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IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF KAARA SOODA SATHU PARPAM (KSSP) – A SIDDHA HERBO MINERAL FORMULATION BY PROTEIN (ALBUMIN) DENATURATION

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ABSTRACT

Inflammation is a complex biological response that involves various molecular mechanisms, including protein denaturation. Uncontrolled inflammation can lead to chronic diseases, current synthetic anti-inflammatory drugs have side effects. Therefore, there is a need for potent anti-inflammatory agents from medicinal plants. Kaara Sooda Sathu Parpam (KSSP) is a Siddha Herbo mineral formulation used in traditional medicine to treat inflammatory disorders. This study aimed to evaluate the in vitro anti-inflammatory activity of KSSP by investigating its ability to inhibit protein (albumin) denaturation. The results showed that KSSP significantly inhibited heat-induced albumin denaturation, with a maximum inhibition of 47.9% at 500 µg/ml, comparable to the standard anti-inflammatory drug diclofenac sodium (92.59% at 100 µg/ml). The findings of this study suggest that KSSP's anti-inflammatory activity may be attributed to its ability to inhibit protein denaturation, a key event in the inflammatory cascade. These results support the traditional use of KSSP in Siddha medicine and

highlight its potential as a therapeutic agent for inflammatory disorders. Further investigation is warranted to fully understand KSSP's anti-inflammatory mechanisms and therapeutic potential. This study provides a foundation for the development of KSSP as a novel anti-inflammatory agent, offering a promising alternative to synthetic drugs.

KEYWORDS: Kaara Sooda Sathu Parpam (KSSP), Siddha Herbo mineral formulation, Anti-inflammatory activity, Protein denaturation, Albumin, Inflammation, Traditional medicine, Diclofenac sodium, In vitro study, Therapeutic potential.

INTRODUCTION

Inflammation is defined as a localized protective response induced by injury or destruction of tissues. When the tissue is injured by mechanical or chemical factors, some substances are released from the affected area. Chemical substances such as histamine, serotonin, leukotrienes, prostaglandins and bradykinin, which are released from damaged tissues cause vasodilatation and erythema in the affected area, many leukocytes, particularly neutrophils and monocytes infiltrate the affected area. Vasodilator substances released in the affected area increase the permeability of capillary membrane, resulting in oozing out of fluid from blood into interstitial space.[6]

The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species (ROS) from activated neutrophil and macrophages. Free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation.[12]

Protein denaturation is defined as a process where due to external factors such as heat, strong acid or strong base; an organic solvent or a concentrated inorganic salt causes the protein to denature that means the protein’s tertiary structure and secondary structure is disoriented. Enzymes lose their activity since the substrates are able to no longer attach to the active site.

The anti-inflammatory, analgesic and anti-pyretic properties of non-steroidal anti-inflammatory drugs (NSAIDs) are particularly useful in treating rheumatoid and other musculoskeletal disorders.[7] These non-steroidal anti-inflammatory drugs have demonstrated strong antimicrobial property when tested against a large number of Gram-positive and Gram-negative bacteria[8] and are now referred to as “non-antibiotics”[9] To isolate bacteria causing UTIs, detection of antibacterial activity of some NSAIDs (diclofenac sodium)[10] But

long-term use of NSAIDS is also associated with side effects like stomach bleeding, allergic reactions, kidney problems, heart problems etc.,[11]

Traditional Siddha Indian Medicine has many such herbomineral medicines indicated for the treatment of Neerkaduppu(UTI), etc,.. This research paper deals with the in-vitro anti-inflammatory screening of such a medicine documented in Classic Siddha text, "Sikitcha rathina deepam".

AIM AND OBJECTIVE

The primary objective of the study is to determine the in-vitro anti-inflammatory activity of the chosen Siddha formulations KAARA SOODA SATHU PARPAM (KSSP) using protein (Albumin) denaturation method.

MATERIALS AND METHODS

The Siddha formulation KSSP was selected from SIKITCHA RATHINA DEEPAM PART-2" by KANNUSAMIPILLAI.[3] The test sample KSSP was prepared after the purification of the ingredients.

Table 1: Ingredients of kssp.

Sl. No	*Name*	*Botanical name/ Chemical name*	*Quantity*
1.	*Vengaram*	*Borax,Sodiyam biborate*	*1 palam (35 gms)*
2.	*Karpoora silasathu*	*Gypsum, Calcium sulfate dihydrate*	*1 palam(35 gms)*
3.	*Lemon juice*	*Citrus limon. Linn[18]*	*Required quantity*

Collection of raw material

The indigeneous herbal and mineral raw drugs were procured from a reputed raw drug store, identified and authenticated by the Botanist of Government Siddha Medical College, Chennai, (Voucher number GSMC/MB- 630) and HOD of the Department of Gunapadam, Government Siddha Medical College, Chennai, Tamilnadu – 106, respectively.

Purification of raw drugs[4,5]

Vengaram

The salt is fried in an earthen plate until the moisture content in it completely gets evaporated.[4]

Karpoora silasathu

Karpoora silasathu was boiled in tender coconut water, then washed and dried.[5]

Preparation of kara sooda sathu parpam[3]

- Grind the above 2(Table1) purified raw materials with lime juice.
- Then make pelletes of grind material and dry it well.
- Prepare the crucible and its lid with limestone.
- Then disintegrate the dried pelletes collect into the crucible and sealed with mud cloth and dry it well.
- After that it incinerated with 6 cow dung cakes.
- Then collect the inside material of crucible and grind into fine powder with stone mortar and pestle.
- Then, store it in an air tight glass container.

***Dosage*:** 500 mg twice a day for 15 days.

Adjuvant: Honey.

Indications: Neerkaduppu(UTI), Neeradaippu, Kalladaippu, Sathaiyadaippu, kiricharam.

Albumin denaturation assay procedure

In-vitro anti-inflammatory activity of the sample KSSP was studied using albumin denaturation technique. The reaction mixture consisting of bovine serum albumin (5% aqueous solution) and the test sample KSSP at varying concentration ranges from 100 to 500 µg/ml along with 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 formula.[16,17]

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

Statistical analysis

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

Table 2: Final result.

Concentration in µg/ml	Percentage inhibition of protein denaturation
KSSP 100	4.757 ± 3.241
KSSP 200	11.9 ± 3.324
KSSP 300	23.62 ± 1.642
KSSP 400	37.86 ± 1.38
KSSP 500	47.9 ± 1.789
Diclofenac sodium (100 µg)	92.59 ± 0.517

Each value represents the mean ± SD. N=3

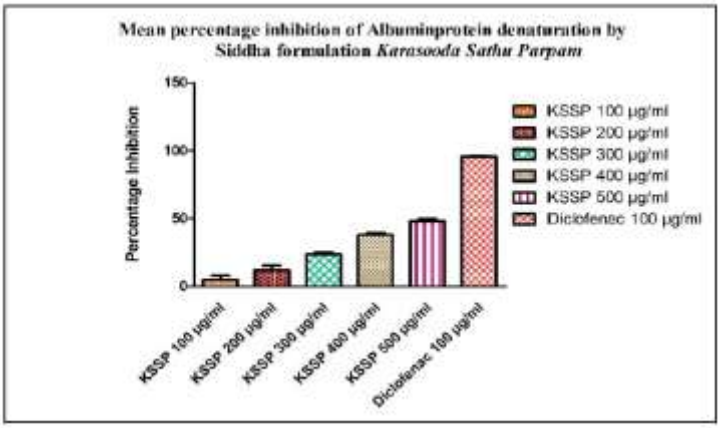

Figure 1: ***Percentage Inhibition of Protein Denaturation by KSSP and Standard.***

RESULTS ANALYSIS

The result obtained from the present study clearly indicates that the test drug KSSP was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 47.9 ± 1.789 % was observed at 500 µg/ml, when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 92.59 ± 0.517 % at the concentration of 100 µ g / ml. (Table 2)

DISCUSSION

Inhibition of albumin 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.[1] As part of the investigation on the mechanism of the anti-inflammation activity, ability of KSSP to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation.

An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. Currently used synthetic anti-inflammatory drugs are associated with some side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary from medicinal plants origin.[13,14]

The anti-inflammatory activity of KSSP was evaluated through in vitro methods. The assessment was done using the Inhibition of albumin denaturation assay, with diclofenac sodium as a reference. The results of the study showed that KSSP effectively inhibited heat-induced albumin denaturation, with a maximum inhibition of 47.9% at a concentration of 500 µg/ml. In comparison, diclofenac sodium, a standard anti-inflammatory agent, achieved a maximum inhibition of 92.59% at 100 µg/ml.

The results of this study suggest that KSSP shows promise as a Herbo mineral Siddha formulation for alleviating inflammation and pain, similar to the effects of diclofenac sodium.

CONCLUSION

In conclusion, the Herbo mineral siddha formulation KSSP has been found to be effective in reducing inflammation as demonstrated by its ability to significantly inhibit protein denaturation in vitro. These results are similar to those seen with the standard anti-inflammatory drug diclofenac sodium. Therefore, it can be concluded that KSSP is a promising Herbo mineral siddha formulation for combating inflammation and pain.

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