

Evaluation of anti-inflammatory effects of green tea and black tea: A comparative *in vitro* study

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J. Adv. Pharm. Tech. Res.

ABSTRACT

The present study was conducted to evaluate and compare the anti-inflammatory effects of aqueous extracts of green tea and black tea leaves (*Camellia sinensis*) against the denaturation of protein *in vitro*. The test extracts at different concentrations were incubated with egg albumin under controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present results exhibited a concentration-dependent inhibition of protein (albumin) denaturation by both the tea extracts. From the present findings it can be concluded that both green and black tea leaves possessed a marked anti-inflammatory effect against the denaturation of protein, *in vitro*. Green tea was found to be more active than black tea, plausibly due to the higher flavonoid contents of green tea.

Key words: Anti-inflammatory, green tea, polyphenols, protein denaturation

INTRODUCTION

The inflammatory process is the response to an injurious stimulus evoked by a wide variety of noxious agents, for example, infections, antibodies or physical injuries. Inflammation is a bodily response to injury, infection or destruction, characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants, and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells.^[1] The commonly used drug for the management of inflammatory conditions

are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to the formation of gastric ulcers.^[2,3] Natural products have contributed significantly towards the development of modern medicine. Of late, traditional medicine is being re-evaluated worldwide, by extensive research on different plant species and their active therapeutic principles. The rich wealth of the plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost.

Tea, a product made from the leaf and bud of the plant, *Camellia sinensis*, is the second most consumed beverage in the world.^[4] *Camellia sinensis* is a large evergreen shrub indigenous to Eastern Asia, where it is cultivated extensively. The dried, cured leaves of *C. sinensis* have been used to prepare beverages for more than 4000 years. The method of curing determines the nature of the tea to be used for infusion. 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.^[5] Tea has been used medicinally for centuries in the Traditional Chinese Medicine (TCM). In recent times, there has been renewed interest in green tea, for the prevention of several disease risks and other important health benefits.^[6] Previous researchers have reported the effects of several pharmacological and toxicological properties of green tea and black tea on animals and

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Access this article online

Quick Response Code:



Website:

www.japtr.org

DOI:

10.4103/2231-4040.97298

humans, including anti-inflammatory activities.^[7-12] In the present study it has been found worthwhile to evaluate and compare the possible anti-inflammatory effect of green tea and black tea against the denaturation of protein, *in vitro*, as their anti-inflammatory effects have not been studied in this model so far.

MATERIALS AND METHODS

Plant Materials

Packaged green tea and black tea leaves were procured in the month of July, 2011, from Desai and Sons, Ezra Street, Kolkata 700001, India. Just after procurement, both type of tea leaves were ground mechanically into fine powder and kept into an air-tight container for use in the study.

Preparation of Extracts

The powdered plant materials (50g) were extracted with distilled water (350 mL) by boiling under reflux for 30 minutes. The extracts were filtered and evaporated to dryness to yield the dry extracts of green tea (AQGT, yield: 51.28%) and black tea (AQB, yield: 17.45%). The dry extracts were kept in a vacuum desiccator until use.

Evaluation of Anti-inflammatory Effect *in vitro*

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 AQGT and AQB so that final concentrations became 31.25, 62.5, 125, 250, 500, 1000 µg/mL. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at $37 \pm 2^\circ\text{C}$ in a BOD incubator (Labline Technologies) for 15 minutes and then heated at 70°C for five minutes. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using the vehicle as a blank. Diclofenac sodium in the final concentrations of (78.125, 156.25, 312.5, 625, 1250, 2500 µg/mL) was used as the reference drug and treated similarly for the determination of absorbance.^[13] The percentage inhibition of protein denaturation was calculated by using the following formula: % inhibition = $100 \times [V_t / V_c - 1]$

Where, V_t = absorbance of the test sample, V_c = absorbance of control.

The extract concentration for 50% inhibition (IC_{50}) was determined by the dose-response curve.

RESULTS AND DISCUSSION

There are certain problems associated with the use of animals in experimental pharmacological research, such as, ethical issues and the lack of rationale for their use when other suitable methods are available, or can be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of the anti-inflammatory property of the aqueous extracts of green

tea and black tea (AQGT and AQB). Denaturation of the 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 proteins *in vivo*.^[14,15] Therefore, using agents that can prevent protein denaturation would be worthwhile for anti-inflammatory drug development.

In the present investigation, the *in vitro* anti-inflammatory effect of AQGT and AQB was evaluated against denaturation of egg albumin. The results are summarized in Table 1. The present findings exhibited a concentration-dependent inhibition of protein (albumin) denaturation by AQGT and AQB, throughout the concentration range of 31.25 to 1000 µg/mL. Diclofenac sodium, in the concentration range of 78.125 to 2500 µg/mL, was used as a reference drug which also exhibited concentration-dependent inhibition of protein denaturation [Table 2]. The IC_{50} values are summarized in Table 3. Here, AQGT was found to be more effective than AQB; however, the effect of diclofenac sodium was found to be quite low when compared with both the test tea extracts. This was further confirmed by comparing their IC_{50} values [Table 3].

The increments in the absorbance of the test samples, with respect to the control, indicated stabilization of protein, that is, inhibition of protein (albumin) denaturation or an anti-denaturation effect by the tea extracts and the reference drug, diclofenac sodium.^[16]

Tea leaves contain varying amounts of polyphenols

Table 1: Effect of AQGT and AQB against protein denaturation.

Concentration (µg/mL)	% Inhibition (AQGT)	% Inhibition (AQB)
Control	-	-
31.25	220	140
62.5	320	180
125	620	220
250	1100	700
500	2480	1540
1000	4980	2480

AQGT: Aqueous extract of green tea, AQB: Aqueous extract of black tea

Table 2: Effect of diclofenac sodium against protein denaturation

Concentration (µg/mL)	% Inhibition
Control	-
78.125	12.5
156.25	12.5
312.5	25
625	50
1250	212.5
2500	812.5

AQGT: Aqueous extract of green tea, AQB: Aqueous extract of black tea

Table 3: IC₅₀ values of AQGT, AGBT and diclofenac sodium

Treatments	IC ₅₀ (µg/mL)
AQGT	7.10
AGBT	11.16
Diclofenac sodium	625

AQGT: Aqueous extract of green tea, AGBT: Aqueous extract of black tea

particularly flavonoids. Polyphenols are well-known natural products known to possess several notable biological properties.^[17] Black tea and green tea both contain almost a similar amount of flavonoids, however, they differ in their chemical composition; green tea contains more catechins (simple flavonoids), while the oxidation undergone by the leaves, in order to make black tea, polymerizes these simple flavonoids into theaflavins and thearubigins (polymerized flavonoids).^[8,18] 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 the synergistic effect rather than a single constituent.

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).^[19] Therefore, from the results of the present preliminary study, it can be concluded that both green and black tea leaves possess a marked anti-inflammatory effect against the denaturation of protein *in vitro*. Previous researchers have reported the anti-inflammatory activity of both green tea and black tea.^[9-12] The present findings corroborated this property *in vitro*, against this protein denaturation model. This correlation is further strengthened by the fact that the present study was performed in a different model, in which it has not been studied earlier. It is suggested that the anti-inflammatory effect of tea leaves could be further evaluated in other experimental models.

ACKNOWLEDGEMENT

The authors are thankful to the authority of the Bengal School of Technology (A College of Pharmacy), Sugandha, Hooghly, 712102, West Bengal, India, for providing the necessary facilities for the present study.

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How to cite this article: Chatterjee P, Chandra S, Dey P, Bhattacharya S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative *in vitro* study. J Adv Pharm Tech Res 2012;3:136-8.

Source of Support: Nil, **Conflict of Interest:** Nil.