

Anti-inflammatory activity of Isolated Allicin from Garlic with Post-Acoustic Waves and Microwave Radiation

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ABSTRACT

In this present study the isolated allicin from fresh garlic cloves was screened for anti-inflammatory activity in carrageenan induced paw edema in rats. The allicin was isolated with effect of post-acoustic waves and microwave radiation. The effect was assessed by difference in paw edema volume, after oral administration of allicin in rats. Anti-inflammatory activity was compared with standard drug. These observations helped us to ascertain the effective dose of allicin which was isolated with effect of post-acoustic waves and microwave radiation.

Keywords: Anti-inflammatory, carrageenan, paw edema, sonication, microwave extraction.

INTRODUCTION

Inflammation is the tissue reaction to infection, irritation or foreign substance. It is a part of the host defense mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins. The development of non-steroids in overcoming human sufferings such as rheumatoid arthritis has evoked much interest in the extensive search for new drugs with this property. [1] It is believed that current drugs, such as opioids and NSAIDs are not useful in all cases of inflammatory disorders, because of their side effects, economy and potency. [2]

Garlic (*Allium sativum* Linné) is a widely distributed plant and is used in all parts of the world not only as a spice or food, but also as a popular remedy for pathological states.[1] Numerous studies have previously demonstrated that garlic may be useful for the prevention of carcinogenesis, cardiovascular, and age-related diseases.[2] Especially, it has been strongly suggested that its medicinal and beneficial properties are attributed to specific organosulfur compounds.[3-6] It is known that garlic contains three

ζ -glutamyl peptides, i.e. ζ -L-glutamyl-S-(2-propenyl)-L-cysteine (GSAC), ζ -L-glutamyl-S-(*trans*-1-propenyl)-L-cysteine (GSPC), and ζ -L-glutamyl-S-methyl-L-cysteine (GSMC); their corresponding sulfoxide derivatives, viz., (+)-S-(2-propenyl)-L-cysteine sulfoxide (alliin), (+)-S-(*trans*-1-propenyl)-L-cysteine sulfoxide (isoalliin), (+)-S-methyl-L-cysteine sulfoxide (methiin) and (1*S*,3*R*,5*S*)-5-methyl-1,4-thiazane-3-carboxylic acid 1-oxide (cycloalliin). Sulfur compounds isolated from garlic exert anti-inflammatory properties.[3] There are numerous reports indicating the efficacy of garlic in the prevention and treatment of a variety of diseases and for validating its traditional uses. For instance, garlic has been described to exhibit antimicrobial, antitumor, antithrombotic, antiarthritic, hypolipidemic and hypoglycemic activities. Moreover, garlic has been reported to be effective against diverse parasites such as amoeba, leishmania, trypanosoma.[4, 8-11] The present study is aimed at investigating the possible effects of allicin isolated from fresh garlic cloves with effect of post-ultrasonic sound and microwave radiation on the inflammatory response in carrageenan induced paw edema in rats.

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MATERIALS AND METHODS

Sodium chloride was purchased from Qualigens, Mumbai, dichloromethane from Merck Specialities Pvt. Ltd., Mumbai, Methanol and water of HPLC grade

was procured from Merck Specialties' Pvt. Ltd., Mumbai.

Fresh garlic cloves (*Allium sativum* L.) were obtained from the cultivated fields of Asansol area in West Bengal, India which was authenticated (Authentication no. CNH/ 1-1/ (201)/ 2013/ Tech. II/35) by head, Shibpur Botanical Garden, Shibpur, Howrah, West Bengal, India.

Garlic samples

30 gm of garlic cloves in 300 ml of distilled water were crushed for 1 min in a blender mixer.

Microwave Extraction

The above mixture was transformed to a specially designed round bottom reactor with which a condenser was fitted. The entire assembly was finally kept inside the microwave extractor (Catalyst System, Pune, Model no. CATA 2R). The mixture was kept for 10 min at 41 °C. Next, it was processed to get the pure allicin.

Probe sonication

This was done in a frontline probe sonicator (Frontline, Ahmedabad, Model no. Sonicator FS 600) by transferring the same quantity of mixture in an Erlenmeyer flask. The process was repeated twice for 20 minutes with an interval of 10 minutes between each sonication.

Bath sonication

Bath ultrasonic assisted extraction was carried out in an ultrasonic bath (Enertech Electronics Pvt. Ltd., Mumbai, Model no. 2K 205035). Keeping the other conditions similar with that of probe sonication. The temperature was kept constant (25 ± 1 °C) by periodical addition of ice in the bath.

Cold maceration

The same quantity of mixture was kept for cold maceration in a well closed container separately for 3 h with frequent mechanical stirring.

Purification of each extract

Each aqueous extract was filtered and diluted to 500 ml with water. The diluted extract (10 ml) was saturated with sodium chloride and subsequently extracted with dichloromethane (3×10 ml). All the

fractions were pooled, dried over anhydrous sodium sulphate, and desiccated by means of rotary-evaporation.

Acute toxicity test

The acute toxicity (LD_{50}) of all the isolated allicin were determined in mice by the method of Lorke (1983)[5] using the oral and intraperitoneal routes.

Anti-inflammatory activity by carrageenan-induced rat paw edema test [6,7]

Male Wistar albino rats, weighing 150-200 g maintained under standard husbandry conditions (temperature 23±2° C, relative humidity 55±10% and 12 h light: 12 h dark cycle) were used for all experiments. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocols approved by institutional animal ethics committee (Regd. No.-955/a/06/ CPCSEA), Gupta College of Technological Sciences, Asansol, West Bengal, India. The rats were divided into six groups containing five rats in each group (one control, one standard & four test groups) acute inflammation was induced according to edema assay. The extracts were suspended in 2.0 % tween 80 & administered orally (250 mg/kg/b.w) to rats 1 hour before carrageenan injection. Diclofenac Sodium (10 mg/kg b.w) is given to standard group. Carrageenan was prepared as 1% w/v solution in 0.9 % w/v NaCl & inject 0.1 ml underneath the planter region.

Control group 1: Carrageenan + 2% Tween 80 (10 ml /kg b.w)

Standard group 2: Carrageenan + Diclofenac Sodium (10 mg/kg b.w)

Test group1: Carrageenan + Allicin from microwave extraction (250 mg/kg b.w)

Test group2: Carrageenan + Allicin from probe sonication extraction(250 mg/kg b.w)

Test group3: Carrageenan + Allicin from bath sonication extraction (250 mg/kg b.w)

Test group 4: Carrageenan + Allicin from maceration extraction (250 mg/kg b.w)

The paw volume was measured with Plethysmograph in a gap of every 1 hour from 0 to 5 hours after

Carrageenan injection. The percentage of inhibition of edema was calculated using formula:

$$\% \text{ Inhibition of edema} = (V_c - V_t / V_c) \times 100$$

where V_t = Paw volume in test group animals.

V_c = Paw volume in control group animals.

RESULTS AND DISCUSSION

In our earlier work, it was observed that the yield of allicin was much higher while opting for post acoustic waves and microwave radiation assisted extraction rather the convention extraction process. The above fact in turn supports microwave cooking system too. In anti-inflammatory activity by carrageenan-induced rat paw edema test, it was seen that all the isolated allicin have a challenging anti-inflammatory property compared with control and standard drug (Table 1). In between that, the microwave extracted allicin was showed the highest potency against carrageenan-induced inflammation in rat paw and both the bath and probe sonication extracted allicin were also showed their anti-inflammatory property but the bath sonication extracted allicin was found to be a linear potency like simple maceration extracted allicin. But a sudden decreasing order of percentage inhibition of carrageenan-induced inflammation in rat paw was noted for probe sonication extracted allicin (Fig. 1). From this data it can be concluded that the isolation technique using microwave radiation yielding a more chemically effective allicin from fresh garlic cloves.

CONCLUSION

With different types of radiations so far employed on the extraction of allicin garlic cloves clearly indicated the presence of pure and more potent allicin with a higher yield. Therefore, grounding these techniques in pharmaceutical industry may provide a ray of hope in getting things things done in lesser time and low cost. Most interestingly, the methods ruled out possibilities of degradation of organo-sulfur compounds and showed a challenging result against inflammation and arthritis as well.

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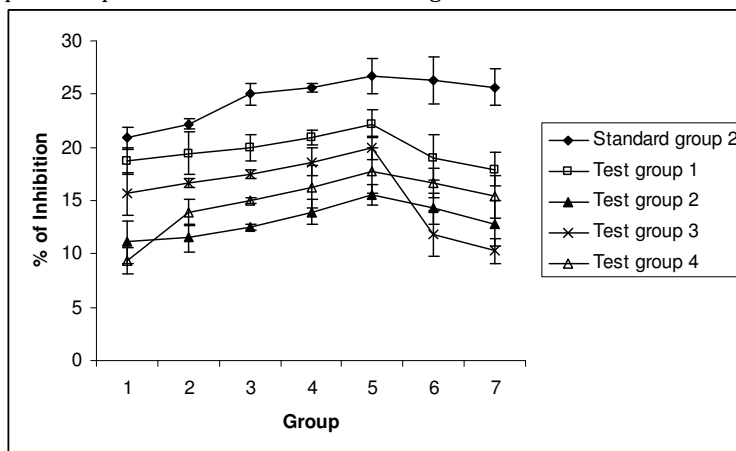
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Table 1: Percentage of inhibition of carrageenan-induced inflammation in rat paw

Groups	% of Inhibition						
	0h	1h	2h	3h	4h	5h	6h
Control Group 1	-	-	-	-	-	-	-
Standard Group 2	20.88±1.01	22.22±0.46	25.00±1.03	25.58±0.37	26.67±1.65	26.19±2.14	25.64±1.76
Test Group 1	18.75±1.23**	19.44±2.01**	20.00±1.25**	20.93±0.67**	22.22±1.34**	19.05±2.10**	17.95±1.56**
Test Group 2	11.11±2.00**	11.50±0.45**	12.50±0.43**	13.95±1.32**	15.56±1.11**	14.29±2.11**	12.82±1.22**
Test Group 3	15.63±2.01**	16.67±1.32**	17.50±0.23**	18.60±1.21**	20.00±1.02**	11.90±1.44**	10.26±2.15**
Test Group 4	9.38±1.21**	13.87±1.22**	15.00±0.34**	16.28±2.01**	17.78±2.13**	16.67±1.33**	15.38±2.00**

Probability values (calculated as compared to standard using one way-ANOVA followed by Dunnet's Test):

**P<0.001. All values are means of individual data obtained from five rats ($n = 5$)

Fig. 1: Graphical Explanation of inhibition of carrageenan-induced inflammation in rat paw

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