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RESEARCH ARTICLE

Antiasthmatic Potential of *Justicia gendarussa*: A Preclinical Study

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Abstract

Background: The chronic respiratory lung illness known as asthma is reversible. Hyperresponsiveness and inflammation of the airways are characteristics of asthma. The pathogenesis of asthma is dominated by Th2 cell immune responses. *Justicia gendarussa* Burm. *f* (*J. gendarussa*) is an evergreen plant belonging to the *Acanthaceae* family. The presence of flavonoids and phenols gives the basis for the anti-inflammatory activity of the *J. gendarussa.* Traditionally in China, *J. gendarussa* is used to treat coughing diseases. Leaf ash was traditionally used to treat asthma in China. **Aim:** This study aims to assess the antiasthmatic potential of ethanolic extract of *J. gendarussa* leaves (EEJG) using *in vitro* and *in vivo* methods. **Method:** In this study, I have prepared *J. gendarussa* leaves extract by the Soxhlet method and done phytochemical analysis and an acute oral toxicity study. And Pharmacological screening was done by using an *in vitro* method that is DPPH assay and Goat trachea chain method, and *in vivo* leukocytosis and eosinophilia were caused by milk in Swiss albino mice, clonidine induced mast cell degranulation in Wistar rats and ovalbumen induced allergic asthma in Wistar rats. **Result:** The results indicated that the ethanolic extract of *J. gendarussa* drastically showed statistically significant inhibition of milk induced leukocytosis and eosinophilia in a dose-dependent manner, have mast cell stabilizing potential and also lowered BALF's synthesis of Th2 cytokine (IL-4), as well as the numbers of major and total inflammatory cells. Histological investigations showed that significantly reduced the infiltration of inflammatory cells into lung tissues caused by OVA. **Conclusion:** *J. gendarussa* is beneficial for asthma therapy. The results demonstrated that *J. gendarussa* leaves extract exhibits significant antihistaminic, antiallergic, anti-inflammatory, and immunomodulatory actions in animal models used in experiments, successfully reducing asthma symptoms. These findings validate the traditional use of *J. gendarussa* and support the prospective therapeutic application of EEJG in the prevention and intervention of asthma.

Major Findings: In animal models (rats and mice), the study showed that *Justicia gendarussa* had strong antiasthmatic activity. Plant extract treatment decreased bronchial hyperresponsiveness, eosinophil infiltration, and airway inflammation, indicating its promise as a natural therapeutic agent for asthma control.

Keywords: Asthma, *J. gendarussa*, Lung Tissue, Ovalbumen

1. Introduction

Asthma is a recurring, non-communicable and potentially fatal respiratory disease characterised by acute bronchial obstruction. It manifests with symptoms such as coughing, chest tightness, shortness of breath, and wheezing; it can differ in intensity and recurrence[1,2]. These symptoms arise from persistent airway inflammation caused by inhaled allergens, resulting in Airway Hyper-Responsivity (AHR), airway remodelling, and excessive mucus production[3,4]. It impacts nearly 300 million individuals globally, and the incidence is rapidly increasing[1,5].

Due to the debilitating side effects of synthetic medicine and the shift in the therapeutic paradigm towards alternative medicine, herbal medicine is making a spectacular comeback. Both organised (Ayurveda, Unani) and unorganised (folk, tribal, native) types of plants are used as medicinal agents[6-8].

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Justicia gendarussa Burm. *f (J. gendarussa*) is an evergreen shrub belonging to the *Acanthaceae* family. In the conventional medicine system, it is mentioned that it has different therapeutic activities[9]. Pharmacological investigations have revealed that *J. gendarussa* possesses a broad spectrum of biological activities, comprising, although not restricted to, anti-HIV, anti-inflammatory[10], analgesic, anxiolytic, anti-microbial, hepatoprotective[11], anti-fungal, antioxidant, larvicidal, cardioprotective[12], anthelmintic and antiarthritic[13]. *J. gendarussa's* anti-inflammatory properties are based on the presence of flavonoids and phenols[14,15]. Traditionally in China, *J. gendarussa* is used to treat coughing diseases. Leaf ash was used to treat asthma in China tradionally[13,15].

Given the foregoing context, the current study aims to assess the antiasthmatic properties of *J. gendarussa* ethanolic extract. In this study, leaves were extracted by the Soxhlet method and phytochemical analysis and acute oral toxicity study. And Pharmacological screening was done by using an *in vitro* method, that is, DPPH Assay and Goat trachea chain preparation method, and *in vivo* in rats, clonidine caused mast cell degranulation, while in mice, milk caused leukocytosis and eosinophilia.

2. Materials and Methods

2.1 Drugs and Chemicals

Dexamethasone (Zydus Cadila), Clonidine (Neon Laboratories Ltd.), Ketotifen (Sun Pharmaceutical Industries Ltd), DPPH (Analab Fine Chemicals). The drugs and chemicals employed in this research were all of analytical significance.

2.2 Procurement of Plant

Fresh plant of *J. gendarussa* was procured from the Gachwala nursery and garden centre, Kolkata, West Bengal. The Plant specimen (No. BSI/WRC/Iden. Cer./2024/0705240000179) was authenticated by a Botanist, D. L. Shirodkar, Western Regional Centre 7, Koregaon Park, Pune, Botanical Survey of India - 411001.The leaves of the plant were shade-dried for a week and then powdered.

2.3 Extraction[16,17]

The indoors withered leaves, about 350g, were made into coarse powder by reducing their size. The powder was then extracted with 500 mL of ethanol (analytical grade) using a Soxhlet apparatus. Then Marc was acquired, following the ethanolic extraction was extracted and then the extract was dried by the rotatory drum dryer. Following their collection, concentration, and storage in tight desiccators, the extracts were filtered through Whatman filter paper, and the yield % was determined.

2.4 Animals

The National Institute of Biosciences in Pune, India, provided the healthy female Wistar rats (150–200 g) and Swiss albino mice (20–25 g), which were kept in animal houses in polypropylene cages in groups of six. The animals were housed at 25 ± 2°C, 45 to 55% relative humidity, and a 12-hour light-dark cycle. All animals were acclimated to the registered animal household (1554/PO/Re/S/11/CPCSEA) for seven days before the start of the experimental phase. The animals were provided with unlimited access to food and water (Nutrivet Life Sciences, Pune, India).

2.5 Ethical Approval

As directed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the Institutional Animal Ethical Committee (IAEC) accepted the research protocol, which was registered under the number DYPCOP/IAEC/2023/14/07.

2.6 Phytochemical Analysis of EEJG[18,19]

Presence of various phytoconstituents in EEJG were analyzed using Dragendorffs, Mayer's, Hager's, and Wagner's tests for alkaloid, Molisch tests for carbohydrate, Shinoda and zinc hydrochloride tests for flavonoids, Benedicts and foam tests for saponins, Salkowaski and Liebermann– Burchard tests for steroid and triterpenoids, and ferric chloride test, lead acetate test and potassium permanganate tests for tannins.

2.7 Measurement of DPPH Free Radical Assay[20]

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method was used to measure the free radical scavenging activity (RSA) of the diluted leaves extract of *J. gendarussa*. This method was modified from Brand-Williams *et al.* In this process, 0.3 ml of a 0.5 mm

DPPH radical solution in methanol, 3 ml of methanol, and 0.5 ml of the extract were combined. After that, the mixture was allowed to sit at room temperature for half an hour while the tubes were kept in the dark. The antioxidant activity was measured in BHT equivalents, with butylated hydroxytoluene (BHT) serving as the standard. After measuring the absorbance at 517 nm, the following formula was used to determine the proportion of antioxidants, or RSA: % DPPH free radical scavenging ability $=[(Absc\text{-}Abss)]\div(Absc)\times100$

Where, Absc = absorbance of the control

Abs = absorbance of the sample

2.8 Isolated Goat Tracheal Chain Preparation[21,22]

Immediately after the animal was slaughtered, the slaughterhouse provided isolated adult goat tracheal tissue. After cutting the trachea separated into several rings and tied together in series to create a chain. It was suspended in an organ bath that contained Krebs' solution: NaCl-6.9, CaCb-0.28, $MgSO_4$-0.28, KC1-0.35, NaHCO3-2.1, KH2PO4-0.16, and glucose-2.0 gm/litter. The solution was kept at 37 ± 0.5°C and continuously aerated.

After applying a 400g load, the tissue was given 45 minutes to acclimate. For histamine (200µg/ml), a dose-response curve at different concentrations was obtained using a 15-minute time cycle.

A dose-response curve of histamine on the trachea was obtained, and then 0.1 ml of the extract with a concentration of 100 µg/ml was injected into the reservoir and allowed to react for 20 minutes. Afterwards, the identical histamine dosages were administered again. They took note of the DRC's change.

The average percentage contractile response on the ordinate and logarithmic dose of histamine on the abscissa was plotted on a graph to record the histamine dose response curve, both with and without the test formulations.

2.9 Acute Toxicity Studies of Ethanolic Extract of *J. gendarussa*[23,24]

For the present study, an acute oral toxicity test was performed on the extract according to OECD Guideline No. 423 (Acute Oral Toxicity- Class Method). Swiss albino mice weighing 20-25 g were grouped into 4 groups, each containing 3 mice (Figure 1).

2.10 *In Vivo* Method

2.10.1 Milk-Induced Leukocytosis and Eosinophilia[25,26]

Asthma pathology involves the activation of various mediators, with milk proteins known to trigger the release of pro-inflammatory cytokines and exacerbate airway inflammation. Anti-asthmatic activity was estimated using a milk-induced leukocytosis and eosinophilia mouse model. The mice were divided into 6 groups, each containing 6 animals, and followed a treatment schedule (Table 1).

2.1.2 Clonidine-Induced Mast Cell Degranulation in Wistar Rats[25,27,28]

In this animal model, Wistar rats were grouped into 5 groups (every 6 animals): normal control, standard and three EEJG 100mg, 200mg and 400mg (Table 2). For 7 consecutive days, treatment was given as per group.

2.1.3 Ovalbumin-Induced Allergic Asthma in Rats[29,30]

In this animal model, ovalbumen was induced in Wistar rats. Wistar rats were grouped into 6 groups: normal control, induction control, standard and 3 treatment groups (100 mg, 200 mg, 400 mg) (Table 3). The treatment schedule was followed for 30 days (Figure 2).

2.1.3.1 Collection of Blood and Bronchoalveolar Lavage (BAL) Fluid

Standard method was followed for blood and BAL fluid samples collection from all rats that had been euthanized by using a standard procedure[29,30].

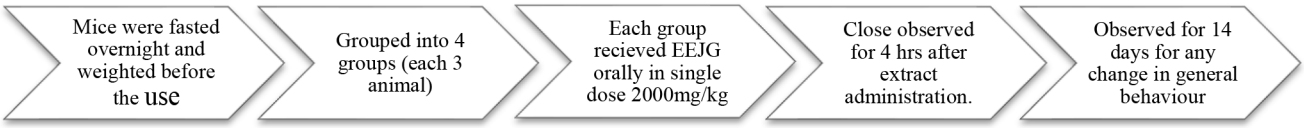


Figure 1. Acute oral toxicity (OECD-423).

2.1.3.2 Histopathological Analysis of Lung Tissue

The lung tissues were embedded in paraffin after being preserved in a 10% formalin solution (pH 7.0). Deparaffinization was performed after thin sections, 3–5 µm thick, were produced and put on sanitised glass slides. Hematoxylin-eosin (HandE) staining was done on the sections so that inflammatory cell infiltration and mucus production could be observed under a microscope. Using the scoring method outlined by Wang *et al.*, both the infiltration of inflammatory cells in H and E-stained sections and the proportion of goblet cells in PAS-dyed sections were assessed. A blindfolded observer assigned scores.

2.1.3.3 Estimation of IL-4 and TNF-α

Cytokine levels, particularly IL-4, were quantified in serum samples using a rat enzyme-linked immunosorbent assay

Table 1. Group and treatment schedule

Group (N=6)	Group Name	Observation
I	Normal Control (4ml/kg, p.o.)	Leukocyte and eosinophile count
II	Induction Control (4ml/kg milk,s.c)	
III	EEJG 100mg/kg (p.o.)	
IV	EEJG 200mg/kg (p.o.)	
V	EEJG 400mg/kg (p.o.)	

Table 2. Groups and treatment schedule

Groups (N=6)	Name of group	Observation
I	Normal control (5ml/kg, Distilled water)	Total degranulated and intact mast cells and per percentage protection.
II	Standard (Ketotifen 5mg/kg, i.p.)	
III	EEJG 100 mg (p.o.)	
IV	EEJG 200 mg (p.o.)	
V	EEJG 400 mg (p.o.)	

Table 3. Groups and treatment schedule

Group (N=6)	Name of group	Observation
I	Normal control	Histopathological change in the lung tissue, Level of IL-4, TNF-α and anti-inflammatory cells
II	Induction control (Ova i.p)	
III	Standard (Dexamethasone.p)	
IV	EEJG 100 mg (p.o.)	
V	EEJG 200 mg (p.o.)	
VI	EEJG 400 mg (p.o.)	

Table 4. Phytochemical screening of EEJG

Sr. No.	Phytochemical Constituents	Ethanolic Extract of *J. gendarussa*
1	Carbohydrates	205.11 mg/g
2	Flavonoids	81.02 mg/g
3	Proteins	-
4	Amino Acids	29.04 mg/g
5	Steriods	-
6	Saponins	+
7	Alkaloids	+
8	Tannins	+
9	Glycosides	+

[Present = (+), Absent= (-)]

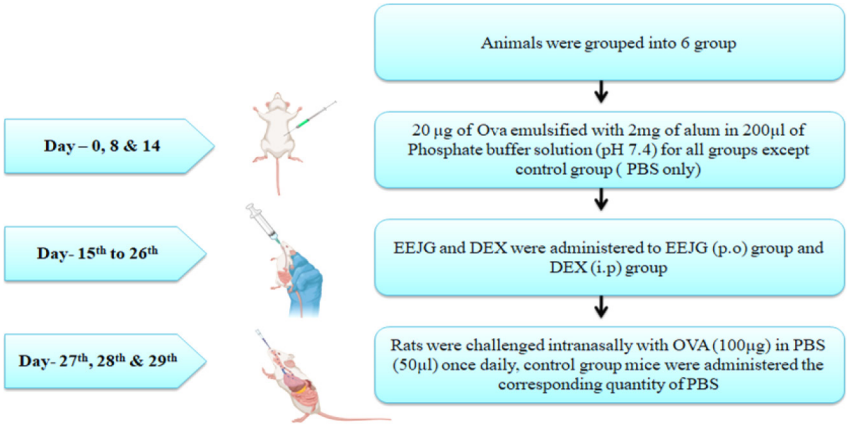


Figure 2. Ovalbumin-induced allergic asthma in rats.

(ELISA) kit. The serum was collected from the blood of rats and sent to PreclinBio Solutions LLP, Research and Development, Pune, for the estimation of IL-4 and TNF-α levels.

2.1.3.4 Statistical Analysis

The mean ± SEM was used to express the data. Dunnett's test and one-way ANOVA were used for statistical comparisons between experimental groups. Statistical significance was established at $p < 0.01$ and $*p < 0.1$. $***p < 0.1$ and $**p < 0.01$.

3. Results

The % yield of extract was calculated and found to be 7.1%.

3.1 Preliminary Phytochemical Studies

The preliminary phytochemical analysis confirmed the presence of various bioactive compounds, including alkaloids, carbohydrates, flavonoids, glycosides, saponins, phenolic compounds, and tannins. These findings are summarised in Table 4.

3.2 DPPH Assay of EEJG

Justicia gendarussa leaf ethanolic extract's antioxidant activity was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) technique. When an odd electron interacts with the free radical DPPH, the highest absorption occurs at 517 nm (purple colour) (Figure 3).

3.3 Effect of EEJG on Goat Tracheal Chain Preparation

As shown in the table showing the maximal percentage of contractile response vs negative log molar concentration of histamine, histamine caused dose-dependent contraction of the goat tracheal chain preparation in the current investigation. The contractile action of histamine was considerably ($P < 0.001$) reduced by the modified physiological salt solution containing EEJG (200 µg/ml) (Figure 4).

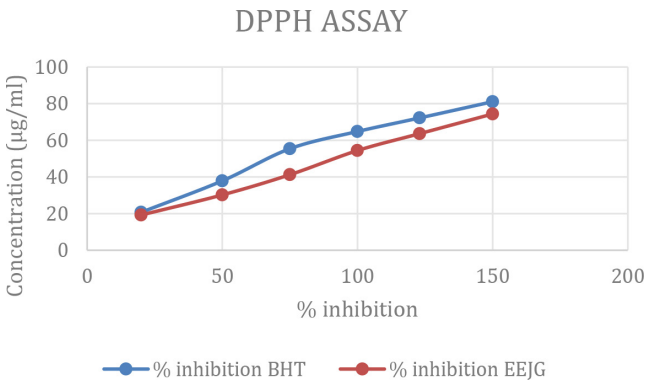


Figure 3. DPPH assay of BHT and EEJG.

3.4 Acute Oral Toxicity

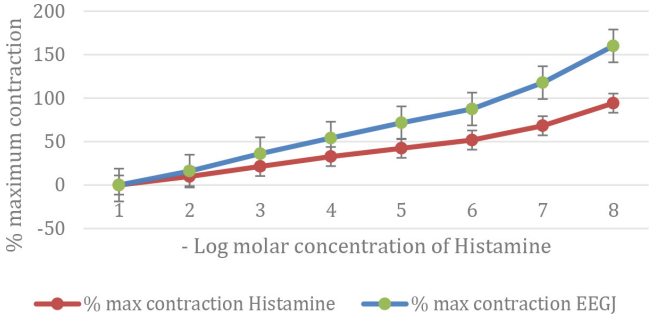
Swiss albino mice (female mice) were used to test the ethanolic extract of *Justicia gendarussa* leaves for acute oral toxicity in accordance with OECD guideline 423. The ethanolic extract did not cause death or any indication of morbidity at the dosage of 2000 mg/kg. Therefore, as low, moderate, and high doses for animal administration, 100 mg/kg (1/20th of 2000 mg/kg), 200 mg/kg (1/10th of 2000 mg/kg), and 400 mg/kg (1/5th of 2000 mg/kg) were chosen. The evaluation of changes is summarised in Table 5.

3.5 *In Vivo*

3.5.1 Effect of EEJG on Milk-induced Leukocytosis and Eosinophilia

3.5.1.1 Effect of EEJG on Total Leukocyte Count

The results showed that subcutaneous injection of milk at a dose of 4 ml/kg produced a significant ($p< 0.001$) increase in the leucocyte count after 24 hr of its administration. In the group of mice pre-treated with EEJG at doses 100, 200 and 400 mg/kg, p.o, showed statistically significant ($***p<0.001$) inhibition of milk-induced leukocytosis 24 hours after treatment in a dose-dependent manner (Figure 5).



Values are expressed as Mean ± SEM. Where n = 5. Control = D.R.C. of Histamine in the absence of the ethanolic extract of *Justicia gendarussa.* EEJG = D.R.C. of Histamine in the presence of ethanolic extract *Justicia gendarussa* (200µg/ml). Statistical analysis was carried out by using Student's t-test. **p< 0.01, *** p< 0.001, significantly different from control.

Figure 4. Effect of EEJG on Histamine induced contraction of isolated goat tracheal chain preparation.

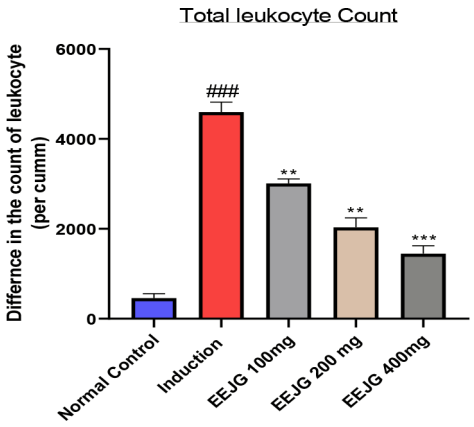
3.5.1.2 Effect of EEJG on Total Eosinophile Count

The results showed that milk (4 ml/kg, s.c.) produced a significant increase (###p< 0.001) in the total eosinophil count. The group pre-treated with EEJG (100, 200 and 400 mg/kg, p.o) showed statistically significant (***p<0.001) inhibition of milk-induced leukocytosis 24 hours after treatment in a dose-dependent manner (Figure 6).

Table 5. Evaluation of changes during acute oral toxicity

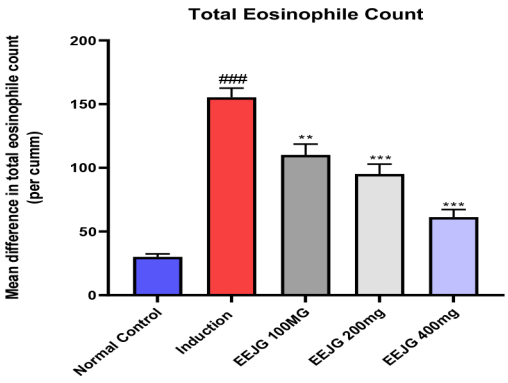
Sr. No.	Parameter	Observation (0-14 days)
1	Convulsions	Nil
2	Righting reflex	Nil
3	Body weight	No change
4	Pinna reflex	Nil
5	Coma	Nil
6	Corneal reflex	Nil
7	Salivation	Nil
8	Diarrhea	Nil
9	Writhing	Nil
10	Snoring	Nil
11	Gripping	Nil
12	Urination	Nil
13	Pain response	Nil
14	Food intake	Nil
15	Water intake	Nil
16	Mortality	Nil

0,1,2,3 – Number of mortality



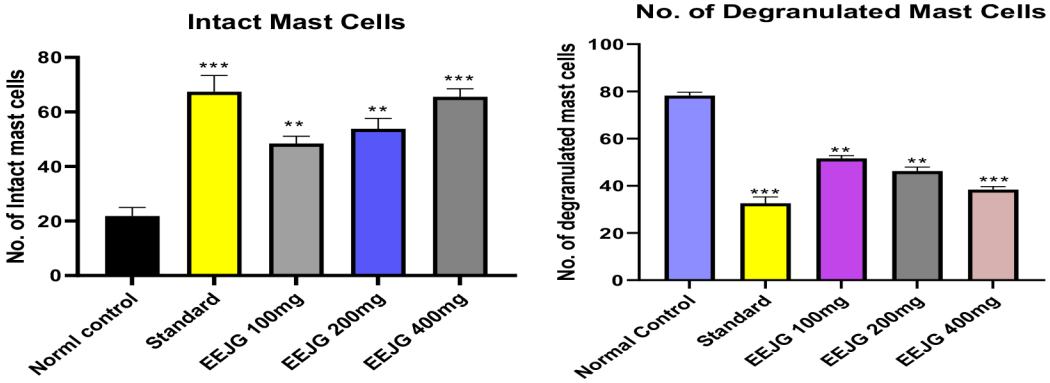
Values are stated as mean ±SEM; n=6; Data analysed by one-way ANOVA test followed by Dunnett's multiple comparison. Level of significance p < 0.001, in contrast with the normal group, also ***p< 0.001, **p< 0.01 when in contrast with the induction control group.

Figure 5. Effect of EEJG on total leukocyte count.



Values are stated as mean ±SEM; n=6; Data analysed by one-way ANOVA test followed by Dunnett's multiple comparison. Level of significance: ###p < 0.001, in contrast with the normal group, also ***p< 0.001, **p< 0.01, when in contrast with the induction control group.

Figure 6. Effect of EEJG on total eosinophile count.



Values are stated as mean ±SEM; n=6; Data analysed by one-way ANOVA followed by Dunnett's multiple comparison. Level of significance: ###p < 0.001, in contrast with the normal group; also ***p< 0.001, **p< 0.01, when in contrast with the Standard group.

Figure 7. **(A).** Effect of EEJG on intact mast cells; **(B).** Effect of EEJG on degranulation of mast cells.

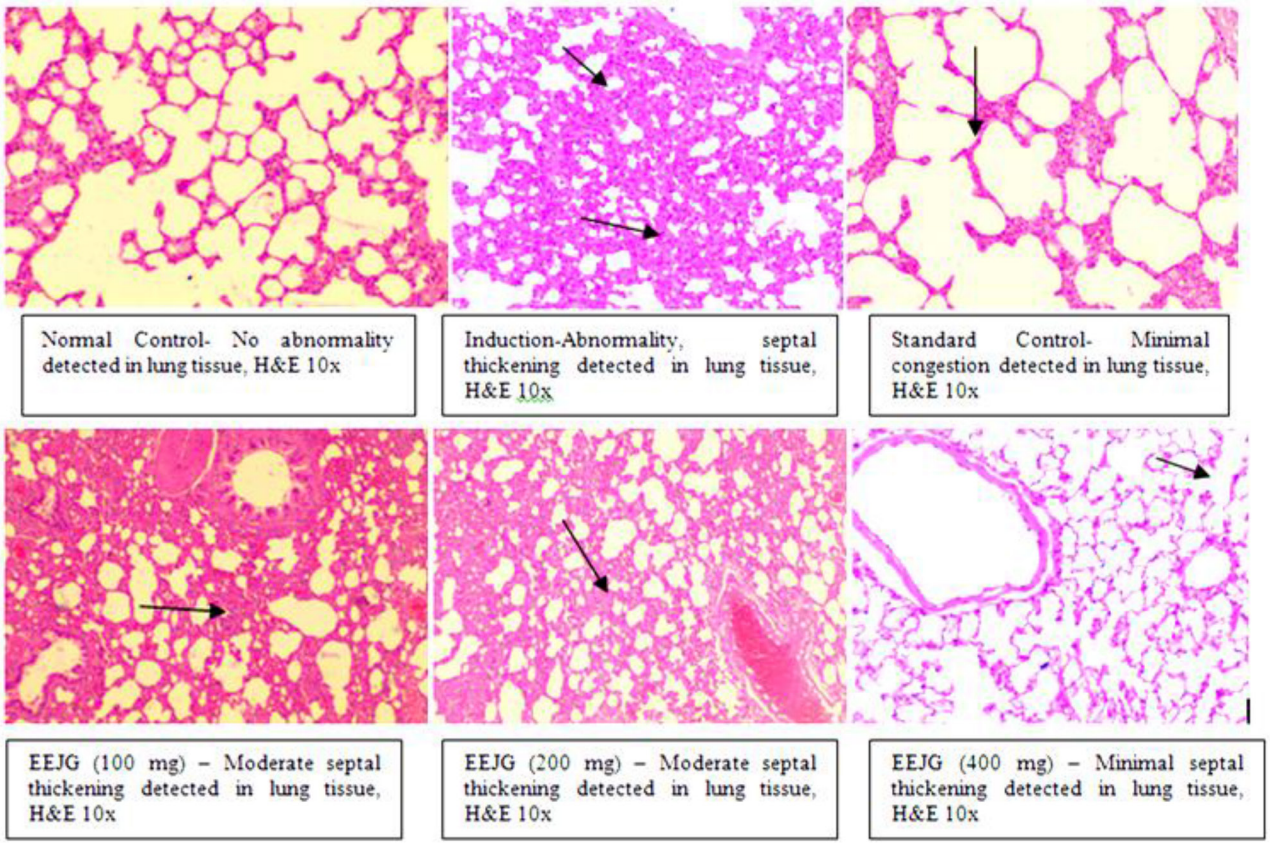


Figure 8. Effect of EEJG on the histopathology of lung tissue in ova-induced allergic asthma rats.

3.5.2 *Effect of EEJG on Clonidine-Induced Mast Cell Degranulation*

The results showed that the treatment group of EEJGexhibited significant mast cell stabilising potential at all three doses (100, 200, 400 mg/kg p.o.), 51.60±1.20, 46.20±1.71, 38.40±1.28, respectively, as compared to the control group. Per cent protection offered by EEJG at all three doses was notably 55%, 59.5%, and 65.2% and was statistically significant. EEJG at 400 mg/kg shows 38.40±1.28 mast cells degranulation (Figure 7) while EEJG at 100 and 200 mg/kg shows 51.60±1.20 and 46.20±1.71 mast cells degranulation, which was not significant.

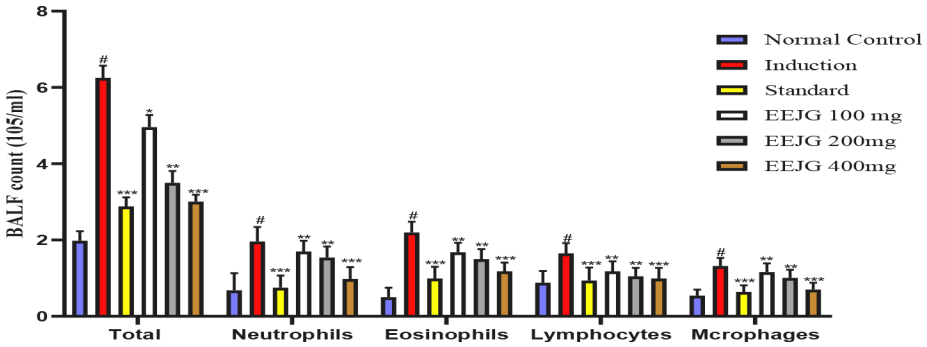
3.5.3 *Effect of EEJG on Ovalbumen-Induced Allergic Asthma in Rats*

3.5.3.1 *Histopathology*

The results of histopathology showed that Normal control rat lung sections indicate no abnormalities in tissue. The OVA-challenged group showed the presence of an abnormal number of inflammatory cells, peribronchial edema and congestion in alveoli. The standard treated group shows absence of abnormalities, whereas; EEJG group shows dose-dependent reduction in peribronchial edema, congestion in alveoli and minimal septal thickening (Figure 8).

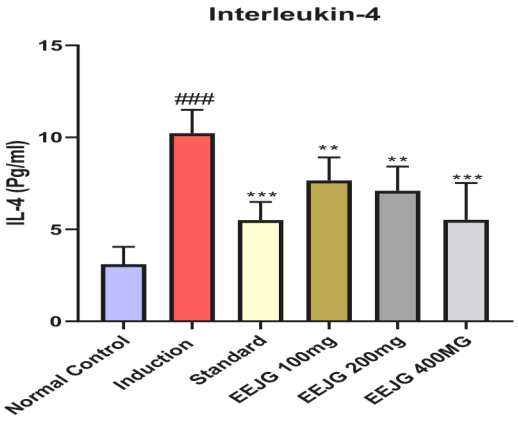
3.5.3.2 *Effect of EEJG on Inflammatory Mediators in BALF*

The total and differential inflammatory cell counts in the bronchoalveolar lavage (BAL) fluid were measured to assess the impact of EEJG on the recruitment of inflammatory cells into the lungs. The results showed, rats challenged with OVA exhibited a significant increase in the total inflammatory cell count in the BAL fluid compared to the control rats (***$p < 0.001$, **$p < 0.01$). However, at all studied doses, EEJG dose-dependently prevented the increase in inflammatory cells compared to the OVA-challenged group (Figure 9). Both DEX and EEJG at 400 mg/kg showed the maximum reduction in the total and differential inflammatory cell counts in the BAL fluid.



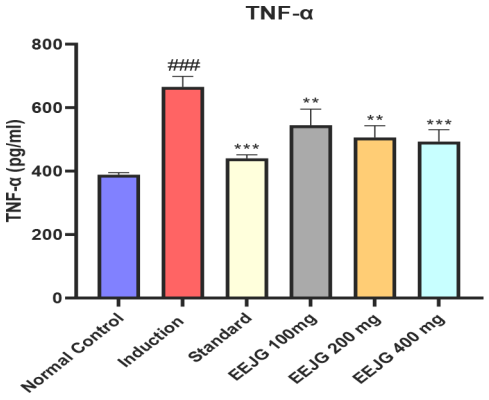
Values are stated as mean ±SEM; n=6; Data analysed by two-way ANOVA test followed by Turkey's multiple comparison. Level of significance: ###p < 0.001, in contrast with the normal group, also ***p< 0.001, **p< 0.01, when in contrast with the induction control group.

Figure 9. Effect of EEJG on inflammatory cells in BALF.



Values are stated as mean ±SEM; n=6; Data analysed by one-way ANOVA test followed by Dunnett's multiple comparison. Level of significance ###p < 0.001, in contrast with normal group also ***p< 0.001, **p< 0.01, when in contrast with induction control group.

Figure 10. Effect of EEJG on IL-4 Level.



Values are stated as mean ±SEM; n=6; Data analysed by one-way ANOVA followed by Dunnett's multiple comparison. Level of significance: ###p < 0.001, in contrast with the normal group, also ***p< 0.001, **p< 0.01, when in contrast with the induction control group.

Figure 11. Effect of EEJG on TNF-α.

3.5.3.3 Effect of the EEJG on the levels of IL4

As shown in (figure.10) the results showed that the level of Interleukin-4 in the serum was significantly increased (### p< 0.001) in the induction group treated with the ovalbumin compared with the normal control group, and the EEJG (100mg and 200 mg) group decreased the IL-4 level significantly (** p<0.01) and EEJG(400mg) and standard decreased the IL-4 level significantly (***p<0.001) compared with the Ova group.

3.5.3.4 Effect of EEJG on TNF-α

As shown in (Figure 11) the results showed that the level of TNF- α was significantly increased (### p< 0.001) in the induction group treated with the ovalbumin compared with the normal control group, and the EEJG (100mg and 200 mg) group decreased the TNF- α significantly (** p<0.01) and EEJG (400mg) and standard decreased the TNF- α level significantly (***p<0.001) compared with the Ova group.

4. Discussion

For this research, we systematically evaluated the anti-asthmatic properties of *J. gendarussa* leaf extract using multiple approaches.

The study began with the authentication of *Justicia gendarussa*. The leaves of *J. gendarussa* were subjected to Soxhlet extraction using ethanol as the solvent. The resulting extracts were dried and utilised for pharmacological studies. A preliminary phytochemical evaluation of the plant was conducted, revealing the existence of carbohydrates, amino acids, alkaloids, glycosides, flavonoids, tannins, and phenolic

compounds. These findings were confirmed through qualitative analysis[16-18].

The ethanolic extract of *Justicia gendarussa* leaves demonstrated significant dose-dependent DPPH scavenging activity, with BHT serving as the standard control. Phytochemical analysis revealed the existence of phenols and flavonoids in EEJG, which are primarily responsible for its radical scavenging activity.

In the present study, we observed a rightward change in the response curves for the dose of histamine in the presence of EEJG. This change showed that histamine caused the isolated goat tracheal chain preparation to contract[21].

A study of acute oral toxicity of ethanolic extract of *Justicia gendarussa* (EEJG) was conducted following OECD guideline 423[23, 24]. No mortality or observable changes were recorded after administration of EEJG at a dose of 2000 mg/kg over 14 days. These findings showed that the median lethal dose (LD50) of EEJG is likely more than 2000 mg/kg body mass in mice. In accordance with these findings, a safe experimental dose of ≤ 200 mg/kg was determined and subsequently used for further screening of the extract.

Studieshave demonstrated that parental administration of milk significantly increases leukocyte and eosinophil counts within 24 hours[25,26]. Pre-treatment with the ethanolic extract of *Justicia gendarussa* (EEJG) at doses of 100, 200, and 400 mg/kg substantially inhibited this response ($p < 0.001$), likely due to the flavonoid and phenolic content of the extract.

In asthma, mast cell activation triggers the rapid liberation of histamine and eicosanoids, leading to airway smooth muscle contraction and mucus secretion[25,27,28]. Flavonoids have been shown to stabilise mast cells more effectively than sodium cromoglycate by inhibiting histamine release. The mast cell-stabilising activity of *Justicia gendarussa* ethanolic extract (EEJG) is attributed to its flavonoid content, highlighting its potential as a therapeutic agent in asthma management.

In our study, OVA-challenged rats exhibited elevated serum IL-4 levels and elevated inflammatory cell counts in BAL fluid. Intervention with EEJG subsequently decreased IL-4 levels and inflammation in a dose-dependent manner, comparable to the effects of dexamethasone. Furthermore, EEJG mitigated peribronchialoedema and alveolar congestion, likely by inhibiting cytokine activity and reducing the inflammatory response.

5. Conclusion

The goal of the research was to evaluate the preclinical anti-asthmatic potential of the ethanolic extract of *J. gendarussa* (EEJG) using various animal models. A comprehensive literature review supported the ethnopharmacological claims regarding the efficacy of *J. gendarussa* in the prevention and treatment of asthma. In this study, preliminary phytochemical analysis was conducted on the ethanolic extract of *J. gendarussa*, confirming the presence of major phytoconstituents within the extract. The results demonstrated that EEJG exhibits significant antihistaminic, antiallergic, anti-inflammatory, and immunoregulatory activities in experimental animal models, effectively alleviating asthmatic conditions. These findings validate the traditional use of *J. gendarussa* and support the potential therapeutic application of EEJG in the prophylaxis and intervention of asthma.

6. Author Contribution

P.S, P.W and N.V.: Conceptualization, design of study. P.S and P.W: Manuscript draft. N.V and P.W: Supervision and revised the manuscript. P.S and V.P: Animal experiments, extraction. D.S and R.S: Analyzation of biochemical and histopathological data. P.S , V.P and G.P: literature review, data curation, and preparation of figures and tables. All authors have read and agreed to the published version of the manuscript.

7. References

1. Grant T, Croce E, Matsui EC. Asthma and the social determinants of health. Ann Allergy Asthma Immunol. 2022; 128(1):5-11. https://doi.org/10.1016/j.anai.2021.10.002
2. Holt P, Sly P. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. Nat Med. 2012; 18(5):726-35. https://doi.org/10.1038/nm.2768
3. Quirt J, Hildebrand KJ, Mazza J, Noya F, Kim H. Asthma. Allergy Asthma Clin Immunol. 2018; 14(Suppl 2):50. https://doi.org/10.1186/s13223-018-0279-0

4. Yadav D, Reshi MS, Uthra C, Shrivastava S, Srivastava N, Narayana SK, Shukla S. Botanical and chemical fingerprinting of medicinal roots of *Justicia gendarussa* Burm *f*. Pharmacognosy research. 2017; 9(2):208.
5. Corris PA, Dark JH. Aetiology of asthma: lessons from lung transplantation. Lancet. 1993; 341(8857):1369-71. https://doi.org/10.1016/0140-6736(93)90941-9
6. Nedungadi P, Salethoor SN, Puthiyedath R, Nair VK, Kessler C, Raman R. Ayurveda research: Emerging trends and mapping to sustainable development goals. J Ayurveda Integr Med. 2023; 14(6):100809. https://doi.org/10.1016/j.jaim.2023.100809
7. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: a comparative overview. Evid Based Complement Alternat Med. 2005; 2(4):465-73. https://doi.org/10.1093/ecam/neh140
8. Sharma R, Amin H, Galib R, Vyas MK, Prajapati PK, *et al.* Efficacy of an Ayurvedic treatment regimen on bronchial asthma. Anc Sci Life. 2012; 31(4):141-6. https://doi.org/10.4103/0257-7941.108596
9. Kirtikar KR, Basu BD. Indian Medicinal Plant. 2nd ed. Vol. III. International Book Distributer; 1869-1897.
10. Kavitha SK, Viji V, Kripa K, Helen A. Protective effect of *Justicia gendarussa*Burm.f. on carrageenan-induced inflammation. J Nat Med. 2011; 65(3-4):471-9. https://doi.org/10.1007/s11418-011-0524-z
11. Mondal M, Hossain MM, Rahman MA, Saha S, Uddin N, Hasan MR, *et al.* Hepatoprotective and antioxidant activities of *Justicia gendarussa* leaf extract in carbofuran-induced hepatic damage in rats. Chem Res Toxicol. 2019; 32(12):2499-508. https://doi.org/10.1021/acs.chemrestox.9b00345
12. PKS, Vijayan FP, Pareeth CM, Padikkala J, Babu TD. Cardioprotective effect of *Justicia gendarussa* on doxorubicin-induced toxicity in mice. J Basic Clin Physiol Pharmacol. 2022; 34(5):669-75. https://doi.org/10.1515/jbcpp-2022-0085
13. Jain T, Singh MP, Bhardwaj H, Gohil KJ. Review on pharmacology activities of *Justicia gendarussa* Burm F. Pharmacol Res Mod Chin Med. 2024; 10:100339. https://doi.org/10.1016/j.prmcm.2023.100339
14. Ali MF, Mahmud S, Mohiuddin RB, Chowdhury K, Mohiuddin AKM. Screening of preliminary phytochemicals, molecular identification, and antimicrobial and anti-inflammatory activity of *Justicia gendarussa*. Evid Based Complement Alternat Med. 2023; 2023:6885353. https://doi.org/10.1155/2023/6885353
15. Rangaraj K, Ananthy V, Singam A, Salwe KJ, Manimekalai K, Samy S, *et al.* Phytochemical analysis and acute toxicity study of seaweed *Halimeda macroloba* using Wistar albino rats. J Complement Integr Med. 2024; 21(4):511-9. https://doi.org/10.1515/jcim-2024-0077
16. Jothimanivannan C, Kumar RS, Subramanian N. Anti-inflammatory and analgesic activities of ethanol extract of aerial parts of *Justicia gendarussa* Burm. Int J Pharmacol. 2010; 6(3):278-83. https://doi.org/10.3923/ijp.2010.278.283
17. Zhang HX, Xia Z, Xu TQ, Chen YM, Zhou GX. New compounds from the aerial parts of *Justicia gendarussa* Burm. *f.* and their antioxidant and anti-inflammatory activities. Nat Prod Res. 2021; 35(20):3478-86. https://doi.org/10.1080/14786419.2019.1710708
18. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud. 2020; 8(2):603-8. https://doi.org/10.22271/chemi.2020.v8.i2i.8834
19. Banoth RK. *In vitro* antioxidant potential, total phenolic and flavonoid contents in *Justicia gendarussa* leaf extracts. Res J Pharm Technol. 2021; 14(12):2707-13. https://doi.org/10.52711/0974-360X.2021.00477
20. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, *et al.* Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules (Basel). 2022; 27(4):1326. https://doi.org/10.3390/molecules27041326
21. Ninave PB, Patil SD. Pharmacological screening of *Acalypha indica* L.: Possible role in the treatment of asthma. J Ethnopharmacol. 2022; 290:115093. https://doi.org/10.1016/j.jep.2022.115093
22. Pandit P, Singh A, Bafna AR, Kadam PV, Patil MJ. Evaluation of antiasthmatic activity of *Curculigo orchioides* Gaertn. rhizomes. Indian J Pharm Sci. 2008; 70(4):440-3. https://doi.org/10.4103/0250-474X.44590
23. OECD. Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing; 2002. https://doi.org/10.1787/9789264071001-en
24. Jonsson M, Jestoi M, Nathanail AV, Kokkonen UM, Anttila M, Koivisto P, *et al.* Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. Food Chem Toxicol. 2013; 53:27-32. https://doi.org/10.1016/j.fct.2012.11.023
25. Kumar D, Bhujbal SS, Deoda RS, Mudgade SC. Bronchodilator activity of aqueous extract of the stem bark of *Ailanthus excelsa* Roxb. Pharmacognosy Res. 2010; 2(2):102-6. https://doi.org/10.4103/0974-8490.62955
26. Mali RG, Dhake AS. Evaluation of effects of *Bauhinia variegata* stem bark extracts against milk-induced eosinophilia in mice. J Adv Pharm Technol Res. 2011; 2(2):132-4. https://doi.org/10.4103/2231-4040.82949
27. Vikhe S, Nirmal S. Antiallergic and antihistaminic actions of *Caesalpinia bonducella* seeds: Possible role in treatment of asthma. J Ethnopharmacol. 2018; 216:251-8. https://doi.org/10.1016/j.jep.2017.12.007
28. Shahzad N, Alzahrani AR, Ibrahim IAA, Soni K, Shahid I, Alsanosi SM, *et al. In vivo* pharmacological testing of herbal drugs for anti-allergic and anti-asthmatic properties.

J Pharm Bioallied Sci. 2021; 13(4):380-6. https://doi.org/10.4103/jpbs.jpbs_454_21
29. Thakur VR, Khuman V, Beladiya JV, Chaudagar KK, Mehta AA. An experimental model of asthma in rats using ovalbumen and lipopolysaccharide allergens. Heliyon. 2019; 5(11):e02864. https://doi.org/10.1016/j.heliyon.2019.e02864
30. Wu D, Li S, Liu X, Xu J, Jiang A, Zhang Y, *et al.* Alpinetin prevents inflammatory responses in OVA-induced allergic asthma through modulating PI3K/AKT/NF-κB and HO-1 signalling pathways in mice. Int Immunopharmacol. 2020; 89(Pt A):107073. https://doi.org/10.1016/j.intimp.2020.107073
31. Patel P, Shirode DS, Wankhade PP, Chautmal AB. Effect of prepared polyherbal formulation on histamine-induced contraction of isolated Goat tracheal chain preparation. Indian J Nat Sci. 2023; 14(81):67107-16.