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Research Article

# ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *ALLIUM SATIUM* LEAVES

**SATARUPA PAN*, APARNA LAKSHMI I, PAMBA PRIYANKA**

**Department of Pharmacology, The Oxford College of Pharmacy, Hongasandra, Bengaluru, Karnataka, India.**
**Email: satarupa.pan@gmail.com**

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**ABSTRACT**

**Objective:** The aqueous extract of *Allium sativum* leaves (AEAL) was investigated for anti-inflammatory activity in albino rats of Wistar strain.

**Methods:** The AEAL was screened for the presence of phytochemical constituents like terpenoids, tannins, carrageenan, flavonoids, anthraquinones, alkaloids, cardiac glycosides, etc. The anti-inflammatory activity of AEAL was evaluated by different animal models like induced paw edema and histamine-induced paw edema, using two different concentrations of AEAL namely 100 mg/kg and 200 mg/kg, respectively.

**Results and Conclusion:** The phytochemical screening of AEAL showed the presence of carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkaloids, steroids, and triterpenes. The two different concentrations of AEAL were able to inhibit the induced edema in experimental animals in a graded fashion.

**Keywords:** *Allium sativum* leaves, Anti-inflammatory, Paw edema.

## INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [1]. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Proinflammatory cytokines (e.g., tumor necrosis factor - α [TNF-α], interleukin [IL-6], and IL-1β) are produced in large quantities by activated macrophages/monocytes that stimulate cellular responses via increasing prostaglandins (PGs) and reactive oxygen species (ROS). In addition, lipid peroxidation (malondialdehyde [MDA]) is produced by free radicals attacking the cell membranes. Thus, inflammatory effect results in the accumulation of MDA [2]. Many studies have indicated that flavonoids in herbs possess anti-inflammatory activities via scavenging ROS and reducing proinflammatory cytokines (e.g., NF-αB, TNF-α, IL-1β, and IL-6), such as ursolic acid [3-5] and lupeol [6].

*Allium sativum* (garlic) belonging to family Alliaceae is a plant containing 1-2% essential oil on a dry basis with wide variation of chemical composition as a function of genetic diversity, habitat, and agronomic treatment of culture. Garlic has a long folklore history as a treatment for cold, cough, and asthma and is reported to strengthen immune system. It has many medicinal effects such as lowering of blood cholesterol level [7], antiplatelet aggregation [8], anti-inflammatory activity [9], and inhibition of cholesterol synthesis [10]. Garlic has been long known to have antibacterial [11-13], antifungal [8], anticancer [14,15], antioxidant, and antiviral activities. Therapeutic effects of garlic is due to the presence of allicin in the cloves, the present study was based on the presence of allicin in the garlic leaves and we have evaluated the anti-inflammatory activity based on the same property by using aqueous extract of garlic leaves.

## METHODS

### Plant material

Fresh green leaves of *A. sativum* plant (garlic) have been purchased from the local market, Howrah, West Bengal, India. The plant specimen is submitted to Botanical Survey of India, Shibpur, Howrah for authentication (CNH/7/2012/Tech. Ц/815).

### Preparation of aqueous extract of garlic leaves

Fresh garlic leaves (100 g) were blended in 50 ml sterile distilled water, centrifuged at 5000 rpm and sterilized by filtration (0.45 µm). By subtracting the weight of the insoluble material from the weight of the original leaves, the final concentration of the garlic extract in solution was determined to be 52.1% (w/v). Aliquots were stored at −20°C until required.

### Preliminary phytochemical screening

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites using standard procedures [16].

### Acute toxicity studies

Acute toxicity study was carried out using wistar strain rats. The rats were fasted overnight and the weight of each rat was recorded just before use. Animals were divided randomly into groups *viz.*; a control and treatment groups, each group consisting of six rats. Control group received only the vehicle and each treatment group received orally the 52.1% aqueous leaf extract of garlic in a dose of 100, 200, 400, 600, 800, and 1000 mg/kg. Animals were kept under close observation for 4 hrs after administration of the extract, and then they were observed daily for 3 days for any change in general behavior and/or other physical activities [17].

### Grouping of animals

Albino rats of Wistar strain (weighing 170-200 g) were used for the study. Rats used for the study were obtained from the Oxford college of pharmacy, Bengaluru. All experimental procedures and protocols used in the study were reviewed by the "Institutional Animal Ethical Committee" and Committee for the Purpose of Control and Supervision of Experiments on Animals. Animals were allowed to free access of water and standard chow diet up to the end of the experimental period and divided into following groups.

Each group consists of six rats:

Group I: Served as control group, received the vehicle (saline) only,
Group II: Served as standard group treated with indomethacin at dose of 10 mg/kg
Group III: Served as test group treated with Aq. Extract of garlic leaves (100 mg/kg p.o)

GROUP IV: Served as test group treated with aqueous extract of garlic leaves (200 mg/kg p.o).

**Carrageenan-induced rat paw edema**

The rats weighing 160-180 g were divided into four groups, and each group consisting of six animals. Paw edema was induced by subplantar injection of 0.1 ml of freshly prepared 1% carrageenan suspension into the right hind paw of each rat. The paw volumes were measured using a plethysmometer before as well as 30, 60, 120, 180, and 240 minutes after the injection of carrageenan [18]. The aqueous extract of leaves of *A. sativum* at 100 and 200 mg/kg were administered orally to group three and four. The first and second group of rats received saline and 10 mg/kg indomethacin as drug control for comparative pharmacological assessment respectively. Test drugs and vehicle were given 1 hr before the injection of carrageenan. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation.

% inhibition = 100 – (edema volume in the treated – edema volume in the control) × 100

**Histamine induced paw edema**

The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% histamine sulfate in normal saline (0.9% w/v NaCl) was injected to the subplantar region of the right hind paw. The *Allium sativum* leaves (AEAL) was administered to the rats 1-hr before histamine injection in two different concentrations, i.e., 100 and 200 mg/kg for group three and four, respectively. The paw volume was measured initially and at 30, 60, 120, 180, and 240 minutes after histamine injection, using plethysmograph, inflammation was calculated for comparison [19]. The first and second group of rats received saline and indomethacin (10 mg/kg) respectively.

**Statistical analysis**

Results of all parameters were expressed as mean ± standard error of mean for each group. The statistical significance was measured by one-way ANOVA followed by Dunnett test.

## RESULTS

**Preliminary phytochemical screening**

The phytochemical screening of AEAL has revealed the presence of carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkaloids, steroids, and triterpenes.

**Acute toxicity studies**

In the acute toxicity studies, death was recorded by administering graded doses of the crude 52.1% aqueous extract of garlic leaves up to a dose of 1000 mg/kg body weight. The animals did not show significant changes in general behaviors like alertness, motor activity, breathing, restlessness, diarrhea, convulsions, and coma. Mortality was not observed up to 800 mg/kg, whereas, 100% mortality was noticed in the dose of 1000 mg/kg. From the above toxicity studies, the $LD_{50}$ dose was found to be 1000 mg/kg body weight. Therefore, one-tenth of this dose (100 mg/kg) was selected as the therapeutic dose for the evaluation of anti-inflammatory activity.

The anti-inflammatory activity of AEAL in two different concentrations, i.e., (100 mg/kg and 200 mg/kg) on carrageenan-induced paw edema was depicted and compared in Table 1. AEAL was able to show a significant decrease in carrageenan-induced paw edema at a concentration of 200 mg/kg at the end of 4$^{th}$ hr when compared with negative control saline (i.e., 1.45±0.52** [74.09]). The percentage inhibition exhibited by 100 mg/kg and 200 mg/kg of AEAL extract (68.1 and 74.09, respectively) was similar to that of indomethacin, which has shown 92.2% of inhibition.

The effect of AEAL on histamine-induced paw edema was depicted in Table 2. The AEAL has significantly reduced the paw edema induced by histamine at two different concentration 100 mg/kg and 200 mg/kg (0.65±0.016* [63.88] and 0.50±0.024* [72.22]) and the values were comparable with the standard indomethacin.

## DISCUSSION

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility [20]. Carrageenan induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinins whereas the second phase is related to the release of PG and slow reacting substances [21]. It has been reported that the second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone, and indomethacin.

Anti-inflammatory activity of AEAL was comparable to indomethacin and probably it would have reduced the edema by inhibiting the release of mediators in the same way as that of indomethacin.

Histamine exists in bound form in granules (mast cell or basophils) and in free form during inflammatory process. The H1R-PKC-ERK pathway may play crucial roles in eliciting cytokine production from bronchial epithelial cells stimulated by histamine, leading to airway inflammation [22]. Upon injury to a tissue, histamine causes local vasodilatation, and leakage of plasma containing mediators of acute inflammation (complement, C-reactive protein), antibodies, and

**Table 1: Effects of AEAL and indomethacin on carrageenan-induced paw edema in rats**

| Groups | Dose | 30 minutes | 1 hr | 2 hrs | 3 hrs | 4 hrs (% inhibition) |
|---|---|---|---|---|---|---|
| Group-I | Control (normal saline) | 3.92±0.13 | 4.51±0.23 | 4.97±0.11 | 5.24±0.71 | 5.52±0.61 |
| Group-II | Indomethacin (10 mg/kg) | 0.73±0.024** | 0.692±0.054** | 0.61±0.034** | 0.54±0.77** | 0.43±0.13** (92.2) |
| Group-III | AEAL (100 mg/kg) | 3.11±0.013** | 2.43±0.014** | 2.13±0.047** | 1.93±0.054** | 1.75±0.014** (68.1) |
| Group-IV | AEAL (200 mg/kg) | 2.59±0.017** | 2.21±0.045** | 1.94±0.039** | 1.76±0.013** | 1.45±0.52** (74.09) |

All the values are expressed as mean±SEM; n=6. Statistical significance: (*p<0.05) and (**p<0.01) one-way ANOVA followed by Dunnett's test. SEM: Standard error of mean, AEAL: Aqueous extract of *Allium sativum* leaves

**Table 2: Effect of AEAL and indomethacin on histamine-induced paw edema**

| Groups | Dose | 30 minutes | 1 hr | 2 hrs | 3 hrs | 4 hrs (% inhibition) |
|---|---|---|---|---|---|---|
| Group-I | Control (normal saline) | 2.24±0.014 | 2.26±0.027 | 2.08±0.19 | 1.88±0.077 | 1.80±0.56 |
| Group-II | Indomethacin (10 mg/kg) | 1.34±0.019** | 0.88±0.014** | 0.74±0.041** | 0.56±0.036** | 0.36±0.023**(80) |
| Group-III | AEAL (100 mg/kg) | 1.87±0.048** | 1.46±0.019** | 1.16±0.08** | 0.89±0.045** | 0.65±0.016*(63.88) |
| Group-IV | AEAL (200 mg/kg) | 1.68±0.074** | 1.23±0.011** | 0.86±0.061** | 0.68±0.037** | 0.50±0.024*(72.22) |

All the values are expressed as mean±SEM; n=6. Statistical significance: (*p<0.05) and (**p<0.01) One-way ANOVA followed by Dunnett test. SEM: Standard error of mean, AEAL: Aqueous extract of *Allium sativum* leaves

inflammatory cells (neutrophils, eosinophils, basophiles, monocytes, and lymphocytes). Injection of histamine causes a rapid rise in edema and peak was observed at 30 minutes. Activation of H1-receptor may lead to activation of the phosphatidylinositol cycle and associated with inflammatory reactions. Hence, H1-receptor antagonist can be act as an anti-inflammatory agent. The aqueous extract showed a significant reduction in paw volume after histamine injection, which was also comparable with the standard drug indomethacin. This result indicates that the extract possess anti-histaminic potential.

## CONCLUSION

On the basis of above experimental results from the present study, it can be concluded that the AEAL may possess anti-inflammatory activity. The anti-inflammatory activity of *A. satium* leaf extract may be attributed to flavonoids which have anti-inflammatory properties. Further exploration of experimental work was needed for the isolation of compounds and pharmacological investigation of plant extract which have many pharmacological activities reported traditionally and for its exact mechanism of action.

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