Evaluation of *In-vitro* Anti-Inflammatory Activity of Sri Lankan Medicinal Pill of Aloe vera, *Centella asiatica,* and *Strychnos potatorum* exacts using Egg Albumin Denaturation Assay

Abstract

Aims: The study aims to evaluate the albumin heat denaturalization inhibition activity of a Sri Lankan Traditional Medicine pill (STMP) as an indirect measure of anti-inflammation

Methodology: The extracts of Aloe vera, *Centella asiatica* (Asiatic pennywort), and *Strychnos potatorum* (Cleaning nut) are the ingredients of the pill. Concentration gradients from 1000 µg/ml to 0.02 µg/ml were prepared using egg albumin and phosphate buffer saline. The pill was incubated in controlled experimental settings at various concentrations, and the absorbance was measured to quantify the level of albumin denaturation. One NSAID (Ibuprofen) and one steroid (Prednisolone) were used as the reference drugs. The percentage inhibition of protein denaturation at each concentration was calculated by using the formula of (Vt / Vc - 1) x 100% where Vt = absorbance of the test sample and Vc = absorbance of control.

Result: The maximum inhibition rate of egg albumin denaturation (46.7%) was seen in the 200 µg/ml concentration, while Prednisolone and Ibuprofen had 2.5% and 24.6% inhibition rates in the same concentration, respectively. Less than 35% inhibition rate of egg albumin denaturation showed in all other concentrations lower than 200 µg/ml (0.1 µg/ml – 100 µg/ml) of this STMP. The inhibition rates of these concentrations did not differ noticeably from those of the reference medications. In addition, the STMP had a 28.3% inhibited rate of egg albumin denaturation in 1000 µg/ml concentration while Prednisolone and Ibuprofen showed low inhibition rates compared to the STMP (3.4% and 13.8% respectively) in this concentration.

Conclusion: This study discovered that 200 µg/ml of the tested Sri Lankan Traditional Medicine pill had the strongest anti-inflammatory efficacy against protein denaturation. Furthermore, the effect against albumin denaturation in high-concentration Sri Lankan STMP (200 µg/ml and 1000 µg/ml) surpassed that of the reference drugs, signifying a more pronounced impact.

Keywords: Anti-inflammatory activity, Aloe vera, *Centella asiatica, Strychnos potatorum,* Albumin denaturation method

Introduction

Inflammation presents itself as a condition where the affected body part becomes visibly reddened, swollen, tender, or pruritic. This response is often a pathophysiological mechanism orchestrated by the body's defense systems, aimed at safeguarding against potential threats such as infections, burns, toxic substances, allergens, or other harmful stimuli (1). Persistent or unregulated inflammation can contribute to the emergence of serious medical conditions. The mediators involved in the inflammatory process can exacerbate or sustain the related disorders, potentially triggering and perpetuating pain responses. In the treatment of inflammatory conditions, non-steroidal anti-inflammatory Drugs (NSAIDs) are commonly

employed (2). These medications offer anti-inflammatory, analgesic, and antipyretic properties, and they also possess the capability to inhibit thrombocyte aggregation (3). However, the use of NSAIDs is accompanied by a range of adverse effects including stomach ulcers, epigastric discomfort, duodenal perforation, and gastrointestinal bleeding (4).

Within the realm of Ayurvedic Medicine, many natural plant compounds have been harnessed for centuries to intervene in inflammatory pathways with minimal side effects (5). Despite this, limited research has been directed towards evaluating the anti-inflammatory attributes of Ayurvedic medicines. The botanical landscape harbors numerous plants with medicinal potential. Among them, Aloe vera, *Centella asiatica, and Strychnos potatorum* were selected for their recognized anti-inflammatory properties.

Aloe vera, belonging to the succulent family and characterized by its stemless and drought-resistant nature, has been utilized for medicinal purposes for over five millennia across Egyptian, Indian, and Chinese cultures. Aloe vera gel, extracted from the leaf pulp of Aloe barbadensis Miller, contains more than 70 biologically active compounds and is acclaimed for its anti-inflammatory, antioxidant, immune-enhancing, anti-cancerous, wound-healing, anti-aging, and anti-diabetic properties (6).

C.asiatica, commonly referred to as Asiatic pennywort, Indian pennywort, Spadeleaf, or Gotukola, pertains to the Umbelliferae/Apiaceae family (7). Employed extensively in traditional Ayurvedic medicine across India and Asia, this plant's aerial parts and roots have demonstrated efficacy in addressing various ailments. Its chemical constituents have bestowed it with therapeutic applications encompassing antimicrobial, anti-inflammatory, anti-cancer, neuroprotective, antioxidant, and wound-healing attributes. *C. asiatica* extracts exhibited inhibition of hypotonicity-induced human red blood cell membrane breakdown and membrane stabilization was observed at different concentrations, comparable to Diclofenac Sodium (8). Furthermore, its anti-inflammatory potential was established in dose-dependent responses against prostaglandin E2-induced inflammation (9).

S. potatorum, another medicinal plant, demonstrated normalization of increased levels of alkaline and acid phosphatases and lipid peroxides, indicating its membrane-stabilizing and free radical scavenging properties. The plant exhibited dose-dependent anti-inflammatory effects in both acute and subacute inflammatory models, comparable to the standard drug diclofenac sodium (10).

The primary objective of this study was to evaluate the anti-inflammatory properties of STMP through the utilization of the egg albumin denaturation inhibition method as an indirect measure. Additionally, the study aimed to conduct a comparative analysis of STMP's anti-inflammatory potential in relation to established pharmaceutical agents, Ibuprofen and Prednisolone.

Materials and methods

Preparation of plant material extracts

Following the acquisition process (11), freshly cultivated Aloe vera and *C. asiatica* leaves, cultivated without the use of pesticides and insecticides, underwent a cleaning procedure using flowing water. The external layers of the Aloe vera leaves were removed, and the internal gel portion was gently mashed into a paste, with 20.0g of this paste being weighed for further analysis. *C. asiatica* leaves were subjected to grinding without addition of water, and the resulting material was filtered using a clean cloth. Subsequently, 20.0 ml of the extract was quantified using a graduated cylinder. Pure and purified *S. potatorum* seeds were gathered, and ground to a fine consistency, and a quantity of 20.0 g of the resultant powder was measured. These three measured samples were then combined and processed into diminutive pellets, as illustrated in Figure 1. The pellets underwent a gradual drying process under partial sunlight conditions.

Figure 1- The prepared smaller pills were dried in the sunlight

Preparation of drug solutions

Ibuprofen and prednisolone were procured from the Osusala, University Hospital of Sir John Kotelawala Defense University, Sri Lanka. All the other chemicals were standardized before actual work.

Preparation of reference drugs and traditional pill stock solution

The experimental procedures involved in this study were conducted with careful consideration for cleanliness, ensuring that soap solution was not introduced to the process. The prepared STMP was finely ground into powder form, and 0.2g of this powder was accurately weighed. This powder was then combined with 20.0 ml of distilled water to create Solution S1. Additionally, tablets of Ibuprofen (S2) and prednisolone (S3) were crushed and 0.2g of their respective powders were weighed. These powders were mixed with 20.0 ml of distilled water to produce separate solutions. All three solutions (S1, S2, and S3) were subjected to a series of five different concentration stages for dissolution.

In this context, each concentration stage was prepared by adding 4.5 ml of distilled water to separate test tubes. Subsequently, 0.5 ml of Solution S1 was introduced into the first test tube, resulting in a solution with a concentration of 1000 µg/ml (referred to as T1). A portion of T1 (0.5 ml) was then transferred to the second test tube, generating a solution with a concentration of 100 µg/ml (referred to as T2). This process was repeated for a total of five concentration levels: 1000 µg/ml, 100 µg/ml, 10 µg/ml, 1 µg/ml, and 0.1 µg/ml. These solutions were prepared using the STMP and collectively designated as $R1_{SLMP}$.

Likewise, a similar sequence of concentration stages was executed using solutions derived from Ibuprofen (S2) and prednisolone (S3) tablets. These reference drug solutions were prepared independently and labeled as $R1_{Ibuprofen}$ and $R1_{Prednisolone}$, respectively.

Furthermore, an additional set of concentration stages was established for the STMP, Ibuprofen, and prednisolone, encompassing concentrations of 200 µg/ml, 20 µg/ml, 2 µg/ml, 0.2 µg/ml, and 0.02 µg/ml. These solutions were designated as $R2_{STMP}$, $R2_{Ibuprofen}$, and $R2_{Prednisolone}$, respectively (12,13,14).

Collection of Egg albumin

A fresh hen's egg was used and the inner white part of the egg was separated into a beaker without disturbing the yellow part. The collected whitish part of the inner egg is the egg albumin.

Phosphate buffer saline (pH 6.4)

Phosphate Buffer Saline (PBS) PH 6.4: Dissolved 8 g of Sodium Chloride (NaCl), 0.2 g of Potassium Chloride (KCl), 1.44 g of Disodium Hydrogen Phosphate (Na_2HPO_4), 0.24 g of Potassium Dihydrogen Phosphate (KH_2PO_4) in 800 ml distilled water. The pH was adjusted to 6.4 using 1N HCl and added the volume to 1000 ml with distilled water (15).

Evaluation of in-vitro anti-inflammatory activity

All the reactions were carried out in clean test tubes without the interruption of soap solution. The test sample (5 ml) consisted of 0.2 ml of egg albumin (from a fresh hen's egg), 2.8 ml of PBS, and 0.2 ml-2 ml of varying concentrations of the series. The test samples were prepared for each concentration of the R1 and R2 series of STMP, ibuprofen, and prednisolone and assessed the anti-inflammatory function of the STMP. The solution consists of 2.8 ml of PBS, 0.20 ml of egg albumin, and 2.0 ml of distilled water served as the control. (Considering the R1 series 15 test samples were prepared; 5 samples for each drug, for R2 also 15 samples were prepared and finally the control sample, collectively 31 samples were prepared.) (10,12).

Procedure

The prepared 31 samples were incubated at (37±2) ^{C} in a water bath for 15 minutes and then at 70^{o}C for 5 minutes. After cooling, their absorbance was measured at 680 nm Calorimetry (WPA) by using distilled water as the control. Ibuprofen and prednisolone at the final concentrations (set one and two) were used as reference drugs and treated similarly to determine the level of absorbance.

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100\times (Vt / Vc - 1)$$

Where, Vt = absorbance of the test sample, Vc = absorbance of control(Chandra *et al*., 2012)

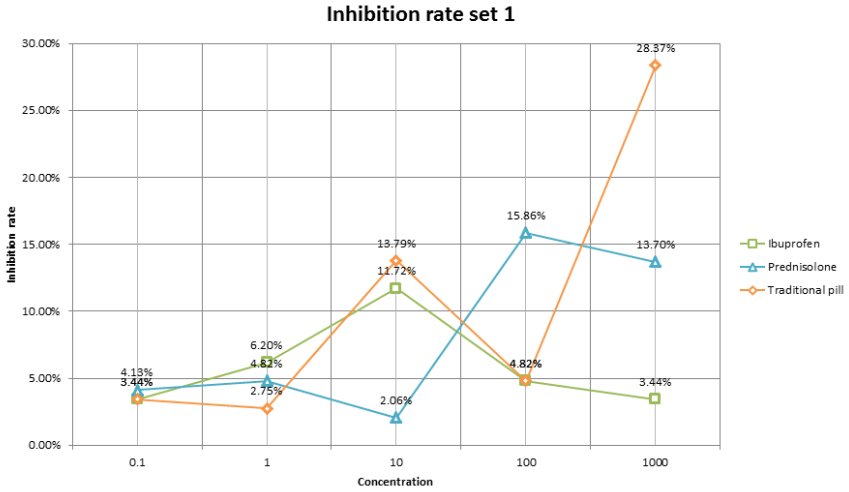
Result

The effectiveness of this specific STMP in reducing inflammation was assessed using the method of denaturation of egg albumin. The highest level of inhibition, amounting to 46.7%, was observed at a concentration of 200 µg/ml. In comparison, when the same concentration was considered, Prednisolone exhibited a much lower inhibition rate of 2.5%, while Ibuprofen showed an inhibition rate of 24.6%.

Across concentrations lower than 200 µg/ml (ranging from 0.1 µg/ml to 100 µg/ml), the referenced STMP demonstrated inhibition rates of egg albumin denaturation consistently below 35%. This pattern held across the various concentrations, aligning with the performance of the reference drugs.

Furthermore, at a concentration of 1000 µg/ml, the STMP displayed an inhibition rate of 28.3% in the egg albumin denaturation process. In contrast, Prednisolone and Ibuprofen demonstrated notably lower inhibition rates at the same concentration, standing at 3.4% and 13.8%, respectively. This underscores the comparatively higher efficacy of the STMP in this specific assessment.

Figure 2: Inhibition rate of Ibuprofen, Prednisolone & STMP set 1 (I) and set 2 (II)



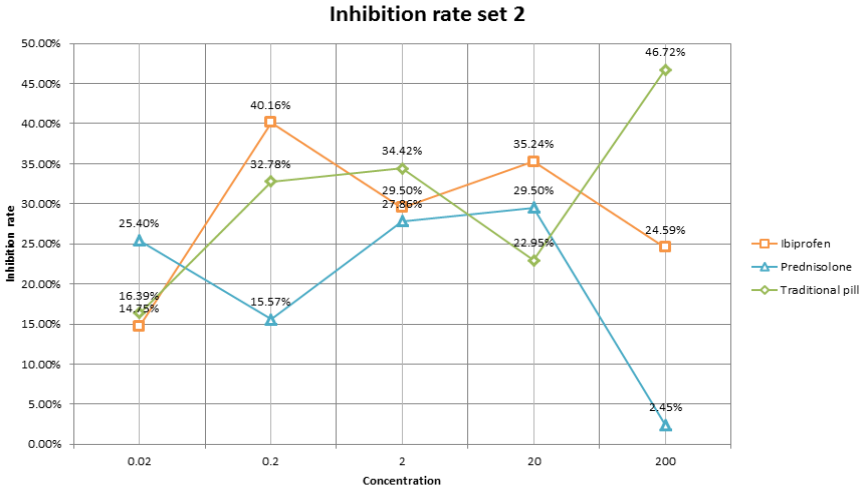
2(I)

2(II).

Discussion

Throughout history and persisting into the contemporary era, medicinal plants have occupied a fundamental role as primary sources of remedies for an extensive spectrum of human ailments, as well as health concerns affecting animals. These botanical entities serve as reservoirs of therapeutic attributes, serving as indispensable foundational elements in both traditional and modern medical practices. Despite their sustained application for remedial purposes in Sri Lanka, the advantageous attributes inherent in these plants have yet to be subjected to comprehensive scientific investigation or formal documentation (16).

The present study directs its attention to a triad of plant species, namely the leaves of Aloe



vera and *C. asiatica*, along with the seeds of *S. potatorum.* These selections were made based on their readily accessible presence within the geographical confines of Sri Lanka. Earlier literature has underscored the noteworthy medicinal characteristics associated with these chosen plants. Notably, Aloe vera is recognized for its encompassing attributes including anti-inflammatory, antioxidant, antimicrobial, anti-helminthic, antifungal, aphrodisiac, antiseptic, and cosmetic properties (17). Additionally, *C. asiatica*, apart from its anti-inflammatory attributes, has exhibited a diverse spectrum of medicinal potentials, encompassing anti-cancer, anti-fungal, anti-bacterial, antioxidant, anti-depressant, wound-healing, cognitive-enhancing, neuroprotective, anti-diabetic, and hepatoprotective effects (18). *S. potatorum* has demonstrated intriguing properties, including anti-diabetic activity, antiulcerogenic potential, hepatoprotective and antioxidant activity, antiarthritic activity, antinociceptive and antipyretic effects, antidiarrheal activity, diuretic activity, contraceptive efficacy (10).

In light of this context, the present study is primarily concerned with evaluating the combined in-vitro anti-inflammatory activity of Aloe vera, *C. asiatica*, and *S. potatorum* extracts. This evaluation is conducted using the Egg Albumin Denaturation Assay. Denaturation, a complex phenomenon, involves alterations in electrostatic hydrogen, hydrophobic, and disulfide bonding of proteins. Upon exposure to heat, proteins generally undergo a loss of their biological properties and functional attributes.

The resulting graphs (Figure I and Figure II) derived from the study exhibit patterns that are neither linear nor curved. Both the extracts of the selected medicinal plants and the reference drugs (Ibuprofen and Prednisolone) demonstrate analogous trends; an increase in concentration corresponds to an increase in absorbance levels.

As depicted in Figure 02, the STMP extracts at higher concentrations exhibit substantial inhibition of egg albumin denaturation. This observation signifies that the STMP possesses appreciable anti-inflammatory properties in comparison to the reference drug control. Among the two reference drugs, Ibuprofen showcases more pronounced anti-inflammatory action in contrast to Prednisolone (which belongs to the category of steroidal drugs).

Traditional medicine is distinguished by its propensity for minimal or negligible side effects, often rendering it more accessible to a broader populace. The constituent herbs employed in the formulation of this particular Traditional Medicinal pill can be sourced from local village gardens and can be prepared with the guidance of experienced practitioners. These three botanical entities have long served as dietary components in the Sri Lankan context. It is widely acknowledged that traditional herbal remedies are universally suitable across various age groups.

Summary

Inflammation serves as the innate immune response to various threats but can lead to pathological conditions if unregulated. NSAIDs are conventionally employed for inflammation management, but utilizing them is associated with adverse effects. Herbal remedies, such as Aloe vera, *C. asiatica,* and *S. potatorum,* present as potential alternatives with fewer side effects.

This study sought to assess the anti-inflammatory efficacy of STMP consisting of Aloe vera, *C. asiatica*, and *S. potatorum* extracts, utilizing the egg albumin denaturation method as an evaluation criterion. The STMP exhibited noteworthy anti-inflammatory properties, particularly at a concentration of 200 µg/ml, surpassing the anti-inflammatory effects of Ibuprofen and Prednisolone. These findings suggest that the STMP holds promise as a readily accessible herbal intervention for managing inflammation. Further research is warranted to validate these results rigorously.

Conclusion

The results obtained from the current investigation demonstrated a correlation between concentration and the inhibition of egg albumin denaturation by the STMP. Notably, the study revealed that the STMP exhibited a dose-dependent response, with the most substantial anti-inflammatory activity against protein denaturation observed at a concentration of 200 µg/ml. Moreover, it was noteworthy that the anti-denaturation effect exhibited by high-concentration STMP (200 µg/ml and 1000 µg/ml) surpassed that of the reference drugs, signifying a more pronounced impact.

In addition to that the egg albumin method provides a cheap alternative way of testing the anti-inflammatory activity of herbal medicine using the denaturation technique and this method should be validated by conducting further studies.

Finally, it is found that the tested STMP has been a very effective and useful one. This can be concluded with a good message that the STMP has a proven anti-inflammatory effect against the egg albumin denaturation method.

Competing Interests

Authors have declared that no competing interests exist.

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