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RESEARCH ARTICLE

Anti-inflammatory activity of ethanolic extract of *Alpinia galanga* in carrageenan induced pleurisy rats

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ABSTRACT

Background: *Alpinia galanga* belonging to Zingiberaceae family was traditionally used to treat numerous inflammatory disorders. The rationale behind its use since ages has to be scientifically validated for developing newer therapeutic agents. **Aims and Objectives:** Anti-inflammatory activity of ethanolic extract of *A. galanga* in carrageenan induced pleurisy rats. **Materials and Methods:** This study was investigated on the ethanolic extract of *A. galanga* rhizome by scientifically validated anti-inflammatory screening technique on rats by carrageenan induced pleurisy. This pleurisy model is considered to be an excellent acute inflammatory model in which fluid extravasations, leukocyte migration and the various biochemical parameters involved in the inflammatory response can be measured easily in the exudates. **Results:** The results obtained indicate that the ethanolic extract had significant activity in rats in all the tested groups *A. galanga* 100, 200 and 400 mg with $P < 0.005$ compared to that of control. **Conclusion:** The study confirms the potential anti-inflammatory activity of ethanolic extract of *A. galanga* rhizome.

KEY WORDS: *Alpinia galanga*; Inflammation; Carrageenan, Pleurisy; Rats

INTRODUCTION

A. galanga from Zingiberaceae family has a very strong ethnobotanical history, endemic in South East Asia and still available as an Ayurvedic preparation for wider therapeutic application, importantly for inflammatory disorders.[1] The Zingiberaceae family consists of 1300 species and among them ginger, and *A. galanga* has 2500 years history of therapeutic use in Ayurvedic and Chinese medicine. It has been used to treat bowel inflammation and rheumatism. Ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase-1 and cyclooxygenase-2 thereby its usefulness in arthritis was understood.[2]

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The inflammatory mediators such as prostaglandins and leukotrienes are formed with the help of enzyme phospholipase A_2; these mediators attract polymorphonuclear leukocytes to the site of inflammation thereby leading to tissue injury. Phospholipase A_2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation.[3,4] *A. galanga* belonging to the family of Zingiberaceae with equally similar traditional claims was not evaluated scientifically, hence selected and investigated for anti-inflammatory activity.

MATERIALS AND METHODS

The *A. galanga* plant rhizome was collected from herbal medicine raw material supplier and the same was authenticated by Siddha central research institute, Chennai, India. The rhizome extract as dry powder is obtained

from Chemiloids, Vijayawada, India. Laboratory grade carrageenan was procured, and the extract was subjected to screen anti-inflammatory activity followed by carrageenan induced pleurisy in rats. Inbred male Wistar lewis rats were selected for the study. The animals were maintained on a 12 h/12 h day/night cycle with free access to food and water. Prior Institutional Animal Ethical Committee approval (Meenakshi Medical College and Research Institute) was obtained for performing the experiments.

Carrageenan Induced Pleurisy in Rats[5]

Various doses of the ethanolic extract of *A. galanga* (100-400 mg/kg) were prepared as a fine suspension in 0.5% CMC and given per oral 30 min before the testing procedure. The animals were given 0.25 ml of an intrapleural injection of 1% carrageenan on the right side of the thorax. The animals were sacrificed 3 h after carrageenan injection by ether inhalation. 1 ml of heparinized Hank's solution was injected into the pleural cavity and gently massaged to mix its contents. The fluid was aspirated out of the cavity, and the exudates were collected. The number of migrating leukocytes in the exudate was determined with Neubauer chamber. The values of each experimental group were expressed as a mean ± SEM and compared with the control group.

Statistical Analysis

The results were analyzed using one-way analysis of variance (ANOVA) followed by paired *t*-test utilizing Graphpad Instat software Version 3.1 (U.S.A). A $P < 5\%$ was considered to be statistically significant.

RESULTS

The results obtained indicate that the ethanolic extract had significant anti-inflammatory activity in rats. The volume of pleural exudates in the control group was 1.25 ± 0.104 ml. In animals treated with the ethanolic extract of *A. galanga* 100, 200 and 400 mg/kg, p.o., the pleural exudates decreased to 0.96 ± 0.103, 0.62 ± 0.144, and 0.38 ± 0.12 ml, respectively, (Table 1) and those treated with Indomethacin (10 mg/kg, p.o.) produced the exudates of 0.27 ± 0.070 ml.

The leukocyte count for the control group was found to be 88.00 ± 1.325×10^6 cells/cavity. In animals treated with the ethanolic extract of *A. galanga* 100, 200 and 400 mg/kg, p.o., count decreased to 76 ± 1.24, 54 ± 0.25 and 32 ± 0.12×10^6 cells/cavity, respectively. The standard produced a leukocyte count of 27 ± 0.026×10^6 cells/cavity (Table 1). The volume of pleural exudates and leukocyte count was significantly decreased in the treatment group.

DISCUSSION

Pleurisy is a well-known phenomenon of exudative inflammation in humans. Carrageenan injection is widely used as an acute, resolving model of inflammation and is a standardized method for the investigation of localized, predominantly PMN leukocyte-driven reactions.[6] In our study, the injection of carrageenan led to a significant increase in the leukocyte count and fluid extravasation in the pleural cavity triggering a typical inflammatory reaction, which can be measured easily. The analysis of results in this study indicated that the ethanolic extract of *A. galanga* caused significant inhibitory effects on total leukocyte influx (Figure 1) in *A. galanga* 200 mg/kg and 400 mg/kg groups and reduction of exudation in the inflammatory edema is observed in dose-dependent manner (Figure 2). This effect could be explained by the presence of flavonoids which was detected in the phytochemical analysis of *A. galanga* in previous studies by Subash et al.[7] Flavonoids have pronounced antioxidant activity and abilities to modulate several enzymes.

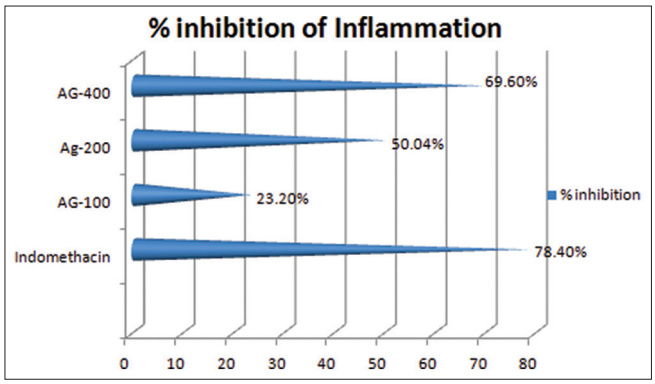

Figure 1: % Inhibition of Inflammation of Alpinia galanga in carrageenan induced pleurisy

Table 1: Effect of ethanolic extract of *A. galanga* on carrageenan induced pleurisy in rats

Treatment group	Dose (mg/Kg, p.o)	Pleural exudates (ml)	Inhibition %	Leukocytes (×10^6 cells/cavity)
Control (normal saline)	-	1.25±0.104	-	88±1.25
Standard-Indomethacin	10	0.27±0.070**	78.4	27±0.26**
Test-I *A. galanga*	100	0.96±0.103**	23.2	76±1.24^{ns}
Test-II *A. galanga*	200	0.62±0.144**	50.04	54±0.25**
Test-III *A. galanga*	400	0.38±0.129**	69.6	32±0.12**

AG: *Alpinia galanga*, ns: Not significant, ** $P<0.01$ versus control

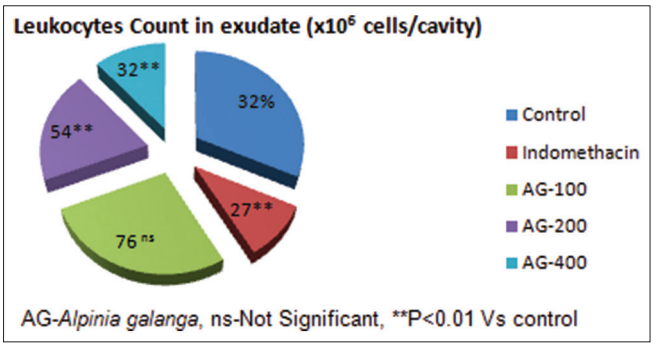

Figure 2: Effect of ethanolic extract of Alpinia galanga in Leukocyte migration in pleural exudates

The role of nitric oxide (NO) overproduction and inducible nitric oxide synthase (iNOS) in the development of acute carrageenan-induced pleurisy is well established.[5] Moreover, there is an important cross-talk between the tissue NO signaling and XOR, a prototypic superoxide-producing enzyme, as XO-derived superoxide can react with excess NO to form peroxynitrite, which has adverse effects on tissue homeostasis.[8] In support to this, the antioxidant assay analysis in an earlier study[9] by *in vitro* antioxidant assay of *A. galanga* revealed pronounced nitric oxide free radical scavenging activity by which the extract is able to reduce NO release, iNOS expression and the production of proinflammatory cytokines through blockade of the NF-kappa B activation pathway.[10,11] The percentage inhibition of inflammation by *A. galanga* 400 mg/kg (69.6%) was almost similar to the standard indomethacin (78.4%) as shown in Figure 1. The anti-inflammatory activity of *A. galanga* alcoholic extract can be partly due to the prevention of prostaglandins biosynthesis via cyclooxygenase blockade.[12]

CONCLUSION

Thus, it can be concluded that the rhizome of the plant *A. galanga* possesses significant anti-inflammatory activity in rats with previous studies supporting the presence of flavonoids and nitric oxide free radical scavenging. Further studies involving the mechanism of action by identification of the active chemical constituents of this extract and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

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