The Protective Role of *Lannea coromandelica* (Houtt.) Merr. against Histamine Release and Action: Insights from *In vitro, In vivo* Investigations

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ABSTRACT

This study aims to evaluate the antihistaminic potential of the plant extract from *Lannea coromandelica* using both in vitro and in vivo models. In vitro antihistaminic effects were studied using isolated guinea pig ileum to assess dose-dependent inhibitory impacts on histamine-induced contractions. Mast cell density was evaluated using a mast cell count model, calculating the average number of mast cells per unit area in the mesentery. For in vivo assessments, a histamine aerosol-induced bronchospasm model in guinea pigs was used, where pre-convulsive dyspnea (PCD) onset time was noted as pre-convulsive time (PCT). Additionally, a clonidine-induced mast cell degranulation model in rats was employed, with cells stained using 1% toluidine blue to count intact and degranulated mast cells. The *Lannea coromandelica* extract exhibited a dose-dependent inhibition of histamine-induced contractions in isolated guinea pig ileum. Similarly, the extract inhibited mast cell degranulation in a dose-dependent manner, with a higher dose of 400 mg/kg proving more effective than a lower dose of 200 mg/kg. Acute toxicity studies confirmed the safety of the extract at moderate doses, revealing no toxic symptoms at a dosage of 2000 mg/kg body weight. Importantly, the extract significantly increased PCT in guinea pigs and reduced the percentage of disrupted mast cells induced by clonidine. *Lannea coromandelica* shows promising antihistaminic properties, effectively inhibiting histamine-induced bronchospasm and mast cell degranulation, which can be an option for the development of antiasthmatic drugs.

Keywords: *Lannea coromandelica,* antihistamine, guinea pigs, Wistar rats.

1. INTRODUCTION:

Asthma is a chronic inflammation that drives the attention of entire mankind because of its severity in patients [1]. The inflammatory response in asthma involves various immune cells, including mast cells, eosinophils, T lymphocytes, and dendritic cells. These cells release pro-inflammatory mediators, such as histamine, leukotrienes, and cytokines, contributing to airway inflammation and hyperresponsiveness [2][3]. Histamine is a bioactive amine released primarily from mast cells and, to a lesser extent, from other cells such as basophils. Histamine is a key mediator in allergic reactions and induces bronchoconstriction, specifically in asthma patients even at low doses. The airway hyperresponsiveness in asthma causes difficulty in breathing, wheezing, and coughing [4]. Controlling histamine release is crucial in managing asthma, given that histamine plays a significant role in eliciting and exacerbating asthma symptoms. Antihistaminic agents, such as H1 receptor antagonists, serve a supportive role in asthma treatment, particularly for symptoms triggered by allergies. They aid in bronchodilation, similar to β2-stimulants, by blocking histamine's action, thus reducing airway smooth muscle contraction in allergic asthma [5].

Lannea coromandelica is a deciduous tree that belongs

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to the family Anacardiaceae. It is popularly known as the Indian ash tree and is native to South and Southeast Asia [6]. The tree is 10-20 meters tall. Leaves are alternate, opposite, estipulate, and swollen at the base. Flowers are unisexual and yellowish-green; the fruit is a drupe with a hard stone and compressed seeds. It is geographically distributed from Sri Lanka to Southern China [7].

The traditional applications of L. coromandelica include treatment for hepatitis, diabetes, ulcers, heart disease, and dysentery [8]. The aerial parts are enriched with phytochemicals such as anthraquinones, flavonoids like (+)-leucocyanidin, rutin, quercetin, morin, and isoquercitrin; flavonoid glycosides like guaijaverin; and tannins like ellagic acid, which have been isolated from various parts of L. coromandelica. Pharmacologically, L. coromandelica is reported to be effective in controlling glucose levels and has cardioprotective, anti-hyperlipidemic, and diverse pharmacological activities. Its gum exhibits drug delivery potential, and the bark extract demonstrates antibacterial and anticancer effects [9][10][11].

L. coromandelica possesses potent anti-inflammatory and immunomodulatory properties, which could make it a promising natural remedy for asthma [12]. This study aims to address this gap by providing more robust evidence for the therapeutic potential of L. coromandelica as an antihistaminic agent for asthma through in vitro and in vivo studies for the development of new and effective treatments for asthma.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

The leaves of *Lannea coromandelica* (Houtt.) Merr. were collected from the forest areas of Tirupati, Andhra Pradesh, India, in February. The leaves were dried, powdered, and subjected to Soxhlet extraction using ethanol. The solvent was removed, and the percentage yield was calculated using the formula:

% yield= Weight of the extract obtained/ Weight of the raw material × 100

2.1. Preliminary phytochemical analysis

Standard protocols were used to qualitatively identify a variety of phytochemicals present in the ethanolic extract of *L. coromandelica* [13][14][15][16].

2.2. Total flavonoid content

The Dowd colorimetric method using $AlCl_3$ was adapted to determine the total flavonoid content in the ethanolic extract of *L. coromandelica* [17].

2.3. *In vitro* antihistaminic activity

Intestines were dissected from anesthetized guinea pigs, and the distal section was divided into 2-3 cm long pieces. They were placed in a 25 mL organ bath containing Tyrode physiological solution at a constant temperature of 37°C, with a steady tension of 1 g and continuous aeration for 30 minutes. Various concentrations of histamine from a 25 µg/mL stock (0.1, 0.2, 0.4, 0.8, 1.6 mL) were used to identify the submaximal response. The concentration that induced a submaximal response was utilized for subsequent testing. Each test sample, including the standard drug (Mepyramine 0.04 µg/mL) and various concentrations of *L. coromandelica* extract (4 mg/mL and 8 mg/mL), was individually added after a five-minute interval to observe their impact on histamine-mediated contraction. The changes in tissue contraction were accurately recorded using a micro dynamometer [18].

2.4. Mesenteric Mast Cell Count

Adult Wistar rats were anesthetized and euthanized through cervical dislocation. The mesentery was dissected from the abdominal cavity and placed in a petri dish containing a 10% neutral buffered formalin fixative solution. Three batches were made, and Ketotifen (10 µg/mL), and 200 mg/mL and 400 mg/mL concentrations of *L. coromandelica* extract were added respectively and incubated for 15 minutes at 37°C. The mesentery was embedded in paraffin and sliced into thin sections measuring 5-6 microns after fixation. These sections were then stained and observed under a microscope, utilizing resolutions of 40x and 60x, to identify and quantify the

mast cells within a specific unit area of the mesentery. The mast cell count was calculated as the average number of mast cells per unit area of the mesentery [19].

2.5. Acute toxicity studies

The acute toxicity of the test extracts was assessed following the guidelines set by the OECD. The "up and down" method was employed with a limit test conducted at a 2000 mg/kg body weight dosage, using a progression factor of 1.3. The animals were monitored for 14 days, and any deaths occurring within this period were documented [20].

2.6. Histamine-induced bronchospasm in guinea pigs

The histamine-induced bronchospasm in guinea pigs model replicates pathophysiological conditions similar to human asthma, characterized by airway inflammation, mucus secretion, and bronchospasm. Bronchospasm was experimentally induced in guinea pigs by exposing them to histamine aerosol. Guinea pigs of both genders were selected and divided into the following groups (n = 5): Group I - Control, Group II - Ketotifen (10 µg/mL), and Group III and IV - Extracts (Low and High concentrations). The animals displayed progressive difficulty breathing (dyspnea) when exposed to histamine aerosol at a pressure of 40 mmHg from a nebulizer placed in the histamine chamber (manufactured by M/s Inco Ambala, India). The time at which pre-convulsive dyspnea (PCD) began was recorded as the pre-convulsive time (PCT), and as soon as PCD occurred, the animals were immediately transferred to an area with fresh air. On the 5th day, the PCT was measured 2 hours after the last administration of the extracts [21].

The percentage increase in PCT was estimated using the equation:

$$\% PCT = (1 - T1/T2) \times 100$$

Where, T1 = time for PCD onset on day 0 , T2 = time for the PCD onset on day 5

2.7. The Clonidine-induced mast cell degranulation in rats

Wistar rats were divided into four groups, each consisting of five rats. Group I received an oral administration of 5 mL/kg of the vehicle. Group II received an intraperitoneal injection of 50 mg/kg of sodium cromoglycate. Group III and IV were orally administered low and high doses of the *L. coromandelica* extract, respectively. On the seventh day, the peritoneal cavity was injected with 10 mL of normal saline solution, followed by a gentle abdominal massage for 90 seconds, and the fluid containing mast cells was aspirated and collected in a siliconized test tube containing 7-10 mL of RPMI-1640 Medium (pH 7.2-7.4). The mast cell suspension, approximately 1×1061 \times 10^61×106 cells/mL, was then challenged with a 0.5 µg/mL clonidine solution and stained with 1% toluidine blue. Under a high-power microscope, 100 cells were counted from various visual areas, and the number of intact and degranulated cells was recorded [22].

3. RESULTS

3.1. Preliminary phytochemical analysis

The percentage yield was found to be 7.5%, and standard protocols were followed to conduct the preliminary phytochemical analysis of *L. coromandelica*. The analysis revealed the presence of various compounds such as alkaloids, glycosides, flavonoids, terpenoids, steroids, tannins, proteins, carbohydrates, amino acids, and saponins (Table 1).

Table 1. Preliminary phytochemical analysis of *L. coromandelica*

Phytochemicals	***Lannea coromandelica***
Alkaloids	+
Glycosides	+
Flavonoids	++
Terpenoids	++
Steroids	+
Tannins	++
Proteins	++
Carbohydrates	++
Amino acids	+
Saponins	+

+ Present,- Absent

3.2. Total flavonoid content

The total flavonoid content in the plant extract was determined utilizing the colorimetric method with $AlCl_3$. The total flavonoid content of the extract was calculated and represented as milligrams of quercetin equivalents (QE) per gram of dry weight sample (mg/g). The ethanolic extract of *L. coromandelica* has a flavonoid content of 40.7±1.53 mg QE/g under the given conditions and procedures.

3.3. *In vitro* antihistaminic activity

The results in Table 2 demonstrate the effect of *L. coromandelica* on histamine-induced contraction in isolated guinea pig ileum. When treated with *L. coromandelica* at a lower dosage, i.e., 4 mg/mL, the mean contraction reduced to 75.17±2.36 units, significantly lower than the control group, suggesting the ability to inhibit histamine-induced contractions. However, treatment with a higher dosage (8 mg/mL) further decreased the mean contraction to 65.93±1.46 units.

Table 2. Effect of *L. coromandelica* on histamine-induced contraction in isolated guinea pig ileum

Concentration of Histamine	Control group	Mepyramine Group	*L. coromandelica* (4mg/mL)	*L. coromandelica* (8mg/mL)
1.6 µg/mL	91.25±1.27	1.48±1.25**	75.17±2.36*	65.93±1.46**

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with $^{***}P<0.001$ and $^{**}P<0.01$, $^{*}P<0.05$ being considered statistically significant.

3.4. Mesenteric Mast Cell Count

The experiment investigated the effect of ethanolic extracts on mast cell degranulation. Ketotifen, a known mast cell stabilizer, shows significant inhibition of mast cell degranulation by 82.61%. *L. coromandelica* at the lower dose shows a 45.18% inhibition of degranulation, which is not statistically significant compared to the control group or Ketotifen. On the other hand, the higher dose of *L. coromandelica* extract (400 mg/mL) shows a 59.37% inhibition of mast cell degranulation, which is higher than the lower dose and is statistically significant compared to the control group (Table 3).

Table 3. Effect of *L. coromandelica* on mast cell degranulation in rats

Treatment	% inhibition of degranulation
Ketotifen	82.61±1.18
L. coromandelica (200mg/ml)	45.18±2.67^{ns}
L. coromandelica (400mg/ml)	59.37±1.64**

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with $^{***}P<0.001$ and $^{**}P<0.01$, $^{*}P<0.05$ being considered statistically significant.

3.5. Acute toxicity studies

Acute toxicity studies, performed according to OECD guidelines using the "up and down" method, showed that *L. coromandelica* does not exhibit any toxicity symptoms in rats at a dose of 2000 mg/kg body weight orally. Therefore, it is considered safe to use the extract at medium doses. The working dose was calculated as 1/5th and 1/10th of the highest safe dose, i.e., 400 and 200 mg/kg body weight, respectively.

3.6. The Clonidine-induced mast cell degranulation in rats

The percentage of disrupted mast cells in the control group was 80.36%. The administration of sodium

cromoglycate significantly reduced the percentage of disrupted mast cells to 25.16%. In the *L. coromandelica* treatment groups, the percentage of disrupted mast cells was 71.55% at 200 mg/kg and 63.19% at 400 mg/kg, suggesting that *L. coromandelica*, at a higher dose, has the potential to reduce mast cell degranulation (Table 4).

Table 4. Effect of *L. coromandelica* on Clonidine-induced mast cell degranulation in rats

Treatment	% of disrupted mast cells
Control	80.36±1.15
Sodium cromoglycate	25.16±0.38
L. coromandelica (200mg/kg)	71.55±1.18^{ns}
L. coromandelica (400mg/kg)	63.19±1.34**

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with $^{***}P<0.001$ and $^{**}P<0.01$, $^{*}P<0.05$ being considered statistically significant.

3.7. Histamine-induced bronchospasm in Guinea pigs

The effects of various extracts on the pre-convulsive time in guinea pigs were investigated. Ketotifen at a concentration of 10 µg/mL significantly increased pre-convulsive time from 101.77±6.94 on Day 0 to 146.55±10 on Day 5.

L. coromandelica, at a dose of 200 mg/kg, did not exhibit a statistically significant effect on pre-convulsive time. However, when the dose was increased to 400 mg/kg, it showed a significant effect with a pre-convulsive time that increased from 104.86±7.15 on Day 0 to 116.02±7.92 on Day 5. This indicates that the higher dose of *L. coromandelica* significantly prolonged the pre-convulsive time in guinea pigs (Table 5).

Table 5. Effect of *L. coromandelica* on pre-convulsive time in Guinea pigs

Treatment	Day 0	Day 5
Control	93.55±6.38	94.48±6.45
Ketotifen (10 µg/ml)	101.77±6.94****	146.55±10****
L. coromandelica (200mg/kg)	97.66±6.66^{ns}	99.71±6.8^{ns}
L. coromandelica (400mg/kg)	104.86±7.15^{ns}	116.02±7.92***

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with $^{***}P<0.001$ and $^{**}P<0.01$, $^{*}P<0.05$ being considered statistically significant.

4. DISCUSSION

The in vitro antiasthmatic activity data demonstrated that *L. coromandelica* mitigated histamine-induced contractions in isolated guinea pig ileum. There was a marked difference between the control group, which displayed a mean contraction, and the standard group, which demonstrated significantly lesser contraction, highlighting pronounced inhibition of histamine-induced contractions. Treatment with *L. coromandelica* at a dosage of 200 mg/kg significantly reduced the mean contraction, indicating a notable decrease compared to the control group. Interestingly, increasing the dosage to 400 mg/kg further reduced the mean contraction, which was statistically significant compared to both the control group and the lower dosage groups. These results suggest a dose-dependent inhibitory effect of *L. coromandelica* against histamine-induced contractions, presenting potential therapeutic benefits in managing asthmatic manifestations. Our findings align with similar

experiments on *Mirabilis jalapa*, which support its traditional applications [23].

The investigation into the effects of *L. coromandelica* extracts on mast cell degranulation revealed that at a lower dose of 200 mg/kg, there was no statistically significant inhibition of mast cell degranulation compared to both the control group and Ketotifen. However, at 400 mg/kg, the extract not only exceeded the inhibitory performance of its lower-dose counterpart but also demonstrated statistically significant mitigation of mast cell degranulation compared to the control group.

In the context of acute toxicity studies, *L. coromandelica* manifested no symptoms of toxicity in rats even at a substantial dose of 2000 mg/kg body weight when administered orally, indicating its safety in medium-dose applications. This finding correlates with similar observations reported by Venkatesham et al., 2019 [24]. The significant reduction in mast cell disruption observed in the *L. coromandelica* treatment groups during clonidine-induced mast cell degranulation reinforces the extract's impact in physiological contexts relevant to mast cell activity and stability. These results are consistent with the research conducted by Kumar et al., 2010, who also reported significant protection against mast cell degranulation when challenged with clonidine, indicating mast cell stabilizing activity [25].

Additionally, phytochemical analyses elucidated that *L. coromandelica* is rich in flavonoids, a class of secondary metabolites often associated with various biological activities, including anti-inflammatory and antioxidant properties. The abundant flavonoid content not only enhances the pharmacological potential of *L. coromandelica* but also potentially elucidates the mechanistic pathways through which the extract exerts its effects on mast cell stabilization and inhibition of degranulation [26].

5. SUMMARY AND CONCLUSION

In vitro studies demonstrated that L. coromandelica extract possesses significant antihistaminic activity by effectively inhibiting histamine-induced contractions in isolated guinea pig ileum. This inhibitory effect showed a dose-dependent response, particularly at the higher dosage of 400 mg/kg. Investigations involving clonidine-induced mast cell degranulation in rats underscored the potential of *L. coromandelica* as an antihistaminic agent by inhibiting clonidine-induced mast cell degranulation. Additionally, histamine-induced bronchospasm experiments in guinea pigs indicated that *L. coromandelica* extract exerted a protective effect against convulsions, as reflected by a slight increase in pre-convulsive time on Day 5.

In conclusion, the in vitro antihistaminic activity of *L. coromandelica* demonstrated its ability to inhibit histamine-induced contractions in isolated guinea pig ileum. The higher dosage of 400 mg/kg exhibited a stronger inhibitory effect compared to the lower dosage of 200 mg/kg, suggesting a dose-dependent response. Furthermore, *L. coromandelica* extract displayed inhibitory effects on mast cell degranulation, particularly at the higher dosage of 400 mg/kg, indicating its potential as a stabilizer. Thus, the results collectively advocate *L. coromandelica* as a candidate worthy of further research and development in the context of therapies involving mast cell stabilization and related antiasthmatic applications.

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Conflict of interest

The authors declare no conflict of interest.

Authors contribution

All the authors contributed to the conception, and design and approved the submission.

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الدور الوقائي للانيا كورومانديليكا (هوت.) مير. ضد إفراز وعمل الهستامين: رؤى من التحقيقات في المختبر وفي الجسم الحي

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ملخص

تهدف هذه الدراسة إلى تقييم القدرة المضادة للهستامين للمستخلص النباتي منلانيا كوروماندليكا باستخدام كليهما في المختبر ونماذج على قيد الحياة. في المختبر تمت دراسة التأثيرات المضادة للهستامين باستخدام اللفائفي المعزول لخنزير غينيا لتقييم التأثيرات المثبطة المعتمدة على الجرعة على الانقباضات الناجمة عن الهستامين. تم تقييم كثافة الخلايا البدينة باستخدام نموذج عدد الخلايا البدينة، وحساب متوسط عدد الخلايا البدينة لكل وحدة مساحة في المساريق. لعلى قيد الحياة التقييمات، تم استخدام تشنج قصبي الهستامين الناجم عن الهباء الجوي في نموذج خنزير غينيا، حيث لوحظ أن وقت بداية ضيق التنفس قبل التشنج (PCD) هو وقت ما قبل التشنج .(PCT) بالإضافة إلى ذلك، تم استخدام نموذج تحلل الخلايا البدينة الناجم عن الكلونيدين في الفئران، مع تلوين الخلايا باستخدام 1% من التولويدين الأزرق لحساب الخلايا البدينة السليمة والمحببة. اللانيا كوروماندليكا أظهر المستخلص تثبيطًا يعتمد على الجرعة للتقلصات التي يسببها الهستامين في اللفائفي لخنزير غينيا المعزول. وبالمثل، فإن المستخلص يمنع تحلل الخلايا البدينة بطريقة تعتمد على الجرعة، حيث أثبتت جرعة أعلى قدرها 400 ملجم / كجم أنها أكثر فعالية من جرعة أقل قدرها 200 ملجم / كجم. أكدت دراسات السمية الحادة سلامة المستخلص عند تناول جرعات معتدلة، ولم تظهر أي أعراض سمية عند جرعة 2000 ملجم/كجم من وزن الجسم. الأهم من ذلك، أن المستخلص زاد بشكل كبير من معاهدة التعاون بشأن البراءات في خنازير غينيا وخفض النسبة المئوية للخلايا البدينة المعطلة الناجمة عن الكلونيدين. لانيا كوروماندليكا يُظهر خصائص واعدة مضادة للهيستامين، حيث يثبط بشكل فعال التشنج القصبي الناجم عن الهيستامين وتحلل الخلايا البدينة التي يمكن أن تكون خيارًا لتطوير الأدوية المضادة للربو.

الكلمات الدالة: لانيا كوروماندليكا، مضادات الهيستامين، خنازير غينيا، فئران ويستار.

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