WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 11, 1423-1430 Research Article ISSN 2278 – 4357

# ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *AGANOSMA CYMOSA* ON FORMALIN INDUCED PAW EDEMA IN WISTAR RATS

**Farmiza Begum[1]*, T. Ravi Chander[1], P. Anitha[2], B. Keerthi[1]**

[1]Department of Pharmacology, Vaagdevi Pharmacy College, Bollikunta, Warangal-506001, Telangana, India.

[2]Department of Pharmacognosy, Vaagdevi Pharmacy College, Bollikunta, Warangal-506001, Telangana, India.

Article Received on
11 Sept. 2018,

Revised on 02 Oct. 2018,
Accepted on 23 Oct. 2018

DOI: 10.20959/wjpps201811-12664

***Corresponding Author**
**T. Ravi Chander**
Department of Pharmacology, Vaagdevi Pharmacy College, Bollikunta, Warangal-506001, Telangana, India.

## ABSTRACT

Inflammation is one of the most important processes involved in the defense of an organism against local injury and infections. However it often progress to painful or chronic harmful diseases requiring pharmacological treatment. Inflammatory response is a series of well coordinated dynamic mechanism consisting of specific vascular humoral and cellular events that is characterized by the movement of fluids plasma and inflammatory leukocytes to the site of inflammation. A variety of chemical mediators or signalling molecules such as histamine, serotonin, leukotrienes, prostaglandins and ROS are produced by inflammatory and Phagocytic cells predominantly in the sequences which participate in onset of inflammation. The Primary objective of this present study is to evaluate the anti-inflammatory activity of ethanol extract of leaves of *Aganosma cymosa* in male wistar rats. In this perspective Ethanolic extract of leaves of *Aganosma cymosa* was taken & Formalin induced paw edema model was selected for inducing inflammation & Diclofenac sodium is used as Standard. Statistical analysis was carried out by one-way analysis of variance followed by Dunnet's test. The anti inflammatory activity of ethanolic extract of *Aganosma cymosa* at the doses of 150 and 300 mg/kg p.o. was evaluated by Formalin induced paw edema model in Rats. The results showed that, the Ethanolic extract significantly decreased the paw edema in a dose dependant manner and exhibited more activity at the dose of 300 mg/kg. The Ethanolic extract exhibited anti inflammatory activity, which may be due to the presence of phytoconstituents present in the

extract. These findings justify the traditional use of this plant in the control and/or treatment of inflammation as the plant is unexplored yet. Further detailed phytochemical investigations are required to identify the phytoconstituent/s responsible for the anti inflammatory activity.

**KEYWORDS:** *Aganosma cymosa,* anti-inflammatory effect, Formalin induced paw edema, Diazepam.

## 1.0. INTRODUCTION

The inflammation is a sequence of events that occurs in response to noxious stimuli, infection, trauma, or injury in the living tissues.[1] The inflammation is initiated by a cascade of events including enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair processes.[2] The inflammation releases white blood cells as a protective measure against injury. These white blood cells synthesize several biomolecules and release them after injury leading to swelling and redness. The inflammation is characterized by induction of pain, redness, and rashes.[3] Prostaglandins are one of the important biomolecules, which play a key role in the induction of inflammatory response as their biosynthesis is significantly increased during inflammation.[4]

Cyclooxygenase, is a prostaglandin endoperoxide synthase enzyme involved in the metabolism of Arachidonic acid and synthesis of prostanoid including potent proinflammatory prostaglandins ($PGE_2$, $PGF_2\alpha$).[5,6] In Mammalian cells, COX exist in atleast two isoforms COX-1 and COX-2.[7-9] The former is expressed in almost all cell types, including platelets and those present in stomach, kidney, vascular endothelium, forebrain and uterine epithelium and is regulated as a house keeping enzyme for various physiological functions, wheareas the later is inducible and expressed during tissue damage or inflammation in response to proinflammatory cytokines such as IL-1β, interferon gamma and TNF-α.[10-12] A crucial proinflammtory role played by the cox has made this enzyme an attractive target for the design and development of novel anti-inflammatory agents.

The inflammatory responses are elicited as a defense mechanism by an organism or tissues; however, sustained inflammation can lead to undesired health effect as a consequence of interplay of various biomolecules that are secreted during the process of inflammation. Inflammation has been indicated in several diseases including cancer.[13,14] The agents that contain or block inflammation may play an important role in treating pathologies associated with inflammatory reactions.[15]

Looking at the present scenario, medicinal compounds derived from plant sources could provide an excellent fountainhead to develop new anti-inflammatory drugs, which could be more efficacious, safer, affordable, and accessible to patients. *Aganosma cymosa* a native of India, china, Bangladesh, Srilanka is a liana that can grow upto 10cm and is the larval host plant for Malabar tree nymph and belongs to the family Apocynaceae. It is commonly known as Manimalaankodi, Sellakkodi in Tamil. The leaves of *Aganosma cymosa* were used traditionally in treating bronchitis, ulcer, aacaris, cough, eye diseases, leprosy, skin diseases, swelling, vomiting, wounds. However, fewer reports are available with respect to the pharmacological properties of the plant. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory studies of ethanolic extract of *Aganosma cymosa* in standard animal models.[16]

## 2.0. MATERIALS AND METHODS

**2.1.Plant Material:** The Leaves of *Aganosma cymosa* were collected from Tirupati, Andhra Pradesh, India. The Leaves were identified and authenticated by Prof. K. Madhava Chetty, Department of Botany, Sri Venkateswara University.

**2.2. Drugs & Chemicals:** Diclofenac sodium (Invision Medi sciences, Bangalore), Formalin (Ranbaxy) was used in this study.

**2.3. Preparation of extracts and preliminary phytochemical screening:** The dried powdered plant material was extracted with ethanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained. The extract was subjected to qualitative chemical tests for various phytoconstituents like Alkaloids, Carbohydrates, Saponins, Tannins, Proteins, Lipids, Flavonoids and Steroids.[17]

**2.4 Animals:** All the study protocol was approved by the institutional animal ethical committee (IAEC). (1533/PO/a/11/CPCSEA) Animals were procured from Sainath Labs, Hyderabad. The animals were housed in groups of 6 in cages with paddy husk as bedding, fed with normal commercial pellet diet, given water *ad libitum* and maintained under laboratory conditions (temperature 24 - 28°C, relative humidity 60 - 70%, and 12h light-dark cycle) and were acclimatized for a minimum of 7 days before experiment was performed. Food was with held for 12h before the start of experiments.

**2.5 Acute oral toxicity studies:** Male Albino Wistar rats were used and the procedure was followed by using OECD 423, annexure D (Acute Toxic Class Method)[18]

### 2.6. Formalin Induced Inflammation

The grouping and other conditions were essentially similar to those described in Table 1**.** The anti-inflammatory activity was assessed as described. Male Wistar Rats were divided into groups of Four. The inflammation was produced by subaponeurotic injection of 0.1 mL of 2% formaldehyde in the right hind paw of the mice on the first and third day. The animals were treated daily with the EEAC and diclofenac intraperitoneally for 10 days. The daily changes in paw size were measured by wrapping a piece of cotton thread around the paw and measuring the circumference with a meter rule. Usually 6 Rats were used for each group.[19,20]

**Table. 1: Grouping of animals for anti-inflammatory activity by Formalin-induced Rat paw edema.**

| Groups(n=6) | Treatment |
|---|---|
| Group I | DMSO |
| Group II | Diclofenac sodium 50 mg/kg b.wt. |
| Group III | EEAC 150mg/kg b.wt. |
| Group IV | EEAC 300 mg/kg b.wt. |

**2.7. Statistical analysis:** The data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test. $p < 0.05$ was considered as statistically significant. The data were expressed as mean ± standard deviation (SD).

## 3.0. RESULTS

**3.1. Preliminary Phytochemical Screening:** The phytochemical screening revealed the presence of triterpenoids, steroids, glycosides, flavonoids, alkaloids and tannins.

### 3.2. Acute Toxicity Study

In the acute toxicity study, In the acute toxicity study, EEAC was found to be safe up to 2000 mg/kg, p.o. So, two doses i.e., 150 and 300 mg/kg body weight can be taken for the studies weight in Rats and EEAC was found to be safe up to 2000 mg/kg, *p.o.* So, two doses i.e., 150 and 300 mg/kg body weight can be taken for the studies.

### 3.3. Evaluation of Formalin Induced Inflammation of ethanolic extract of leaves of *Aganosma cymosa*

Treatment of Rats with EEAC gradually reduced diameter of the paw with time in both the treated and positive control group. The EEAC reduced the inflammatory reactions when compared to the DMSO control group as indicated by the significant reduction in the paw diameter. However, the effect was more pronounced for 300 mg/kg EEAC treatment.

**Table. 2: Effect of ethanol extract of Leaves of *Aganosma cymosa* on the formalin induced inflammation in Rats paw.**

| Group | PAW Size in different days intervals in mm | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 |
| **Control** | 21.33± 0.393 | 21.33±0.393 | 21.33±0.393 | 21.3± 0.37 | 21.33±0.393 | 21.08±0.345 | 21.3± 0.379 | 21.33±0.393 | 21.33±0.393 | 21.33±0.393 | 21.33±0.393 |
| **Standard** | 15.38± 0.157 | 24.62±0.213 | 18.62±0.225 | 31.33±0.352 | 25.37±0.183 | 23.07±0.071 | 22.75±0.143 | 22.62±0.914 | 20.03±0.105 | 18.67±0.266 | 18.30±0.152 |
| **EEAC-150** | 17.3± 0.036 | 22.62±0.119 | 20.62±0.087 | 25.37±0.183 | 23.30±0.106 | 22± 0.456 | 21.32±0.426 | 20.62±0.082 | 19.80±0.367 | 19.73±0.264 | 19.33±0.282 |
| **EEAC-300** | 21.68±0.23 | 26.67±0.328 | 27.37±0.358 | 30.75±0.332 | 27.67±0.284 | 27.05±0.259 | 25.73±0.199 | 24± 0.422 | 23.63±0.108 | 23.07±0.147 | 23.07±0.071 |

n=6, values expressed as Mean ± S.E.M.

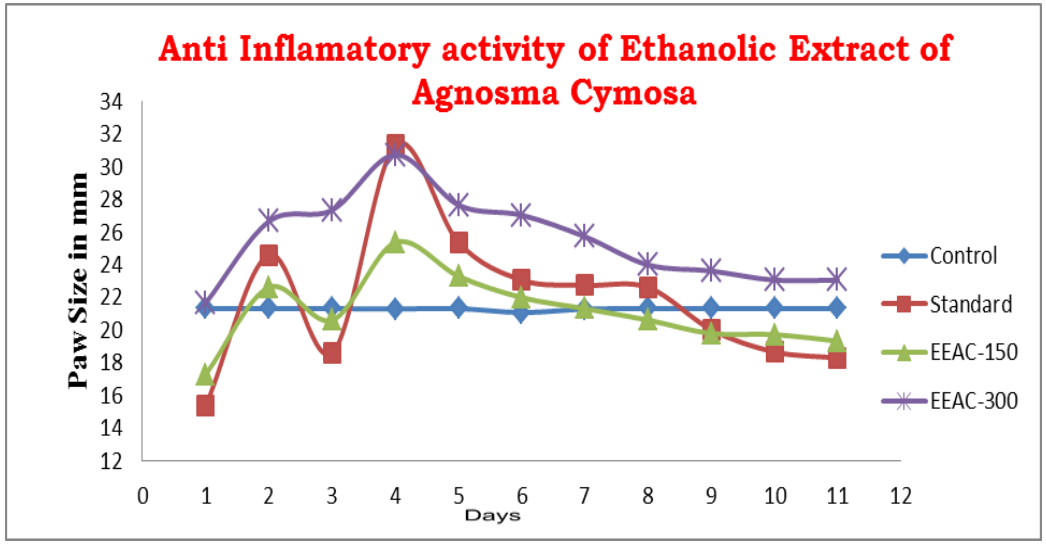


**Fig 1: Anti inflammatory activity of *Aganosma cymosa* on the formalin induced inflammation in rats paw.**

The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent. This edema depends on the participation of kinins and polymorph nuclear leukocytes with their pro-inflammatory factors including prostaglandins. The development of edema in the paw of the rat after the injection of formalin has been a biphasic event. The initial phase, observed around 1 day, is attributed to the release of histamine and serotonin; the second, accelerating, phase of swelling is due to the release of prostaglandin-like substances. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents. In present study the significant activity was observed in the suppression of the first and second phases of formalin-induced inflammation may due to inhibition of the release of the early mediators such as histamine, serotonin and kinins. The action on the second phase may be due to an inhibition of cyclo-oxygenase, a prostaglandin derivative. The extract showed significant reduction of inflammation in both phase in dose dependent manner.

## 4. DISCUSSION

The present investigation describes some unique features of the leaf extract from the plant *Aganosma cymosa* with respect to its potential anti inflammatory capacity in rats. Plant products are largely preferred because of their wide spread availability, non-toxicity, absence of unwanted side effects, and effectiveness as crude preparations. The treated rats showed marked decreased level in paw edema, than control.

The inflammatory activity of any agent can be determined by formalin induced paw edema test. The formalin administration elicits behavioral effects stimulated by nociceptors. The inflammatory phase induced pain evokes a combination of stimuli, including inflammation of peripheral tissues and mechanisms of central sensitization. The central nervous system acting drugs including opioids suppress both phases equally; however drugs that act on peripheral nervous system such as NSAIDs and corticosteroids only inhibit the second phase.[21,22] Our findings indicate that *Aganosma cymosa* extract acts as anti-inflammatory agent as it reduced the formalin induced paw edema in treated rats. *Aganosma cymosa* extract has been effective in both the central and peripheral nervous systems since it was able to desensitize neurons of both central and peripheral nervous systems equally as indicated by the attenuation of pain and inflammation.

The results of the present study showed that *Aganosma cymosa* possesses a definite anti inflammatory action. In formalin induced rat paw edema model the ethanolic extract of the leaves of the plant *Aganosma cymosa* showed significant decrease in paw edema of rats. The exact mechanism of suppression of inflammation by *Aganosma cymosa* is not known. Further studies are required to know it exact mechanism of action.

## 5. CONCLUSION

Ethanolic extract of *Aganosma cymosa* possess significant anti-inflammatory potential. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions.

## ACKNOWLEDGEMENTS

The authors are grateful to the management of Vaagdevi Pharmacy College, Bollikunta, Warangal for providing the lab equipments and facility for carrying out the research work.

## REFERENCES

1. Calixto J.B, Campos M.M, Otuki M.F, and Santos A.R.S. Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Medica, 2004; 70(2): 93–103.
2. Risso W.E, Scarminio I.S, and Moreira E.G. Antinociceptive and acute toxicity evaluation of Vernonia condensata Baker leaves extracted with different solvents and their mixtures. Indian Journal of Experimental Biology, 48(8): 811-816.
3. Opdenakker. G, Fibbe W.E, and Damme J.V. The molecular basis of leukocytosis. Immunology Today, 1998; 19(4): 182-189.
4. Ricciotti. E and FitzGerald.GA. Prostaglandins and inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology, 2007; 31(5): 986-1000.
5. Limongelli V, Bonomi M, Marinelli L. Molecular basis of cyclooxygenase enzymes (COXs) selective inhibition. Proc Nat Acad Sci., 2010; 107(12): 5411-5416.
6. Cao H, Yu R, Choi Y, et al. Discovery of cyclooxygenase inhibitors from medicinal plants used to treat inflammation. Pharmacol Res., 2010; 61(6): 519-524.
7. Hwang IK, Yi SS, Yoo KY, et al. Effects of treadmill exercise on cyclooxygenase-2 in the hippocampus in type 2 diabetic rats: correlation with the neuroblasts. Brain Res., 2010; 1341: 84-92.
8. Chen YJ, Quilley J. Fenofibrate treatment of diabetic rats reduces nitrosative stress, renal cyclooxygenase-2 expression, and enhanced renal prostaglandin release. J

Pharmacol Exp Ther., 2008; 324(2): 658-663.

9. Jianhua F, Eliana L, Gregor F, et al. Cardiac remodeling hinders activation of cyclooxygenase-2, diminishing protection by delayed pharmacological pre-conditioning: role of HIF1a and CREB. Cardiovasc Res., 2008; 78: 98-107.
10. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet, 2010; 376(9746): 1094-1108.
11. Lubberts E. Th17 Cytokines and arthritis. Semin Immunopathol, 2010; 32(1): 43-53.
12. Sabat R. IL-10 family of cytokines. Cytokine Growth Factor Rev., 2010; 21: 315-324.
13. Grivennikov S.I, Greten F.R, and Karin.M. Immunity, inflammation, and cancer. Cell 2010; 140(6): 883-899.
14. Moore M.M, Chua W, Charles K.A, and Clarke S.J. Inflammation and cancer: causes and consequences. Clinical Pharmacology and Therapeutics, 2010; 87(4): 504-508.
15. Sosa. S, Balick M.J, Arvigo.R et al. Screening of the topical anti-inflammatory activity of some Central American plants. Journal of Ethnopharmacology, 2010; 81(2): 211-215.
16. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci., 1996; 55: 225-69.
17. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Last 25 Years. Journal of Natural Products, 2007; 70(3): 461-477.
18. Saxena R.S, Gupta B, Saxena K, Singh R.C, and Prasad D.M. Study of anti-inflammatory activity in the leaves of Nyctanthes arbor tristis Linn. An Indian medicinal plant. Journal of Ethnopharmacology, 1984; 11(3): 319-330.
19. Lalrinzuali K, Vabeiryureilai M, and Ganesh Chandra Jagetia. Investigation of the Anti-Inflammatory and Analgesic Activities of Ethanol Extract of Stem Bark of Sonapatha Oroxylum indicum In Vivo. International Journal of Inflammation 2016; http://dx.doi.org/10.1155/2016/8247014.
20. Shibata. M, Ohkubo T, Takahashi H, and Inoki R. Modified formalin test: characteristic biphasic pain response. Pain, 1989; 38(3): 347-352.
21. LeBars D, Gozariu M, and Cadden S.W. Animal models of nociception. Pharmacological Reviews, 2001; 53(4): 597-652.