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RESEARCH ARTICLE

Evaluation of Anti-Inflammatory Effect of Cinnamaldehyde – an *in vitro* Study

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ABSTRACT

The present study was conducted to evaluate the anti-inflammatory effect of Cinnamaldehyde (CM) compound isolated from Cinnamomum tamala, against the denaturation of protein *in vitro*. The test compound was incubated with egg albumin at different concentrations to study its anti-inflammatory nature. Acetaminophen was used as reference standard drug. Present study narrated the Concentration dependent inhibition of protein denaturation by CM. The current study can thus be summarized as, CM possess marked anti-inflammatory effect against *in-vitro* protein denaturation. This effect plausibly accounts to the total anti-inflammatory nature of the plant in addition to valid contribution of flavor and odour and can be used for various other curative ailments.

KEYWORDS

Cinnamaldehyde, Anti-Inflammatory, Protein Denaturation, Acetaminophen

INTRODUCTION

Inflammation is a reaction of a part of the body to injury or infection, characterized by swelling, heat, redness, and pain.[1] The process includes increased blood flow with an influx of white blood cells and other chemical substances that facilitate healing. Inflammation is a body response to inactivate or destroy the invading organism to remove and set the tissues for repair. It is triggered by the injured tissue chemical mediators and migrating cells. Inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.[2] Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. The commonly used drug for the treatment of anti inflammatory are non steroidal – anti inflammatory drugs which posses many adverse side effects.[3] Recently traditional medicines are used to treat diseases based on their active therapeutic principles. The plant kingdom have novel source of newer compounds with significant anti inflammatory activities.

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MATERIALS AND METHOD

Cinnamomum tamala is an evergreen tropical tree, belonging to Lauraceae family. It is mainly used for flavouring foods and widely used in pharmaceutical preparations because of its hypoglycemic, stimulant and carminative properties. Essential oil from cinnamomum tamala used to treat rheumatism, colic, diarrhea, nausea, vomiting, diuretic, anti –flatulent effects and useful in treating heart abnormalities.[4] Cinnamomum tamala also possess various pharmacological activities such as anti microbial[5], anti inflammatory[6], antioxidant[7], anti tumour, and Immunomodulatory effects.[8]

Cinnamaldehyde (CM) is one of the biologically active compounds found in cinnamomum tamala. CM is the organic compound that gives cinnamon its flavour and odour.[9] This Pale yellow, viscous liquid occurs naturally in the bark of cinnamon trees and other species of the genus Cinnamomum. The essential oil of cinnamon bark contains about 90% CM. The CM compound is highly found in bark and little amount in leaf.

The present study was conducted to evaluate the *in vitro* anti inflammatory effect of CM against the denaturation of protein.

Drugs and Chemicals

Cinnamaldehyde and Acetaminophen were procured from Sigma Aldrich, Mumbai, India. All other chemicals used were of analytical grade obtained commercially.

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 compound, so that final concentrations become 2.5,5,10,20,40 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[10,11]. The percentage of inhibition of protein denaturation was calculated by using the formula:

$$\% \text{ inhibition} = 100 \times [Vt / Vc - 1]$$

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

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

RESULTS

The present investigation summarizes the *invitro* bioassay of anti inflammatory effect of CM, assessed against denaturation of egg albumin. The results are summarized in Table1, 2 and 3.

Table 1: Influence of Cinnamaldehyde, CM against protein Denaturation

Concentration (µg/ml)	% Inhibition (CM)
2.5	7.18
5	14.352
10	33.79
20	63.37
40	116.49

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 CM and Acetaminophen against Protein Denaturation

Treatments	IC_{50} (µg/ml)
Acetaminophen	228.879
CM	16.519

DISCUSSION

In the present study the protein denaturation bioassay was selected for *in-vitro* assessment of anti-inflammatory property of CM. Denaturation of tissue protein is one of the well documented causes of anti inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of tissue protein *in vivo*[12,13]. Agents that can prevent protein denaturation therefore, would be worthwhile for anti inflammatory drug development.

In the present study the *in vitro* anti inflammatory effect of CM was evaluated against the denaturation of protein (Table 1). The present study exhibited a concentration dependent inhibition of protein (albumin) denaturation by the test compound at varying concentration ranges of 2.5 to 40 µg/ml. Acetaminophen (at the concentration ranges of 62.5 to 1000 µg/ml) was used as the reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table2); However, the effect of CM was found to be more as compared with that of Standard Acetaminophen. This was further confirmed by comparing their IC_{50} values. It has been reported earlier that Hydroalcoholic extract of Plectranthus amboinicus with potent stress combating potential also showed valid anti-inflammatory nature against protein denaturation.[14,15]

The increments in absorbance of test sample with respect to control indicated stabilization of protein i.e., inhibition of protein (albumin) denaturation or anti denaturation effect by the test extract and the reference drug acetaminophen.

CONCLUSION

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.[2,16,17] Therefore, from the findings of the present preliminary experiment it can be concluded that the CM has marked anti-inflammatory effect against the *in-vitro* denaturation of protein. Further, this study gives an idea that this compound of the plant Cinnamomum tamala can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation.[18]

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