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Research article

In-vitro protein denaturation inhibition assay of *Sivappu thailam* an anti-inflammatory Siddha therapeutic oil.

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ABSTRACT

The needs for herbal source of anti-inflammatory formulations are important to ease the pain of individuals. This could possibly due the known ill effect of currently available non-steroidal anti-inflammatory drugs (NSAIDS). This study is conducted to evaluate the In-vitro anti-inflammatory activity of *Sivappu thailam* (ST). The Objective of this study is to assess the anti-inflammatory of *Sivappu thailam* using Invitro Protein denaturation assay. The test drug *Sivappu thailam* at varying concentration ranges from 100 to 500µg/ml is incubated and heated with Egg albumin in controlled experimental conditions. The percentage inhibition of the protein denaturation is calculated using standard methods where Diclofenac sodium is used as reference standard. The result from the present study clearly states that the drug *Sivappu thailam* is effective in inhibiting heat induced protein denaturation. Turbidity developed was measured spectrophotometrically at 660nm and maximum percentage of inhibition of 50.91 ± 0.99 was observed at 500 µg/ml when concern with the Diclofenac sodium as 97.19 ± 3.80.

Keywords:

Herbal, anti-inflammatory, Sivappu thailam, Siddha external medicine.

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INTRODUCTION

Inflammation is a complex process which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes physical agents or chemical agents the injury is in the form stress. Inflammation of tissue is due to response to stress[1]. It is a defensive response that is characterized by redness pain heat and swelling and loss of function in the injured area.

Loss of function occurs depends on the site and extent of injury. Since inflammation[2] is one of the body's nonspecific internal systems of defence the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage caused by burns due to heat radiation bacterial or viral invasion When tissue cells become injured they release kinins prostroglandins and histamine[3]. These work collectively to cause increased vasodilation (widening of blood capillaries) and permeability of the capillaries. This leads to increased blood flow to the injured site. These substances also act as chemical messengers that attract some of the body's natural defense cells through chemotaxis mechanism[4].

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response involving the local vascular system the immune system and various cells within the injured tissue.

Prolonged inflammation known as chronic inflammation leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Several experimental protocols of inflammation are used for evaluating the potency of drugs. The management of inflammation related diseases is a real issue in the rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal herbs.

Sivappu thylam is one among the poly herbal formulation[5] contains 8 ingredients which is mentioned in siddha text book of Pharmacopoeia of hospital of Indian medicine. This drug used for all kind of skin diseases. The drug review of *'Sivappu thylam'* is a poly herbal formulation gives evidence for its therapeutic action mentioned in literatures and research studies.

MATERIALS AND METHODS

Plant materials

Source of raw drugs:

The required raw drugs for the siddha medicine *Sivappu thailam* purchased from a well reputed country raw drug shop and drugs was authenticate by the competent authority Medicinal Botany and Central council for Research in Siddha Chennai. After that the raw drugs was purified separately then the trial drugs prepared in Gunapadam laboratory of National Institute of Siddha Chennai.

Ingredients of *Sivappu thailam*

The sivappu thailam are prepared by the mentioned ingredients with the ration of *Pungan Ver (Pongamia pinnata Pierre)-4kg, Manjitti (Rubia cordifolia Linn.)- 62.5gm, Nannari (Hemidesmus indicus R.Br.) -62.5gm, Manjal Mezhugu (Cera wax)- 62.5gm, Vellai Kungiliyam (Vateria indica Linn.) - 62.5gm, Chevvallikkodi (Dioscorea purpurea)- 20gm, Surul Pattai (Cinnamomum verum.Juss.)- 30gm* and *Coconut Oil (Cocos nucifera Linn.) - 1 Lr.* The individual descriptions about the ingredients were clearly explained in Table. 1.

Preparation of Sivappu thailam

The Manjitti, Nannari, Chevvallikodi and Pungan Ver are taken individually and coarsely powdered which is suitable for making heat extracts (i.e decoction) and adding 8 times of water then boiled and reduce the quantity of mixed water into 1/8. Then equal quantity of oil should be mixed with the decoction and again to be boil. The yellow wax should be cut into pieces and added them into the melted thick consistency. After melting it will be taken from the oven in the texture of sand. Then the pulverized *Surulpattai* (lavangapattai) added into it and stir well. Then the filter and kept it for the routine clinical usage to treat the skin ailments.

Figure.1 Prepared Sivappu thylam

Table.1 Information about ingredients of *Sivappu thylam*

Botanical name	Tamil name/ English name	Parts used	Phytochemistry	Action	Medicinal uses in Siddha
Pongamia pinnata Pierre	*Pungu/Indian beech*	Root	Demethoxy-Kanugin Glabrin KanuginKarangin flavonoids Flurophenylalaline Vinblastin incristine (Sulphate)Teniposide Fluoxetine	Astringent Alterative Antiseptic Parasiticide	Skin diseases Inflammations Diabetic
Rubia cordifolia.Linn	*Manjitti /Indian madder*	Root	Purpurin, Alizarin, Mollugin, Manjistin	Emmenagogue	Skin diseases Wound Inflammation Diabetic
Hemidesmus indicus R.Br	*Nannari / Indian Sarasaparilla*	Root	Glucose hemidesmol 2hydroxy-4 -methoxy benzaldehyde glucoside resin acid sterol and tannins	Alternative Tonic Demulcent Diuretic Diaphoretic	Skin diseases Inflammation Syphilis Urinary disorders
Manjal mezhugu	*Cera wax*	Wax	Cerotic Acid Myricin	Demulcent	Skin diseases Wound Inflammations
Vateria indica Linn	*Kungiliam /Sal tree*	Resin	Triterpene Hydrocarbons ketones sesquiterpenes	Stimulant Diuretic	Skin diseases Nervous disorder Arthritis
Dioscorea purpurea / Dioscorea alata	*Chevvallikodi*	Bark	Alkaloids Carbohydrates Flavonoids Glycosides Phenols Saponins Tannins Terpenoids Anthraquinones And Triterpenoids	Anthelmintic	Skin diseases
Cinnamomum verum.Juss	*Surul pattai*	Bark	Camphene Sabinene Myrcene Limonene Terpinolene Eugenol	Stimulant Carminative	Insecticidal Inflammation
Cocos nucifera Linn	*Thengai / Coconut*	Oil	Phenols Tannins Leucoanthocyanidins flavonoids Triterpenes Steroids	Stomachic Diuretic Demulcent	Insecticidal Disinfectant Appetizer

Assessment of In-vitro anti-inflammatory activity
Inhibition of albumin denaturation assay

In-vitro anti-inflammatory activity ST was studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample ST 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,

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

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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's Multiple comparison test (control Vs test) using the Graph Pad Prism.

RESULTS

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

Figure.2 Sample Description

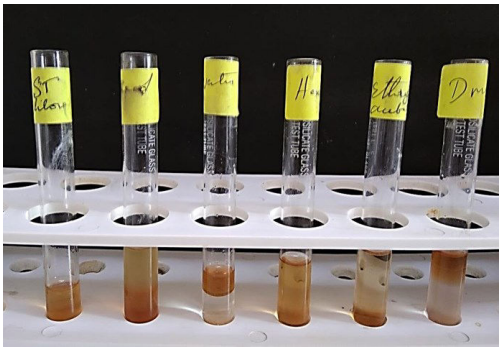


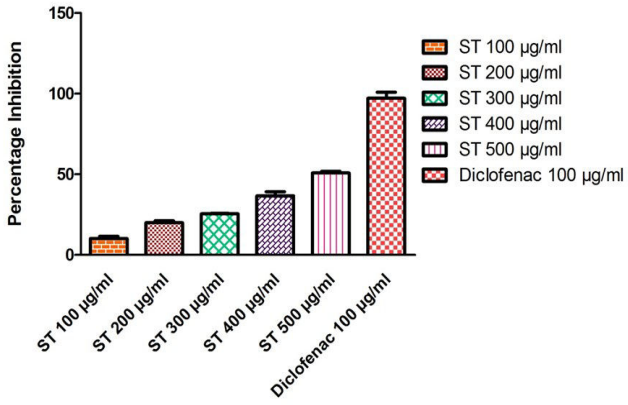
Table.1 Solubility Profile of *Sivappu thailam*

Solvent Used	Solubility / Dis-
Chloroform	Soluble
Ethanol	Insoluble
Water	Insoluble
Ethyl acetate	Soluble
Hexane	Soluble
DMSO	Insoluble

Sample Preparation: Chloroform Extract of the sample ST were been used for the assay

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
ST 100	10.21 ± 1.45
ST 200	20 ± 1.38
ST 300	25.54 ± 0.26
ST 400	36.71 ± 2.47
ST 500	50.91 ± 0.99
Diclofenac sodium (100 µg)	97.19 ± 3.80

Figure.3 Mean Percentage Inhibition of Protein Denaturation study on *Sivappu thailam*



DISCUSSION

The result obtained from the present clearly indicates that the test drug ST was effective in inhibiting heat induced albumin denaturation. In 100 µg/ml concentration the protein denaturation was inhibited around 10.21 ± 1.45 %. In a concentration of 200 µg/ml, the inhibition of denaturation was about to 20± 1.38 %. In 300 µg/ml concentration, the inhibition of denaturation was about 25.54 ± 0.26 %. In 400 µg/ml concentration, the inhibition of denaturation was about 36.71 ± 2.47 %. In 500 µg/ml concentration, the protein denaturation was inhibited by 50.91 ± 0.99 %. Whereas 100 µg/ml concentration of Diclofenac sodium exhibits a maximum of percentage inhibition at it low level. For herbal medications, the five-fold of increase in dosage, exhibits it half of its activity which is safe and effective for long term usage.

Maximum percentage inhibition of about 50.91 ± 0.99 % was observed at 500 µg/ml when compare to that of the Diclofenac sodium a standard anti-inflammatory agent with the maximum inhibition 97.19 ± 3.80 at the concentration of 100 µg/ml.

Diclofenac sodium is also a known NSAIDS, which exerts its action by inhibition of prostaglandin synthesis by inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) pathway[6]. In this way, *Sivappu thailam* also predicted that, it has been acting as an anti-inflammatory agent by inhibiting this COX-1 and COX-2 pathways.

CONCLUSION

From the result of the study it was concluded that the test drug *Sivappu thailam* possess promising anti-inflammatory property in protein denaturation assay through COX-1 and COX-2 inhibitory mechanism. The drug *Sivappu thailam* is safe to use as an external therapeutic agent and could be used for long term use without any ill effects.

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