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IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF SIDDHA FORMULATION PANCHATHIKTHA GHRITHAM BY PROTEIN (ALBUMIN) DENATURATION ASSAY

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Abstract

Background: Siddha medicine is a traditional system of healing that emphasizes the use of natural remedies for the management of various ailments. Rooted in ancient wisdom, it is believed to have been developed by the Siddhars, with particular applications in the prevention and treatment of childhood disorders. Among them Panchathiktha Ghritham which is mentioned in Siddha literature Chikicha rathnadeepam which is used for the management of psoriasis in children. The siddha formulation , composed of 23 ingredients, has been clinically evaluated, and the constituent drugs present in Panchathiktha Ghrithm have demonstrated the ability to regulate inflammatory responses, thereby showing therapeutic potential in the management of Kalanjagapadai (Psoriasis in children).
Method: The drug screened for Anti-inflammatory activity.
Results: The result obtained from the present study clearly indicates that the test drug Panthathiktha ghritham was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 39.24 ± 5.27 % was observed at 500µl, when compared to that Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 90.51 ± 1.70 % at the concentration of 100 µg/ml.
Conclusion: The results of the study revealed that the Siddha formulation Panchathiktha Ghritham complies with the established standards and may be considered for clinical management of Kalanjagapadai. However, further investigations focusing on its anti-inflammatory activity in the context of Kalanjagapadai are warranted. Based on the findings, it can be concluded that the test drug Panchathiktha Ghritham exhibits a promising anti-inflammatory effect, as demonstrated by the protein denaturation assay.

Keyword: Anti-inflammatory, Panchathiktha ghritham, Kalanjagapadai

Introduction:

The children of today represent the future citizens of a nation, and their health plays a pivotal role in shaping a progressive society. The Siddha system of medicine provides a comprehensive and scientific perspective on child health, encompassing aspects such as growth and development across various stages of life, the potential health challenges encountered during childhood along with their management, preventive measures against such conditions, and guidance on adopting an appropriate way of living[1]. Most of the drugs in the *Panchathiktha Ghritham* possess the anti-inflammatory, immunomodulator, antioxidant and antimicrobial properties. It is effective in the treatment of *kalanjagapadai* (Psoriasis in children).

Psoriasis is a chronic, inflammatory, immune-mediated papulosquamous disorder affecting all age groups. Psoriasis in children significantly affects the quality of life of the child and family members. Childhood psoriasis is a well-recognized entity, yet its true prevalence is not known. The worldwide prevalence of childhood psoriasis ranges from 0.1% to 1.37%. In another study from South India, childhood psoriasis comprised 0.6% of total outpatient cases and 17.8% of total psoriatic patients. Various epidemiological studies of childhood psoriasis in India have shown the prevalence of psoriasis ranging between 0.5% and 2%. Indian studies have shown most children developed psoriasis at 6–14 years of age and girls outnumbered the boys[2].

Materials and methods:

The test drug *Panchathiktha Ghritham* has been purchased from GMP Certified Pharmacy, Chennai, Tamilnadu. It is a poly herbo mineral Siddha preparation prepared from twenty-eight ingredients.

Table 1: Ingredients of *Panchathiktha Ghritham*

Sl.No	Common Name	Botanical Name
1.	*Veppampattai*	*Azadirachta indica*
2.	*Seenthil kodi*	*Tinospora cordifolia*
3.	*Adathodai samoolam*	*Adhatoda vasica*
4.	*Peypudal*	*Trichosanthes cucumerina*
5.	*Kandankathiri*	*Solanum surrattense*
6.	*Sittrarathai*	*Alpinia officinarum*
7.	*Vaividangam*	*Embelia ribes*
8.	*Devadaru*	*Cedrus deodara*
9.	*Yanaithippili*	*Scindapsus officinalis*
10.	*Chukku*	*Zingiber officinale*
11.	*Maramanjal*	*Coscinium fenestratum*
12.	*Athimathuram*	*Glycyrrhiza glabra*
13.	*Chevviyam*	*Black pepper root*
14.	*Koshtan*	*Costus speciosus*
15.	*Milagu*	*Piper nigrum*
16.	*Vetpaalarisi*	*Wrightia tinctoria*
17.	*Ommam*	*Carum copticum*
18.	*Chithiramoola verpattai*	*Plumbago zeylanica*
19.	*Kadugurogani*	*Picrorhiza scrophulariflora*
20.	*Tamarai kizhangu*	*Nelumbo nucifera*
21.	*Manjitti*	*Rubia cordifolia*
22.	*Vasambu*	*Acorus calamus*
23.	*Modi*	*Piper longum*
24.	*Athividayam*	*Aconitum heterophyllum*
25.	*Sivathai ver*	*Operculina turpetham*
26.	*Kurosani omam*	*Hyoscyamus niger*
27.	*Kukki*	*Shorea robusta*
28.	*Evacharam*	*Potassium carbonate*

Albumin Denaturation method: Albumin Denaturation assay procedure

In-vitro anti-inflammatory activity PTG as studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample (PTG) in ethanol (100 mg/mL stock) at varying concentration ranges from 100 to 500 µl and standard Diclofenac sodium at the concentration of100 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

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

Discussion:

The present study evaluated the in vitro anti-inflammatory activity of the Siddha formulation *Panchathiktha Ghritham* (PTG) using the protein (albumin) denaturation assay. Protein denaturation has been well documented as one of the causes of inflammation, and agents that inhibit this process are considered to possess anti-inflammatory potential.

In this study, PTG exhibited dose-dependent inhibition of albumin denaturation, with a maximum inhibition of 39.24 ± 5.27% at 500 µl. Although the inhibition was lower when compared with the standard anti-inflammatory drug Diclofenac sodium (90.51 ± 1.70% at 100 µg/ml), the results clearly indicate that PTG possesses measurable and convincing anti-inflammatory properties. The gradual increase in activity with increasing concentration further confirms the potential of PTG to modulate inflammatory processes.The relatively lower activity of PTG compared to Diclofenac may be attributed to the fact that PTG is a complex polyherbal formulation, containing multiple constituents with diverse pharmacological actions, whereas Diclofenac is a pure synthetic compound with established potency. Nevertheless, the observation that PTG could inhibit nearly 40% of protein denaturation at higher concentrations suggests that it has a significant anti-inflammatory effect that may contribute to its traditional use in conditions like *Kalanjagapadai* (Psoriasis in children).

These findings support the traditional claims of Siddha medicine and highlight the potential of PTG as a natural anti-inflammatory agent. However, since this is an in vitro study, further research involving in vivo models, phytochemical analysis, and clinical evaluation will be essential to validate its therapeutic applicability and safety profile.

Statistical analysis:

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

Results Analysis:

The results clearly indicate that the test drug Panchathikatha Ghritham (PTG) was effective in inhibiting heat-induced albumin denaturation in a dose-dependent manner. The maximum percentage inhibition observed was 39.24 ± 5.27% at 500 µl, compared to 90.51 ± 1.70% for the standard anti-inflammatory drug Diclofenac sodium at 100 µg/ml. Although the inhibitory effect of PTG was lower than that of Diclofenac, the progressive increase in activity with increasing concentrations confirms its potential anti-inflammatory property.

Table 2: Percentage inhibition

Concentration in µl	Percentage Inhibition of Protein Denaturation
PTG 100	4.945 ± 4.59
PTG 200	9.736 ± 5.23
PTG 300	18.05 ± 1.45
PTG 400	26.73 ± 1.68
PTG 500	39.24 ± 5.27
Diclofenac sodium (100 µg)	90.51 ± 1.70

Each value represents the mean ± SD. N=3

Percentage Inhibition of Protein Denaturation by PTG and Standard

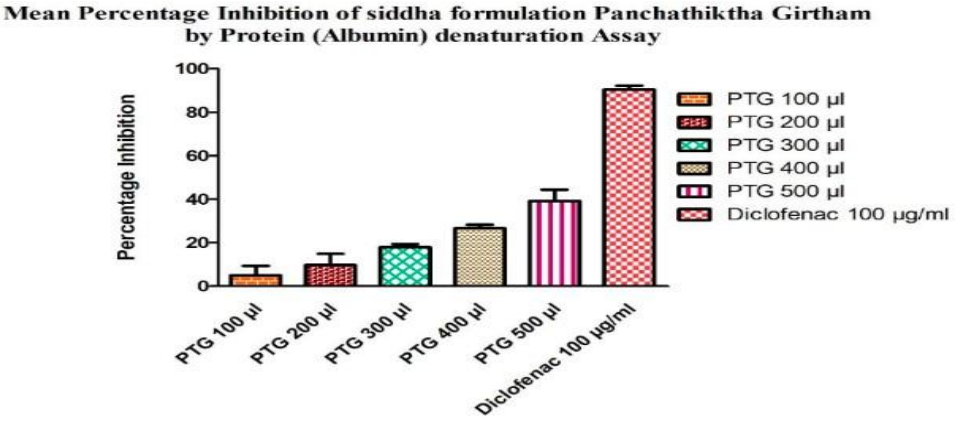

Figure 1: Protein Denaturation by *Panchathiktha Ghritham* and Standard

Conclusion:

From this study, it can be concluded that Panchathikatha Ghritham possesses significant anti-inflammatory activity, as demonstrated by its ability to inhibit protein denaturation in vitro. While not as potent as Diclofenac sodium, the formulation showed convincing dose-dependent activity, thereby supporting its traditional therapeutic claim as an anti-inflammatory agent.

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