



**EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF SIDDHA  
FORMULATION AKKARAM ENNAI BY PROTEIN (ALBUMIN)  
DENATURATION ASSAY**

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**ABSTRACT**

Stomatitis is one of the most common oral mucosal painful ulcerative lesions which can significantly affect the life quality of patients. This condition is associated with some pathological complications along with similar clinical demonstrations as round or elliptic recurrent lesions in oral mucosa. The most frequently used drugs for treatment of stomatitis are topical steroid which can cause several side effects on continuous application. Herbal drugs belonging to this category are considered alternative agents and have been used for several years around the world. Siddha system of medicine offers unique therapeutic remedies for the treatment of recurrent inflammation like stomatitis. One such novel formulation is Akkaram Ennai (AKE) intended for inflammatory condition of mucosa as per literature. The main objective of the present investigation is to evaluate the anti-inflammatory potential of AKE by using protein (albumin) denaturation assay. The result obtained from the present clearly indicates that the test drug AKE was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about  $64.16 \pm 1.14\%$  was observed at  $500 \mu\text{g/ml}$  when compare to that of the diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition  $97.91 \pm 1.18$  at the concentration of  $100 \mu\text{g/ml}$ . From the result of the study it was concluded that the siddha drug AKE possess significant anti-inflammatory property in protein denaturation assay and the same shall be utilised clinically upon successful validation of the drug with proper clinical validation.

**KEY WORDS:** Stomatitis, Siddha, Akkaram Ennai, Anti-inflammatory, Protein denaturation assay, Diclofenac

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## 1. Introduction

Stomatitis is one of the most common problems recognized by doctors, skin specialists, and dentists. This problem is seen in children and adults. Studies showed that the occurrence of RAS ranges from 2% to 50% between different population groups. In high-risk population such as students and soldiers, the occurrence is reported to reach up to 50–60% [1,2] there are several etiologic reasons responsible for this problem [3].

Stomatitis is a common oral disorder of uncertain etiopathogenesis, and presently, its management is largely focused on symptomatic treatment [4,5]. Stomatitis causes considerable pain and distress for patients and presents a difficult management challenge for clinicians [6]. Different classes of chemical and biochemical products have been reported to be of some benefit in the management of stomatitis, but no definitive treatment is yet available. Management of the pain of stomatitis using various herbal preparations has also been reported [7,8].

Inflammation is generally referred to as a complex biological response of vascular tissues to harmful stimuli. As well, inflammation is associated with pain, and it involves in an increase of protein denaturation, an increase of vascular permeability, and membrane alteration, among others [9]. Inflammation is also described as the body response to inactivate or eliminate the invading stimuli or organisms, to remove the irritants and set the stage for tissue repair, and the process is accelerated by the release of chemical mediators from injured cells or tissues and migrating cells [10]. The migration of leukocytes from the venous systems to the site of damage, and the release of cytokines, are known to play a crucial role in the inflammatory response [11].

Despite the availability of sufficient drugs, the side effects of analgesic and anti-inflammatory agents, which include gastrointestinal upset, gastric ulcer, bleeding, and liver damage, are a major concern in clinical use. Because of this, the search for safe and effective newer agents is growing. As one of research areas, screening herbs with claimed anti-inflammatory effect may create the opportunity of discovering new compounds with more safety and efficacy [12]. Traditionally, different practices are routinely used to manage pain and inflammation in various countries.

Herbal remedies are widely used in developing countries to manage pain and inflammation because of their cost, accessibility, and eco-friendly advantages [13].

Siddha system of medicine pioneers the treatment of inflammation and other degenerative disorders, most of the siddha proration are composed of herbal ingredients which has novel therapeutic values. Herbal supplements are known for its wide safety index in addition to that pharmacologically phytocomponents excel the healing mechanism by adequately strengthening the cellular biochemical pathway. As per the literature it was evident that the siddha drug Akkaram Ennai (AKE) claimed to have potential anti-inflammatory property but till date there is no proper documentary evidence substantiating this claim, Hence the main objective of the present investigation is to evaluate the anti-inflammatory potential of AKE by using protein (albumin) denaturation assay.

## 2. Materials and Methods

### 2.1. Albumin Denaturation Assay Procedure [14,15]

In-vitro anti-inflammatory activity AKE as studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and chloroform extract of AKE at varying concentration ranges from 100 to 500 µg/ml and standard Diclofenac sodium at the concentration of 100 µg /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 formulae

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

### 2.2. Statistical analysis

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

### 3.Results

#### 3.1. Effect of AKE on Protein Denaturation

Protein denaturation assay is considerably an excellent model to evaluate the anti-inflammatory property of drugs and formulations. The ability of drug to halt the progression of denaturation indirectly taken as a sign of hindering inflammation. The result obtained from the present clearly indicates that the test drug AKE was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 64.16±1.14% was observed at 500 µg/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 97.91 ±1.18 at the concentration of 100 µg/ml.

**Table 1: Percentage Inhibition of Protein Denaturation by AKE and Standard**

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
AKE 100	12.36 ± 1.31
AKE 200	30.47 ± 2.12
AKE 300	49.7 ± 1.75
AKE 400	59.85 ± 0.94
AKE 500	64.16 ± 1.14
Diclofenac sodium (100 µg)	97.91 ± 1.18

Each value represents the mean ± SD. N=3

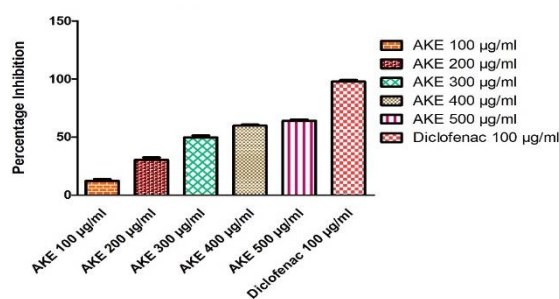


Figure 1: Inhibition of Protein Denaturation by AKE and Standard

### 4.Discussion

The prevalence of stomatitis depends on the race and socio-economic condition, but an overall 10-20% of the general population is affected by the disease. The onset of the stomatitis is usually in childhood and the severity and number of lesions will decreased with aging [16]. Different studies have introduced different factors affecting the disease such as genetic predisposition, immune disorders, drugs, foods, hormonal changes, lack of vitamins and so on.

However, the etiology of this illness is unknown [17]. So far, many treatments have been introduced such as administration of antibiotics, anti-inflammatories and immune system modulators, traditional and home complementary remedies.

Denaturation of protein molecules is well documented in the literature, and it is due to an inflammation process. One of the main mechanisms of action of NSAIDs is the protection against protein denaturation. Inhibition of protein denaturation may play an important role in the anti-inflammatory activity of NSAIDs. The ability of drugs or formulation to prevent thermal and hypotonic protein denaturation maybe responsible for their anti-inflammatory properties [18].

Various nonsteroidal anti-inflammatory drugs can reduce pain and inflammation by blocking the metabolism of arachidonic acid by isoform of cyclooxygenase enzyme (COX-1 and/or COX-2), thereby reducing the production of prostaglandin. Unfortunately, there are many side effects associated with the administration of nonsteroidal anti-inflammatory drugs. However, there are herbal supplements with anti-inflammatory therapeutic effects with low or no side effects. The result obtained from the present clearly indicates that the test drug AKE was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 64.16±1.14% was observed at 500 µg/ml when compare to that of the diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 97.91 ±1.18 at the concentration of 100 µg/ml.

Herbs which have analgesic and anti-inflammatory activity contain mainly alkaloids, flavonoids, saponin, tannins phenolic compound, glycosides, coumarins, and triterpenoids chemical constituents [19]. Tannins, flavonoids, and saponins are well known for their ability to inhibit pain perception and anti-inflammatory properties due to inhibition of enzymes involved in inflammation, especially arachidonic acid metabolic pathway, and synthesis of prostaglandins [20]. Tannins could affect the inflammatory response via free radical scavenging properties and inhibition of iNOS in macrophages [21]. Saponins, on the other hand, inhibit pain and inflammation via NO inhibition [22]. Therefore, the presence of saponins, alkaloids, flavonoids, tannins, terpenoids, and phenols in the formulation Akkaram Ennai may be responsible for

the anti-inflammatory effect in the tested protein denaturation model.

## 5. Conclusion

Herbal therapy, although still an unwritten science, is well established in some countries and traditions and has become a way of life in almost 80% of population in rural areas. At present, although synthetic drugs are dominating the market but element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration the most common being gastrointestinal bleeding and peptic ulcers. Consequently there is a need to develop a new anti-inflammatory agent with minimum side effects from the traditional origin. From the result of the study it was concluded that the siddha drug Akkaram Ennai possess significant anti-inflammatory property in protein denaturation assay and the same shall be utilized clinically upon successful clinical validation

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