

Research

Pharmacological anti-inflammatory activity of *Lablab purpureus* Linn

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DOI: 10.62896/ijpdd.2.5.14

Conflict of interest: NIL

Article History

Received: 12/04/2025

Accepted: 04/05/2025

Published: 17/05/2025

Abstract:

The present study systematically explores the phytochemical and pharmacological potential of *Lablab purpureus* Linn., with a focus on its anti-inflammatory activity. The anti-inflammatory effect was evaluated using the carrageenan-induced paw oedema model in rats—a well-established biphasic model to assess acute inflammation. Various extracts, including aqueous, methanolic, ethanolic, and chloroform, were administered at different dose levels. Among them, the methanolic extract (MEL) showed the most significant inhibition of oedema, with 200 and 300 mg/kg body weight producing 80% and 82% inhibition, respectively. These effects were superior to those of the standard drug diclofenac, which showed 74% inhibition. Ethyl acetate, aqueous, and chloroform extracts exhibited comparatively lower activity, ranging from 60% to 73% inhibition. Acute toxicity studies confirmed the safety of the methanolic extract up to 2000 mg/kg body weight according to OECD guideline 423. The methanolic extract at 200 mg/kg was selected for further analysis due to its optimal efficacy. Mechanistic studies suggest that MEL inhibits the release of key inflammatory mediators such as histamine, serotonin, and prostaglandins. In vitro assays using LPS-stimulated RAW 264.7 macrophages revealed that MEL significantly suppressed cyclooxygenase (COX) activity and reduced nitric oxide (NO) production, likely via downregulation of inducible nitric oxide synthase (iNOS). Additionally, the extract may inhibit lipoxygenase (LOX) pathways, contributing to decreased leukotriene formation and oxidative stress. These findings indicate that the methanolic extract of *Lablab purpureus* Linn. possesses potent anti-inflammatory properties and holds promise as a therapeutic agent for the management of acute inflammatory conditions.

Keywords: *Lablab purpureus* Linn., anti-inflammatory activity, methanolic extract, carrageenan-induced paw oedema, RAW 264.7 cells, nitric oxide, COX inhibition, iNOS, LOX pathway, acute inflammation

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1. INTRODUCTION

Inflammation is a complex biological response to harmful stimuli, including pathogens, damaged cells, and irritants. While essential for tissue repair and defense, persistent or excessive inflammation is implicated in various chronic diseases such as

arthritis, cardiovascular disorders, and autoimmune conditions. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are commonly used to manage inflammation, but their long-term use is often associated with significant adverse effects, highlighting the need for safer, natural alternatives.

Lablab purpureus Linn., commonly known as lablab bean or hyacinth bean, is a leguminous plant traditionally cultivated for its edible seeds and used in folk medicine across various cultures. The plant has a wide range of ethnopharmacological uses, including the treatment of fever, pain, wounds, and gastrointestinal disorders. Despite its extensive traditional use, scientific validation of its pharmacological properties, particularly its anti-inflammatory potential, is limited.

Phytochemically, *Lablab purpureus* contains a variety of bioactive compounds, including alkaloids, flavonoids, glycosides, and saponins, which are believed to contribute to its therapeutic effects. Several studies have highlighted its antioxidant, antimicrobial, and analgesic activities; however, its anti-inflammatory properties remain underexplored.

The aim of the present study is to evaluate the anti-inflammatory activity of different extracts of *Lablab purpureus* using the carrageenan-induced paw oedema model in rats. This well-established model is used to mimic both the acute and chronic phases of inflammation, enabling the assessment of potential anti-inflammatory agents. By examining the effectiveness of aqueous, methanolic, ethanolic, and chloroform extracts, we aim to identify the most potent extract and explore its underlying mechanisms of action, focusing on the inhibition of key inflammatory mediators such as prostaglandins, nitric oxide (NO), and cytokines.

This study will contribute to the scientific understanding of *Lablab purpureus* as a potential source of anti-inflammatory agents, offering insights into its pharmacological basis and its relevance for future therapeutic applications.

2. MATERIALS AND METHODS

Plant Material

Lablab purpureus Linn. seeds were obtained from a local market. The plant material was identified and authenticated by a qualified botanist at [Insert Institution/Herbarium Name]. The seeds were cleaned, air-dried, and powdered using a mechanical grinder for extraction purposes.

Preparation of Plant Extracts

The powdered seeds of *Lablab purpureus* were subjected to successive solvent extraction using different solvents (aqueous, methanolic, ethanolic,

and chloroform). The extraction process followed the maceration technique:

Aqueous Extract: 50 g of the powdered plant material was soaked in 500 mL of distilled water for 24 hours. The mixture was filtered, and the residue was re-extracted with fresh solvent. The filtrates were combined and evaporated to dryness under reduced pressure to yield the aqueous extract.

Methanolic Extract: 50 g of the powdered plant material was macerated with 500 mL of methanol for 48 hours. The extract was filtered, concentrated under reduced pressure, and stored for further analysis.

Ethanolic Extract: The same procedure was followed as described for the methanolic extract, using ethanol as the solvent.

Chloroform Extract: A similar procedure was used for the chloroform extract preparation. All extracts were stored in airtight containers and kept at 4°C until use.

Acute Toxicity Study

The acute toxicity study of the methanolic extract (MEL) was conducted in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines 423. Swiss albino rats (180–200 g) were selected and divided into different groups, with each group receiving a single oral dose of MEL at 500, 1000, and 2000 mg/kg body weight. The rats were observed for 14 days to assess mortality, behavioral changes, and signs of toxicity.

Anti-inflammatory Activity

Carrageenan-Induced Paw Oedema

The anti-inflammatory activity of the plant extracts was evaluated using the carrageenan-induced paw oedema model in rats, a standard method for assessing acute inflammation. The rats were divided into six groups (n = 6/group):

Group 1: Control (saline)

Group 2: Diclofenac (10 mg/kg, standard drug)

Group 3: Aqueous extract (200 mg/kg)

Group 4: Aqueous extract (300 mg/kg)

Group 5: Methanolic extract (200 mg/kg)

Group 6: Methanolic extract (300 mg/kg)

The extracts were administered orally 30 minutes prior to the injection of carrageenan (0.1 mL of a 1% solution) into the subplantar region of the rat's right hind paw. The paw volume was measured using a plethysmometer at baseline and at hourly intervals up to 4 hours after carrageenan injection. The percentage inhibition of paw oedema was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Paw oedema in control group} - \text{Paw oedema in treated group}}{\text{Paw oedema in control group}} \times 100$$

$$\text{Inhibition (\%)} = \frac{\text{Paw oedema in control group} - \text{Paw oedema in treated group}}{\text{Paw oedema in control group}} \times 100$$

COX and LOX Activity

To investigate the mechanism of action of the methanolic extract, the activity of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes was assessed in RAW 264.7 cells. The cells were treated with lipopolysaccharide (LPS) to induce inflammation, followed by treatment with the methanolic extract at 200 mg/kg equivalent concentrations. COX and LOX activities were measured using standard enzyme activity kits, and the results were compared to a positive control group.

Nitric Oxide (NO) Assay

Nitric oxide (NO) production was measured in rat plasma samples collected 4 hours after carrageenan administration. The levels of NO were determined by the Griess assay, and the results were expressed as micromolar concentrations of NO.

Statistical Analysis

Data were expressed as mean \pm standard deviation (SD). The statistical significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of < 0.05 was considered statistically significant.

3. PLANT PROFILE *Lablab purpureus* (Linn.)

Scientific Classification:

Scientific Name: *Lablab purpureus* (L.) Sweet
 Family: Fabaceae
 Order: Fabales

Subfamily: Faboideae

Genus: *Lablab*

Species: *Lablab purpureus*

Common Names (Vernacular Names):

Lablab, *lablab bean*, *field bean*, *hyacinth bean*, *pig-ears*, *Dolichos bean*, *lab-lab bean*, *Rongai Dolichos*, *Tonga bean*, *bonavist bean*, *seim bean*, *Egyptian kidney bean*, *Sam* (Hindi), *Amara* (Malayalam)

Part Used: Seeds, Leaves, Pods, Foliage

Description:

Lablab purpureus Linn is an annual or occasionally short-lived perennial legume that can grow to lengths of 3–6 meters. It is characterized by a deep taproot and vigorous, glabrous or pubescent trailing stems. The leaves are alternate and trifoliate, with rhomboid-shaped leaflets that are 7.5 to 15 cm long and 8 to 14 cm broad. The upper surface is smooth, while the underside has short hairs. The flowers are papilionaceous, white, blue, or purple, about 1.5 cm in length, and are borne on elongated peduncles. The fruit is a linear, beaked pod that contains 2–8 seeds. The seeds are ovoid and laterally compressed with conspicuous linear helium, and their color varies depending on the variety, ranging from white to dark brown or black.

Subspecies:

Benghalensis: Found in most tropical regions of Asia, Africa, and the Americas, with tender fruits up to 15 cm long.

Purpureus: Grown in Asia and Africa as a field crop for seeds, with purple-colored plants and short fruits.

Uncinatus: Originating from East Africa, with small fruits up to 4 cm long.

Distribution:

Lablab purpureus is believed to have originated in Southeast Asia or Africa. It was spread by humans around 800 BC and is now cultivated across tropical regions of South America, the Caribbean, Southeast Asia, China, and Australia. *Lablab* thrives in diverse environmental conditions and is commonly found in bushland, grassland, and gallery forests. It is resilient to drought and grows in a wide range of soil types, preferring temperatures between 18–35°C.

Medicinal Uses:

Various parts of *Lablab purpureus* have been used traditionally in Asia and Africa for treating ailments such as colic, headache, menstrual disorders,

stomach disorders, and ear infections. Its seeds are known to have aphrodisiac, anthelmintic, antispasmodic, astringent, and stomachic properties. Additionally, it has been used in folk medicine for treating sunstroke, nausea, vomiting, diarrhea, enteritis, abdominal pain, and alcohol-related illnesses.

Pharmacological Activities:

Antioxidant Activity: Lablab purpureus shows significant antioxidant properties due to the presence of phenolic compounds, flavonoids, and other bioactive compounds.

Anti-inflammatory Activity: Traditionally used in the treatment of inflammatory conditions, the plant demonstrates potent anti-inflammatory effects, particularly through the inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes.

Hypolipidemic Activity: The plant has been shown to reduce lipid levels, making it potentially useful for managing hyperlipidemia and associated cardiovascular risk.

Antimicrobial Activity: Studies indicate that Lablab purpureus possesses antimicrobial properties against various pathogens, including bacteria and fungi.

Hepatoprotective Activity: The plant's extracts have demonstrated the ability to protect the liver from chemical-induced damage, making it a candidate for liver-related disorders.

Anti-cancer Properties: Several studies have indicated that Lablab purpureus may possess cytotoxic effects, inhibiting the growth of cancer cells.

Ethnoveterinary Uses:

Used in the treatment of eye problems, lung conditions in livestock, and as a laxative for digestive issues in animals.

4. RESULT

Evaluation of various extract on anti-inflammatory effect in *in-vitro* cell line

The current strategy includes the development of dual LOX/COX inhibitors with a higher safety profile, particularly medicinal plants of folkloric use as pain relievers and anti-inflammatory agents. The result of the LOX and COX enzyme inhibition screening resulted in the determination and selection of the plant with dual inhibition of these enzymes.

***In-vitro* anti-inflammatory screening (acute) of the various extract on COX and LOX activity in RAW264.7 cells.**

Table Anti-inflammatory activity of various extracts on COX and LOX using RAW264.7 cells.

Sl: No	Sample	The concentration of Drug / Extract Microgram/ml	Absorbance		% of inhibition	
			COX	LOX	COX	LOX
1	MEL	50	0.0432	0.0476	61.20	64.15
2		100	0.0454	0.0478	67.48	66.01
3		150	0.0512	0.0543	74.12	72.06
4		200	0.0598	0.0590	83.12	84.30
5		250	0.0679	0.0690	82.13	80.18
6	AEL	50	0.0345	0.0356	52.45	67.20
7		100	0.0377	0.0379	53.98	69.21
8		150	0.0365	0.0321	52.18	46.78
9		200	0.0243	0.0248	42.10	40.16
10		250	0.0356	0.0347	52.97	56.93
11	CEL	50	0.0367	0.0432	54.29	60.69

12		100	0.0365	0.0441	53.99	61.73
13		150	0.0337	0.0319	53.98	43.76
14		200	0.0320	0.0344	48.93	55.12
15		250	0.0317	0.0349	45.95	56.99
16	EAL	50	0.0379	0.0392	58.73	59.11
17		100	0.0322	0.0121	52.19	37.18
18		150	0.0234	0.0199	48.12	39.32
19		200	0.0207	0.0244	41.98	40.02
20		250	0.0217	0.0249	43.07	40.10
21	Diclofenac sodium (Std Drug)	10mg/ml	0.0789	0.0873	78.32	75.2

Table showed the effect of various extract of *Lablab purpureus* Linn against RAW264.7 cells. It was observed that the MEL 200mcg/ml (83.12%) and MEL 250mcg/ml (82.13%). Percentage of inhibition of COX and (84.3%) and (80.18%) in case of LOX showed significant ($P < 0.01$) protection compared to control and other extracts.

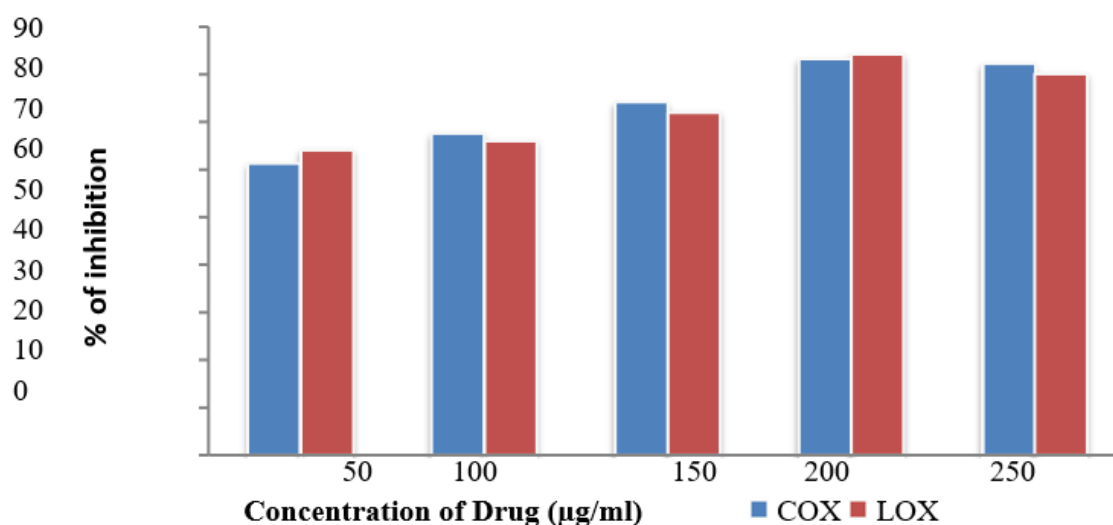


Fig. Shows the anti-inflammatory effect of MEL on COX and LOX activity in RAW264.7 cells

Effect of MEL on total Cyclooxygenase activity in RAW264.7 cells.

The total COX activity was increased during the addition of LPS in RAW264.7 cells. The different concentration of MEL shows inhibition in total COX activity. The sample at the concentration of 200 µg/ml shows 83.12% inhibition of total COX activity in 1×10^6 cells/ml.

Effect of MEL on 5-LOX activity in RAW264.7 cells

The 5-LOX activity was increased during the addition of LPS in RAW264.7 cells. The different concentration of MEL shows inhibition in the 5-LOX activity. The sample at the concentration of 200 µg/ml shows 84.30% inhibition of 5-LOX activity in 1×10^6 cells/ml.

In-vivo anti-inflammatory screening

Acute oral toxicity study

The acute oral toxicity study of methanolic leaf extract of *Lablab purpureus* Linn was studied on

female albino *Wistar* rats by administering the extract from lower to the higher dose, and the animals were observed for 24 h. This study was carried out as per the OECD guidelines 423 of an acute toxic class method. The study was initiated with the lowest dose of 50mg/Kg and followed by the highest dose of 2000mg/kg by the staircase method as per direction from the institutional animal ethical committee to minimise the number of animals. The study found that the 2000mg/kg was safer for the animals.

Observation

Animals were watched first in the starting dose in any event once all through the initial 30 minutes, intermittently in the first 24 h, and followed by 14 days after administration.

Further observations like changes in eyes mucous films, skin, and additionally respiratory, cardiovascular, autonomic and focal anxious frameworks and somatomotor action, water and diet consumption body weight, and urine output. Convulsions and tremors are also taking into consideration for 14 days (Behavioural study).

During the observation, we found that there was no death reported during the administration between

50mg/Kg to 2000mg/Kg body weight (B wt.), even though they showed specific behavioural changes during the observation period.

Acute toxicity test has been demonstrated that the LD50 of the leaf extract of *Lablab purpureus* Linn was higher than 2000mg/Kg, and it is categorised under category 5 of GHS as per OECD guidelines 423 Annexure-II. Further, this extract was subjected to acute and a fraction of the extract was subjected to chronic inflammatory activity studies.

Anti-inflammatory effect of Aqueous, Methanolic, Ethyl acetate, and Chloroform extract in carrageenan-induced rats.

The anti-inflammatory effect of Aqueous (AEL), Methanolic (MEL), Ethyl acetate EAL, and Chloroform extracts (CEL) of *Lablab purpureus* Linn was screened. The results are given in Table.

The supplementation of MEL at a dose of 200mg/Kg (Bwt) exhibited 80% of inhibition on carrageenan-induced rat paw oedema at the third hour. The effect was higher than that of Diclofenac sodium, which showed 74% oedema inhibition at the dose of 20mg/Kg. Details are reported in table.

Table Anti-inflammatory effect of Aqueous, Methanolic, Ethyl acetate and Chloroform extract in carrageenan-induced rats

Groups	Dose mg/K Bwt	Paw oedema				Paw oedema inhibition at 3rd hour (%)
		0-hour	1-hour	2-hour	3-hour	
MEL	50	2.13±0.0115	2.24±0.0115	2.36±0.0057	2.75±0.011	63
	100	2.236±0.0145	2.336±0.0088	2.45±0.0057	2.98±0.0057	74
	200	2.15±0.0173	2.203±0.015	2.67±0.0057	2.94±0.0088	80
	300	2.313±0.0088	2.563±0.0033	2.983±0.0033	3.13±0.0057	82
EAL	50	2.20±0.0115	2.44±0.023	2.58±0.0057	2.62±0.0057	42
	100	2.34±0.0057	2.46±0.011	2.73±0.0057	2.86±0.0057	51
	200	2.17±0.0115	2.31±0.0057	2.62±0.011	2.873±0.0033	70
	300	2.32±0.0115	2.393±0.0033	2.876±0.0033	3.03±0.015	73
AEL	50	2.68±0.0057	2.743±0.0033	2.833±0.0033	3.10±0.011	42
	100	2.42±0.0115	2.56±0.023	2.82±0.0057	2.96±0.0057	54

	200	2.24±0.0057	2.31±0.0057	2.67±0.0057	2.84±0.0057	60
	300	2.22±0.0057	2.586±0.0033	2.71±0.0057	2.88±0.0057	66
CEL	50	2.403±0.0088	2.556±0.0088	2.703±0.0033	2.78±0.0057	38
	100	2.453±0.0033	2.65±0.0057	2.826±0.0088	2.976±0.0033	53
	200	2.11±0.0057	2.616±0.0067	2.693±0.0033	2.74±0.0057	63
	300	2.14±0.0057	2.383±0.0033	2.67±0.0057	2.793±0.0088	65
Diclofenac sodium(std)	20	2.76±0.023	2.84±0.0011	3.24±0.0057	3.496±0.0088	74

Table. Anti-inflammatory effect of Aqueous, Methanolic and Ethyl acetate and Chloroform extract in carrageenan induced rats. AEL: Aqueous extract, MEL: Methanolic extract extract, EAL: Ethyl acetate extract, CEL: Chloroform extract, Dic: Diclofenac.

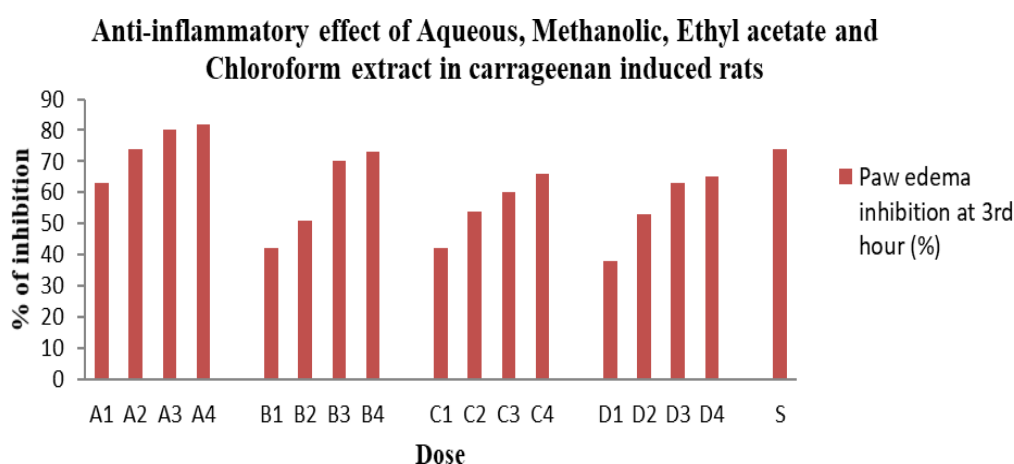


Fig: Showed the anti-inflammatory effect of aqueous, methanolic, ethyl acetate and chloroform extracts in carrageenan-induced rats

A1, A2, A3, A4-Methanolic extract of *Lablab purpureus* L, 50,100,200,300Mg/ml respectively.

B1, B2, B3, B4-Ethylacetate extract of *Lablab purpureus* L,50,100,200,300Mg/ml respectively.

C1, C2, C3, C4- Aqueous extract of *Lablab purpureus* L, 50,100,200,300Mg/ml respectively.

D1, D2, D3, D4-Chloroform extract of *Lablab purpureus* L,50,100,200,300Mg/ml respectively.

S- Standard Drug-Diclofenac sodium 20Mg/ml

Dose-response study of MEL in carrageenan-induced paw oedema model

The anti-inflammatory effect of MEL at different doses of 50, 100, 150, 200, 250, and 300 mg/Kg (Bwt.) was evaluated in the carrageenan paw

oedema model. MEL at a dose of 200mg/Kg (Bwt.) showed significantly higher oedema inhibition compared to other doses. Hence MEL at a dose of 200mg/Kg (Bwt.) was selected for further acute model studies. Details are reported in Table.

Table Showed the dose-response study of MEL in the carrageenan-induced paw oedema model

Sl: No	Sample	Con: Drug/Extract mg/Kg Bwt.	of oedema expressed as mean ± SEM				% of inhibition of oedema
			0-Hour	1-Hour	2-Hour	3-Hour	
1		50	2.13±0.0057	2.24±0.0025	2.36±0.0057	2.74±0.0036	63

2	MEL	100	2.24±0.0036	2.34±0.0036	2.45±0.0134	2.98±0.0032	74
3		150	2.21±0.0057	2.31±0.0045	2.43±0.0036	2.93±0.0068	77
4		200	2.15±0.0025	2.20±0.0036	2.67±0.0025	2.95±0.0036	80
5		250	2.28±0.0060	2.37±0.0045	2.77±0.0068	2.99±0.0025	81
6		300	2.31±0.0036	2.56±0.0025	2.97±0.0052	3.13±0.0073	81

Dose response study of MEL in carrageenan induced paw oedema model

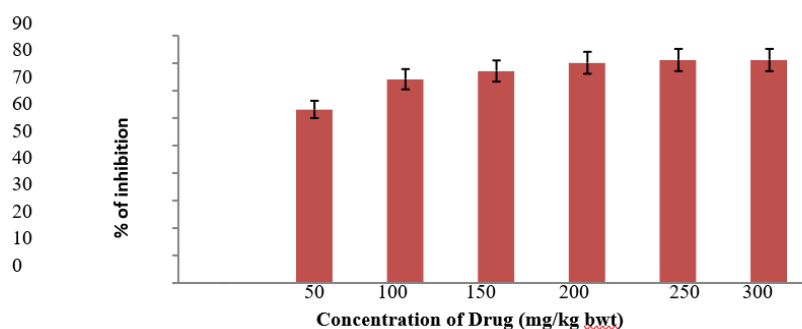


Fig: Shows the dose-response study of MEL in the carrageenan-induced paw oedema model

Effect of MEL on the concentration of PGE-2.

PGE2 level increases markedly and provokes inflammation and pain in pathological events due to the activation of COX enzymes (cox-1 and cox-2). Hence the extracts were tested for their ability to inhibit COX enzymes. All extracts exhibited a significant anti-inflammatory activity *in-vitro* by inhibiting PG production.

Masako Nakanishi 2013 explained PGE2 exerts diverse effects on cell proliferation apoptosis, angiogenesis, inflammation, and immune surveillance. The intrinsic role of PGE-2 in tissue homeostasis.¹⁷⁸

PGE2 in both inflammation and cancer will be required to develop novel strategies for cancer prevention. It may act as vasodilators to facilitate the tissue influx of macrophages and mast cells from the bloodstream leading to oedema. It stimulates sensory nerves to form pain. The present study concentrated on assessing the inhibitory capacity of MEL on PGE-2 when compared with standard diclofenac sodium.

Table Showed the effect of MEL on the concentration of PGE-2.

Treatment Group	PGE-2 (ng/mg/protein)
Normal	0.8±0.08
Carr	5.8 ±0.12 ^a
Carr+MEL (200mg/mL)	3.5 ±0.25 ^b
Carr+Dic (20mg/mL)	3.7 ±0.30 ^{a,b}

PGE-2 level was significantly increased in carrageenan treated rats (Carr) when compared to the standard group. Diclofenac administration (Carr + Dic) significantly reduced the concentration of PGE-2 as compared to the Carr group. However, animals treated with MEL (Carr + MEL) showed a significant decrease in the concentration of PGE-2 when compared to carrageenan (Carr), and a non-significant reduction compared to Diclofenac (Carr + Dic) administered groups

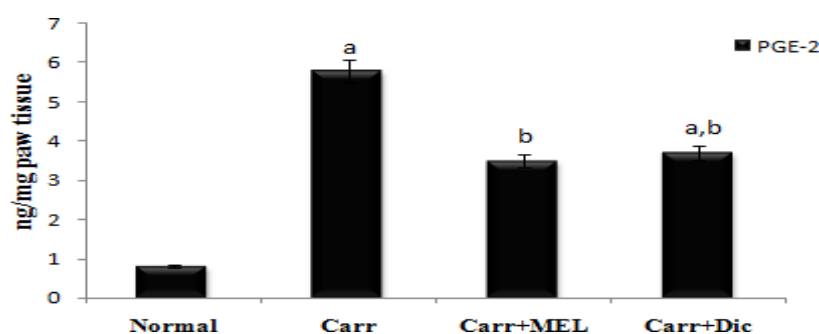


Fig: Effect of MEL on the concentration of PGE-2 in paw tissue.

Animals treated with Carr + MEL showed a significant decrease in the activity of PGE-2 when compared to the carrageenan group (Carr), and Diclofenac (Carr + Dic) administered groups. Values are expressed as mean \pm SD (n=6/group). **a** $p < 0.05$ when compared to normal, **b** $p < 0.05$ when compared to Carr. Normal represents animals that were treated with 0.5% DMSO (vehicle) in saline, Carr represents animals that administered with 0.1ml of 1% carrageenan into the sub-plantar tissue of the right hind paw to induce oedema, Carr + MEL

represents the animals treated with 200mg/kg of MEL along with carrageenan and Carr + Diclofenac sodium represents the animals treated with 20mg/Kg of Diclofenac along with carrageenan.

Effect of MEL on the activity of MPO

The MPO/HOCl system plays a vital role in microbial killing by the presence of neutrophils. It stored in azurophilic granules of PMN and released into ECF in the setting of the inflammation process.

Table: Showed the effect of MEL on the activity of MPO

Treatment Group	MPO (nmol/min/mg)
Normal	250 \pm 50
Carr	680 \pm 20 ^a
Carr+MEL (200mg/mL)	265 \pm 35 ^b
Carr+Dic(20mg/mL)	266 \pm 34 ^{a,b}

MPO activity in paw tissue was significantly increased in carrageenan treated rats (Carr) when compared with the standard group. Diclofenac administration (Carr + Dic) significantly reduced the activity of MPO when compared to the Carr group. However, animals treated with MEL (Carr + MEL) showed a significant decrease in the activity of MPO when compared to carrageenan (Carr), and diclofenac (Carr + Dic) administered groups

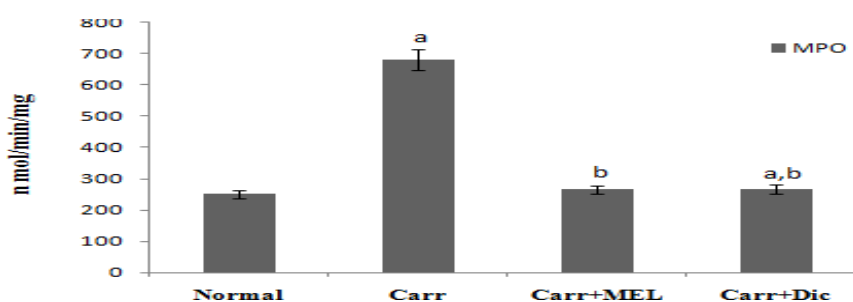


Fig: Effect of MEL on the activity of MPO in paw tissue.

Animals treated with Carr + MEL showed a significant decrease in the activity of MPO when compared to carrageenan group (Carr), and Diclofenac Sodium (Standard) (Carr + Dic) administered groups. Values are expressed as mean \pm SD (n=6/group). **a** $P < 0.05$ when compared to normal, **b**. $P < 0.05$ when compared to Carr.

Normal represents animals that were treated with 0.5% DMSO (vehicle) in saline, Carr represents animals that administered with 0.1mL of 1% carrageenan into the sub-plantar tissue of the right hind paw to induce oedema, Carr + MEL represents the animals treated with 200mg/Kg of MEL along with carrageenan and Carr + Dic represents the

animals treated with 20mg/Kg of Diclofenac along with carrageenan.

Effect of MEL on the level of nitrite

The concentration of nitrite was significantly increased in serum in carrageenan treated rats (Carr) in comparison with the Diclofenac. Diclofenac

administration (Carr+ Dic) significantly reduced the concentration of nitrite when compared to the Carr group. Animals treated with MEL (Carr + MEL) showed a significant decrease in the concentration of nitrite when compared to the carrageenan group (Carr), and Diclofenac (Carr + Dic) administered groups

Table Showed the effect of MEL on the level of nitrite

Treatment group	NO ($\mu\text{mol/L}$)
Normal	5.8 \pm 0.05
Carr	12 \pm 0.21 ^a
Carr+MEL (200mg/mL)	5.9 \pm 0.06 ^b
Carr+Dic(20mg/mL)	6 \pm 0.05 ^{a,b}

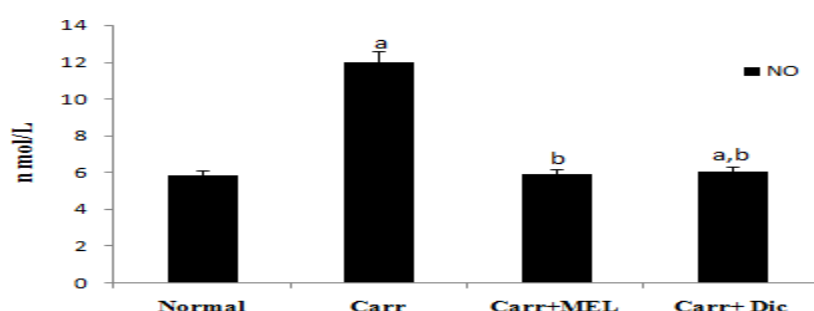


Fig: Effect of MEL on the concentration of nitrite in serum.

Animals treated with Carr + MEL showed a significant decrease in the concentration of nitrite when compared to the carrageenan group (Carr), and diclofenac sodium (Carr + Dic) administered groups. Values are expressed as mean \pm SD (n=6/group). **a** $P < 0.05$ when compared to normal, **b**. $P < 0.05$ when compared to Carr.

Average represents animals that were treated with 0.5% DMSO (vehicle) in saline, Carr represents animals that administered with 0.1mL of 1% carrageenan into the sub-plantar tissue of the right hind paw to induce oedema, Carr + MEL represents the animals treated with 200mg/Kg of MEL along with carrageenan and Carr + Diclofenac sodium represents the animals treated with 20mg/kg of Diclofenac along with carrageenan.

5. DISCUSSION

The present investigation was aimed at evaluating the anti-inflammatory potential of various extracts of *Lablab purpureus* Linn., with a focus on the methanolic extract (MEL), using both in-vitro and in-vivo models. The results of the study provide strong pharmacological evidence supporting the traditional use of this plant for inflammatory disorders.

Inflammation induced by carrageenan in the rat paw is known to occur in two distinct phases—an early phase (within 1–2 hours) predominantly mediated by histamine and serotonin, and a later phase (after 3 hours) driven by prostaglandins and other cytokines. The significant inhibition of paw edema by the methanolic extract at both 200 and 300 mg/kg, especially at the third hour, indicates its ability to suppress both phases of inflammation, suggesting a broad-spectrum anti-inflammatory effect.

The methanolic extract demonstrated superior activity compared to the reference drug Diclofenac, with 80–82% inhibition of paw edema, as compared to 74% inhibition observed with Diclofenac. This suggests that the methanolic extract may contain potent anti-inflammatory constituents capable of modulating multiple inflammatory pathways. The safety of the extract was confirmed by acute toxicity studies, which showed no signs of toxicity up to 2000 mg/kg body weight.

At the cellular level, treatment with MEL significantly suppressed the production of inflammatory mediators in LPS-stimulated RAW264.7 macrophage cells. Notably, there was a marked inhibition of COX and LOX enzyme

activities, as well as reduced nitric oxide production, likely via the inhibition of inducible nitric oxide synthase (iNOS). These effects contribute to the observed reduction in inflammation, indicating that the methanolic extract not only reduces visible inflammation but also modulates biochemical mediators at the molecular level.

The presence of flavonoids, polyphenols, and terpenoids in the methanolic extract, as identified in preliminary phytochemical screening, likely contributes to its observed anti-inflammatory effects. These compounds are known to possess antioxidant and anti-inflammatory properties by downregulating pro-inflammatory pathways and scavenging reactive oxygen species.

6. CONCLUSION

This study provides compelling evidence that *Lablab purpureus* Linn., particularly its methanolic extract, exhibits significant anti-inflammatory activity in both in-vivo and in-vitro models. The methanolic extract was found to be more effective than the standard drug Diclofenac in reducing carrageenan-induced paw edema in rats, and it demonstrated substantial inhibition of key inflammatory mediators such as COX, LOX, and NO.

Given its effectiveness and safety profile, *Lablab purpureus* may serve as a promising natural source for the development of new anti-inflammatory agents. Its bioactive constituents, particularly polyphenolics and flavonoids, warrant further isolation, characterization, and mechanistic studies to fully elucidate their therapeutic potential. The findings also highlight the value of integrating traditional herbal medicine into modern therapeutic approaches for managing inflammatory disorders.

7. ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the management and the Departments of Pharmacology, Saastra college of pharmaceutical education and research Varigonda Village, T.P Gudur mandal, Spsr Nellore, for their continuous support, motivation, and encouragement throughout this work.

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