



IN-VITRO EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF NOVEL SIDDHA FORMULATION PAVAZHA PARPAM BY ALBUMIN PROTEIN DENATURATION ASSAY

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ABSTRACT

Inflammation is biological response of the living being towards specific insults. Inflammation is characterized by pain, redness, swelling and dysfunction of the tissues and organs, is the normal result of host protective responses to tissue injury caused by numerous stimuli. Inflammation leads to the upregulation of a series of enzymes and signaling proteins in affected cells and tissues. The commonly used drugs for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to the formation of gastric ulcers. Herbs have been used in traditional medicine since many years and the use of herbal-derived natural products as a

therapeutic tool has been increasing considerably. Herbs become the integral part of the siddha system of traditional medicine since it has unique feature of counteracting the oxidative stress there by preventing the generation of free radicals. It was evident through several research outcome that the oxidative stress worsen the condition of the inflammation by promoting synthesis and release of cytokines. Pavazha parpam (PP) is a novel siddha formulation as indicated for the treatment of bronchitis, tuberculosis, diarrhea etc as per the

literature. But still now there is no documentary evidence on briefing the inflammatory potential of PP. Hence this study is the first of its kind attempting for in-vitro anti-inflammatory evaluation of the formulation PP using protein denaturation assay. The result obtained from the present study has clearly indicates that the test drug PP was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 38.41 % was observed at 500 µg/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 95.98 % at the concentration of 100 µg/ml.

KEYWORDS: Inflammation, Herbal-derived, Siddha, Pavazha parpam, Anti-inflammatory, Diclofenac sodium.

1. INTRODUCTION

Inflammation constitutes body's response to injury and is characterized by a series of events that mainly occur in three distinct phases. The first phase is caused by an increase in vascular permeability resulting in exudation of fluids from the blood into the interstitial space; the second phase involves the infiltration of leukocytes from the blood into the tissue and third phase is characterized by granuloma formation and tissue repair.^[1] Inflammation is a defense response of our body to hazardous stimuli such as allergens and/or injury to the tissues; on the other hand, uncontrolled inflammatory response is the main cause of a vast continuum of disorders including allergies, cardiovascular dysfunctions, metabolic syndrome, cancer, and autoimmune diseases imposing a huge economic burden on individuals and consequently on the society.^[2]

Inflammation is commonly associated with pain as a secondary process, resulting from the secretion of analgesic mediators.^[3] To protect against outer stimuli or tissue injury, various pro-inflammatory mediators, including tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6) and nitric oxide (NO), are released by the host cellular immune response system.^[4] However, the excessive release of pro-inflammatory mediators may activate the inflammatory cascade reaction, leading to systemic inflammatory response syndrome (SIRS).^[5] In addition, prostaglandin E2 (PGE2), a major pain enhancing inflammatory mediator, can be induced by cyclooxygenase 2 (COX-2) in the process of inflammation. Previous investigations have demonstrated that it is beneficial for treating inflammatory diseases to down-regulate the expression of TNF- α , IL-6 and COX-2.^[6-8]

Complementary and alternative medicine for the treatment of various diseases is gaining popularity globally, at a faster pace since the past two decades^[9,10] and the studies revealed a worldwide market for herbal supplements for the management of inflammatory dysfunction/diseases, which is presently at around 83% and is expected to reach 95% in the forthcoming years.^[11,12] Several herbal-derived natural compounds significantly affect cellular mechanisms and evidence of the benefic effects of herbal-derived natural products in inflammatory diseases has been increasing.^[13] The main aim of the present study is to evaluate the anti-inflammatory potential of the siddha formulation pavazha parpam in albumin protein denaturation assay in order to access the efficacy and to provide the documentary evidence on the folklore claim of this novel formulation.

2. MATERIALS AND METHODS

2.1. Source of raw drugs: The Required raw materials were procured from a well reputed indigenous drug shop from Chennai, Tamil Nadu, India. Herbal drugs were authenticated by the Botanist, Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India.

2.2. Ingredients

1. Purified red coral rocks (Pavazha puttru) – Q.S.
2. Adhatoda (Adhatoda zeylanica Medik) leaf juice – Q.S.

2.3. Formulation of Pavazha Parpam

Purified red coral rocks were been broken in to small pieces and it was placed in mud pot with the addition of sufficient Adhatoda leaf juice followed by sealing and calcine. Then the resultant product was ground well with addition of Adhatoda leaf juice with necessary process of calcine twice or thrice until to obtain fine dark coloured ash calx.

2.4. Indication: Indicated for dyspnoea, tuberculosis, diarrhoea, cough, bronchitis, haemoptysis.

2.5. Dosage: 100 to 200 mg twice daily with water, milk, butter, ghee or butter milk.

3. RESULTS

3.1. Effect of Pavazha parpam on percentage inhibition of protein denaturation

Result analysis of the albumin protein denaturation assay has clearly shown that the mean inhibition of protein denaturation of the test drug PP was found to be 38.41, 32.51,

28.23, 21.52 and 9.94% observed for doses of 500, 400, 300, 200 and 100 $\mu\text{g}/\text{mL}$ respectively, whereas, for the standard anti-inflammatory drug diclofenac sodium it was found to be 95.98% at the concentration of 100 $\mu\text{g}/\text{mL}$. As shown in Table 1 and Figure 1.

Table. 1: Mean percentage inhibition of protein denaturation by Pavazha parpam.

Concentration in $\mu\text{g}/\text{ml}$	Percentage Inhibition of Protein Denaturation
PP 100	9.94 \pm 5.86
PP 200	21.52 \pm 6.57
PP 300	28.23 \pm 6.54
PP 400	32.51 \pm 7.52
PP 500	38.41 \pm 6.28
Diclofenac (100 μg)	95.98 \pm 4.54

Each value represents the mean \pm SD. N=3

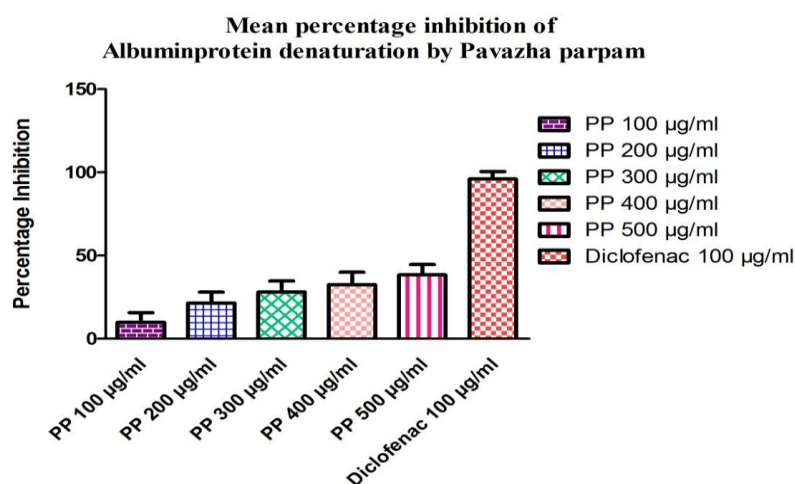


Figure. 1: Graphical representation of percentage protein denaturation by Pavazha parpam.

4. DISCUSSION

Inflammation is a part of the immune response that can prevent infection through production of pro-inflammatory cytokines and generation of inflammatory mediators in response to microbial products.^[14] Although inflammation is crucial to maintaining the health and integrity of an organism, when the inflammatory process is poorly controlled, it can cause massive tissue destruction and a series of chain reactions.^[15-17] The current treatment of inflammatory disorders involves extensive use of nonsteroidal anti-inflammatory drugs and corticosteroids. Although use of modern drugs for inflammation has a relieving effect, it is still unsatisfactory.^[18]

Inflammation is a natural response that promotes the survival of a host in the presence of a variety of internal and external insults.^[19] However, abnormal regulation of inflammatory processes can result in the destruction of cells, or may disturb cellular metabolism and thus contribute to the development of chronic diseases. Macrophages play a central role in the innate immune response and chronic inflammation processes by secreting pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor- α (TNF- α).^[20] Macrophages activate inflammation-related genes that are regulated via nuclear factor- κ B (NF- κ B) signaling.^[21] Activated NF- κ B translocates to the nucleus, where it induces the expression of pro-inflammatory mediators and cytokines, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), TNF- α , IL-1 β , and IL-6.^[22] Expression of these proteins elicits further immune responses.

Nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, and immunosuppressant drugs that have been used conventionally against all forms of inflammatory conditions^[23] are associated with adverse effects like ulceration, perforation, gastric irritation, haematochezia^[24], angioedema, hepatic failure, headache, hemolytic anemia, hyperglycemia, osteoporosis, immunodeficiency-related problems, and others.^[25] Considering these potential adverse effects of these drugs and their limited ability to provide long-term remission, complementary and alternative medicinal products that are generally considered safe are continuously being explored for their anti-inflammatory potential.^[26] Presently interest in herbal medicine as a path to drug development increased greatly in the early 1980s.^[27] This could be due to the inefficiency of conventional medicine (e.g. cytotoxicity, side effects and ineffectiveness of synthetic drugs), abusive and incorrect use of synthetic drugs and most importantly, the high cost involved in conventional medicine and the fact that a large percentage of the world's population does not have access to conventional pharmacological treatment. Result analysis of the albumin protein denaturation assay has clearly shown that the mean inhibition of protein denaturation of the test drug PP was found to be 38.41, 32.51, 28.23, 21.52 and 9.94% observed for doses of 500, 400, 300, 200 and 100 μ g/mL respectively, whereas, for the standard anti-inflammatory drug diclofenac sodium it was found to be 95.98% at the concentration of 100 μ g/mL.

5. CONCLUSION

Medicinal plants and their secondary metabolites are progressively used in the treatment of diseases as a complementary medicine. Inflammation is a pathologic condition that includes a

wide range of diseases such as rheumatic and immune-mediated conditions, diabetes, cardiovascular accident, and etcetera. Oxidative stress has been implicated in many chronic diseases, and its pathological processes are closely related to those of inflammation, in that one can be easily induced by another. In fact, it is known that inflammatory cells liberate a number of reactive species at the site of inflammation leading to oxidative stress, which demonstrates the relationship between oxidative stress and inflammation. From the results of the present investigation it was concluded that the anti-inflammatory potential of the siddha formulation Pavazha parpam may be due to its strong profile of phytochemicals present in it further in-vivo studies has to be carried out to extrapolate the in-vitro data's in near future.

6. REFERENCES

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