Int. J. Pharm. Investigation, 2025; 15(4):1175-1183.
https://www.jpionline.org

Original Article

Assessment of Anti-Inflammatory Properties of Methanolic Root Extract of *Plumeria alba* Linn.: An *in vitro* and *in vivo* Approach

Harshitha Nagaraj*, Kusu Susan Cyriac, Deepak Kumar Jha

Department of Pharmacology, Karnataka College of Pharmacy, Bengaluru, Karnataka, INDIA.

ABSTRACT

Background: Inflammation is the body's innate defence mechanism that is initiated in reaction to injury or on exposure to detrimental agents. *Plumeria alba* Linn, a small laticiferous tree rich in phytochemicals and antioxidants is traditionally used to handle a variety of ailments, including ulcers, rheumatism, bronchitis. **Objectives and Methodology:** The purpose of the study was to investigate the anti-inflammatory potential of *Plumeria alba* methanolic root extract. Following their collection, authentication, and extraction, the roots were evaluated using a variety of *in vitro* and *in vivo* experimental models, including HRBC membrane stabilization, heat-induced paw edema, proteinase inhibitor assay, inhibition of albumin denaturation, paw edema, and cotton pellet granuloma respectively. Pro-inflammatory cytokines IL-6 and TNF-α as well as antioxidants SOD, LPO were biochemically analysed. Histopathological examinations of the thigh region of rats were performed to assess structural alterations in the tissue. **Results:** The results revealed that*Plumeria alba* significantly reduced the paw edema and granuloma comparable to positive control group. Both the doses of the extract (250 and 500 mg/kg) demonstrated a reduction in pro-inflammatory cytokines, indicating its potential to treat inflammation. Biochemical analyses of antioxidants SOD and LPO showed improvements as well. *In vitro* analyses showed the extracts have stabilized HRBC membrane, and have anti-haemolytic, anti-proteinase and anti-albumin denaturation properties. Histological assessments revealed that *Plumeria alba* at 500 mg/kg preserved the structural architecture of the thigh tissue showing reappearance of blood supply and reduced granuloma when compared to the control group. **Conclusion:** The study concludes that *Plumeria alba* root extract exhibits promising anti-inflammatory properties, likely attributed to its improvement of pro-inflammatory cytokines and antioxidants. These effects suggest that the extract could be used to treat inflammation.

Keywords: *Plumeria alba*, Carrageenan, Cotton pellet, Granuloma, Paw edema, Tumor Necrosis Factor, Herbal Medicine.

Correspondence:
Ms. Harshitha Nagaraj
Department of Pharmacology, Karnataka College of Pharmacy, Bengaluru, Karnataka, INDIA.
Email: harshithagowda6620@gmail.com

Received: 17-01-2025;
Revised: 09-04-2025;
Accepted: 27-06-2025.

INTRODUCTION

Inflammation is a natural, physiological, defensive mechanism of the body to injury or deleterious stimuli. It aims to neutralize or minimize the dissemination of toxic substances, to remove dead cells and tissues, and to stimulate tissue repair, but inflammation can also be pathogenic, causing anaphylaxis or formation of bands and adhesions. Inflammation is primarily designed to identify and eliminate dangerous agents, concurrently to stimulate tissue healing. Roman physicians Celsus and Galen, in the 1st and 2nd centuries AD, respectively, characterized the symptoms of inflammation: rubor (redness), calor (heat), tumor (swelling), dolor (pain), and functio laesa (loss of function) (Mohan H., 2015). In response to detrimental stimuli, the body initiates acute inflammation, which is marked by prominent vasodilation, heightened permeability, the influx of neutrophils, and development of edema (Huang MH *et al*., 2011). Persistent acute inflammation can evolve into chronic inflammation, specifically distinguished by the proliferation of tissue, development of granulomas, and the activation of repair processes (Eddouks M *et al*., 2012). Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are comprehensively being employed to manage inflammation and pain. These NSAIDs currently in use are linked with adverse effects such as gastrointestinal disturbances and renal failure. Attributed to these undesirable effects, there is a need to shift to plant based drugs to treat inflammation. Plants have been a primary source of medicine since antiquity. About 75-80% of individuals in developing nations utilize herbal medicine as their fundamental healthcare approach due to its cultural relevance,

DOI: 10.5530/ijpi.20250149

affordability, compatibility with human body, and reduced side effects. Furthermore, there has been a substantial rise in natural remedies among populations in nations over the last few years (Sangita K *et al*., 2011; Karim A *et al*., 2011).

For centuries, plants have served as natural treatments for pain and inflammation. *Plumeria alba* Linn. is one such plant, which is known to have multiple uses in traditional healthcare. *Plumeria* often called as Frangipani is a category of flowering plants belonging to the Apocynaceae family, consisting mainly of deciduous shrubs and small trees. It is a small lactiferous shrub, growing up to 5-8 feet, primarily cultivated for its fragrant and ornamental flowers. *Plumeria alba* is typically from Central America, Mexico, the Caribbean, and has spread to tropical and sub-tropical regions of the globe, including Asia. The flowers are arranged in groups that emerge from the ends of the branches on a long, thick stem, and they are viewed as one of the most auspicious flowers used in prayers throughout the Indian subcontinent (Goyal RK *et al*., 2012; Raju RA., 2000). *Plumeria alba* has diverse bioactive compounds including sterols, carbohydrates, tannins, triterpenoids, and iridoid glycosides. The plant also contains a blend of amyrins, β-sitosterols, copotein, iridoids like isoplumericin, and various forms of plumeride and plumeride coumerate glucoside (Imrana M *et al*., 2020). The plant parts, such as its leaves, bark, flowers, and latex, have revealed to exhibit a diverse range of pharmacological activities such as antioxidant, antibacterial, antitumor, and hypoglycemic effects. Conventionally, a preparation of the bark and roots is applied to manage asthma, soothe constipation, decrease fever, and eliminate parasitic worms. In Ayurveda,*Plumeria* species are applied in the treatment of ulcers, leprosy, rheumatism, bronchitis, cholera, diarrhoea, dysentery, and typhoid (Hasan RU *et al*., 2023).

Through evaluating the anti-inflammatory potential of *Plumeria alba*, this study aims to ascertain whether the plant can alleviate stress, and tissue damage. It has been an approach to find plant-based products that can manage inflammation with minimal to no side effects. This study is particularly important given that the drugs currently in use for inflammation cause adverse effects and only a few anti-inflammatory agents are available that have minimal side effects.

MATERIALS AND METHODS

Drugs and Chemicals

The roots of *Plumeria alba* were obtained from Subramanya Nursery of Doddaballapur region, Bengaluru Rural, Karnataka, India. Dr. V. Rama Rao, Research Officer (Botany) from Central Ayurveda Research Institute, identified and authenticated the plant specimen, and specimens were retained for reference under CARI Acc. No 970. Indomethacin was obtained from a local medical supply store in Bengaluru. The chemicals employed in this research were the entire highest analytical standard and created fresh on the day of the examination.

Preparation of Extract of *Plumeria alba*

The roots of *Plumeria alba* were thoroughly rinsed with tap water and then parched in shade at 25°C. Once dried, the roots were finely ground into powder form. This powdered material was subsequently passed through a 1 mM sieve to prepare for extraction.

Method of Extraction

A quantity of 440 g of this powder was employed for extraction with 1.2 L of methanol using a Soxhlet apparatus. The resulting extract was then evaporated to dryness, yielding a constant extract weight of 11.3%. The final extract was stored in the refrigerator until further use.

Experimental animals

In this study, Wistar rats weighing 150-200 g were utilized. They were maintained in standard laboratory environment with access to regular feed pellets and water. Experimental techniques cohered the guidelines set forth by CCSEA, India, and received approval from the IAEC.

Dose Selection for Pharmacological Activity

The selected doses for evaluating the anti-inflammatory potential of *Plumeria alba* and Indomethacin were determined based on findings from acute toxicity studies available in the literature. The doses were set at 250 mg/kg and 500 mg/kg (Tessou KZ *et al*., 2013), while Indomethacin was administered at 10 mg/kg.

Phytochemical Analysis

The concentrated extracts were used for phytochemical analysis of preliminary phytoconstituents and were found to be: Flavonoids, alkaloids, tannins, sterols and phenols.

Experimental Design

In vitro methods

Human Red Blood Cell (HRBC) Membrane Stabilization

For the experiment, a Human Red Blood Cell (HRBC) suspension was prepared to examine the anti-inflammatory potential of the crude drug. Human blood was centrifuged for 10 min at 3000 rpm with an equivalent volume of Alsever's solution to produce the packed cells. Following an isosaline washing, the cells were diluted to a 10% solution. Distilled water was used to prepare the crude drug solution at a concentration of 50 mg/mL. The sample consisted 1 mL of the extract which was combined with 1 mL of 2 mM phosphate buffer, 2 mL of hyposaline, and 0.5 mL of HRBC solution. The mixture was centrifuged for 20 min at 3000 rpm following 30 min of incubation at 37°C. Spectrophotometry was used to measure the supernatant at 560 nm. The reference

standard was diclofenac, and the control was made without extracts (Murthuza S *et al.*, 2018).

Percentage Protection=100-(Abs test/Abs control)×100

Heat Induced Haemolysis

The experiment employed 1 mL of the test drug (50 mg/mL) and 1 mL of HRBC solution made up the 2 mL total volume reaction mixture. Diclofenac (50 mg/mL) was used as the reference, and saline was the placebo. The mixtures were cooled and centrifuged for 5 min at 2500 rpm following a 30 min incubation period at 56ºC in a water bath. The absorbance of the supernatants at 560 nm was used to assess the test drug's anti-inflammatory properties (Murthuza S *et al.*, 2018).

Percentage inhibition=(Abs control-Abs sample)×100/Abs control

Proteinase Inhibitor Assay

The reaction mixture consisted 1 mL of 20 mM Tris-HCl buffer (pH 7.4), 1 mL of the test sample, and 0.06 mg/mL trypsin. Saline was used to prepare control (Murthuza S *et al.*, 2018). 1 mL of 0.8% (w/v) casein was added after the mixture had been incubated for 5 min at 37ºC. It was then incubated again for 20 min. The reaction was halted by adding 2 mL of 70% perchloric acid, and the resultant cloudy solution was centrifuged for 5 min at 3000 rpm. The absorbance of the supernatant was measured at 210 nm in order to evaluate its anti-inflammatory capabilities:

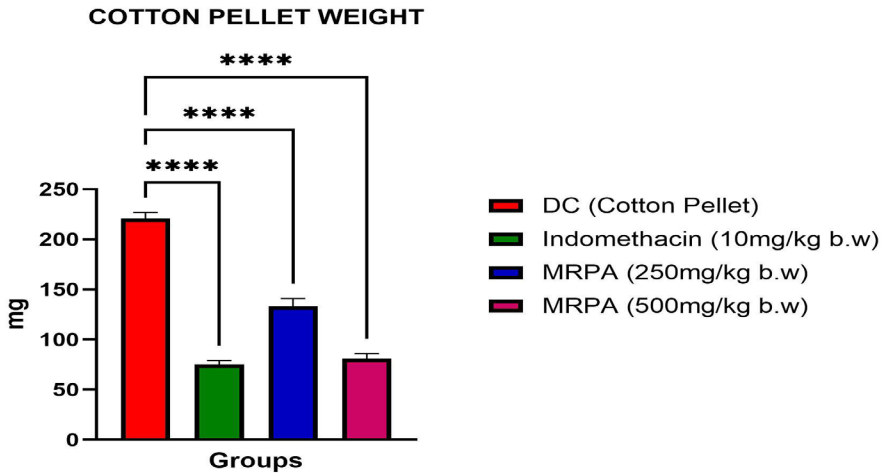

Figure 1: Effect of MRPA on Cotton pellet induced granuloma. Values are expressed as Mean±S.E.M (*n*=6). ****p<0.0001, compared with DC using one-way ANOVA followed by Tukey's multiple comparison test.

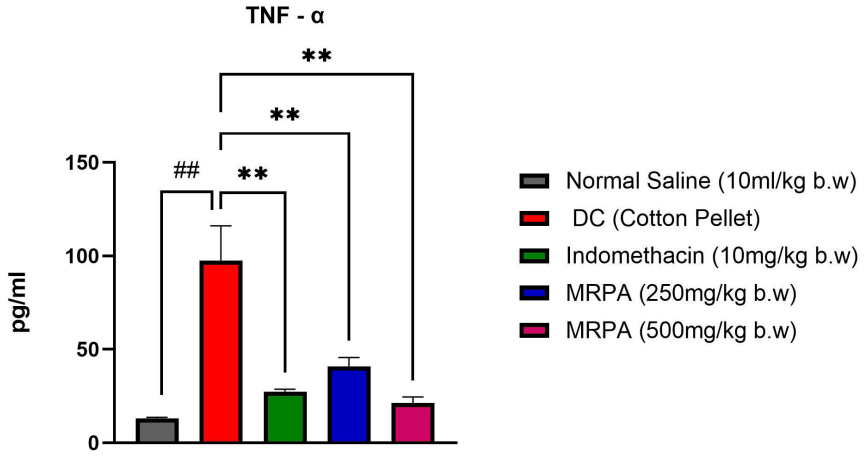

Figure 2: Effect of MRPA on TNF- α. Values are expressed as Mean±S.E.M (*n*=6) ##p<0.005, compared with NC, **p<0.005, *p<0.01 compared with DC using one-way ANOVA followed by Tukey's multiple comparison test. NC- Normal Saline 10 mL/kg.b.w., DC- Disease Control: Cotton pellet implantation S.C. Std: Indomethacin (10 mg/kg).

Percentage inhibition=(Abs control-Abs sample)×100/Abs control

Inhibition of Albumin Denaturation Assay

2 mL of the reaction mixture was made up of 1 mL of the test sample (50 mg/mL) and 1 mL of a 1 mM albumin solution in 0.2 M phosphate buffer (pH 7.4) (Murthuza S *et al.*, 2018). The standard Diclofenac (50 mg/mL) was also prepared in the same manner. After mixing the samples, they were incubated at 27°C for 10 min. The drug-albumin interaction was subsequently evaluated by measuring turbidity using spectrophotometry at 660 nm.

Percentage inhibition=(Abs control -Abs sample)×100/Abs control

In vivo methods

Carrageenan induced paw edema in rats

Experimental rats were categorised into 5 groups, n=6/group

Group I received Normal saline (10 mL/kg, orally) Group II received carrageenan sub-plantar injection (0.1 mL of 1% w/v suspension) (Chouhan YS *et al.*, 2014). Group III received Indomethacin (10 mg/kg, orally), administered 30 min after carrageenan. Group IV received *P. alba* extract (250 mg/kg, orally), administered 30 min after carrageenan. Group V received *P. alba* extract (500 mg/kg, orally) administered 30 min after carrageenan.

Paw volume was recorded using a plethysmometer right after the injection and again at 3, 6, and 24 hr post challenge. The percentage inhibition of edema was determined by:

% inhibition of edema=[1-Vt/Vc]×100

Vt is the increase in paw volume in the treated group and Vc is the increase in paw volume in the control group.

Cotton pellet induced Granuloma

Wistar rats weighing approximately 200 g were used for the experiment (Sultana A *et al.*, 2019). After anesthetizing the rats with phenobarbital, the back skin was shaved and disinfected, followed by a lumbar incision to place sterilized cotton pellets in subcutaneous tunnels created in the scapular region. The rats received *Plumeria alba* extracts orally for seven days. After treatment, the animals were sacrificed, and the cotton pellets were dried until a constant weight was achieved. The percentage inhibition of inflammation was determined by:

% inhibition=100×(1-Wt/Wc)

Where,

Wt is the difference in pellet weight in the drug-treated group and Wc is the difference in pellet weight in the untreated group.

Experimental rats were categorised into 5 groups, *n*=6/group

Group I received Normal saline (10 mL/kg, orally) for seven days. Group II received cotton pellet implantation S.C. Group III received Indomethacin (10 mg/kg, orally) for seven days after cotton pellet implantation. Group IV received *P. alba* extract (250 mg/kg, orally) for seven days after cotton pellet implantation. Group V received *P. alba* extracts (500 mg/kg, orally) for 7 days after cotton pellet implantation.

Biochemical and Tissue Histology Assessments

After the treatment period, all experimental animals were anesthetized with phenobarbital (50 mg/kg, I.P), and blood samples were obtained by means of cardiac puncture. Serum and plasma samples were preserved at -20°C for biochemical analysis. The same animals were then euthanized with an overdose of phenobarbital (100 mg/kg, I.P). Cotton pellets, along with surrounding tissue from the thigh region, were removed and weighed, then placed in 10% formalin for histopathological

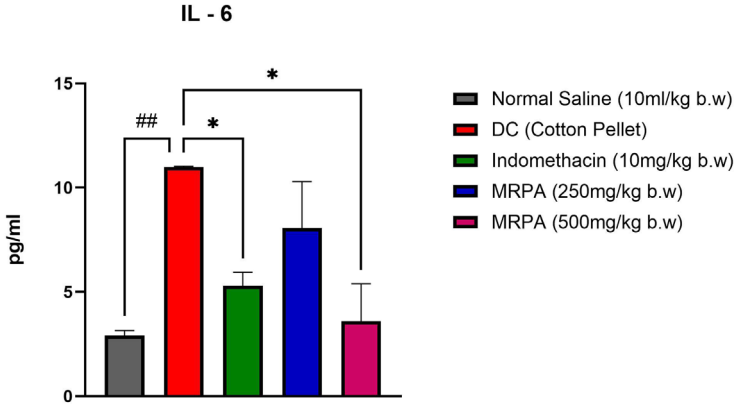

Figure 3: Effect of MRPA on IL-6. Values are expressed as Mean±S.E.M (*n*=6). ##*p*<0.01, compared with NC, *p<0.05, compared with DC.

analysis. Cytokines such as TNF-α and IL-6 assessments were evaluated. The study aimed to assess serum antioxidant enzymes and their effects on inflammation. Pro-inflammatory cytokines like TNF- α and IL-6 were studied by a Sandwich ELISA (Mohan CV *et al.*, 2022). Plasma samples were collected under mild anesthesia, and protein content was normalized using the Lowry method (Lowry OH *et al.*, 1951). IL-6 and TNF-α concentrations were studied by antigen capture ELISA. Lipid peroxidation was measured using *Ohkawa et al's* method, which involved adding Thiobarbituric acid, SDS, and acetic acid to a 10% serum solution. Absorbance of malondialdehyde was assessed at 532 nm, and indicated as nmol/mg of protein (Ohkawa H *et al.*, 1997). SOD activity was measured by adding adrenaline and observing shift in absorbance at 480 nm. Sucrose served as a blank, and SOD was indicated in units per milligram of protein (Okado-Matsumoto A, Fridovich I. 2001). Histopathological analysis involved preserving The tissue surrounding the cotton pellets in neutral buffered formalin, dehydrating it in ethanol, clearing it with xylene, and embedding it in paraffin wax. Portions were cut, processed, stained with H&E, and examined in a microscope for histopathological evaluation (Kareem AA *et al.*, 2022).

Statistical Analysis

The outcomes were articulated as Mean±SEM, with each group consisting of 6 rats (n=6). Statistical analyses were performed using GraphPad Prism (version 10). Group differences were assessed through ANOVA, followed by Tukey's test. Statistical significance was established by comparing the untreated control group with the other groups, where p-values lower than 0.001 were regarded highly significant.

RESULTS

In vitro

MRPA at 500 mg/kg shows significant HRBC Membrane stabilization, anti-haemolytic, anti-proteinase and anti-albumin denaturation properties when compared to standard diclofenac (Table 1).

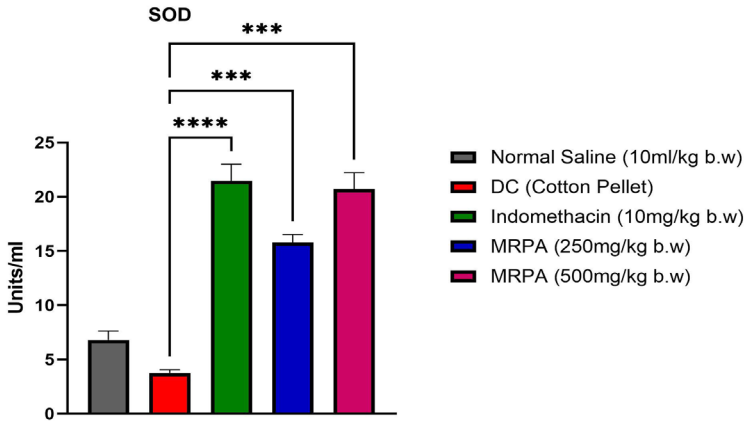

Figure 4: Effect of MRPA on SOD. Values are expressed as Mean±S.E.M (n=6). ****p<0.0001, ***p<0.001 compared with DC.

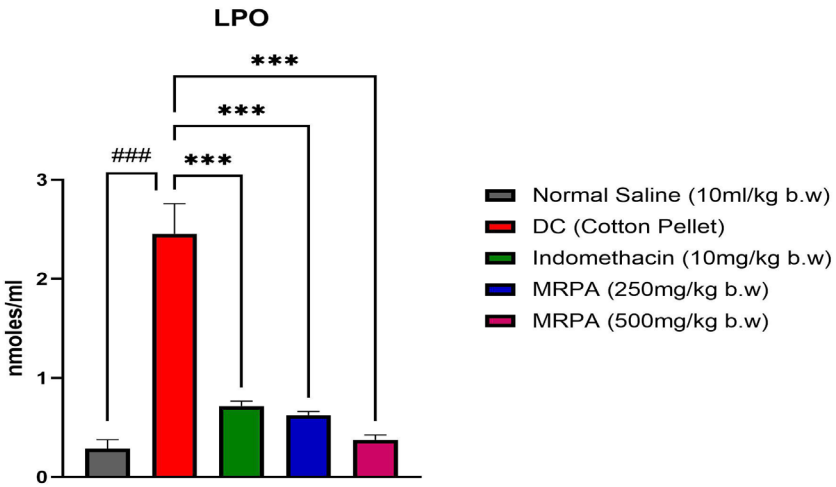

Figure 5: Effect of MRPA on LPO. Values are expressed as Mean±S.E.M (i=6). ###p<0.0005, compared with NC, ***p<0.0005, compared with DC.

In vivo

The results of carrageenan induced edema show that the 500 mg/kg of MRPA show anti-inflammatory activity at 24-hr interval similar to standard drug indomethacin. It showed a percentage inhibition of 84.21% that was similar to standard indomethacin depicting 89.48%.

In the cotton pellet induced granuloma, MRPA 500 mg/kg significantly reduced the amount of granuloma formed. Percentage inhibition shown by 500 mg/kg MRPA was 63.4% similar to that of standard indomethacin 66.1% (Figure 1).

The anti-inflammatory potential of Methanolic Root extract of *Plumeria alba* (MRPA) was assessed by analysing various

Table 1: Effect of methanolic root extract of *P. alba* on HRBC membrane stabilization, heat-induced haemolysis, Proteinase inhibitor assay and Albumin denaturation assay.

Sample	*In vitro* Anti-inflammatory Methods			
	HRBC membrane stabilization (% Protection)	Heat-induced haemolysis (% Inhibition)	Proteinase Inhibitor Assay (% Inhibition)	Inhibition of Albumin Denaturation Assay (% Inhibition)
Control	-	-	-	-
MRPA 250 mg/kg	8.39%	41.82%	26.68%	33.3%
MRPA 500 mg/kg	27.2%	65.38%	36.19%	45.92%
Diclofenac	38.36%	77.78%	50.98%	53.96%

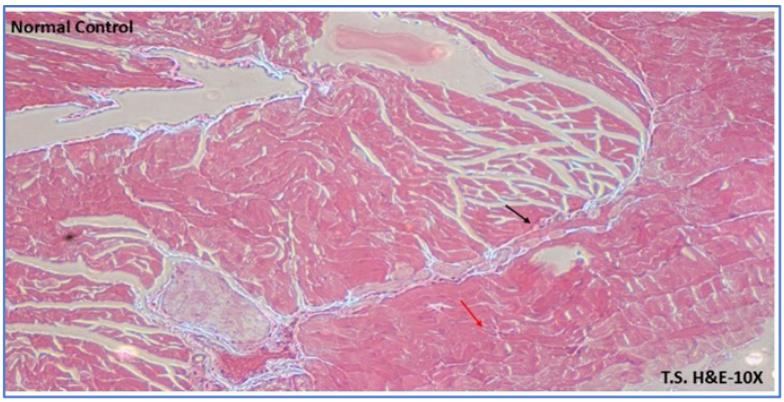

Figure 6: Normal Control. The image shows healthy skin tissue with tightly packed collagen fibres (red arrow), blood vessels (black arrow) for nourishment, and no signs of inflammation.

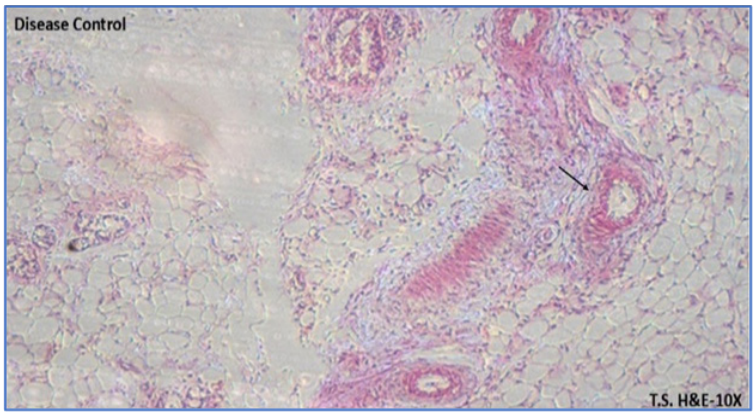

Figure 7: Disease Control. The image shows abnormal skin architecture with mild signs of inflammation or granuloma formation (small, round cells with dark nuclei-black arrow). There's an unusual arrangement of skin layers and the presence of inflammatory cells.

biochemical parameters. TNF-α (**p<0.005), IL-6 (*p<0.05), SOD (***p<0.001) and LPO (***p<0.0005) were significantly improved in contrast to untreated group. Therapy involving Indomethacin (10 mg/kg) and MRPA 500 mg/kg significantly lowered pro-inflammatory cytokines TNF-α and IL-6. Contrarily, 250 mg/kg methanolic extract did not demonstrate a considerable variation in comparison with disease control group. TNF-α and IL-6 were substantially raised in the disease group (p<0.005, p<0.01, Figures 2 and 3) comparative to normal group. Therapy with Indomethacin (10 mg/kg) and 500 mg/kg MRPA effectively prevented the increase in these cytokine levels (p<0.005, p<0.05) corresponding to disease group. Conversely, the lower 250 mg/kg methanolic extract did not demonstrate a pivotal difference.

Additionally, cotton pellet implantation resulted in decrease in the antioxidant defense system, as evidenced by lower levels of SOD (p<0.001, Figure 4) increased lipid peroxidation (p<0.0005, Figure 5) correlative to untreated group. Both Indomethacin (10 mg/kg) and 500 mg/kg MRPA considerably lowered LPO (p<0.0005) and restored SOD levels (p<000.1) contrasted to the disease group.

Histopathological examinations of the tissues from disease group shows significant granuloma formation with ruptured blood vessels, abnormal skin architecture and presence of inflammatory cells. There was an unusual arrangement of skin layers comparable to untreated group (Figures 6 and 7). In contrast, animals that received Indomethacin (Figure 8) and MRPA treatment (Figures 9 and 10) showed preserved tissue architecture, better maintained vascularity and appeared less fibrotic and dense. Overall, the treatment effectively reduced abnormalities in the tissues with reduced inflammatory cells, showcasing its anti-inflammatory potential.

DISCUSSION

The study effectively addressed its objective of investigating the anti-inflammatory potential of *Plumeria alba* Methanolic Root extract (MRPA) through a combination of *in vitro* and *in vivo* models. MRPA exhibited significant anti-inflammatory activity by stabilizing HRBC membranes, inhibiting proteinase activity, and preventing albumin denaturation, which align with the mechanisms typically observed in anti-inflammatory agents (Ahmed M *et al*., 2014; Winter CA *et al*., 1962). These findings are consistent with prior reports on plant-derived compounds known for their ability to stabilize membranes and inhibit inflammatory enzymes (Tatiya AU *et al*., 2012; Vane JR *et al*., 1998). Furthermore, MRPA's efficacy at 500 mg/kg, comparable to the standard drug diclofenac, underscores its potential as a plant-based alternative for managing inflammation while minimizing side effects (Singh G *et al*., 2016). *In vivo* evaluations demonstrated MRPA's ability to reduce granuloma formation, a hallmark of chronic inflammation, which suggests its efficacy in controlling persistent inflammatory conditions. The significant reduction in pro-inflammatory cytokines TNF-α and IL-6 observed in MRPA-treated group's highlights its modulatory effect on inflammatory pathways (Kirtikar KR *et al*., 1993). Moreover, the extract improved antioxidant defenses by reducing LPO and enhancing SOD activity, corroborating its antioxidative role in combating oxidative stress, a key factor in the progression of inflammation (Kirtikar KR *et al*., 1993). These effects may be attributed to bioactive constituents such as flavonoids, sterols, and iridoid glycosides, known for their anti-inflammatory and antioxidant properties (Tiwari A. 2014). Histopathological observations further validated these findings, showing reduced fibrosis, restoration of vascular architecture, and normalized blood supply in tissues treated with MRPA, indicating effective

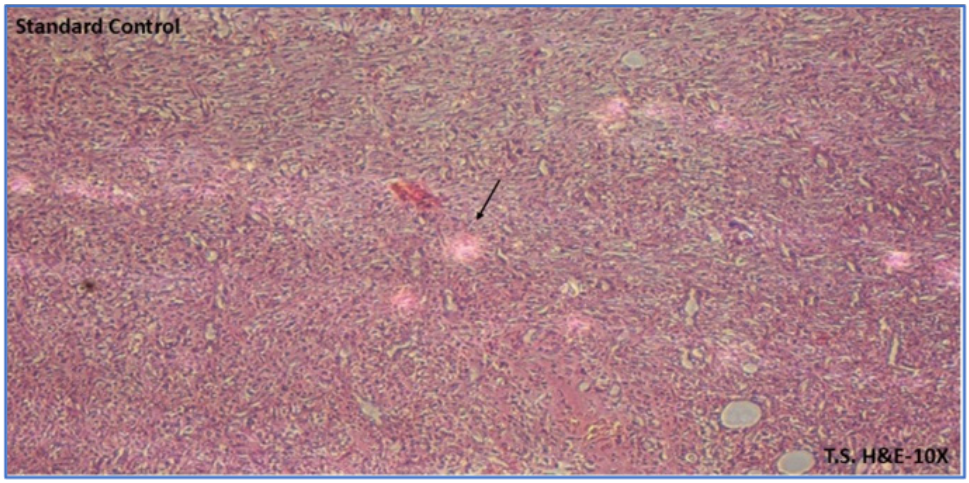

Figure 8: Standard Control. The image shows moderate fibrosis (black arrow), a mild inflammatory response, and preserved tissue architecture. This suggests that the standard treatment promotes a balanced tissue response with controlled fibrosis and inflammation. The vascular structures are better maintained, indicating less disruption and better tissue health compared to the disease.

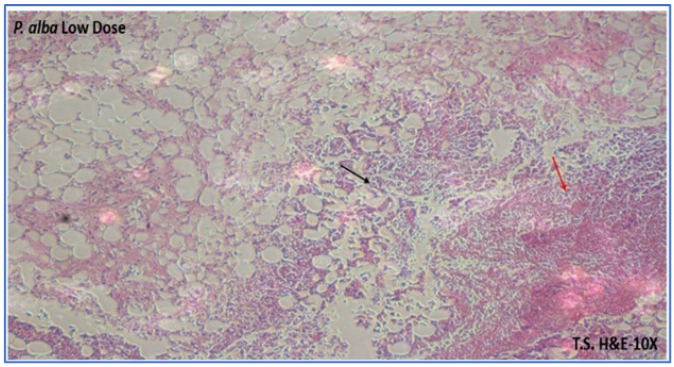

Figure 9: *P. alba* (250 mg/kg). The image shows dense connective tissue, indicating significant fibrosis and tissue remodelling. The dense fibrotic tissue is characterized by a high number of cells, suggesting an intense inflammatory response. Ruptured blood vessels (black arrow) and mild inflammation is seen (red arrow).

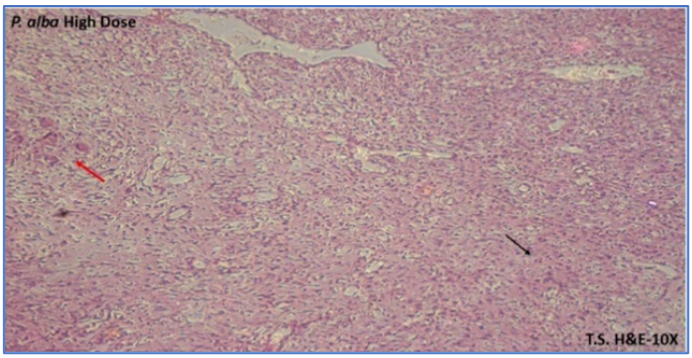

Figure 10: *P. alba* (500 mg/kg). The image shows moderate fibrosis, a mild inflammatory response (red arrow), and preserved tissue architecture. The tissue appears less dense and less fibrotic. Vascular structures are visible, indicating the tissue has maintained some normal architecture and blood supply (black arrow).

resolution of inflammation and promotion of tissue repair. The results align with previous studies on medicinal plants containing similar phytoconstituents, which have demonstrated significant anti-inflammatory and tissue-protective effects. Overall, the findings highlight MRPA as a promising natural alternative to conventional NSAIDs, offering effective management of acute and chronic inflammation with reduced risk of adverse effects. This suggests that MRPA not only mitigates inflammation but also promotes tissue repair and regeneration. The existence of bioactive compounds such as sterols, flavonoids, and iridoid glycosides likely contributes to these observed effects, making MRPA a potent natural anti-inflammatory agent. Collectively, these findings emphasize MRPA's potential as a safer, effective alternative to conventional NSAIDs, offering a holistic approach to managing inflammation while minimizing adverse effects.

CONCLUSION

The study on anti-inflammatory potential of *P. alba* has illustrated a significant dose-dependent anti-inflammatory effect. The *in vitro* assessments depicted stabilized HRBC membranes, anti-haemolytic, anti-proteinase and anti-albumin denaturation properties. The granuloma formation was reduced with a significant effect on antioxidant enzymes in drug treated rats. The anti-inflammatory effect of MRPA 500 mg/kg was showing more prominent results similar to standard indomethacin. The anti-inflammatory activity of *P. alba* could be due to the presence of antioxidant active polyphenolic components like flavonoids, glycosides and tannins.

ACKNOWLEDGEMENT

The corresponding author acknowledged her gratitude to Karnataka College of Pharmacy, Bengaluru, for providing the facilities necessary to conduct the research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SOD: Superoxide dismutase; **LPO:** Lipid Peroxidation; **TNF-α:** Tumour Necrosis Factor α; **IL-6:** Interleukin-6; **HRBC:** Human Red Blood Cell; **MRPA:** Methanolic Root extract of *Plumeria alba*; **NC:** Normal Control.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol was approved by IAEC, Karnataka College of Pharmacy, Bengaluru-560064. IAEC registration number: KCP-IAEC/14/23-24/05/28/03/24.

THE EXPERIMENTAL APPROACH IS DIVIDED INTO *IN VITRO* AND *IN VIVO* MODELS

In vitro methods include HRBC stabilization, heat-induced hemolysis, proteinase inhibitor assay, and inhibition of albumin denaturation assay. Carrageenan-induced paw edema (acute inflammation model): Carrageenan is injected sub-plantar to induce localized inflammation, with MRPA administered to evaluate anti-inflammatory efficacy. Cotton pellet granuloma (chronic inflammation model): Oral administration of MRPA is tested for its ability to reduce granuloma formation caused by cotton pellet implantation.

Highlight Results: Key findings (reduction in edema, cytokine levels, granuloma formation).

REFERENCES

Ahmed, M., Shafiq, M., Kashif, M., Ali, M. S., & Ahmad, S. (2014). Anti-inflammatory potential of plant secondary metabolites: A review. International Journal of Pharmacy and Pharmaceutical Sciences, 6(3), 1–8.

Chouhan, Y. S., Kataria, H. C., & Gosawmi, C. S. Anti-inflammatory activity of methanolic extract of *Brassica juncea* seed on carrageenan induced paw edema in rats. IJPSR.2014;5(9):3849–3851.

Eddouks, M., Chattopadhyay, D., & Zeggwagh, N. A. (2012). Animal models as tools to investigate antidiabetic and anti-inflammatory plants. Evidence-Based Complementary and Alternative Medicine: eCAM, 2012, Article 142087. https://doi.org/10.1155/2012/142087

Goyal, R. K., Goyal, G., Goyal, S., & Mittal, S. (2012). Pharmacognostical evaluation of bark of *Plumeria alba Linn*. Int J Nat. Prod. Sci., 1, 178.

Hasan, R. U., Aisha, A., Nanda, J., Rizvi, A., Alam, S., & Asif, M. (2023). A study on diverse pharmacological potential of *Plumeria alba linn*. European Chemical Bulletin, 12(3), 5257–5266.

Huang, M.-H., Wang, B.-S., Chiu, C.-S., Amagaya, S., Hsieh, W.-T., Huang, S.-S., Shie, P.-H., & Huang, G.-J. (2011). Antioxidant, antinociceptive, and anti-inflammatory activities of Xanthii fructus extract. Journal of Ethnopharmacology, 135(2), 545–552. https://doi.org/10.1016/j.jep.2011.03.057

Imrana, M., & Asif, M. (2020, April 14). Morphological, ethnobotanical, Pharmacognostical and pharmacological studies on the medicinal plant *Plumeria alba linn*. (*apocynaceae*). Arabian Journal of Medicinal and Aromatic Plants, 6(1), 54–84.

Kareem, A. A., Aziz, T. A., Ahmed, Z. A., Othman, H. H., & Hussain, S. A. (2022). Anti-inflammatory activity of gingko biloba extract in cotton pellet-induced granuloma in rats: A comparative study with prednisolone and dexamethasone. Iraqi Journal of Pharmaceutical Sciences, 31(1), 184–193. https://doi.org/10.31351/vol31iss1pp184-193

Karim, A., Nouman Soh, M. N., Munir, S., & Sattar, S. (2011). pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. International Journal of Pharmacology, 7(4), 419–439. https://doi.org/10.3923/ijp.2011.419.439

Kirtikar, K. R., & Basu, B. D. (1993). Indian medicinal plants. Bishen Singh Mahendra Pal Singh.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193(1), 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Mohan, C. V., Chatterjee, S., & Jha, D. K. (2022, November 29). Pharmacological evaluation of Moringa oleifera on collagen-induced arthritis in rats. Journal of Pharmaceutical Research International, 34(54B), 11–32. https://doi.org/10.9734/jpri/2022/v34i54B7240

Mohan, H. (2015). Inflammation and healing. Textbook of pathology (7th ed.) (pp. 116–164). Jaypee Brothers Medical Publishers (P) Ltd.

Murthuza, S., & Manjunatha, B. K. (2018). *In vitro* and *in vivo* evaluation of anti-inflammatory potency of *Mesua ferrea, Saraca asoca, Viscum album* and *Anthocephalus Cadamba* in murine macrophages raw 264.7 cell lines and Wistar albino rats. Beni-Suef University Journal of Basic and Applied Sciences, 7(4), 719–723. https://doi.org/10.1016/j.bjbas.2018.10.001

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Okado-Matsumoto, A., & Fridovich, I. (2001). Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu,Zn-SOD in mitochondria. Journal of Biological Chemistry, 276(42), 38388–38393. https://doi.org/10.1074/jbc.M105395200

Pietta, P. G. (2000). Flavonoids as antioxidants. Journal of Natural Products, 63(7), 1035–1042. https://doi.org/10.1021/np9904509

Raju, R. A. (2000). A textbook of wild plants of Indian subcontinent and their economic use p. 145. CBS Publishers.

Sangita, K., Shukla, G., & Sambasiva Rao, A. (2011). The present status of medicinal plants-Aspects and prospects. Int. J. Res Pharm. Biomed Sci., 2, 19–22.

Singh, G., Kumar, P., & Jat, R. K. (2016). Herbal anti-inflammatory agents: A review. Journal of Intercultural Ethnopharmacology, 5(4), 308–316.

Sultana, A., Afroz, R., Yasmeen, O., Aktar, M. T., & Yusuf, M. A. (2019). Anti-inflammatory effect of ethanolic extract of *Carica papaya* leaves and indomethacin in cotton pellet induced granuloma in animal model. Journal of Current and Advance Medical Research, 6(1), 2–5. https://doi.org/10.3329/jcamr.v6i1.40773

Tatiya, A. U., Saluja, A. K., & Surana, S. J. (2012). Anti-inflammatory and membrane stabilizing properties of ethanol extract of stem bark of *Mangifera indica* L. Indian Journal of Natural Products and Resources, 3(1), 50–55.

Tessou, K. Z., Lawson-Evi, P., Metowogo, K., Diallo, A., Eklu-Gadegkeku, K., Aklikokou, K., & Gbeassor, M. (2013). Acute and subacute toxicity studies of Plumeria alba linn. (*Apocynaceae*) Hydroalcoholic Extract in Rat. International Journal of Biomedical Science, 9(4), 255–259. https://doi.org/10.59566/IJBS.2013.9255

Tiwari, A. (2014). Phytochemical and pharmacological properties of *Plumeria alba* Linn.: A review. Journal of Pharmacognosy and Phytochemistry, 3(4), 58–62.

Vane, J. R., & Botting, R. M. (1998). Mechanism of action of anti-inflammatory drugs. American Journal of Medicine, 104(3), 2–8.

Winter, C. A., Risley, E. A., & Nuss, G. W. (1962). Carrageenin-induced edema in the hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine, 111(3), 544–547.

Cite this article: Nagaraj H, Cyriac KS, Jha Dk. Assessment of Anti-Inflammatory Properties of Methanolic Root Extract of Plumeria *alba* Linn.: An *in vitro* and *in vivo* Approach. Int. J. Pharm. Investigation. 2025;15(4):1175-83.