

Evaluation of anti-inflammatory activity of *Camellia sinensis* extract using protein denaturation assay

C. Cinthura, R. V. Geetha*

ABSTRACT

Introduction: This study was conducted to evaluate and compare the anti-inflammatory effects of aqueous extracts of green tea against the denaturation of protein *in vitro*. Different concentrations of test extracts were incubated with egg albumin under controlled experimental conditions and determination of absorbance was done to assess the anti-inflammatory property. The results exhibited a concentration-dependent inhibition of protein (albumin) denaturation by green tea extract. **Materials and Methods:** Method used was protein (albumin) denaturation assay. **Results:** The results of this study showed that the green tea extract at different concentrations exhibited anti-inflammatory activity. **Conclusion:** The study proves that the extract of green tea possessed potential anti-inflammatory property which is equally good than its chemical counterparts with an added benefit of causing lesser or no side effects. However, there should always be a quest for newer plant extracts.

KEY WORDS: Anti inflammatory, Green tea, Free radical, Lipid peroxidation, Protein denaturation

INTRODUCTION

Camellia sinensis also known as green tea contains a wide array of organic compounds, such as polyphenols and catechin, which can potentially reduce the risk of cardiovascular and neurodegenerative disorders in addition to substances with anticancer properties.^[1]

Catechin, represented by epicatechin, epicatechin 3-gallate, 3-epigallocatechin, and epigallocatechin 3-gallate, is the most important flavonoids in tea, and is colorless, water-soluble compounds which contribute to the characteristic bitterness and astringent property of tea.^[2,3] It is also considered to be anti-inflammatory.^[4]

Inflammation is induced by endogenous or exogenous stimulation of the vascularized connective tissue which, in turn, produces and releases chemical mediators with the purpose of repairing tissue injury.^[5] In chronic inflammation, thrombi may be formed due to lipid peroxidation causing vascular occlusion or neoplasms may develop.^[6,7]

According to Ryu and Chung,^[8] free radical scavenger activity in addition to inhibiting lipoperoxidation due to the presence of polyphenols (especially catechin), alkaloids, vitamins, and mineral salts with antioxidant, chemoprotective, anti-inflammatory, and anticarcinogenic properties is also seen.^[9-11]

Polyphenols are natural products known to possess notable biological properties,^[12] green tea contains these. In the present study, the higher anti-inflammatory effect of green tea can be attributed to its higher flavonoids (catechin) content. The effect may be due to synergistic effect rather than a single content in green tea.

MATERIALS AND METHODS

Preparation of Aqueous Extract

The green tea was procured. The tea powder was added to 200 ml of water in a beaker. This was followed by heating and filtering it to obtain the extract.

Methodology

Inhibition of albumin denaturation assay

The anti-inflammatory activity of *C. sinensis* was studied using inhibition of albumin denaturation technique which was studied according to Mizushima

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Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

*Corresponding author: R. V. Geetha, Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600 077, Tamil Nadu, India. Tel.: +91-9710456203. E-mail: rvgeetha2015@gmail.com

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and Kobayashi^[13] and Sakat *et al.*^[14] followed with minor modifications. The reaction mixture consisted of test extracts in different concentrations and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using 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. The experiment was performed in triplicate.

The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

RESULTS

In the present study, the protein denaturation assay was selected for *in vitro* assessment of the anti-inflammatory property of the aqueous extracts of green tea. 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 also be due to denaturation of proteins *in vivo*. Therefore, using agents that can prevent protein denaturation would be worthwhile for anti-inflammatory drug development. The absorbance values were as follows:

Table 1 shows the values of inhibition percentage of green tea extracts in different concentrations using albumin denaturation assay. There is evidence that the percentage inhibition is proportional with the increasing concentrations. Hence, there is a proof that *C. sinensis* extract has anti-inflammatory activity.

DISCUSSION

Response to an injurious stimulus is always an inflammation which is evoked by a wide variety of noxious agents such as infections, antibodies, or physical injuries. Inflammation is a normal protective response to tissue injury and is the body's way for tissue repairing, triggered by the release of chemical mediators from injured tissue and migrating cells.^[15] The commonly used drug for the management is nonsteroidal anti-inflammatory drugs which usually have several adverse effects, leading to the formation of gastric ulcers.^[16]

Table 1: Inhibition (%) observed at different concentrations of green tea

Concentration of green tea (ug/ml)	Absorbance at 660 nm	% inhibition
40	0.20±0.02	47
60	0.16±0.03	57
80	0.13±0.01	65
100	0.11±0.07	71

Traditional medicine is being reevaluated worldwide, by extensive research on plant species and their active therapeutic principles. The major benefits of natural medicine are low side effects, low cost, and easy availability.

C. sinensis is the second most consumed beverage in the world.^[17] Green tea is a type of cured tea that is “non-fermented” and produced by drying and steaming the fresh leaves; whereas, black tea leaves are withered, rolled, fermented, and then dried.^[18]

In the present study, *in vitro* anti-inflammatory effect of aqueous extract of green tea was evaluated against denaturation of egg albumin. The results are summarized in Table 1. The values suggest a concentration-dependent inhibition of protein (albumin) denaturation throughout the concentration range of 40–100 µg/mL. In another study, diclofenac sodium, in the concentration range of 78.125–2500 µg/mL, was used which also exhibited concentration-dependent inhibition of protein denaturation. Here, aqueous green tea extract was found to be more effective than diclofenac sodium.^[19]

CONCLUSION

From the results of our study reveal that extracts of green tea are capable of inhibiting denaturation of protein denaturation of albumin. Our present studies also indicate that the extract of green tea exhibits strong anti-inflammatory property so it could be a potential source of antiarthritic property.^[20]

The inhibition of albumin denaturation can also be studied with membrane lysis stabilization in further study to establish the mechanism of antiarthritic activity of green tea. The results are suggestive that the extracts of green tea exhibiting anti-inflammatory activities might be due to the presence of active principles such as polyphenolic content, triterpenoids, alkaloids, and flavonoids.^[21]

From the results of the study, it can be concluded the extract of green tea possessed potential anti-inflammatory which is equally good than its chemical counterparts with the added benefit of lesser or no side effects made available at lower cost for all. However, there should always be a quest for newer plant extracts with better benefits.

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