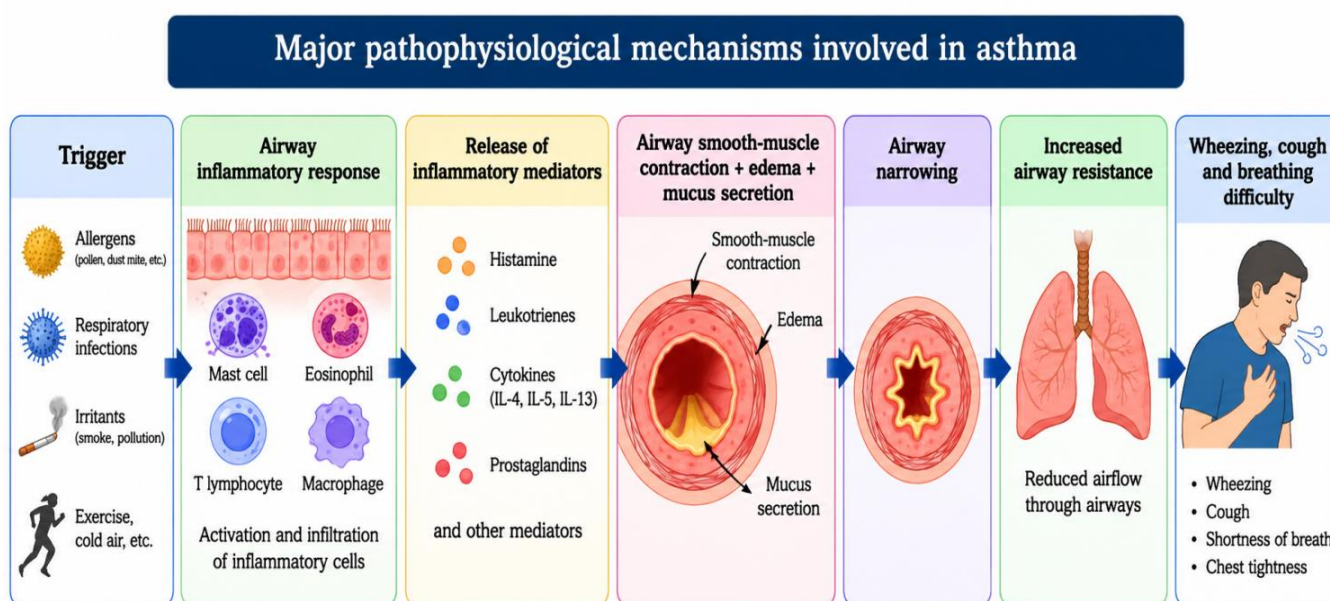
	airway-wall swelling	
Airway remodeling	Structural changes in airway wall and smooth muscle	Persistent alteration of airway function

References: [5,7,8]

Pathophysiology of Asthma

The pathophysiology of asthma is complex and involves interactions among airway smooth muscle, airway epithelium, immune cells, inflammatory mediators and structural components of the airway. The resulting physiological abnormalities include bronchoconstriction, airway inflammation, airway hyperresponsiveness, mucus hypersecretion and, in persistent disease, airway remodeling [7,8].

Figure 1.1. Major pathophysiological mechanisms involved in asthma



Trigger → Airway inflammatory response → Release of inflammatory mediators → Airway smooth-muscle contraction + edema + mucus secretion → Airway narrowing → Increased airway resistance → Wheezing, cough and breathing difficulty

Source: Adapted conceptually from [5,7,8].

Broncho Constriction

Bronchoconstriction is one of the major functional abnormalities responsible for the reduction in airway calibre during asthma exacerbations. Activation of airway smooth muscle by mediators such as histamine, leukotrienes and cholinergic mechanisms results in contraction of the bronchial smooth muscle. The resulting narrowing of the airway increases resistance to airflow and contributes to wheezing and dyspnea [7,8].

Because airway smooth-muscle contraction can be measured directly in isolated airway preparations, bronchoconstriction provides an important experimental endpoint for the preliminary pharmacological screening of potential bronchodilator agents.

Airway Inflammation

Airway inflammation involves a complex interaction among immune and structural cells, including mast cells, eosinophils, lymphocytes, macrophages and airway epithelial cells. Different inflammatory mediators and cytokines contribute to airway edema, mucus production, bronchial hyperresponsiveness and structural changes [5,7].

Although the isolated tracheal preparation does not reproduce the complete inflammatory environment of asthma, inhibition of agonist-induced airway contraction provides useful information regarding direct effects on airway smooth-muscle function.

Airway Hyperresponsiveness

Airway hyperresponsiveness refers to an exaggerated bronchoconstrictor response to stimuli that produce a lesser response in individuals without asthma. It is influenced by airway inflammation, epithelial dysfunction and changes in airway smooth-muscle properties [5,7].

Experimental airway preparations can be used to investigate the responsiveness of airway smooth muscle to defined contractile agonists such as histamine.

Mucus Hypersecretion and Airway Edema

Excessive mucus production and secretion can contribute substantially to airway obstruction, particularly during exacerbations. Inflammatory processes can also increase vascular permeability, resulting in airway-wall edema and further reduction in airway calibre [7,8]. These components are important in the complete pathophysiology of asthma but cannot be fully reproduced using an isolated tracheal preparation.

Airway Remodeling

Persistent airway inflammation can be associated with structural changes collectively described as airway remodeling. These may include alterations in airway smooth-muscle mass, subepithelial fibrosis, changes in extracellular matrix components and vascular alterations [7]. Airway remodeling represents a chronic process and is therefore outside the principal scope of an acute isolated goat tracheal chain experiment.

Role of Histamine in Airway Bronchoconstriction

Histamine is an important biological mediator involved in allergic and inflammatory responses. It acts through several histamine receptor subtypes, among which the H1 receptor has an important role in immediate allergic responses. Activation of H1 receptors in airway smooth muscle can promote contraction and contribute to bronchoconstriction [9,10].

Histamine H1 receptors are G-protein-coupled receptors associated predominantly with Gq/11 signalling. Receptor activation stimulates phospholipase C, resulting in formation of inositol trisphosphate and diacylglycerol, followed by an increase in intracellular calcium and activation of smooth-muscle contraction [9,10].

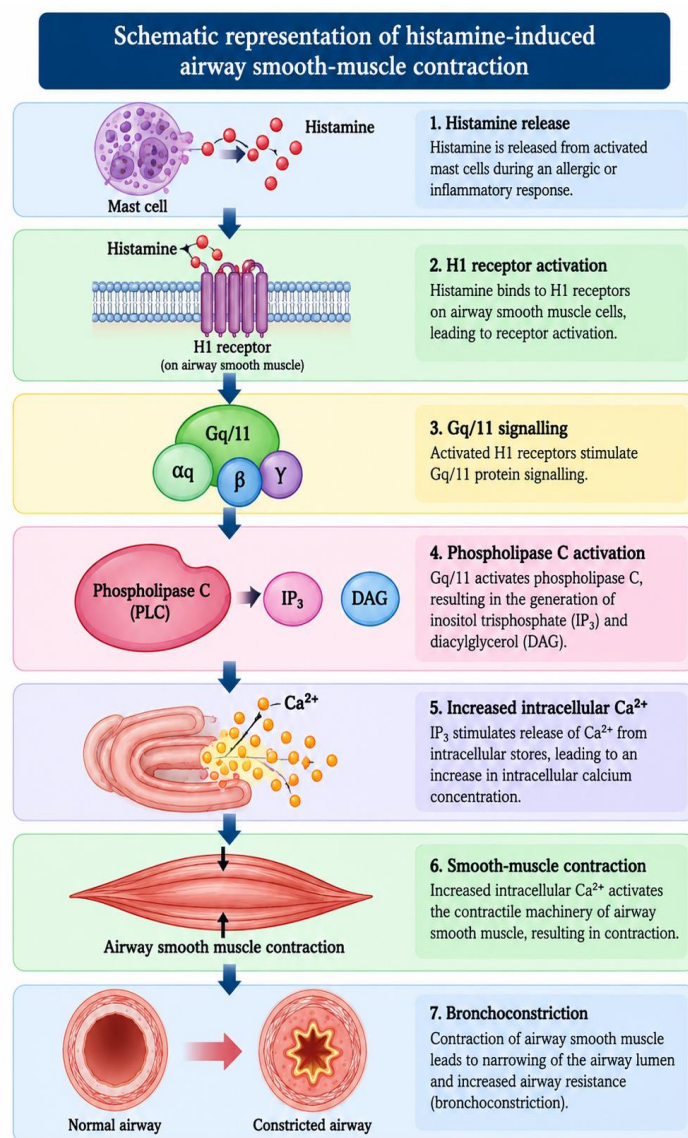
Table 1.2. Role of histamine in airway smooth-muscle contraction

Step	Pharmacological event	Result
1	Histamine is released during an allergic/inflammatory response	Increase in local histamine concentration
2	Histamine interacts with H1 receptors	H1-receptor activation
3	Gq/11-mediated signalling is activated	Phospholipase C activation

4	IP ₃ -mediated calcium mobilisation occurs	Increase in intracellular Ca ²⁺
5	Airway smooth muscle contracts	Reduction in airway calibre
6	Airway resistance increases	Bronchoconstrictor response

References: [9,10]

Figure 1.2. Schematic representation of histamine-induced airway smooth-muscle contraction



Histamine → H1 receptor → Gq/11 → Phospholipase C → IP₃/DAG → ↑ intracellular Ca²⁺ → Smooth-muscle contraction → Bronchoconstriction

Source: Based on histamine receptor pharmacology reported in [9,10].

This pharmacological pathway provides the rationale for using **histamine-induced contraction of isolated goat tracheal tissue** as an experimental model for evaluating the airway smooth-muscle relaxant potential of the ethanolic extract of *A. calcarata* rhizome.

Pharmacological Management of Asthma

The pharmacological management of asthma is directed primarily toward controlling airway inflammation,

preventing exacerbations and relieving airflow limitation. Contemporary asthma management emphasizes anti-inflammatory treatment, particularly inhaled corticosteroid-containing therapy, with treatment tailored according to disease severity, symptom pattern and individual response [5].

β₂-Adrenoceptor Agonists

β₂-adrenoceptor agonists produce relaxation of airway smooth muscle through activation of β₂-adrenoceptors and subsequent increases in intracellular cyclic AMP. Short-acting agents such as salbutamol provide rapid bronchodilation, whereas long-acting agents such as formoterol have a longer duration of action. Current asthma management generally uses long-acting β₂ agonists in appropriate combination with inhaled corticosteroid therapy rather than as monotherapy [5,11].

Inhaled Corticosteroids

Inhaled corticosteroids are important anti-inflammatory drugs in asthma management. They reduce airway inflammation and airway hyperresponsiveness and decrease the risk of exacerbations [5,11].

Leukotriene Modifiers

Leukotrienes contribute to bronchoconstriction, mucus secretion and inflammatory responses. Leukotriene receptor antagonists such as montelukast can therefore provide therapeutic benefit in selected patients [11].

Antimuscarinic Agents

Cholinergic stimulation of muscarinic receptors contributes to airway smooth-muscle contraction. Antimuscarinic agents such as ipratropium and tiotropium inhibit muscarinic-mediated bronchoconstriction and have a role in selected clinical situations [5,11].

Systemic Corticosteroids

Systemic corticosteroids have potent anti-inflammatory activity and are used particularly for the management of significant asthma exacerbations. Their prolonged use is limited by systemic adverse effects [5,11].

Table 1.3. Major pharmacological classes used in asthma management

Drug class	Principal action	Examples
Inhaled corticosteroids	Suppression of airway inflammation	Budesonide, fluticasone
β ₂ -adrenoceptor agonists	Airway smooth-muscle relaxation	Salbutamol, formoterol
Leukotriene receptor antagonists	Inhibition of leukotriene-mediated effects	Montelukast
Antimuscarinic agents	Reduction of cholinergic bronchoconstriction	Ipratropium, tiotropium
Systemic corticosteroids	Potent systemic anti-inflammatory action	Prednisolone
Methylxanthines	Bronchodilator activity through phosphodiesterase-related mechanisms	Theophylline

References: [5,11]

Medicinal Plants as a Source of Potential Antiasthmatic Agents

The limitations associated with adverse effects, variable response and long-term use of conventional medicines have encouraged continuing investigation of natural products as sources of pharmacologically active compounds. This does not imply that herbal preparations are inherently safer than conventional medicines; rather, their potential should be established through systematic pharmacological and toxicological evaluation.

Plant-derived compounds may influence several biological processes relevant to airway disease, including smooth-muscle contraction, oxidative stress and inflammatory signalling. Therefore, medicinal plants traditionally used for respiratory disorders represent a rational source for preliminary investigation of potential airway-relaxant or antiasthmatic activity.

The present investigation focuses on *Alpinia calcarata* because its rhizome has a history of traditional use in respiratory disorders and previous pharmacological studies have demonstrated biological activities that provide a basis for further investigation [1–4].

Alpinia Calcarata Roscoe

Alpinia calcarata Roscoe belongs to the family Zingiberaceae. It is a rhizomatous medicinal plant traditionally used in several regions of South and Southeast Asia. The rhizome is the principal plant part used in traditional medicinal preparations.

The plant has been reported in traditional medicine for conditions including cough, bronchitis and respiratory complaints. In addition to its traditional applications, experimental investigations have demonstrated several pharmacological activities of *A. calcarata* preparations [1,3,4].

Detailed botanical description, taxonomy, geographical distribution, vernacular names, morphology and other botanical characteristics should be presented separately in **Chapter 3: Plant Profile**, rather than repeating the complete plant description in the Introduction.

Phytochemical Significance of *Alpinia calcarata*

Phytochemical investigations have demonstrated the presence of several classes of constituents in *A. calcarata* rhizomes. Reported constituents include polyphenolic compounds, flavonoids, tannins, steroid glycosides and alkaloids [1,2].

These constituents are pharmacologically relevant because several phytochemical groups, particularly phenolic and flavonoid compounds, have been associated with antioxidant and anti-inflammatory effects. However, the presence of a particular phytochemical group alone does not establish that it is responsible for the observed pharmacological activity. Such relationships require appropriate pharmacological and, where possible, bioactivity-guided chemical investigation.

Table 1.4. Major phytochemical groups reported from *Alpinia calcarata* rhizomes

Phytochemical group	Reported occurrence	Possible pharmacological relevance
Flavonoids	Reported	Antioxidant and anti-inflammatory potential
Phenolic compounds	Reported	Antioxidant and biological activity
Tannins	Reported	Diverse biological activities
Steroid glycosides	Reported	Potential pharmacological activity

Phytochemical group	Reported occurrence	Possible pharmacological relevance
Alkaloids	Reported in phytochemical investigations	Diverse pharmacological effects
Volatile/terpenoid constituents	Reported particularly in essential oil	Potential biological activity

References: [1,2,3]

Pharmacological Significance of *Alpinia calcarata*

Previous experimental studies have reported multiple biological activities of *A. calcarata*. A review of the pharmacological literature identified antioxidant, antifungal, antibacterial, antinociceptive, anti-inflammatory, gastroprotective, antidiabetic and other activities associated with different preparations of the plant [1].

Arawwawala et al. reported anti-inflammatory activity of hot-water and hot-ethanolic preparations of *A. calcarata*, supporting the biological activity of the rhizome and providing additional pharmacological rationale for investigating its potential in inflammatory disorders [3].

The essential oil of the rhizome has also demonstrated antioxidant and antifungal activity, with 1,8-cineole reported as an important constituent in one investigation [4].

Table 1.5. Reported pharmacological significance of *Alpinia calcarata*

Pharmacological property	Evidence	Relevance to present research
Anti-inflammatory	Demonstrated experimentally in rhizome preparations	Relevant to inflammatory component of asthma
Antioxidant	Demonstrated in rhizome/essential-oil investigations	May support biological activity
Antifungal	Demonstrated experimentally	Evidence of biological activity
Antibacterial	Reported in pharmacological investigations	Demonstrates antimicrobial potential
Antinociceptive	Demonstrated experimentally	Indicates broader pharmacological activity
Traditional respiratory use	Reported for cough, bronchitis and respiratory ailments	Provides ethnopharmacological rationale

References: [1,3,4]

Rationale for Ethanolic Extraction

The biological activity of a plant preparation depends strongly on the extraction process and solvent used. Ethanol is frequently used for extraction of medicinal plant material because it can extract a broad range of moderately polar phytochemicals, including several phenolic and flavonoid constituents.

The present study therefore employs an **ethanolic extract of *A. calcarata* rhizome (EEAC)** for pharmacological investigation. The use of a defined extraction procedure also facilitates reproducibility

between experimental preparations.

Ex Vivo Isolated Goat Tracheal Chain Preparation

An isolated airway preparation provides a controlled experimental system in which the direct effects of pharmacological substances on airway smooth muscle can be evaluated. In the present study, the **isolated goat tracheal chain preparation** is selected as the ex vivo experimental model.

The goat tracheal chain has been used previously for pharmacological evaluation of medicinal plants with potential antiasthmatic or antihistaminic activity. For example, Pandit et al. investigated *Curculigo orchioides* using an isolated goat tracheal chain and reported inhibition of histamine-induced contraction. Similar goat tracheal chain approaches have been reported for *Ailanthus excelsa* and other medicinal plants [12–15].

Table 1.6. Published examples of goat tracheal chain models in medicinal-plant research

Medicinal plant	Preparation	Experimental model	Principal response
<i>Curculigo orchioides</i>	Rhizome extract	Isolated goat tracheal chain	Inhibition of histamine-induced contraction
<i>Ailanthus excelsa</i>	Aqueous stem-bark extract	Isolated goat tracheal chain	Inhibition of histamine-induced contraction
<i>Acalypha indica</i>	Plant extract	Goat tracheal chain	Investigation against histamine/acetylcholine-induced contraction
<i>Abrus precatorius</i>	Leaf extract	Histamine-induced goat tracheal chain	Antiasthmatic-related activity investigated

References: [12–15]

Published studies support the use of goat tracheal tissue as an **ex vivo airway smooth-muscle preparation** for preliminary screening of plant extracts against agonist-induced contraction.

Importantly, this preparation should not be described as a complete experimental model of asthma. It primarily evaluates **direct effects on airway smooth-muscle contractility** under controlled ex vivo conditions.

Table 1.7. Advantages and limitations of the isolated goat tracheal chain model

Parameter	Advantage / limitation	Significance
Direct measurement of airway contraction	Advantage	Provides a functional measure of airway smooth-muscle activity
Histamine-induced contraction	Advantage	Provides a defined bronchoconstrictor stimulus
Controlled experimental conditions	Advantage	Minimizes systemic physiological variables
Concentration-response assessment	Advantage	Permits pharmacological comparison

Systemic absorption	Limitation	Cannot be evaluated in isolated tissue
Metabolism and pharmacokinetics	Limitation	Not represented
Complete immune response	Limitation	Cannot reproduce whole-organism allergic inflammation
Airway remodeling	Limitation	Chronic structural changes are not represented
Clinical therapeutic efficacy	Limitation	Ex vivo activity cannot establish clinical efficacy

Histamine-Induced Contraction of Goat Trachea

Histamine-induced contraction provides a pharmacologically defined method for investigating the contractile response of airway smooth muscle. When histamine activates H1 receptors in airway tissue, intracellular signalling promotes smooth-muscle contraction [9,10].

If a test extract reduces the magnitude of histamine-induced contraction, the finding may indicate **airway** smooth-muscle relaxant or bronchodilator potential. Depending on the experimental design and appropriate pharmacological controls, such an effect may also support investigation of antihistaminic activity.

However, inhibition of histamine-induced contraction alone should not be interpreted as definitive evidence of selective H1-receptor antagonism. Receptor antagonism, intracellular calcium modulation, smooth-muscle relaxation and other mechanisms can potentially contribute to the observed response and require specific mechanistic experiments for confirmation.

This distinction is important for the interpretation of the present study.

Research Gap

Although *A. calcarata* has been investigated for several pharmacological activities, available evidence has not established all aspects of its potential activity on airway smooth muscle. Previous research has reported anti-inflammatory and other biological activities of the plant, while goat tracheal chain studies with other medicinal plants demonstrate the suitability of this ex vivo preparation for preliminary antiasthmatic-related screening [1–4,12–15].

The present study therefore seeks to investigate the effect of the ethanolic extract of *A. calcarata* rhizome on histamine-induced contraction of isolated goat tracheal chain.

The research gap can be summarized as follows

1. Calcarata has a traditional association with respiratory disorders.
2. Its rhizome contains several classes of pharmacologically relevant phytochemicals.
3. Anti-inflammatory and antioxidant activities have been experimentally reported.
4. Histamine is an established mediator capable of producing airway smooth-muscle contraction.
5. Goat tracheal chain preparations have been used for preliminary investigation of medicinal plants against histamine-induced airway contraction.
6. The specific effect of the **ethanolic rhizome extract of *A. calcarata* in the selected goat tracheal chain model** requires systematic evaluation.

Rationale of the Present Study

The rationale of the present investigation is based on the convergence of ethnomedicinal evidence, phytochemical evidence and pharmacological evidence.

Alpinia calcarata rhizomes have traditional respiratory applications, while experimental studies have demonstrated several biological activities of the plant [1,3,4]. Histamine-mediated airway contraction provides a measurable pharmacological response associated with airway smooth-muscle activation [9,10]. Furthermore, previous medicinal-plant investigations have demonstrated the use of isolated goat tracheal chain preparations for evaluating inhibition of histamine-induced contraction [12–15].

Therefore, evaluation of an ethanolic extract of *A. calcarata* rhizome using an isolated goat tracheal chain provides a rational preliminary approach for determining whether the extract possesses airway smooth-muscle relaxant/bronchodilator potential.

Significance of the Present Study

The present investigation may contribute preliminary pharmacological evidence regarding the activity of *A. calcarata* rhizome extract on airway smooth muscle.

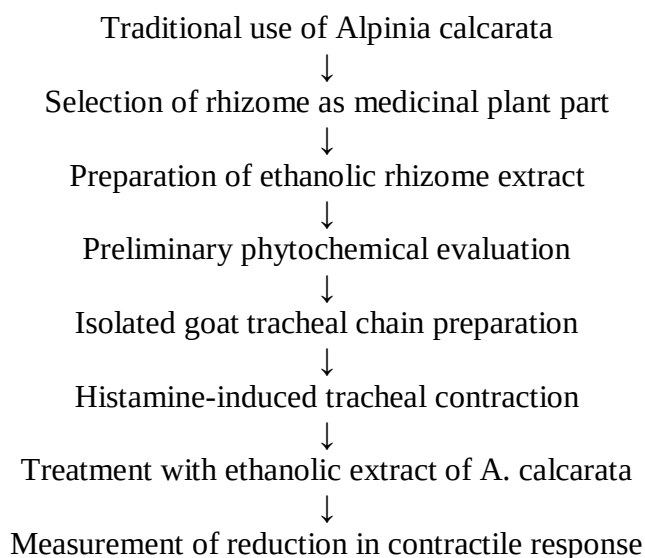
The significance of the study includes:

- evaluation of a traditionally used medicinal plant using an experimental pharmacological model;
- investigation of the direct effect of the ethanolic rhizome extract on airway smooth muscle;
- assessment of the ability of the extract to attenuate histamine-induced tracheal contraction;
- generation of preliminary evidence for possible bronchodilator/airway smooth-muscle relaxant activity;
- providing a basis for subsequent studies involving isolated receptors, molecular mechanisms, inflammatory models and in vivo asthma models.

The findings should be interpreted as preliminary pharmacological evidence and should not be directly equated with clinical efficacy in patients with asthma.

Conceptual Framework of the Present Study

Figure 1.3. Conceptual framework of the present investigation



↓
Pharmacological interpretation
↓

Preliminary assessment of airway smooth-muscle relaxant / bronchodilator potential

Table 1.8. Relationship between study components and expected pharmacological information

Study component	Purpose
A. calcarata rhizome	Source of test material
Ethanollic extraction	Preparation of test extract
Preliminary phytochemical screening	Identification of major phytochemical groups
Goat tracheal chain	Ex vivo airway smooth-muscle model
Histamine	Contractile agonist
Ethanollic extract of A. calcarata	Test substance
Tracheal contraction	Primary pharmacological response
Percentage inhibition/attenuation	Quantitative assessment of extract effect
Statistical analysis	Determination of significance
Overall interpretation	Assessment of preliminary airway smooth-muscle relaxant potential

RESULT AND DISCUSSION

Collection and Authentication of Plant Material

The rhizomes of *Alpinia calcarata* Roscoe were collected and authenticated prior to extraction. The relevant authentication certificate of the plant material is provided in the **Annexure**.

Extraction of Plant Material

The collected rhizomes of *Alpinia calcarata* were cleaned thoroughly, washed and shade-dried. The dried rhizomes were reduced to coarse powder and subjected to Soxhlet extraction using ethanol. The resulting extract was concentrated to obtain the dried ethanollic extract of *Alpinia calcarata* rhizomes (EEAC).

A total of **250 g of powdered rhizome** yielded **43 g of dried ethanollic extract**.
The percentage yield was calculated using the following formula:

$$\begin{aligned} \text{Percentage yield (\% w/w)} &= \frac{\text{Weight of dried extract}}{\text{Weight of powdered plant material}} \times 100 \\ &= \frac{43}{250} \times 100 = 17.2\% \end{aligned}$$

Thus, the percentage yield of the ethanollic extract was found to be **17.2% w/w**.

Table 7.1 Extraction yield of ethanolic extract of *Alpinia calcarata* rhizome

S. No.	Parameter	Observation
1	Weight of powdered rhizome	250 g
2	Extraction method	Soxhlet extraction
3	Solvent used	Ethanol
4	Weight of dried extract	43 g
5	Percentage yield	17.2% w/w

Preliminary Phytochemical Screening of EEAC

The ethanolic extract of *Alpinia calcarata* rhizomes was subjected to preliminary phytochemical screening to detect different classes of phytoconstituents.

The extract showed positive tests for **carbohydrates, proteins, steroids, cardiac glycosides, flavonoids, alkaloids, tannins and phenolic compounds**. Tests for amino acids, fats and oils, anthraquinone glycosides, saponin glycosides, cyanogenic glycosides and coumarin glycosides were negative.

Table 7.2 Preliminary phytochemical screening of ethanolic extract of *Alpinia calcarata* rhizome

6	Phytoconstituent	Result
1	Carbohydrates	+
2	Proteins	+
3	Amino acids	—
4	Fats and oils	—
5	Steroids	+
6	Cardiac glycosides	+
7	Anthraquinone glycosides	—
8	Saponin glycosides	—
9	Cyanogenic glycosides	—
10	Coumarin glycosides	—
11	Flavonoids	+
12	Alkaloids	+
13	Tannins	+
14	Phenolic compounds	+

+ = Present; **—** = Absent

The phytochemical screening demonstrated the presence of several classes of primary and secondary phytoconstituents in EEAC. The presence of flavonoids, phenolic compounds, tannins and alkaloids indicates a chemically diverse ethanolic rhizome extract. The original experimental record reports the detection of several of these constituents in the ethanolic extract.

Acute Oral Toxicity Study

The acute oral toxicity study of the ethanolic extract of *Alpinia calcarata* rhizomes was performed according to **OECD Guideline 423** using **Swiss albino mice**.

The animals were observed following oral administration of the extract for any signs of toxicity or abnormal behavioural changes. Particular attention was given to changes in motor activity, salivation, convulsions, coma and mortality during the observation period.

No mortality or apparent morbidity was observed at the highest tested dose of **2000 mg/kg**. Based on the absence of mortality at this dose, **100 mg/kg and 200 mg/kg** were selected as experimental doses.

Table 7.3 Summary of acute oral toxicity study of EEAC

Parameter	Observation
Guideline followed	OECD 423
Experimental animals	Swiss albino mice
Route of administration	Oral
Highest tested dose	2000 mg/kg
Mortality	Not observed
Apparent morbidity	Not observed
Selected experimental doses	100 and 200 mg/kg

The experimental record likewise reports that administration of EEAC up to 2000 mg/kg produced neither mortality nor signs of morbidity and that 100 and 200 mg/kg were selected for further experimental work.

Ex Vivo Evaluation of Antiasthmatic Activity

The ex vivo airway smooth-muscle activity of EEAC was evaluated using an **isolated goat tracheal chain preparation**.

Goat tracheal tissue was obtained from a nearby slaughterhouse and transported to the laboratory in cold Krebs–Henseleit solution. The experimental procedure was initiated within approximately one hour of tissue collection. The isolated tracheal preparation was maintained in Krebs–Henseleit solution and continuously aerated with carbogen at **37 ± 0.5°C**.

The preparation was allowed to equilibrate for at least **40 minutes** before commencement of the experiment. Krebs–Henseleit solution was replaced at approximately 15-minute intervals.

Histamine at a concentration of **10 µg/mL** was used to induce contraction. The response to different administered volumes of histamine was recorded in the absence and presence of EEAC. EEAC was evaluated at a concentration of **1 mg/mL**. **Chlorpheniramine maleate (1 µg/mL)** was used as the standard drug.

The contractile response was expressed as percentage maximum contraction. The results were expressed as

Mean $\pm$ SEM, with **n = 6**. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test.

Histamine-Induced Contraction of Isolated Goat Tracheal Chain

Histamine produced a progressive increase in the contractile response of the isolated goat tracheal chain with increasing administered volume of the histamine solution.

The contractile response increased from **38.46 $\pm$ 1.58%** at 0.1 mL to **100.00 $\pm$ 1.07%** at 3.2 mL. The response at 3.2 mL was considered the maximum contractile response.

Table 7.4 Histamine-induced contraction of isolated goat tracheal chain

S. No.	Histamine (10 μ g/mL), volume (mL)	Maximum contraction (%)
1	0.1	38.46 $\pm$ 1.58
2	0.2	53.48 $\pm$ 4.23
3	0.4	61.50 $\pm$ 3.89
4	0.8	73.07 $\pm$ 2.32
5	1.6	84.60 $\pm$ 2.13
6	3.2	100.00 $\pm$ 1.07

Values are expressed as Mean $\pm$ SEM; n = 6.

The findings demonstrated a clear histamine-induced contractile response in the isolated goat tracheal preparation and provided the control response for evaluating the effects of EEAC and chlorpheniramine maleate.

Effect of EEAC on Histamine-Induced Contraction

The effect of EEAC on histamine-induced contraction was investigated at a concentration of **1 mg/mL**.

EEAC attenuated the contractile response induced by histamine at all tested histamine volumes. At 0.1 mL histamine, the control response was **38.46 $\pm$ 1.58%**, whereas the response in the presence of EEAC was **30.76 $\pm$ 1.32%**.

At 0.2, 0.4, 0.8, 1.6 and 3.2 mL histamine, the corresponding control responses of **53.48 $\pm$ 4.23%**, **61.50 $\pm$ 3.89%**, **73.07 $\pm$ 2.32%**, **84.60 $\pm$ 2.13%** and **100.00 $\pm$ 1.07%** were reduced to **46.15 $\pm$ 2.91%**, **53.48 $\pm$ 3.31%**, **65.30 $\pm$ 1.76%**, **69.20 $\pm$ 1.09%** and **76.92 $\pm$ 2.11%**, respectively, in the presence of EEAC.

The reductions in contractile response were statistically significant compared with the corresponding histamine control responses. The response was significant at **p < 0.01** for histamine volumes of 0.1–1.6 mL and at **p < 0.05** for 3.2 mL.

Table 7.5 Effect of EEAC on histamine-induced contraction of isolated goat tracheal chain

S. No.	Histamine volume (mL)	Control: Histamine (%) maximum contraction	Histamine + EEAC (1 mg/mL) (%) maximum contraction	% Inhibition
1	0.1	38.46 $\pm$ 1.58	30.76 $\pm$ 1.32**	20.02

2	0.2	53.48 ± 4.23	46.15 ± 2.91**	13.71
3	0.4	61.50 ± 3.89	53.48 ± 3.31**	13.04
4	0.8	73.07 ± 2.32	65.30 ± 1.76**	10.63
5	1.6	84.60 ± 2.13	69.20 ± 1.09**	18.20
6	3.2	100.00 ± 1.07	76.92 ± 2.11*	23.08

**Values are expressed as Mean ± SEM; n = 6. *p < 0.05, p < 0.01 versus corresponding control. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test.

The percentage inhibition of histamine-induced contraction was calculated using:

$$\% \text{ Inhibition} = \frac{\text{Control response} - \text{Test response}}{\text{Control response}} \times 100$$

The percentage inhibition produced by EEAC ranged from **10.63% to 23.08%**. The highest calculated inhibition was observed at 3.2 mL histamine (**23.08%**).

Although EEAC attenuated histamine-induced contraction at all tested histamine volumes, the inhibitory response did not show a progressive or monotonic increase with increasing histamine volume. Therefore, the findings are interpreted as **attenuation of histamine-induced tracheal contraction under the tested experimental conditions**, rather than as a dose-dependent inhibitory effect.

Effect of Chlorpheniramine Maleate on Histamine-Induced Contraction

Chlorpheniramine maleate was used as the **standard drug** at a concentration of **1 µg/mL** in the isolated goat tracheal chain preparation.

Chlorpheniramine attenuated histamine-induced contraction at all tested histamine volumes. At 0.1 mL histamine, the control response of **38.46 ± 1.58%** was reduced to **32.76 ± 1.52%** in the presence of chlorpheniramine.

Similarly, at 0.2, 0.4, 0.8, 1.6 and 3.2 mL histamine, the control responses of **53.48 ± 4.23%**, **61.50 ± 3.89%**, **73.07 ± 2.32%**, **84.60 ± 2.13%** and **100.00 ± 1.07%**, respectively, were reduced to **48.35 ± 2.30%**, **55.48 ± 3.80%**, **67.32 ± 2.56%**, **71.21 ± 3.19%** and **81.18 ± 3.10%**, respectively.

Table 7.6 Effect of chlorpheniramine maleate on histamine-induced contraction of isolated goat tracheal chain

S. No.	Histamine volume (mL)	Control: Histamine (%) maximum contraction	Histamine + Chlorpheniramine (1 µg/mL) (%) maximum contraction	% Inhibition

1	0.1	38.46 ± 1.58	32.76 ± 1.52**	14.82
2	0.2	53.48 ± 4.23	48.35 ± 2.30**	9.59
3	0.4	61.50 ± 3.89	55.48 ± 3.80**	9.79
4	0.8	73.07 ± 2.32	67.32 ± 2.56**	7.87
5	1.6	84.60 ± 2.13	71.21 ± 3.19**	15.83
6	3.2	100.00 ± 1.07	81.18 ± 3.10*	18.82

**Values are expressed as Mean ± SEM; n = 6. *p < 0.05, p < 0.01 versus corresponding control.

The calculated percentage inhibition produced by chlorpheniramine ranged from **7.87% to 18.82%**. The maximum inhibition was observed at 3.2 mL histamine, where the response was reduced from 100.00% to 81.18%, corresponding to **18.82% inhibition**.

Comparative Evaluation of EEAC and Chlorpheniramine Maleate

The percentage inhibition produced by EEAC and chlorpheniramine maleate was compared descriptively to assess their effects on histamine-induced contraction of the isolated goat tracheal chain.

Table 7.7 Comparative percentage inhibition produced by EEAC and chlorpheniramine maleate

Histamine volume (mL)	EEAC (1 mg/mL) (% inhibition)	Chlorpheniramine (1 µg/mL) (% inhibition)
0.1	20.02	14.82
0.2	13.71	9.59
0.4	13.04	9.79
0.8	10.63	7.87
1.6	18.20	15.83
3.2	23.08	18.82

EEAC produced numerically greater inhibition than chlorpheniramine at each of the tested histamine volumes. The maximum calculated inhibition was **23.08% for EEAC** and **18.82% for chlorpheniramine** at 3.2 mL histamine.

However, EEAC and chlorpheniramine were evaluated at different concentrations, and the present analysis was based on comparison of each treatment with the corresponding histamine control. Therefore, the numerical difference should **not be interpreted as evidence that EEAC is more potent or more efficacious than chlorpheniramine**. A direct statistical comparison between the two treatments would be required to establish such a conclusion.

Statistical Analysis

The experimental observations were expressed as **Mean $\pm$ SEM**, with **n = 6** for each experimental group.

The data were statistically analysed using **one-way analysis of variance (ANOVA)** followed by **Dunnett's multiple-comparison test**. The treated groups were compared with the corresponding histamine control.

The level of statistical significance was considered as:

- ***p < 0.05** — statistically significant
- ****p < 0.01** — highly statistically significant

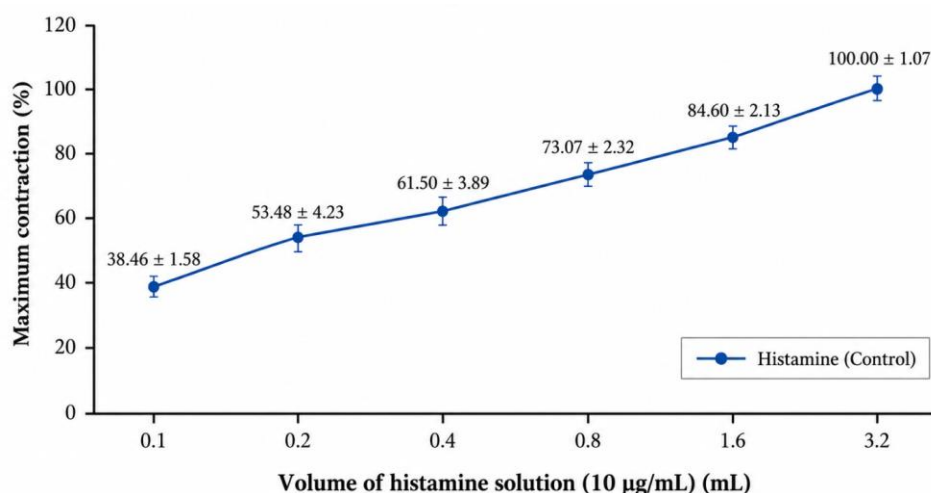
EEAC at 1 mg/mL produced statistically significant attenuation of histamine-induced contraction at all tested histamine volumes. The observed response was significant at $p < 0.01$ at histamine volumes of 0.1, 0.2, 0.4, 0.8 and 1.6 mL, and at $p < 0.05$ at 3.2 mL.

Chlorpheniramine maleate at 1 μ g/mL also produced statistically significant attenuation of histamine-induced contraction at all tested histamine volumes.

The statistical methodology and significance notation are consistent with the original experimental record.

Graphical Presentation of Ex Vivo Findings

Figure 7.1 Histamine-induced concentration/volume-response curve in isolated goat tracheal chain



Values are expressed as Mean $\pm$ SEM ($n = 6$).

The graph present the relationship between the administered volume of histamine solution (10 μ g/mL) and the percentage maximum contraction of the isolated goat tracheal preparation.

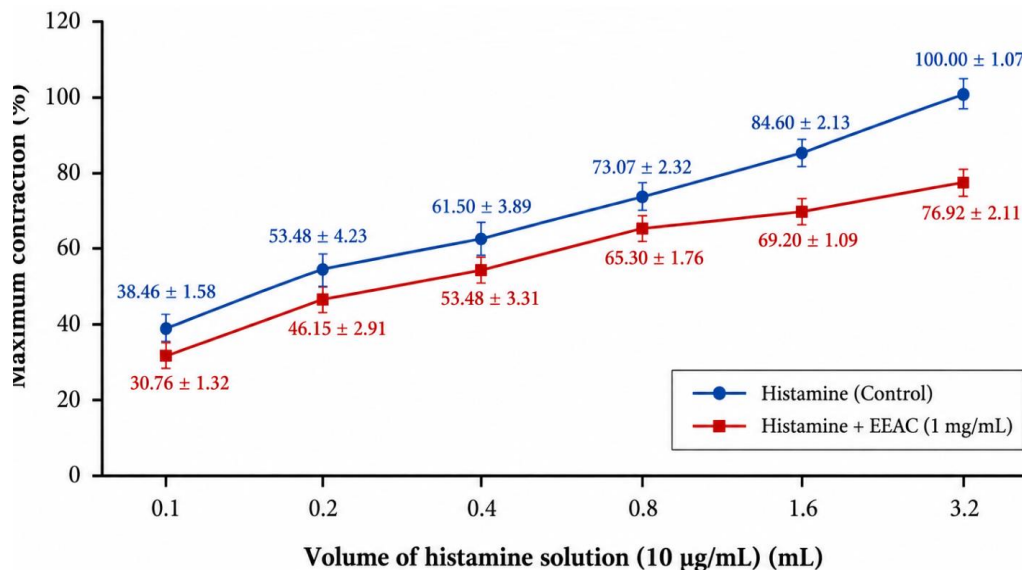
X-axis: Volume of histamine solution (mL)
Y-axis: Maximum contraction (%)

The control values to be plotted are

Histamine volume (mL)	Control response (%)
0.1	38.46
0.2	53.48
0.4	61.50

0.8	73.07
1.6	84.60
3.2	100.00

Figure 7.2 Effect of EEAC on histamine-induced contraction of isolated goat tracheal chain



Values are expressed as Mean ± SEM (n = 6).

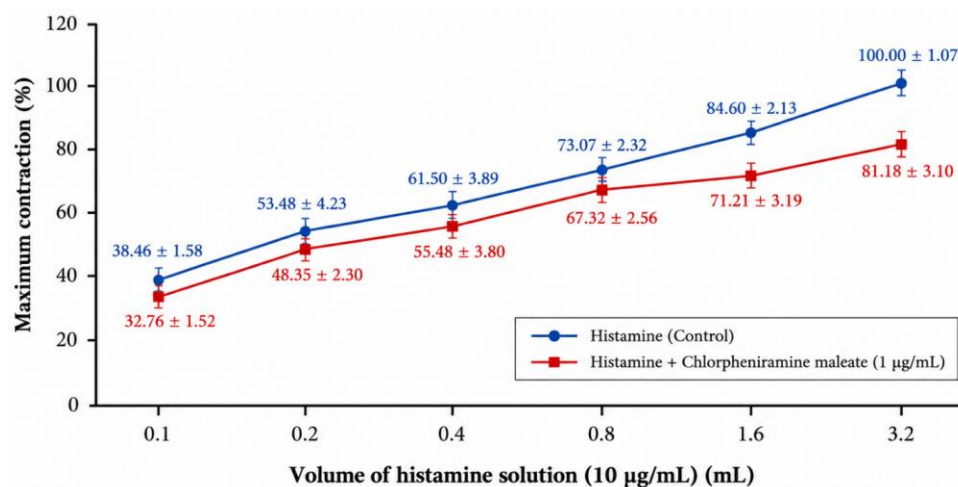
The graph compares the histamine control with histamine + EEAC (1 mg/mL).

X-axis: Volume of histamine solution (mL)

Y-axis: Maximum contraction (%)

The original experimental dataset contains the corresponding control and EEAC responses.

Figure 7.3 Effect of chlorpheniramine maleate on histamine-induced contraction of isolated goat tracheal chain



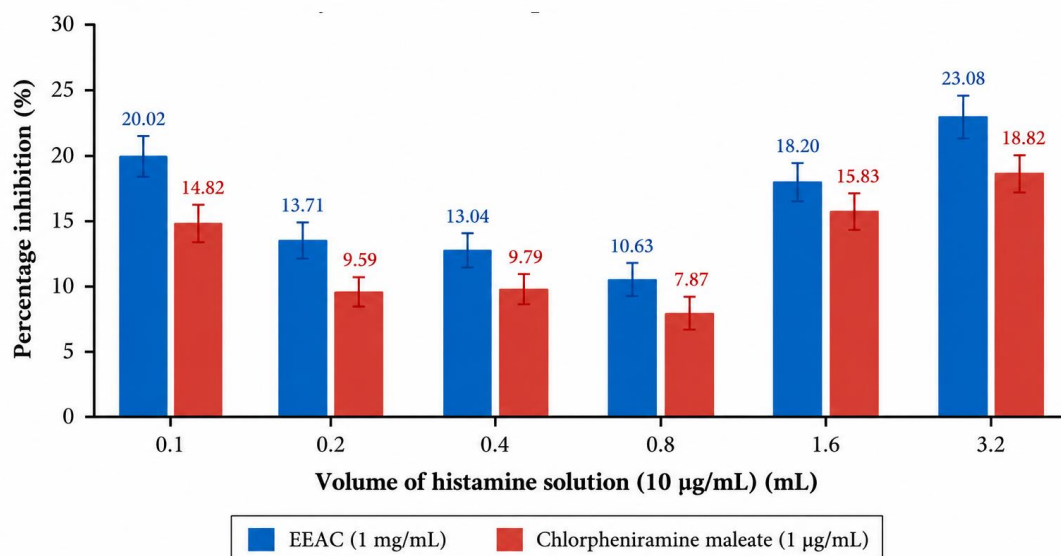
Values are expressed as Mean ± SEM (n = 6).

The graph compare the histamine control with histamine + chlorpheniramine maleate (1 µg/mL).

X-axis: Volume of histamine solution (mL)

Y-axis: Maximum contraction (%)

Figure 7.4 Comparative percentage inhibition of histamine-induced contraction by EEAC and chlorpheniramine maleate



Values are expressed as Mean $\pm$ SEM ($n = 6$).

The graph presents the calculated percentage inhibition produced by:

- EEAC — 1 mg/mL
- Chlorpheniramine maleate — 1 µg/mL

at each tested histamine volume.

SUMMARY OF RESULTS

The present investigation yielded the following principal findings

1. Soxhlet extraction of **250 g of powdered *Alpinia calcarata* rhizome** using ethanol yielded **43 g of dried extract**, corresponding to a percentage yield of **17.2% w/w**.
2. Preliminary phytochemical screening of EEAC demonstrated the presence of **carbohydrates, proteins, steroids, cardiac glycosides, flavonoids, alkaloids, tannins and phenolic compounds**.
3. The acute oral toxicity study conducted according to **OECD Guideline 423** in Swiss albino mice revealed **no mortality or apparent morbidity at 2000 mg/kg**.
4. Based on the acute toxicity findings, **100 mg/kg and 200 mg/kg** were selected as experimental doses.
5. Histamine at **10 µg/mL** produced a progressive contractile response in the isolated goat tracheal chain preparation.
6. EEAC at **1 mg/mL** **significantly attenuated histamine-induced contraction** at all tested histamine volumes.
7. The calculated percentage inhibition produced by EEAC ranged from **10.63% to 23.08%**.
8. Chlorpheniramine maleate at **1 µg/mL**, used as the standard drug, also significantly attenuated histamine-induced contraction.

9. The calculated percentage inhibition produced by chlorpheniramine ranged from **7.87% to 18.82%**.
10. EEAC showed numerically greater inhibition than chlorpheniramine at the tested histamine volumes; however, this represents a **descriptive comparison** and does not establish greater potency or efficacy.
11. The ex vivo findings demonstrate that EEAC possesses **airway smooth-muscle relaxant activity against histamine-induced contraction in the isolated goat tracheal chain preparation**, supporting its potential antihistaminic/bronchodilator activity.

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