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INVESTIGATION OF ANTI-INFLAMMATORY ACTION ETHANOLIC EXTRACT OF PORTULACARIA AFRA LEAF

Correspondence author's: [1]Mr. Adarsh Dawane , [2]Mr. Vishal Shrivastav, [3]Mrs. Manju Prajapati, [4]Dr. Akhlesh Kumar Singhai

1. Research Scholar, School of Pharmacy, LNCT University Bhopal
2. Associate Professor, School of Pharmacy, LNCT University Bhopal
3. Associate Professor, School of Pharmacy, LNCT University Bhopal
4. Director School of Pharmacy, LNCT University Bhopal

ABSTRACT

Anti-inflammatory drugs can interfere in the pathophysiological process of inflammation, to minimize tissue damage and provide greater comfort to the patient. Therefore, it is important to note that due to the existence of a large number of species available for research, the successful development of new naturally occurring anti-inflammatory drugs depends mainly on a multidisciplinary effort to find new molecules. Hence the current research study is focused at evaluation of anti-inflammatory potential of *Portulacaria afra*. The leaves of *Portulacaria afra* were subjected to solvent extraction with ethanol. The best screened extract was further evaluated for it phytochemical profile by qualitative and quantitative phytochemical analysis. The results of the study suggest that *Portulacaria afra* possess significant anti-inflammatory potential. With further mechanistic studies it can be proved as a better source of natural anti-inflammatory agents.

Keywords: *Portulacaria afra*, Inflammation, Carageenan induced rat paw edema. method

INTRODUCTION

Inflammation

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes[1]. Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents. There are mainly two types of inflammation which are as follows:

a. **Acute inflammation:** It is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes.

b. Chronic inflammation: It is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis.

Inflammation is a common clinical conditions and rheumatoid arthiritis (RA) is a chronic debilitating autoimmune disorder[2] that affects about 1% of the population in developed countries[3]. The classic signs of inflammation are local redness, swelling, pain, heat and loss of function[4].

Chemical Mediators of Inflammation

An array of chemical mediators from circulation system, inflammatory cells, and injured tissue aggressively contribute to and regulate the inflammatory response. These chemical mediators are released on to the site of injury and include vasoactive amines such as histamine and serotonin, peptide (e.g., bradykinin), and eicosanoids (e.g., thromboxanes, leukotrienes, and prostaglandins).

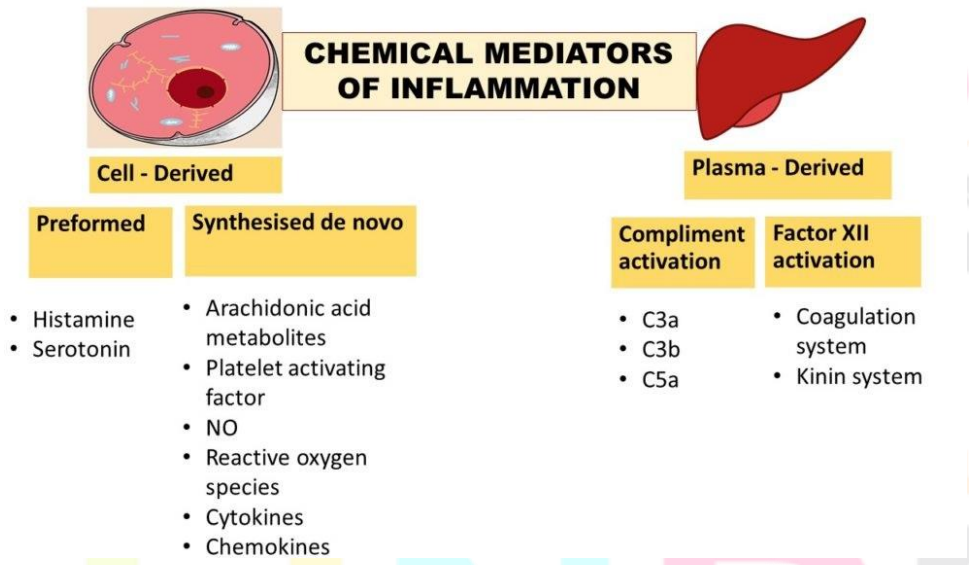

Figure 1.1: Chemical mediators of inflammation

Histamine is released in very small quantity from basophils to maintain acute-phase response during the inflammatory events. Serotonin is produced via decarboxylation of tryptophan, and it is stored in the platelets. Bradykinin is a nanopeptide produced from plasma Kinin–Kallikrein system. All these mediators can increase the synthesis of prostaglandins and produces pain locally.

Arachidonic acid, which represents the main component of membrane phospholipids in all the cells, is one of the most important substrates in the synthesis of biologically active mediators of the inflammation called eicosanoids. The latter includes the products of 5-lipoxygenase (leukotriene and 5-hydroxyeicosatetraenoic acid), cyclooxygenases (prostaglandins and thromboxanes), and 12-1ipoxygenase (12-hydroxyeicosatetraenoic acid).

Cyclooxygenase is an enzyme that is involved not only in the synthesis of proteinoids including the potent proinflammatory prostaglandins but also in the metabolism of arachidonic acid[5]. This enzyme is known to exist in at least two isoforms: cyclooxygenase-1 and -2. Prostanoids, formed by cyclooxygenase-l, are important in many physiological functions including regulation of platelet aggregation as thromboxane-2 induces platelet aggregation while PGh exhibits antiaggregatory properties. In the alimentary canal, prostaglandin-h and prostaglandin E2 inhibit secretion of gastric acid, employ an uninterrupted vasodilator effect on the blood arteries and veins of the gastric mucosa, and induce the viscous mucus creation which represents a protective barrier[6]. In the kidney, vasodilator prostaglandins (prostaglandin-h, prostaglandin E2, and prostaglandin D2) account for a significant portion in dilating of renal vascular beds, improving organ perfusion, regulating of renal blood flow, and shrinking of vascular resistance[7].

Monocytes are known to be the most principle immune effector cells and are available fundamentally in all tissues. They can differentiate, in the process of growth or development, from the peripheral mononuclear cells in blood circulating system and move to any cells in the "steady state" and/or in reaction to inflammatory induction[8].

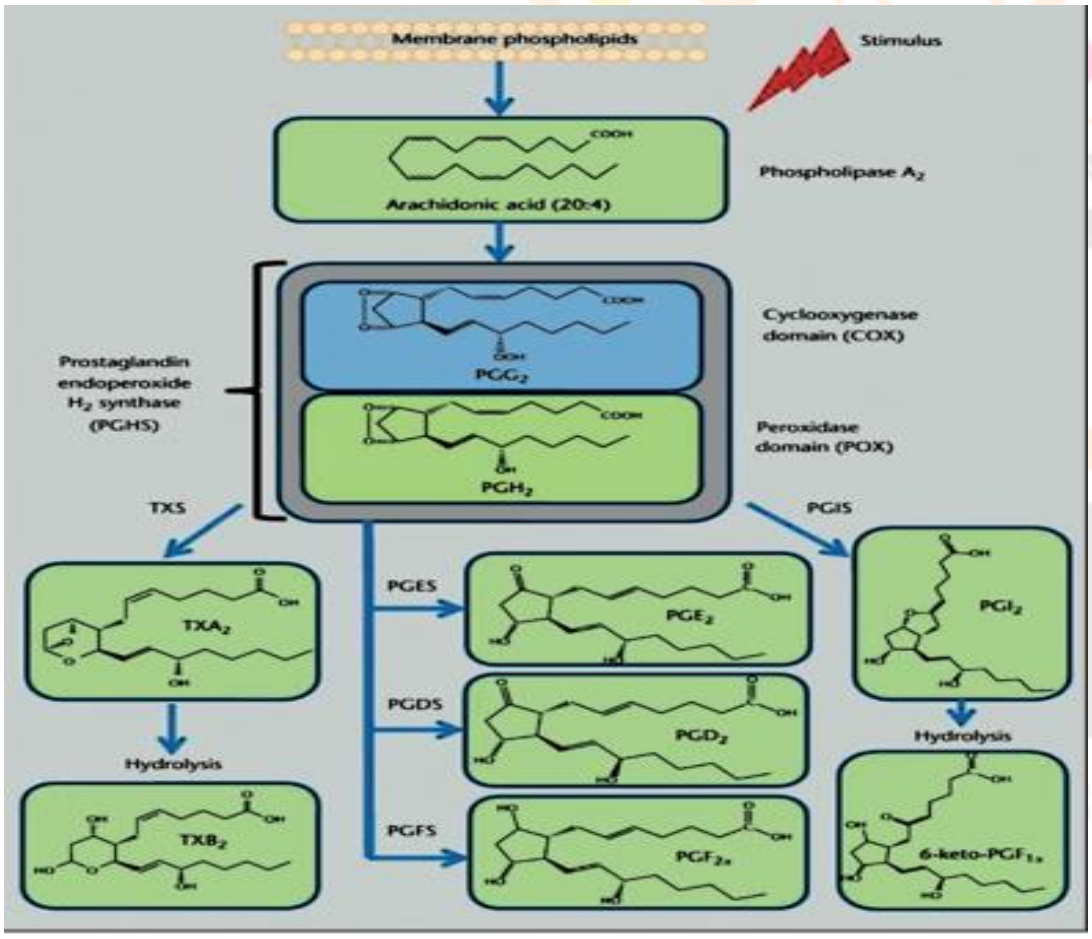

Figure 1.2: Cyclooxygenase pathway of inflammation

Monocytes possess a significant role in both adaptive and innate immunity through their interacting with many immunological and non-immunological cells to trigger feat inflammatory response and clearance of foreign elements. Upon stimulus, monocytes and resident macrophages activate and remove tissue debris and produce inflammatory signals that promote the inflammatory response. Macrophages produce a wide

array of cytokines, chemokines, and growth factors that promote inflammation, its regulation, and the successful restoration of tissue[9].

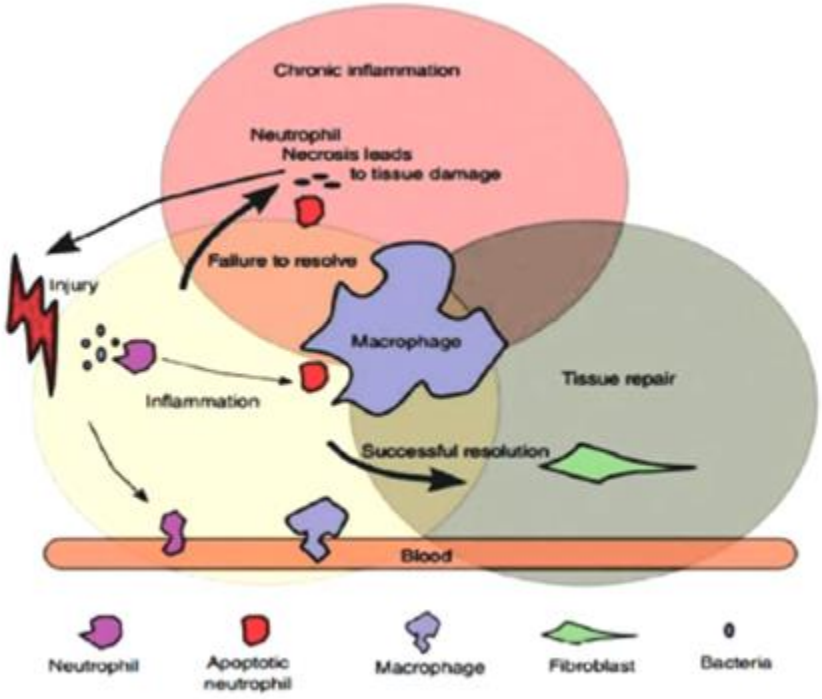

Figure 1.3: Macrophage roles in inflammation and tissue repair

2. MATERIAL AND METHODS

2.1 Collection and Identification of the Plant

The plants of *Portulacaria afra* were purchased from Shubham Nursery, Bhopal, Madhya Pradesh.

2.2 Preparation of the plant material for extraction

The leaves of the plant were washed with distilled water and dried in shade. The dried leaf has been powdered using slow speed blender and is kept in closed airtight container.

2.3 Extraction of leaf[10-15]

The extraction was carried out using ethanol as the solvent by hot continuous extraction method using a soxhlet apparatus. Briefly, 100 g of powder was packed in a thimble and the thimble was placed in the extractor of the soxhlet apparatus. 200 mL of pure ethanol was flown down the extractor in to the round bottom flask. The flask was heated at 15°C to carry out the extraction process. The extraction was carried out for 11 hours (complete extraction of contents was confirmed by the clear solution in the siphon tube of the extractor). The extract was filtered hot through Whatman filter paper in order to remove any impurity. The extract was then concentrated on boiling water bath to obtain the oleo-resinous residue. The oleo-resinous extract was collected and placed in desiccators to remove the excessive moisture**.** The dried extract was weighed and stored in desiccator for further analysis.

2.4 Phytochemical Screening of the extract[12, 16]

The ethanolic extract was evaluated by phytochemical qualitative reactions for identifying the presence or absence of usual plant secondary metabolites. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.

2.5 Total Phenolic Content[17]

The total phenolic content in the leaf extract would be determined quantitatively using Folin-Ciocalteu reagent method, using gallic acid as the reference standard. For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After incubating at room temperature for 15 min, 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture was allowed to incubate at room temperature for 2 h. The absorbance of the solution was measured at 760 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of thesamples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

2.6 Acute Toxicity Study[18]

A total of three animals were used which received a single oral dose (2000mg/kg) of extract. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, heart rate, blood pressure, salivation, perspiration, urinary incontinence, defecation and central nervous changes. Mortality, if any, was also observed over the period of 2 weeks.

2.7 Anti-inflammatory action using carageenan induced rat paw edema method

Animals

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water. The protocol was approved by the institutional ethical committee.

Carageenan induced rat paw edema method

The carageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of the ethanolic leaf extract of *Portulacaria afra* (ELEPA). Paw oedema was induced by subcutaneous injection of 0.1mL (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat. The test sample was administered in dose of 10 mg/kg in different groups of animals, 30 min prior to

carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard anti-inflammatory drug which was administered 30 min prior to carrageenan injection. Animals were divided into 4 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III– ELEPA was administered in dose of 100 mg/kg.

Group – IV– ELEPA was administered in dose of 200 mg/kg.

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter at 1, 2, 4 and 6 h using vernier caliper. The results obtained were compared with control group. The percentage inhibition of paw inflammation exhibited by each group was calculated by using following formula:

$$\% \text{ inhibition} = C\text{-}T/\ C \times 100$$

C= Paw volume (mm) in vehicle treated group (control)

T= Paw volume (mm) in drug treated group

3. RESULTS AND CONCLUSIONS

3.1 Pharmacognostic Study

The leaf of bright green in color, grow opposite on the stem and egg shaped (elephant ear shape), fleshy without a clear petiole (Figure 3.1).

Figure 3.1 Leaf of *Portulacaria afra*

The transverse section of the leaf was observed under microscope and the following features were found. The transverse section of the leaf exhibits the presence of epidermis on both the surfaces. The outer walls of the epidermis have thin cuticle. The mesophyll (tissue enclosed between both the epidermis) consists of elongated or columnar cells called as the palisade parenchyma. Irregularly arranged cells with large intercellular space are also present. They are called as the spongy parenchyma. The vascular bundles are visible in the center of the leaf (Figure 3.2).

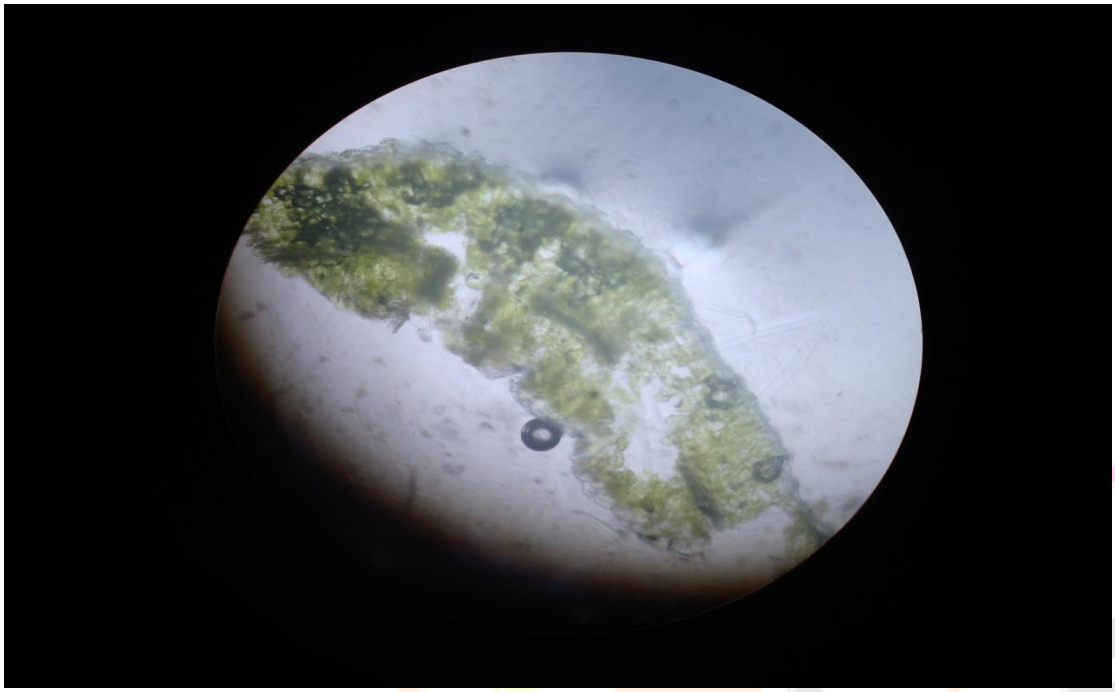

Figure 3.2 Transverse section of *Portulacaria afra* leaf

3.2 Result of Extraction yield

The extract of leaf was found to be dark brown in color and was oleo-resinous in nature. The dry weight (%) of the extract with reference to the weight of dry leaf powder was found to be 13.3%.

3.3 Result of Phytochemical screening of ELEPA

A small fraction of the dried extracts were subjected to the phytochemical screening for detecting the presence alkaloids, glycosides, tannins, saponins, flavonoids and terpenoids. The results of phytochemical screening are presented in table 3.1.

Table 3.1 Phytochemical screening of ELEPA

Phytochemical	Test	Observation	Inference
Alkaloid	*Mayer's reagent*	cream colour precipitate	Alkaloid Present
	Hager's reagent	yellow colour precipitate	

	Wagner's reagent	reddish brown precipitate	
	Dragendorff's reagent	reddish brown precipitate	
Flavonoid	*Shinoda test*	red color	Flavonoid Present
	Alkaline reagent test	Yellow color that turns red on acidification	
	Zinc HCl reduction test	red color	
Glycoside	*Froth Test*	No Frothing	Glycoside absent
	Bontrager's Test	Rose pink or red color in the ammonical layer not found	
	Keller-Kiliani Test	No color in acetic acid layer	
Phenolic and Tannins	*Ferric chloride*	Blue green color	Phenolics and Tannins present
	Gelatin Solution	White precipitate	
	Alkaline reagent test	Yellow to red precipitate	
	Vanillin HCl test	Purplish red color	
Proteins	*Millon's Test*	no precipitate	Protein absent
	Ninhydrin Test	No coloration	
Carbohydrates	*Molisch's Test*	Purple ring	Carbohydrate present
Triterpenoids	*Salkwoski Test*	Yellow color in lower layer	Triterpenes present

3.4 Result of Total Phenolic Content

ELEPA was evaluated for quantification of the total phenolic content concentration in extract. Standard curve of gallic acid was plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be y =

0.004x - 0.003 with a R^2 value of 0.995. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of ELEPA was found to be 34.31±0.26 GAE/mg.

3.5 Acute toxicity

The acute toxicity test was performed by using the dried ELEPA at concentration of 2000 mg/kg to the test animal, administered orally. No animal died and hence the dose of upto 2000 mg/Kg was considered to be safe. As none of the animals died, the LD_{50} was considered to be more than 2000 mg/Kg and any dose less than 2000 mg/Kg would be considered for evaluation of anti-inglammatory action.

3.6 Carageenan Induced rat paw edema measurement

Carrageenan-induced acute inflammation is one of the most suitable test procedures for screening of anti-inflammatory agents. ELEPA was able to reduce the inhibition after the third hour significantly ($p<0.01$). The maximum inhibition of edema by ELEPA at 100 mg/kg dose was 51.37% at the end of the 4th hour while that with 200 mg/kg dose was 61.84%. The inhibition of edema was found to be dose dependent and at every time point the inhibition of the higher dose was better than that of the lower dose.

Table 3.2 Rat paw edema in rats

Group	Change in Paw thickness (mm)			
	1h	2h	3h	4h
Normal Saline	1.476 ± 0.025	3.20 ± 0.072	3.82 ± 0.086	4.01 ± 0.047
Ibuprofen	0.48 ± 0.007**	0.93 ± 0.01**	0.96 ± 0.014**	0.77 ± 0.025**
ELEPA (100 mg/kg)	1.24 ± 0.048	2.51 ± 0.107	2.61 ± 0.054*	1.97 ± 0.083*
ELEPA (200 mg/kg)	1.09 ± 0.025	2.14 ± 0.014*	2.23 ± 0.01*	1.53 ± 0.025**

Results are reported as mean ± SD (n=6); *p<0.01; **p<0.001

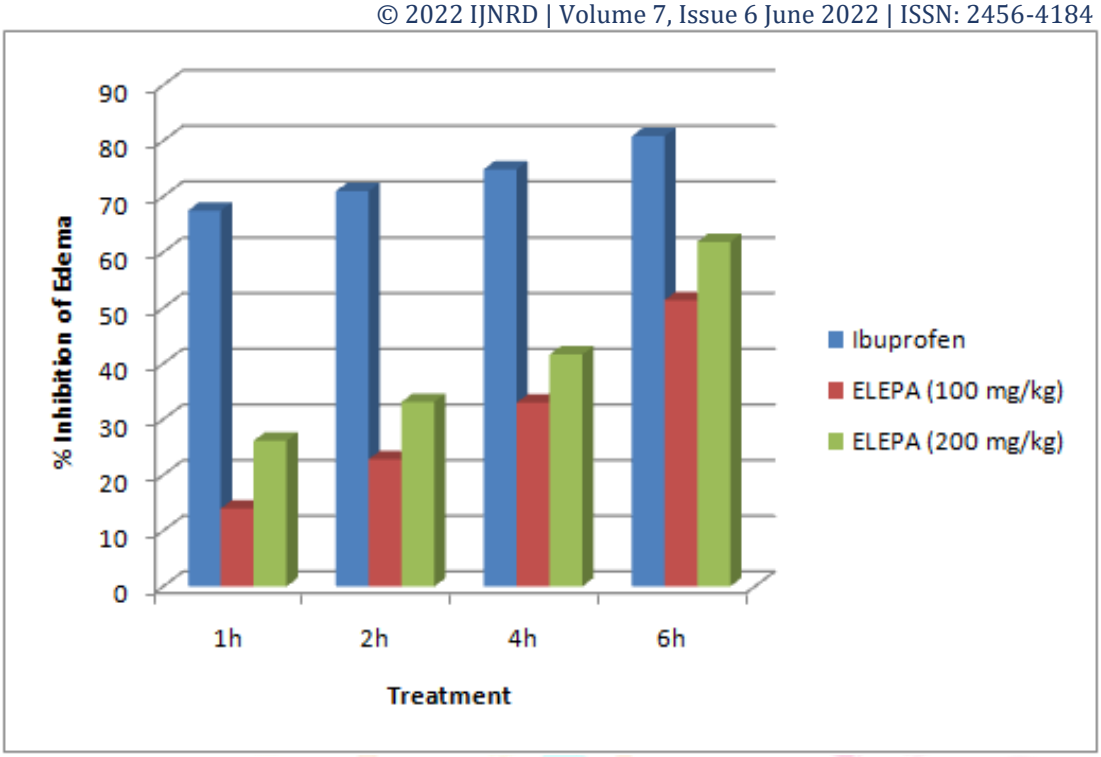

Figure 3.3 Comparison of anti-inflammatory effect of ibuprofen and ELEPA

Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. As shown in the table, ELEPA was not able to inhibit edema significantly in the early hours but was able to inhibit edema considerably at 4h. The anti-inflammatory effect of ELEPA was less as compared to Ibuprofen.

Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis. Therefore, it can be inferred that the inhibitory effect of ELEPA on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

SUMMARY

The leaf of bright green in color, grow opposite on the stem and egg shaped (elephant ear shape), fleshy without a clear petiole. The transverse section of the leaf exhibits the presence of epidermis on both the surfaces. The outer walls of the epidermis have thin cuticle. The extract of leaf was found to be dark brown in color and was oleo-resinous in nature. The dry weight (%) of the extract with reference to the weight of dry leaf powder was found to be 13.3%. The findings of preliminary phytochemical analysis suggest the presence of alkaloids, phenolics, terpenoids, flavonoids and carbohydrates in the ethanolic extract of *Protulacaria afra*.

The total phenolic content of ELEPA was found to be 34.31±0.26 GAE/mg. The LD_{50} of ELEPA was found to be more than 2000 mg/Kg. The maximum inhibition of edema by ELEPA was 50.87% at the end of the 4^{th} hour.

CONCLUSION

The objective of the present study was to assess the anti-inflammatory potential of ethanoilc extract of the leaf of *Protulacaria afra* using the carrageenan induced rat paw edema method. The ethanolic extract of the plant was found possess anti-inflammatory action. Further investigations need to be carried out for determining the active principle in extract responsible of the anti-inflammatory action.

REFERENCES

1. Kumar S, Bajwa BS, Singh K, Kalia AN. Anti-Inflammatory Activity of Herbal Plants: A Review. International Journal of advances in Pharmacy, Biology and Chemistry. 2013; 2(2): 2277-4688
2. Nadkarni AK. Indian Materia Medica. Popular Prakashan, Bombay. 2006
3. Cardinali PD, Esquifino IA. Circardian disorganization in experimental arthritis. Neuro-Signals. 2003; 12: 267-282.
4. Pervical M. Understanding the natural management of pain and inflammation. Clinical Nutrition insights. 1999, 4: 1-5
5. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. Annual Reviews in Pathology. 2008; 3: 279-312.
6. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nature Immunology. 2001; 2(7): 612-619.
7. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual antiinflammatory and pro-resolution lipid mediators. Nature Reviews Immunology. 2008; 8(5): 349-361.
8. Majno G and Joris I (1996). Cells, Tissues and diseases. Principles of general pathology. Blackwell Science Publications, Cambridge MA, pp. 429-463.
9. McEver RF (1997). Perspective Series: Cell adhesion in vascular biology: Role of PSGL-1, binding to selectins in leucocyte recruitment, J. Clin. Invest., 100: 485.
10. Muller LU (1996). Molecular and cellular interactions in rheumatoid synovium, Curr. Opin rheumatol., 8: 210.
11. Kumar S, Bajwa BS, Singh K, Kalia AN. Anti-Inflammatory Activity of Herbal Plants: A Review. International Journal of advances in Pharmacy, Biology and Chemistry. 2013; 2(2): 2277-4688
12. Zahan R, Nahar L, Nesa ML. Antinociceptive and anti-inflammatory activities of flower (*Alangium salvifolium*) extract. *PJBS. 2013;16(19): 1040-5.*
13. Hepcy KD, Dinakar A, Kumar SN. Antidiabetic activity of ethanolic extracts of *Alangium salvifolium* and *pavonia zeylanica* in streptozotocin induced diabetic rats. Int J Pharm Pharm Sci. 2012; 4(5): 337-339.
14. Sharma AK, Agarwal V, Kumar R, Balasubramaniam A, Mishra A, Gupta R. Pharmacological studies on seeds of *Alangium salvifolium* Linn. Acta Pol Pharm. 2011; 68(6): 897-904.
15. https://en.wikipedia.org/wiki/Portulacaria_afra#Medicinal; assessed on19/12/2021
16. https://www.magiminiland.org/Portulacaria.html#Taxonomy; assessed on 19/12/2021

17. Mathur Y, Jain A. Screening of antidepressant potential of leaves of Araucaria columnaris. Journal of Pharmacology and Biomedicine 2021; 5(4): 360-365.
18. Khalid S, Shahzad A, Basharat N, Abubakar M, Anwar P. Phytochemical screening and analysis of selected medicinal plants in Gujarat. Journal of Phytochemistry and Biochemistry 2018; 2(1): 1000108.
19. Singh B, Jain A. Evaluation of wound healing action of Delonix regia leaf extracts in rats. Journal of Pharmacology and Biomedicine 2021; 5(4): 374-382.
20. Khandelwal KR. 2005. Practical PharmacognosyTechniques and Experiments, 14th ed. NiraliPrakashan, Pune, pp. 153
21. Rajshree RS, Ittiyavirah SP, Naseef PP, Kuruniyan MS, Anisree GS, Elayadeth-Meethal M. An Evaluation of the Antioxidant Activity of a Methanolic Extract of Cucumis melo L. Fruit (F1 Hybrid). Separations 2021; 8: 123.
22. Tabassum S, Ahmad S, Khan KuR, Tabassum F, Khusheed A, Zaman Q, Bukhari NA, Alfagham AA, Hatamleh AA, Chen Y. Phytochemical Profiling, Antioxidant, Anti-Inflammatory, Thrombolytic, Hemolytic Activity In Vitro and In Silico Potential of Portulacaria afra. Molecules 2022; 27: 2377.
23. Erhirhie EO, Ihekwreme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdisciplinary Toxicology 2018; 11(1): 5-12.