RESEARCH ARTICLE

Evaluation of Anti inflammatory effect of Plectranthus amboinicus leaf extract - An invitro study

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ABSTRACT

The present study was conducted to evaluate the anti-inflammatory effect of hydroalcoholic extract of leaves from Plectranthus amboinicus (HAEPA) against the denaturation of protein in vitro. The plant extracts were incubated with egg albumin at different concentrations to study the anti-inflammatory nature of the extract. Acetaminophen was used as reference standard drug. Present study narrated the Concentration dependent inhibition of protein denaturation by the HAEPA. The current study can thus be summarized as the HAEPA possess significant anti-inflammatory effect against invitro protein denaturation. The effect may be due to Polyphenolic content and may be synergistic activity rather than a single compound.

Keywords: Anti –inflammatory, Plectranthus amboinicus, Protein Denaturation.

INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants. It is the result of concerted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti inflammatory action. [1] It is one of the first and most important responses of the immune system after infection. Inflammation, a necessary evil, also called a two sided sword, is a protective attempt from the organism to remove the injurious stimuli and to initiate the healing process also.

Thus, the progressive destruction of the tissue would intransigent, the survival of the organism. Chronic inflammation can also lead to diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis, etc. The classic signs of inflammation have long been recognized; the tissues become red, swollen, tender, or painful, there is local heat and the patient may be febrile. However, when the inflammation process goes away and continues to simmer in our body for a prolonged period and starts to become detrimental, it is known as chronic inflammation. Inflammation can be categorized mainly as Chronic and Acute inflammatory. [2]

Acute and chronic inflammatory diseases are still one of the most important 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. For this reason there is a need to find and develop new anti-inflammatory drugs with low side effects. [3]

Plectranthus amboinicus, otherwise called as Coleus amboinicus or Coleus aromaticus commonly known as country borage is a dicotyledonous plant of Lamiaceae family [4, 5]. The plant is distributed throughout India and is as folklore medicinal plant used to treat malaria, common cold, bronchitis and epilepsy. The phytochemical study reveals the presence of various flavonoids like quercetin, luteoliln, apigenin, salvigenin and genkwanin. [6] Since many flavonoids have remarkable anti inflammatory activity, [7] the current study targets on the anti-inflammatory effect of Hydroalcoholic extract of Plectranthus amboinicus.

MATERIALS AND METHODS

Drugs and Chemicals: Acetaminophen was procured from Sigma Aldrich, Mumbai, India. All other

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chemicals used were of analytical grade obtained commercially.

Collection of Plant materials: Fresh leaves of *Plectranthus amboinicus* were collected from local garden in Chennai during the month of August. The leaves were authenticated by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai, India. A voucher specimen (No: PARC/2013/2063) was deposited in the institute.

Preparation of Hydroalcoholic Extract of *Plectranthus amboinicus* (HAEPA): The leaves were shade dried for a period of 2 weeks. The dried leaves were further chapped into small pieces and reduced to fine powder using mechanical grinder. 10gm of leaf powder sample was weighed and soaked with 100 ml of Hydroalcohol (70:30) in a conical flask. The flask was covered with cotton plug and aluminium foil to prevent the solvent from evaporation, placed in shaker for 24 hours and filtered using 8 layered muslin cloth. The filtrate was concentrated in a rotary evaporator (Roteva) to get crude leaf extract. The extract was lyophilized and diluted appropriately and stored in air tight container under refrigeration. This diluted extract was used for further studies.

Evaluation of anti-inflammatory activity:

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the test extract (HAEPA) so that final concentrations become 31.25, 62.5, 125, 250, 500 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2ºC in a BOD incubator for 15 minutes and then heated at 70ºC for 5 minutes. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Acetaminophen at the final concentration of (62.5, 125, 250, 500, 1000 µg/ml) was used as reference drug and treated similarly for determination of absorbance. [8,9,] The percentage inhibition of protein denaturation was calculated by using the formula:

% inhibition = 100 × [Vt / Vc – 1]

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined from the dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS AND DISCUSSION

The Present Investigation summarizes the invitro bioassay of anti inflammatory effect of HAEPA, against denaturation of egg albumin. The results were summarized in Table1,2 and IC50 values summarized in Table 3.

Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of tissue proteins in vivo. [10, 11] Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

The standard reference drug and the plant extract showed percentage dependent inhibition of protein denaturation. However the effect of acetaminophen was found to be less when compared with HAEPA extract, which was also confirmed with IC_{50} values. The increments in absorbance's of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by HAEPA and reference drug Acetaminophen. [12] From the IC50 values it becomes evident that HAEPA was more active than Acetaminophen, being effective in lower concentrations.

Preliminary Phytochemical screenings have revealed the presence of alkaloids, phenols, flavanoids and saponins in Plectranthus amboinicus leaves. Flavonoids are well known for significant biological and medicinal values in plants. Various results have also proved that they act through different mechanisms to prevent and suppress inflammatory response and serve as potent cardioprotective, neuroprotective, nephroprotective and

chemopreventive agents. [13] The invitro anti-inflammatory effect of the HAEPA may also be due to its polyphenolic content, and it may be synergistic activity rather than a single compound.

It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH (pH: 6.2-6.5). [14, 15] Several herbal remedies and available drugs have also shown their ability to inhibit protein denaturation [16]. Further studies will help to ascertain the invivo mechanism of action.

Table 1: Influence of HAEPA against protein Denaturation

Concentration (µg/ml)	% Inhibition (HAEPA)
31.25	**14.98**
62.5	**29.67**
125	**61.567**
250	**135.976**
500	**289.967**

Table 2: Influence of Acetaminophen against protein Denaturation

Concentration (µg/ml)	% Inhibition (Acetaminophen)
62.5	11.23
125	26.746
250	52.45
500	116.789
1000	226.79

Table 3: IC50 values of HAEPA and Acetaminophen against protein denaturation

Treatments	IC50 values (µg/ml)
Acetaminophen	228.879
HAEPE	98.286

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