



## Evaluation of *in-vitro* anti-inflammatory activity of Athimadhura chooranam, a combination of Eight medicinal plants

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### Abstract

The present study was to evaluate the *in vitro* anti-inflammatory effect of Athimadhuram chooranam against the denaturation of protein. The chooranam at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the chooranam. Maximum inhibition,  $51.47 \pm 14.19\%$  was observed at  $500\mu\text{g/ml}$ . Diclofenac sodium, a standard anti-inflammatory drug showed the maximum inhibition,  $86.69 \pm 0.56\%$  at the concentration of  $100\mu\text{g/ml}$ . From the present study it can be concluded that Athimadhuram chooranam possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein.

**Keywords:** Siddha, Athimadhuram chooranam, Diclofenac sodium, *invitro* anti-inflammatory.

### Introduction

Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceuticals research. Approximately 3000 plants species are known to have medicinal properties in India (Prakasha *et al.*, 2010). Our traditional systems of medicines, viz., Ayurveda, Unani, Siddha and Homeopathy etc., uses the herbs for treatment of various disease and disorders (WHO, 2003).

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. In many inflammatory disorders there is excessive activation of phagocytes, production of  $\text{O}_2$ , OH radicals as well as non-free radical species ( $\text{H}_2\text{O}_2$ ) which can harm surrounding tissue either by powerful direct oxidizing action or indirectly with hydrogen

peroxide (H<sub>2</sub>O<sub>2</sub>) and OH radicals formed from O<sub>2</sub> (Gilham *et al.*, 1997), which initiates lipid peroxidation resulting in membrane destruction. Tissue damage then provokes inflammatory response by production of mediators and chemotactic factors (Lewis, 1989). The reactive oxygen species are also known to activate matrix metalloproteinase (e.g. collagenase) causing increased destruction of tissues e.g. collagenase damage seen in various arthritic reactions.

Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use. For this reason, there is a need for anti-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence (Vijaya *et al.*, 2013).

Therefore, the present study focused on the *in vitro* anti-inflammatory activity of selected herbal drugs such as Athimadhuram (*Glycirrhiza glabra*), Elam (*Elettaria cardamomum*), Elavangapattai (*Syzygium aromaticum*), Senbaga Mokku (*Michelia champaca*), Kottam (*Costus speciosus*), Sukku (*Zingiber officinale*), Nar Seeragam (*Cuminum cyminum*), Korai Kizhangu (*Cyperus rotundus*) to provide scientific evidence for their antiinflammatory effect.

## Materials and Methods

### Drug collection, Identification and Authentication

All the ingredients were obtained from Country drug shop, Ramasamychetti, Parrys Chennai, Tamilnadu, India. All the raw drugs were identified and authenticated at Central Research Institute (CRI), Arumbakkam, Chennai-106

### Ingredients of chooranam

The medicinal plants Athimadhuram (*Glycirrhiza glabra*), Elam (*Elettaria cardamomum*), Elavangapattai (*Syzygium aromaticum*), Senbaga Mokku (*Michelia champaca*), Kottam (*Costus*

*speciosus*), Sukku (*Zingiber officinale*), Nar Seeragam (*Cuminum cyminum*), Korai Kizhangu (*Cyperus rotundus*) and Sugar composed in Athimadhuram chooranam were prepared.

### Purification of raw drugs

Raw drugs are purified as mentioned in Sikicharathna Deepam Sarakku Suthi Muraigal.

### Preparation of chooranam

The purified above first 8 raw drugs are made into fine powder as mentioned in the literature and finally sugar added and they were stored properly and used for further studies.

### *In-vitro* Anti-Inflammatory Activity by Protein (Albumin) denaturation Assay

*In-vitro* anti-inflammatory activity Athimathura chooranam (AC) was studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample AC at varying concentration ranges from 100 to 500 mcg/ml and standard diclofenac sodium at the concentration of 100 mcg/ml of final volume. pH was adjusted by using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate. The Percentage protection from denaturation is calculated by using the formula

$$\left[ \frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \right] \times 100.$$

### Statistical analysis

Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis Of Variance (ANOVA) followed by Dungan Multiple comparison test

## Results and Discussion

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of Athimadhuram chooranam.

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of

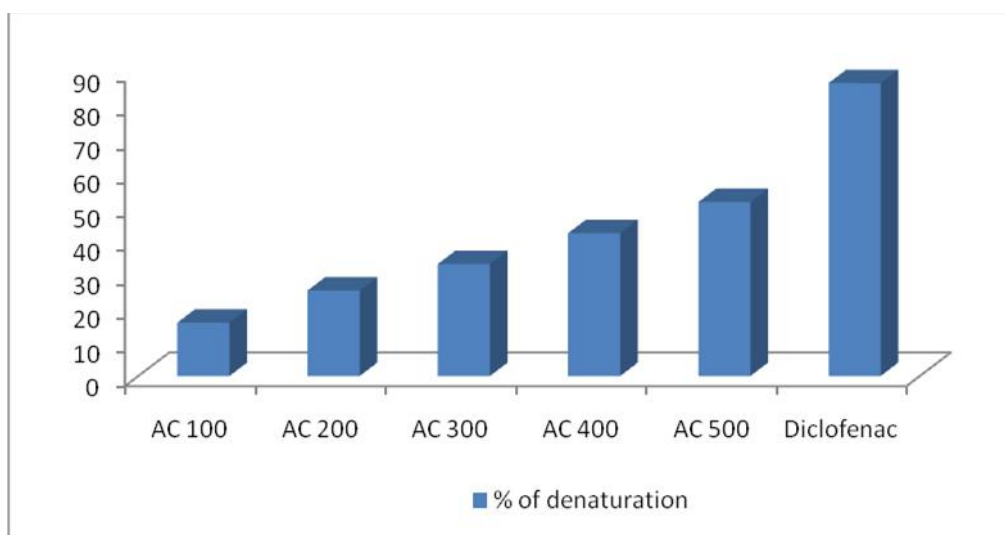
proteins is a well documented cause of inflammation. Phenylbutazone, salicylic acid, flufenamic acid (anti-inflammatory drugs) etc, have shown dose dependent ability to thermally induced protein denaturation (Leelaprakash and Mohan Dass, 2010). As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of Athimadhuram chooranam to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table 1. Maximum inhibition,  $51.47 \pm 14.19\%$  was observed at  $500\mu\text{g/ml}$ . Diclofenac sodium, a standard anti-inflammatory drug showed the maximum inhibition,  $86.69 \pm 0.56\%$  at the concentration of  $100\mu\text{g/ml}$ .

**Table 1: *In vitro* anti-inflammatory activity of Athimadhuram chooranam**

Concentration in $\mu\text{g/ml}$	Absorbance	Percentage Inhibition of Protein Denaturation
Control	$0.885 \pm 0.04$	--
AC 100	$0.647 \pm 0.10$	$15.8 \pm 4.55$
AC 200	$0.563 \pm 0.12$	$25.3 \pm 8.36$
AC 300	$0.493 \pm 0.10$	$33.15 \pm 6.99$
AC 400	$0.413 \pm 0.12$	$42.27 \pm 8.49$
AC 500	$0.333 \pm 0.17$	$51.47 \pm 14.19$
Diclofenac sodium (100 $\mu\text{g}$ )	$0.016 \pm 0.01$	$86.69 \pm 4.30$

Each value represents the mean  $\pm$  SD. N=3

**Fig 1: *In vitro* anti-inflammatory activity of Athimadhuram chooranam**



The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by Athimadhuram chooranam and reference drug diclofenac sodium. This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation (Anson and Mirsky, 1932).

Although, the viscosities of the test samples (extract/drug), of all concentrations were always less than that of control. This decrease in viscosities may be due to decrease in concentration of test extract/drug in reaction mixture, which resulted in decreased viscosity; and/or other uncertain physico-chemical factors. Nevertheless, the viscosity data indicated inhibition of protein (albumin) denaturation. The effect of concentration of test agent on viscosity behaviour of denatured protein

The denaturation is used loosely to designate the change of proteins from a soluble to an insoluble form brought about by a large variety of chemical and physical agents, including acids, alkalies, alcohol, acetone, salts of heavy metals and dyes (Mann, 1906), and heat, light, and pressure (Robertson, 1918). Chick and Martin consider heat denaturation as a reaction between protein and water which implies in all probability a hydrolysis (Chick and Martin, 1910). Mechanism of denaturation is involved in alteration of electrostatic force, hydrogen, hydrophobic and disulphide bonds. Several author anti-inflammatory drugs have shown dose dependent ability to inhibit the thermally induced protein denaturation (Grant *et al.*, 1970).

The data of our studies suggests that Athimadhuram chooranam showed significant anti-inflammatory activity. Therefore our studies support the isolation and use of active constituents of Athimadhuram chooranam in treating inflammation.

## References

- Anson, M.L. and Mirsky, A.E. 1932. The effect of denaturation on the viscosity of protein systems. *Gen Physiol.* 15: 341-350.
- Chick, H. and Martin, C.J. 1910. On the heat coagulation of protein. *J Physiol.* 4: 404-430.
- Gilham, B., Papachristodoulou, K. and Thomas, J.H. 1997. In: *Wills Biochemical Basis of Medicine*, Oxford: Butterworth-Heinemann. 351.
- Grant, N.H., Alburn, H.E. and Kryzanasuskas, C. 1970. Stabilization of serum albumin by anti-inflammatory drugs, *Biochemical pharmacology.* 19(3):715-722.
- Leelaprakash, G. and Mohan Dass, S. 2010. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*, *International Journal of Drug Development & Research.* 3:189-196.
- Lewis, D.A. 1989. In: *anti-inflammatory drugs from plants and marine sources.* Basel: Birkhauser Verlag. 135.
- Mann, G. 1906. *Chemistry of the proteids*, London and New York. 336 - 344.
- Murugesamudhaliyar, K.S. Maru. Pon. Sironmani Kuzhanthai maruthuvam (Bala vagadam), Paadal no. 41, Page no. 479
- Prakasha, H.M., Krishnappa, M., Krishnamurthy, Y.L., Poornima, S.V. 2010. Folk medicine of NR Pura Taluk in Chikamagalur district of Karnatka. *Indian Journal of Traditional Knowledge;* 9(1):55-60.
- Robertson T.B. 1918. *The physical chemistry of the proteins*, New York and London.
- Vijaya, P.P., David Wilson, Aswan Ali, A.M., Yogananth, N., Syed Ali, M., Anuradha, V. and Kalitha Parveen, P. 2013. Evaluation of *in-vitro* anti-inflammatory and antimicrobial properties of *Pergularia daemia* and *Solanum xanthocarpum*. *International Journal of Current Microbiology and Applied Sciences.* 2 (1): 94-99.

World Health Organization. 2003. Guidelines for the Assessment of Herbal Medicine Programme on Traditional Medicine.Doc. WHO/TRM/91.4.WHO, Geneva.

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