Testing the Anti-inflammatory Activity of Sri Lankan traditional medicine pill using albumin denaturation method

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Abstract- Inflammation is a useful protective mechanism in response to infections and injuries. It attempts to remove the initial causes of tissue damages by inactivating and destroying them. Non-Steroidal Anti Inflammatory Drugs (NSAIDs) and steroids were commonly used to reduce pain and inflammation when it is at an undesirable level. However, few numbers of studies have been conducted to evaluate those anti-inflammatory activities. The objective of this study was to evaluate the albumin heat denaturalization inhibition activity of a Sri Lankan Traditional Medicine pill as an indirect measure of anti-inflammation.

Aloe vera, *Centella asiatica* (Asiatic pennywort) and *Strychnos potatorum* (Clearing nut) are the ingredients of the pill. Concentration gradient from 1000 µg/ml to 0.02 µg/ml were prepared using egg albumin and phosphate buffer saline. The pill at different concentrations was incubated in controlled experimental conditions and the absorbance was measured to assess 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 test sample and Vc = absorbance of control.

The highest inhibition rate of egg albumin denaturation (46.7%) showed in 200 µg/ml concentration while Prednisolone and Ibuprofen had 2.5% and 24.6% inhibition rate respectively in the same concentration. 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 traditional pill. However, the inhibition rates of these concentrations were not significantly difference with reference drugs. In addition, Traditional pill had 28.3% of inhibited rate of egg albumin denaturation in 1000 µg/ml concentration while Prednisolone and Ibuprofen showed low inhibition rates compared to traditional pill (3.4% and 13.8% respectively) in this concentration.

Present study identified that 200 µg/ml of the tested Sri Lankan Traditional Medicine pill showed the highest anti-inflammatory activity against the denaturation of protein. Furthermore, the effect against albumin denaturation in high concentration Sri Lankan Traditional Medicine pill (200 µg/ml and 1000 µg/ml) was significantly larger than reference drugs.

Keywords: Anti-inflammatory activity, Traditional medicine pill, Albumin denaturation method

I. INTRODUCTION

Inflammation is a condition in which the affected part of the body is generally red, swollen and sores or itchy. It might be pathophysiological responses of the defense mechanisms. However, it makes enable the body to protect against infection, burn, toxic chemicals allergens or any other harmful stimuli (Rahman *et al.,* 2015). A persistent inflammation or uncontrolled inflammation could result in many severe illnesses and the mediatory involved in the inflammation can maintain or worsen many diseases. In addition, it can initiate and continue the pain reaction. Non-steroidal Anti-inflammatory drugs (NSAID) are presently used for the treatment of inflammatory conditions (Kanagasanthosh *et al.,* 2015). But it gives many other problems to the patient such as stomach ulceration, epigastric discomfort, duodenal perforation and gastro intestinal bleeding in general (Marliyah & Ananthi, 2015; Tripathi, 2008) Acute and chronic inflammatory diseases are still one of the most significant health problems in the world. Although several agent known to treat inflammatory disorders, their prolonged use often leads to gastric intolerance, bone marrow depression, water and salt

retention. Due to this reason there is a need to find and develop new anti-inflammatory drugs with low side effects (Scott, 2004).

Protein 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.

The traditional medicine occupies an essential place in the field of the treatment of various types of diseases. Usage of herbal medicine and natural products has increased in recent years due to low-cost and has a lesser amount of side effects.

People from all countries the historical period they have used herbs to treat the patients. The herb is a plant or a part of a plant, part valued for its medicinal, aromatic or savory qualities. These plants have medicinal value with active chemical substances that acts on the body when treated with it (Matthew *et al.,* 2013).

The native physicians have understand about the values and herbal extracts were Collected and medicines were; prepared from them, similarly poly herbal extract has been made from various plants. *Aloe vera, centella ascatica and Strychons potatorum* seed are used to prepare poly herbal extract to purpose medicines for various types of illness.

In Aurvedic Medicine, many of natural plant compounds used to inhibit inflammatory pathways for centuries with low side effects (Maroon *et al.,* 2010). However, few numbers of studies have been conducted to evaluate those anti-inflammatory activities. The objective of this study was to evaluate the anti-inflammatory activity of a Sri Lankan Traditional Medicine (SLTM) pill using egg albumin denaturation inhibition as an indirect measure of anti-inflammation.

II. METHODOLOGY

A . Collection of plant material and extraction procedures

Aloe vera, *Centella asiatica* (Asiatic pennywort) and *Strychnos potatorum* (Cleaning nut) were taken to prepare the SLTM pill.

1) Aloe vera

A fresh with luxuriant growth leaves of *Aloe vera* was taken. Leaves were cleaned and washed with water. Outer parts of the leaves were removed with a knife. The gel part in the center was taken and 20.0 g of gel was weighted and taken. This gel was slightly grinded.

2) Centella asiatica

The *Centella asiatica* was cleaned and washed in running water. The whole part of the *Centella asiatica* were selected and put into the grinder. Grinding had been done without adding water until it turned as a good paste. Then the paste was taken and filtered with clean pure cloth. After that 20.0 mL of *Centella asiatica* extract was put in a clean dried graduated cylinder.

3) Strychons potatorum

Cleaned, pure, *Strychons potatorum seeds* were taken and grinded until it become as a fine powder. 20.0g was taken with the help of weighing machine.

Then all three ingredients were mixed well and made small pill like beads. These pills were dried in the partial sun light and collected before fungal contaminations.

B. Preparation of reference drugs and traditional pill solutions

2.8 mL of phosphate buffer saline (PBS, pH 6.4) and 0.2 ml of egg albumin was added to the same test tube. Then 2.0 mL of Sri Lankan traditional pill emulsion was added. Two NSAID drugs namely Ibuprofen (non-steroidal) and Prednisolone (steroidal) were used as the reference drugs. Same procedure was used to make solutions of reference drugs. For control samples, distilled water was added in to the test tube.

C. Serial dilutions and readings of absorbance

All samples were contained 5.0 ml of total volume. Concentration gradients of the traditional pill and reference drugs were prepared from 1000 µg/mL to 0.02 µg/mL using egg albumin and phosphate buffer saline.

Then the mixtures were incubated at (37±2) ^{o}C in a water bath for 15 minutes and then at 70^{o}C for 5 minutes (Chandra *et al*., 2012). After cooling, their absorbance was measured at 680 nm Colon imetry by using distilled water as blank. The absorbance of SLTM pill and reference drugs were taken before and after denaturation. The inhibition rate of protein denaturation at each concentration was calculated by using the formula of (Vt / Vc - 1) x 100% where, Vt = absorbance of test sample and Vc = absorbance of control(Chandra *et al*., 2012) .

III. RESULT

Anti-inflammatory activity of this SLTM pill was evaluated against that denaturation of egg albumin method. The effect against albumin denaturation in high

concentration traditional pill was larger than reference drugs. The highest inhibition rate of egg albumin denaturation (46.7%) showed in 200 µg/mL concentration while Prednisolone and Ibuprofen had 2.5% and 24.6% inhibition rate respectively in the same concentration. 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 traditional pill. However, the inhibition rates of these concentrations were not significantly difference with reference drugs. In addition, Traditional pill had 28.3% of inhibited rate of egg albumin denaturation in 1000 µg/mL concentration while Prednisolone and Ibuprofen showed low inhibition rates compared to traditional pill (3.4% and 13.8% respectively) in this concentration.

Table 1. The percentage of inhibition rate of Protein denaturation with five different concentration (0.02µg/mL to 200 µg/ML)

Concentration	Rate of Inhibition		
(µg/mL)	SLTM	Prednisolone	Ibuprofen
0.02	16.39%	25.40%	14.75%
0.2	32.78%	15.57%	40.16%
2	34.42%	27.86%	29.50%
20	22.95%	29.50%	35.24%
200	46.72%	2.45%	24.59%

Table 2. The percentage of inhibition rate of Protein denaturation with another five different concentration (0.1µg/ml to 1000 µg/ml)

Concentration	Rate of Inhibition		
(µg/mL)	SLTM	Prednisolone	Ibuprofen
0.1	3.44%	3.44%	4.13%
1	2.75%	6.20%	4.82%
10	13.79%	11.72%	2.06%
100	4.82%	4.82%	15.82%
1000	28.27%	3.44%	13.79%

IV. DISCUSSION AND CONCLUSION

Denaturation has a complex mechanism which involves alteration in electrostatic hydrogen, hydrophobic and disulphide bonding. The most of the proteins lose their biological properties and their functions when they heat denatured. In this study, the result graphs has not linear or a curved one. The Sri Lankan Traditional Medicine pill and the reference drugs (Ibuprofen and the Prednisolone) also shown similar pattern of graph. When the concentration increases the amount of absorbance would be increased.
But here the tables show that the SLTM pill in high concentration has given a high inhibition of egg albumin denaturation. It has exhibited that this traditional pill has very good anti-inflammatory property than the reference drug control. Within two reference drugs, Ibuprofen has shown high anti-inflammatory action than the Prednisolone (Steroidal). The traditional medicine has less or no side effects and also available in affordable cost. The herbs that have been used to prepare this particular SLTM pill can be collected in the village gardens and could be prepared with a support from an experienced person. These three plants have been used as dietary ingredients for centuries in Sri Lanka. It is a common understanding that the traditional herbal medicines are suitable for all age groups.

The findings of the present study exhibited a concentration dependent inhibition of egg albumin denaturation by Sri Lankan Traditional Medicine pill. This study identified that 200 µg/ml of the tested Sri Lankan Traditional Medicine pill showed the highest anti-inflammatory activity against the denaturation of protein. Furthermore, the effect against albumin denaturation in high concentration Sri Lankan Traditional Medicine pill (200 µg/ml and 1000 µg/ml) was significantly larger than reference drugs.

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

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

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