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Research Article

EVALUATION OF IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF *CHANDRAPRAKASA MATHIRAI* (CPM) BY PROTEIN DENATURATION ASSAY

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ABSTRACT

Chandraprakasa mathirai (CPM) is a classical Siddha herbo-mineral formulation traditionally used in inflammatory conditions such as undifferentiated polyarthritis and rheumatoid arthritis. However, scientific validation of its anti-inflammatory activity remains limited. The present study aimed to evaluate the in-vitro anti-inflammatory potential of CPM using a protein denaturation assay. CPM was prepared according to standard Siddha procedures using purified *Aconitum ferox, Piper nigrum,* and sodium biborate. The anti-inflammatory activity was assessed by inhibition of heat-induced denaturation of bovine serum albumin at concentrations ranging from 100 to 500µg/ml. Diclofenac sodium (100µg/ml) was used as the reference standard. Absorbance was measured at 660 nm, and percentage inhibition was calculated. Data were expressed as mean ± standard deviation and analysed using one-way ANOVA followed by Dunnett's test. CPM exhibited a concentration-dependent inhibition of protein denaturation, with values increasing from 10.7 ± 2.22% at 100µg/ml to 45.78 ± 1.84% at 500µg/ml. The standard drug diclofenac sodium showed significantly higher inhibition (85.19 ± 4.06%). However, CPM did not achieve 50% inhibition within the tested concentration range. In conclusion, CPM demonstrates moderate in-vitro anti-inflammatory activity, supporting its traditional use. Further in-vivo and clinical studies are required to establish its therapeutic efficacy and safety profile.

INTRODUCTION

Chandraprakasa mathirai (CPM) is a Siddha herbomineral formulation indicated for Undifferentiated polyarthritis and rheumatoid arthritis[1]. Its constituents are *Naabi* (*Aconitum ferox*), *Vengaram* (Sodium biborate), *Milagu* (*Piper nigrum*). Genetic susceptibility and triggers like smoking, hormonal changes and infections lead to epigenetic changes. This is followed by activation of antigen presenting cells and formation of auto-antibodies. It culminates in release of pro-inflammatory cytokines like IL-6, TNF-α, INF-γ and synovial hyperplasia [2].

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The role of NSAIDs in inflammatory arthritis is to provide symptomatic relief [3]. The present study aims to evaluate the in-vitro anti-inflammatory activity of CPM by protein denaturation assay.

Principle of protein denaturation assay

Physical or chemical triggers such as stress, pH level, heat will damage the three-dimensional structure of proteins. This process is called denaturation. Protein denaturation leads to loss of biological function and inflammatory conditions where altered proteins act as auto-antigens. In this assay, a standard protein such as albumin is subjected to heat-induced denaturation. When a test substance is added, its ability to inhibit the denaturation is measured spectrophotometrically. A lower absorbance indicates greater protection against denaturation [4].

MATERIALS AND METHODS

Table 1: Ingredients of CPM

Name of the drug	Part used	Quantity
Naabi (Aconitum ferox)	Root	1 part
Milagu (Piper nigrum)	Dried fruit	5 parts
Vengaram (Sodium biborate)	-	5 parts
Inji (*Zingiber officinale*)	Rhizome	Required quantity

Source of raw drugs

The raw drugs were procured from a reputed raw drug store and authentication was obtained from the Head of the Pharmacology department. The raw drugs were purified as mentioned in the Siddha text, *Marundhu Sei Iyalum Kalayum* [5].

Table 2: Purification of raw drugs:

Name of the drug	Part used	Purification procedure
Naabi (*Aconitum ferox*)	Root	Soaked in cow's urine for 8 days, washed with water, outer layer removed and dried in sunlight. [6]
Milagu (Piper nigrum)	Dried fruit	Dirt and impurities removed.
Vengaram (Sodium biborate)	-	Fried in a pan and powdered.
Inji (Zingiber officinale)	Rhizome	The outer skin is removed.

Preparation of CPM

Powdered *Aconitum ferox*, *Piper nigrum* and sodium biborate were mixed in a stone mortar. Required quantity of Zingiber officinale juice was added and the mixture was ground for six hours. *Kundri alavu* tablets (130 mg) [7] were made and dried in the shade.

Procedure of protein (albumin) denaturation assay

The reaction mixture consisted of 5% aqueous bovine serum albumin and the test sample CPM at varying concentrations (100 - 500 µg/ml). Diclofenac sodium at a concentration of 100µg/ml was used as the standard reference [8]. The pH was adjusted using a small amount of 1N hydrochloric acid. The samples were incubated at 37°C for 20 minutes and then heated at 57°C for 3 minutes. After cooling, 2.5ml of phosphate buffer solution was added to each test tube. The turbidity developed was measured spectrophotometrically at 660nm. For the control, distilled water was used instead of the test sample, while the product control lacked bovine serum albumin [8]. The experiment was performed in triplicate. The percentage protection against protein denaturation was calculated using the formula:

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

Statistical analysis

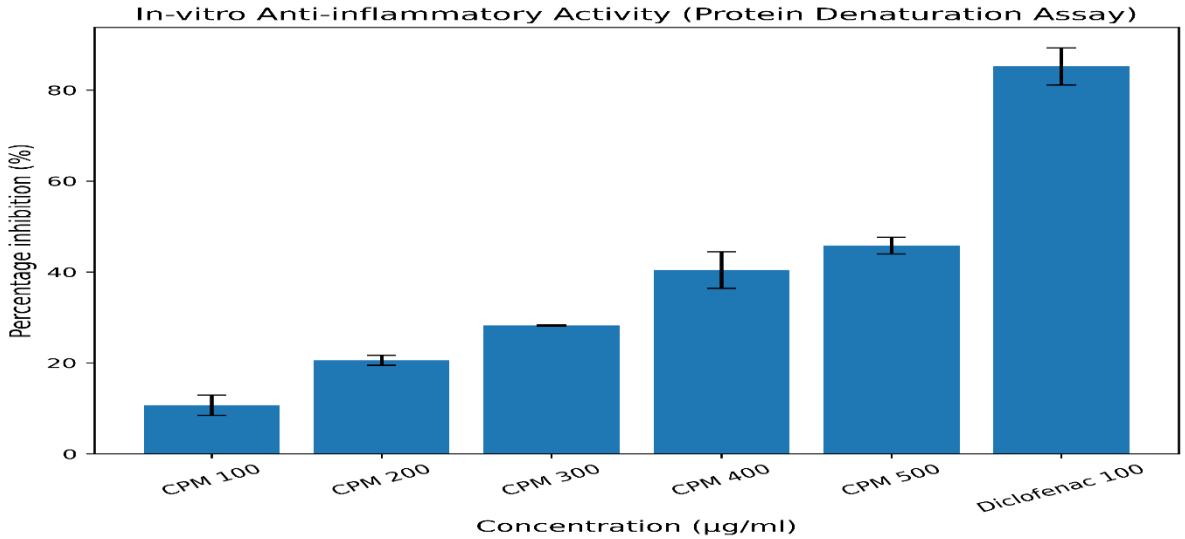
The values were expressed as Mean ± SD. The difference between experimental groups were evaluated by one -way ANOVA (Analysis of Variance) followed by Dunnett's test.

RESULTS

Table 3: Effect of CPM on inhibition of protein denaturation at different concentrations compared with standard Diclofenac sodium

Concentration in µg/ml	Percentage inhibition of protein denaturation
CPM 100 µg	10.7 ± 2.22
CPM 200 µg	20.58 ± 1.09
CPM 300 µg	28.25 ± 0.1
CPM 400 µg	40.4 ± 4.02
CPM 500 µg	45.78 ± 1.84
Diclofenac sodium (100 µg)	85.19 ± 4.06

Figure 1: In-vitro anti-inflammatory activity assessed by protein denaturation assay showing percentage inhibition at varying concentrations with Diclofenac sodium as standard.



DISCUSSION

Steroidal and non-steroidal anti-inflammatory drugs are the first line of treatment in rheumatoid arthritis. They are used to relieve pain and stiffness, by inhibiting the synthesis of prostaglandins. However, NSAIDs are associated with side-effects like gastrointestinal ulcers, peripheral edema and, heart failure [3]. *Chandraprakasa mathirai* (CPM) is a Siddha herbomineral formulation indicated for Undifferentiated polyarthritis and fever. This study evaluated the in-vitro anti-inflammatory activity of CPM by inhibition of protein denaturation.

The present study exhibited that CPM showed concentration dependent inhibition of protein denaturation. However, 50% inhibition was not achieved within the tested concentration range, suggesting moderate potency and indicating the need for evaluation at higher concentrations.

The observed anti-inflammatory activity of CPM may be attributed to its constituents. Purified *Aconitum ferox, Piper nigrum* and *Zingiber officinale* are known for their anti-inflammatory properties [9,10,11]. This study validates the traditional usage of CPM in treating inflammatory arthritis. In vivo studies and clinical trials are essential to explore the therapeutic benefits of CPM in undifferentiated polyarthritis and rheumatoid arthritis.

CONCLUSION

Chandraprakasa mathirai showed concentration -dependent increase in the percentage inhibition of protein denaturation. CPM exhibited a moderate anti-inflammatory activity compared to the standard drug. Further in-vivo studies and clinical studies are necessary to validate its therapeutic potential in inflammatory arthritis.

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