Research Article

In vitro anti-inflammatory activity of *Trichosanthes cucumerina* fruits

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ABSTRACT

Aim: The aim of this study was to evaluvate the in vitro anti-inflammatory potential of *Trichosanthes cucumerina* fruits. **Introduction:** *T. cucumerina* (Family: *Cucurbitaceae*) is reported to show properties such as antidiabetic, antihelmintic, antifebrile, gastroprotective, and antioxidant effects. Inflammation is an adaptive response to harmful stimuli. However it plays major role in the pathogenesis of various diseases. **Materials and Methods:** The anti-inflammatory activity of the ethanolic extract of *T. cucumerina* (TCE) was assessed by human red blood cell membrane stabilization and inhibition of albumin denaturation. **Discussion:** The TCE fruits inhibited the hypotonicity-induced red blood cell membrane lysis and albumin denaturation, which are the measures of anti-inflammatory activity, in a concentration-dependent manner. **Conclusion:** TCE has potent anti-inflammatory activity.

KEYWORDS: Albumin denaturation, Anti-inflammation, Human red blood cell membrane stabilization, *Trichosanthes cucumerina*

INTRODUCTION

Trichosanthes cucumerina is a tropical plant known for its strikingly long fruits which is about 150-cm long in average. It is locally called as snake gourd, serpent gourd, chinchinda, and padwal and is a popular in the cuisines of South Asia and Southeast Asia. It is grown in the home gardens of Africa, and its mature fruits are used as an economical substitute for tomatoes. The shoots, tendrils, and leaves are also edible as greens. The fruit is usually consumed as a vegetable due to its high nutritional value. The plant is a rich source of functional constituents other than its basic nutrients such as flavonoids, carotenoids, phenolic acids, and soluble and insoluble dietary fibers and essential minerals, which makes the plant pharmacologically and therapeutically active. The plant contains proteins, fat, fiber, carbohydrates, minerals, and Vitamins A and E in high levels. The predominant mineral elements are potassium (121.6 mg/100 g) and phosphorus (135 mg/100 g); furthermore, sodium, magnesium, and zinc are found in fairly high amounts. The fruits of this plant become too bitter to eat on maturity. Both the bitter taste and the unpleasant odor of these fruits disappear on cooking. The whole plant including roots, leaves, fruits, and seeds is reported to show medicinal properties such as antidiabetic, antibacterial, anthelmintic, antifebrile, gastroprotective, and antioxidant activity.[1]

T. Cucumerina falls under the family *Cucurbitaceae*. Mixture of snake gourd along with coriander leaves is more effective in treating bilious fever. This juice has been used as an emetic to induce vomiting. It has been found to be effective in treating malarial fever. The vegetable being a low-calorie food makes it an ideal food to keep weight under control yet provide the proper nutrition to people with type II diabetes. It is a strong purgative which helps in flushing off the toxins from our body. The leaves act as an emetic, rid the body of toxins, and also help cleanse the bowels. The seeds of the plants are also used as a moistening agent for treating severe cases of dry constipation. Ingesting 30–60 g doses of the leaves, crushed along with coriander seeds thrice every day, is effective in combating diseases such as jaundice. The extract of *T. cucumerina* (TCE) is a miracle drug for arterial disorders such as palpitation and other conditions such as pain and stress on the heart. They help in improving circulation, thereby ensuring to reduce heart problems.[2]

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Anti-inflammatory is the property of a substance or treatment that reduces inflammation or swelling. Inflammation is localized reaction that produces redness, warmth, swelling, and pain as a result of infection, irritation, or injury. Inflammation being a part of body's immune response is the body's attempt at self-protection to remove harmful stimuli and begin the healing process. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases.[3]

Much work has been done on the other parts of *T. cucumerina* by a very large number of researchers in different parts of the world [4] Several works have been done on the cytotoxic properties of root extract.[5] Other pharmacological properties attributed to this plant are antidiabetic[6], hepatoprotective[7], larvicidal[8], antifertility[9] and gastroprotective[10] activities. However, no studies have been reported on the anti-inflammatory effect of fruits of *T. cucumerina*. Therefore, the present study was conducted to evaluate the anti-inflammatory activity of *T. cucumerina* fruits.

MATERIALS AND METHODS

Test Drug Preparation

The fresh fruits of *T. cucumerina* were collected, washed, dried under shade, and powdered into fine particles. 50 g powder was macerated in 600 ml of 95% ethanol at room temperature for 48 h with occasional shaking at 8 h. It was then filtered by Whatman filter paper (size no. 1) and the filtrate evaporated on a rotary evaporator to concentrate in crude extract form at 40ºC.

In Vitro Anti-inflammatory Activity

Human red blood cell (HRBC) membrane stabilization method

The anti-inflammatory activity of plant extract was assessed by in vitro HRBC membrane stabilization method. Fresh blood was collected and mixed with equal volume of sterilized Alsever solution (containing 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride) and stored at 4°C and used within 5 hrs. Saline at two different concentrations were prepared (isosaline 0.85% and hyposaline 0.25%). 0.5 M phosphate buffer, pH 7.4: Add 19 ml of 0.5 M sodium dihydrogen phosphate solution to 81 ml of 0.15 M sodium hydrogen phosphate solution. Check the pH and adjust with monobasic or dibasic solutions as required. Store at room temperature for 4 weeks. RBC suspension: The blood samples were centrifuged at 3000 rpm at room temperature for 10 minutes and the packed cells obtained were washed with isosaline (pH 7.2) 3 times and 10% (v/v) suspension was made with isosaline. The assay mixture contained different concentration of extract (50, 100, 200, 400, 800 µg/ml) and for standard aspirin (200µg/ml), 1 ml of phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline and 0.5 ml of 10% RBC suspension. In another tube 2ml of distilled water was taken and this is served as the control. All the tubes were incubated at 37ºC for 30 min. Then it was centrifuged and the haemoglobin content in the supernatant was estimated using UV-spectrophotometer at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated using the following formula,

Percentage protection = 100 − OD of Test × 100/OD of Control

Albumin Denaturation

The reaction mixture was consisting of test extracts (50, 100, 200, 400, and 800 µg/ml) and 1% aqueous solution of bovine albumin fraction; the pH of the reaction mixture was adjusted using a small amount of 1 N HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min; after cooling the samples, the turbidity was measured at 660 nm (UV Visible Spectrophotometer Model 371, Elico). The experiment was performed in triplicate.

The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control – Abs Sample) × 100/Abs control.

Values are expressed as mean ± SD ($n = 3$); *$P < 0.001$ as compared with negative control. The IC50 value is 361 µg/ml.

RESULTS AND DISCUSSION

TCE exhibited membrane stabilization effect by inhibiting hypotonicity-induced lysis of erythrocyte membrane [Table 1]. The lysosomal membrane is similar to erythrocyte membrane, and its stabilization implies that the extract may as well stabilize lysosomal membranes.[11] The importance of stabilizing the lysosome membrane is in limiting the inflammatory response by preventing the release of lysomal constituents of activated neutrophil such as proteases and bactericidal enzymes which cause

Table 1: HRBC membrane stabilization

Sample	Concentration (µg/ml)	% Inhibition
Negative control	-	-
TCE	50	20.50±0.99*
	100	32.15±1.1*
	200	49.61±2.1*
	400	65.42±3.2*
	800	79.63±1.9*
Aspirin	200	85.64±3.5*
IC_{50} value	-	203.56

TCE- Trichosanthes cucumrina extract, Results were expressed as Mean±SD of n=3

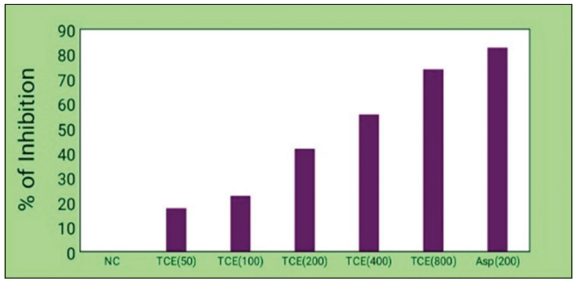

Figure 1: Inhibition of albumin denaturation

further inflammation and damage.[12,13] The activity of the extract is compared was a standard drug aspirin.

Denaturation of the tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of autoantigens in certain arthritic diseases may be due to denaturation of proteins *in vivo*.[14,15] Therefore, using agents that can prevent protein denaturation would be worthwhile for anti-inflammatory drug development. In our investigation, the TCE showed a potent inhibitory activity on protein denaturation in a concentration-dependent manner [Figure 1]. Thus, it is revealed the *in vitro* anti-inflammatory effect of the extract by preventing protein denaturation.

CONCLUSION

The study establishes the *in vitro* anti-inflammatory activity of the extract as evidenced from the HRBC membrane stabilization method and inhibition of protein denaturation.

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