Original Article

Evaluation of acute anti-inflammatory and analgesic activities of green tea decoction on experimental animal models

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ABSTRACT

Background: Green tea has been used as a daily beverage for several years. Anti-inflammatory effect of tea has also been depicted in different papers. Therefore we had set forward this study to examine the potential anti-inflammatory and analgesic activities of green tea in different experimental animal models. **Aims:** Evaluation of anti-inflammatory effects of green tea on rat. Evaluation of analgesic effects of green tea on mice. **Materials and Methods:** Green tea decoction (10% and 20%) was prepared by soaking 20 g of green tea in 100 ml boiled water separately, soaked for 2 mins and thereafter filtered. Acute anti-inflammatory activity of tea decoction was evaluated using carrageenan and dextran whereas central and peripheral analgesic activities were evaluated by tail immersion test and acetic acid-induced writhing test, respectively. **Study Design:** This is an experimental study. **Results:** Green tea decoction (10% and 20%) has shown significant anti-inflammatory effects (65% and 70%) and (50% and 71%), respectively, on carrageenan and dextran-induced acute inflammatory models which can be comparable with the standard drug indomethacin (93% and 98.3%, respectively). In central analgesic model Green tea decoction (10% and 20%) has shown no analgesic action at different hours as the reaction time was less than 10 seconds at all time interval. But at peripheral analgesic model green tea decoction (10% and 20%) has shown 20% and 35.74% inhibition, respectively, as compared to control group. Aspirin shows around 39.81% of inhibition compared to control. **Conclusion:** Taken together, our data indicate that green tea (20%) has a potential anti-inflammatory and peripheral analgesic action and this corroborates with the current trend of tea being promoted as 'health drink'.

Key words: Anti-inflammatory, analgesic animals, green tea

INTRODUCTION

The search for anti-inflammatory agents with analgesic effects coupled with minimal side effects led the attention of Indian workers toward another treasure of remedies -indigenous system of medicine. The importance and utility of medicinal plants in the treatment of any chronic disease is well known as they have the additional advantage of being cheap, and may be used for a prolonged period. Fruits, vegetables and other plant foods tend to be rich in antioxidants and other phytochemicals. Antioxidants consumed in food inhibit damaging reactions within the human body and have a beneficial effect upon health.[1] *Angelica archangelica* Linn. has traditionally been used in mountainous and tropical regions and

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appreciated for centuries; however, its biological properties are only beginning to be elucidated scientifically.[2] Various medicinal plants have been selected for scientific testing and screening of analgesic and anti-inflammatory action.

Camellia sinensis is commonly known as tea which is most consummated beverage in the world.[3] The active components of tea responsible for such biological effects are known to be catechins (known as polyphenols), which constitute seven forms including epigalocatechin-gallate (EGCg). EGCg is a major catechin compound present in tea extracts and is also the most active form in a variety of biological activities.

Inflammation is the response of a tissue and its microcirculation to pathogenic injury. It is characterized by the generation of inflammatory mediators and movement of fluid and leucocytes from the blood into extravascular tissues which gives rise to the four cardinal signs of inflammation, namely rubor (redness), calor (heat), tumor (swelling) and dolor (pain) as described by Anlus Celsus, the Roman encyclopedist, in the second century AD. Inflammation may be classified into acute, sub-acute and chronic or immunological. There are various mediators for these types of inflammation in different stages, which are histamine, 5-hydroxytryptamine, bradykinin, prostaglandins, leukotrienes, etc. Algesia (pain) is always produced along with inflammation as an integral part of the whole process.

This study was undertaken to examine the potential anti-inflammatory and analgesic activities of green tea in various experimental animal models.

Objectives

1. Evaluation of anti-inflammatory effects of green tea decoction in experimental animal models
2. Evaluation of analgesic effects of green tea decoction in experimental animal models

MATERIALS AND METHODS

Animals

The entire study was carried out in IPGMEandR and SSKM hospital, as a part of post-graduate dissertation using Sprague Dawley adult rats of either sex, weighing between 150 and 200 g. and inbred Swiss mice of average weight 15-25 g of either sex. The animals were maintained under standard laboratory conditions with free access to commercial pellet feed and water *ad libitum*. The animals were housed for a period of 7 days for acclimatization prior to the commencement of experimental work at a room temperature of 27°C under fixed 12-hour alternate light and darkness cycle. The protocol was approved and carried out after the permission of Institutional Animal Ethics Committee.

Investigational drugs and dosage preparations.

Drugs and chemicals used for the experiment were Carrageenan (Sigma), Dextran (Sigma), Indomethacin (Inmecin, Sterfillap) Pethidine (Haffkine) Acetic acid (0.6% v/v) (E. Merck). Aspirin (Ecosprin, USV). Green Tea extract.

Preparation of the plant product

Green tea leaves were commercially obtained from P and A Arse, Rajdhani Apt. BIK-I Rg Barua Road, Ganeshguri, Guwahati. For the preparation of tea extract, tea leaves (100 g) were extracted with ethyl acetate using soxhlet assembly. The extract was concentrated in a rotary flash evaporator under reduced pressure to semisolid mass. Tea decoction (20%) was prepared by soaking 20 g of green tea in 100 ml boiled water separately, soaked for 2 mins and thereafter filtered. This filtrate was designated as 'green tea decoction'. The dose of this decoction orally administered to each rat was 0.1 ml/10 g of body weight. Initial pilot study suggested that 10% and 20% of green tea preparation had given significant results. Therefore, we have decided to set forward our study with 10% and 20% green tea decoction.

Models for evaluation of anti-inflammatory activity

In all models, the animals were grouped as follows:

Category of treatment groups	
Group (*n*=12)	Treatment
Group I	Control
Group II	Standard drug, indomethacin (10 mg/kg)
Group III	10% green tea decoction
Group IV	20% green tea decoction

Carrageenan-induced rat paw edema

We followed the method adopted by Winter *et al.*, subsequently modified by Ghosh *et al.*[4,5] 48 rats were taken and divided into four groups of 12 rats each as indicated above.

A fresh solution of 1% carrageenan was prepared by dissolving 50 mg of carrageenan powder in 5 ml of normal saline (0.9% NaCl). All animals were starved for eighteen hours before starting of the experiment. Animals of Group III, and IV were pretreated with the

test drug in the doses mentioned above 30 min prior to the carrageenan injection. All orally administered drugs were given via a rat feeding cannula. Subsequently 30 min after the respective treatments, 0.1 ml of 1% carrageenan was injected subcutaneously into the subplanter region of right hind paw of all animals to induce edema. The paw had been previously marked with ink at the level of the lateral malleolus and the paw volume was measured plethysmographically.[6] The paw volume was measured initially at 0 hrs and subsequently at 1st, 2nd, and 3rd and 4th hr after carrageenan injection. The mean increase in paw volume was measured hourly and at every time point, the percentage inhibition of edema was calculated.[7]

$$\% \text{ Inhibition} = \frac{\text{Mean increase in paw volume of control group} - \text{Mean increase in paw volume of test group}}{\text{Mean increase in paw volume for the control group}} \times 100$$

Dextran-induced rat paw edema

In this model, dextran was used as the phlogistic agent and the methodology of Rowley *et al.*,[8] was used with slight modifications (Ghosh *et al.*).[5] Forty-eight rats were taken and divided into four groups of 12 rats each. The four groups were treated as per the protocol mentioned in the previous model. Indomethacin and tea decoction was administered 30 min prior to the injection of dextran. A volume of 0.1 ml of 2% dextran solution in normal saline was injected into the subplantar tissue of right hind paw of each rat (Vogel, 1997a).[9] The paw volume was recorded by a plethysmometer initially at 0 hrs and subsequently hourly at 1, 2, 3 and 4 hrs after dextran injection. The percentage inhibition of edema at every time point was calculated as per Vetrichelvan and Jegadeesan, 2002.[7]

Method for evaluation of central analgesic activity

The method as described by Ther *et al.* as a modification of earlier publications by D'Armour *et al.*, was followed.[10] Forty-eight rats were taken and they were divided into 4 groups of 12 rats each. The groups were treated as follows:

Category of treatment groups	
Group (***n***=12)	Treatment
Group I	Control
Group II	Standard drug, Pethidine (12 mg/kg)
Group III	10% Green tea decoction
Group IV	20% Green tea decoction

Tail immersion method

This test was based on the method as adopted by Ramabadran *et al.*[11] The albino mice were divided into four groups of 12 animals each. The lower 5.0 cm portion of the tail was then dipped in a beaker of water maintained at 55 ± 0.5°C. The time in seconds required to withdraw the tail clearly out of water was taken as the reaction time. The animals that could not lift its tail out of the water within 10 s were discarded. The basal reaction time for all the animals was recorded as the 0 min observation. The test drug tea decoction was administered orally in doses of 0.1 ml of 10 gm bodyweight while the standard drug, pethidine (12 mg/kg) was administered intraperitoneally to the respective three groups as was maintained for the previous tests. A normal control, without any treatment was also maintained. The reaction time was noted 30, 60, 90, 120 and 150 min after drug administration.

Model for evaluation of peripheral analgesic activity

Acetic acid-induced writhing test

This was based on the method described by Koster *et al.*[12] Albino mice of either sex were divided into four groups of twelve animals each. Green Tea (10% and 20%) at doses of 0.1 ml/10 gm, aspirin (30 mg/kg; standard) was administered respectively to the three groups orally before i.p. injection of 0.6% v/v acetic acid solution in water at a dose of 10 ml/kg. A control group without any drug treatment was maintained, which was also treated with acetic acid. Immediately after administration of acetic acid, the number of writhes or stretches (a syndrome, characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) was counted for 15 min. A reduction in the number of writhes as compared to the control group was considered as evidence for the presence of analgesia, expressed as percent inhibition of writhing, which is calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{\text{Mean number of writhes in control group} - \text{Mean number of writhes in test group}}{\text{Mean number of writhes in control group}}$$

Statistical analysis

The results were expressed as mean ± S.D. and the significance was evaluated by Student's *t*-test versus control, with $P<0.05$ implying significance.

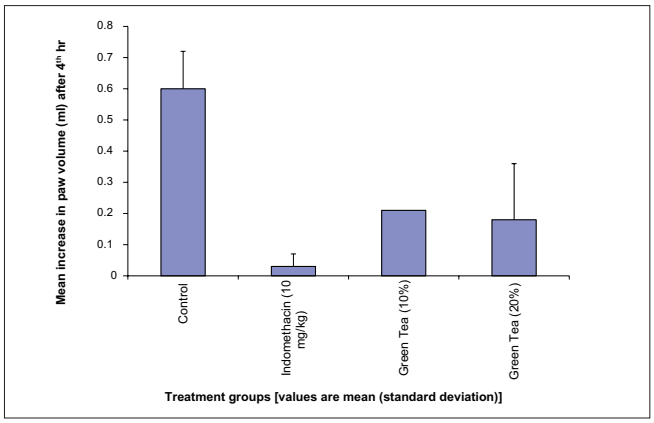

Figure 1: Effects of green tea decoction on carrageenan-induced rat paw edema

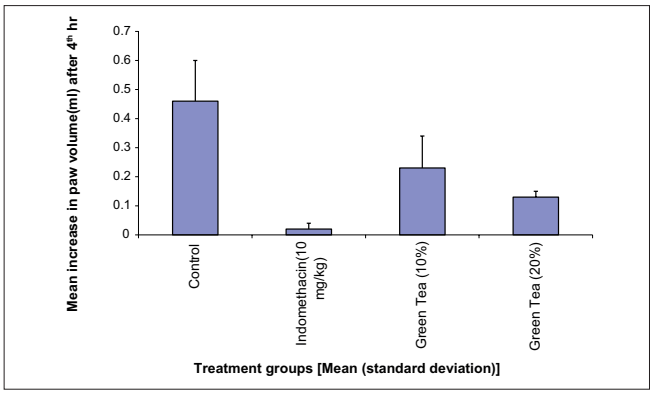

Figure 2: Effect of green tea decoction on dextran-induced rat paw edema

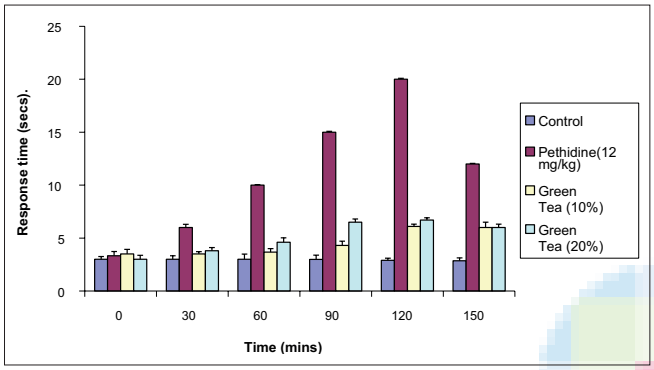

Figure 3: Evaluation of analgesic activity of green tea decoction by tail immersion method

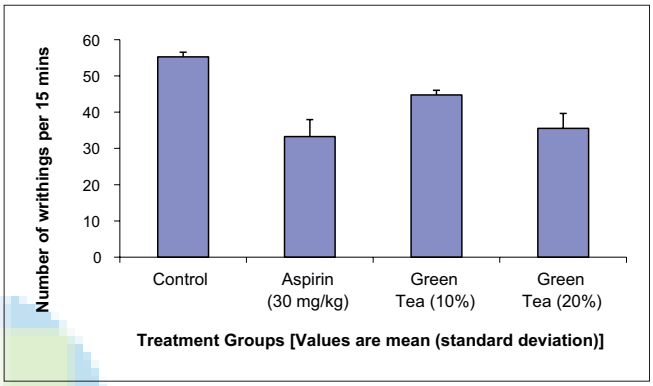

Figure 4: Evaluation of analgesic activity of green tea decoction by acetic acid-induced writhing test

RESULTS

Green tea decoction (10% and 20%) has shown significant anti-inflammatory effects (65%) and (70%) [Figure 1] on carrageenan-induced acute inflammatory models which can be comparable with the standard drug indomethacin (93%). Mean increase in rat paw volume was (0.21 ± 0.12) ml and (0.18 ± 0.18) ml, respectively, with 10% and 20% green tea and (0.03 ± 0.03)ml with indomethacin. In control it was around 0.6 ± 0.12 ml. In dextran-induced inflammatory model, green tea (10% and 20%) fed rats showed mean increase in paw volume of (0.23 ± 0.11) ml and 0.13 ± 0.02 ml, respectively. [Figure 2] (0.02 ± 0.02) ml increase was observed with indomethacin in comparison to 0.46 ± 014 with control. Time and dose-dependent inhibition was observed with Green tea (10% and 20%) being 50% and 71%, respectively, at 4th hr. Indomethacin showed a greater degree of inhibition around 98.3%. All the results were statistically significant as compared to controls (P<0.001).

In central analgesic model, the reaction time was taken at 0, 30, 60, 90, 120 and 150 min in the control group. The standard drug pethidine showed statistically significant analgesic activity from 60 min onwards. Green tea decoction (10% and 20%) has shown no analgesic action at different hours as the reaction time was less than 10 secs at all time intervals [Figure 3]. But in Peripheral analgesic model, number of writhes observed in the normal control group was 55.25 ± 1.26 per 15 mins. Green tea decoction (10% and 20%) pretreated cases; the number of writhes observed was 44.75 ± 1.29 and 35.5 ± 4.12, respectively, per 15 mins. In aspirin-pretreated cases it was 33.25 ± 4.7. Percentage inhibition of number of writhes in the group pretreated with green tea decoction (10% and 20%) [0.1 ml/10 gm of mice] showed 20% and 35.74% of inhibition, respectively, as compared to control group. Aspirin showed around 39.81% of inhibition [Figure 4]. All the results were statistically significant as compared to controls (P<0.001).

DISCUSSION

Plant products are in use for a long time in Ayurvedic and folklore medicine for the cure of ailments with minimal side effects and comparable efficacy. The plant kingdom has been explored for drugs relieving

pain and inflammation as well. Chopra *et al*. pioneered the usefulness of indigenous drugs in inflammatory arthritic conditions. In our laboratory also Nag *et al*., and many other workers have screened active principles of a number of plants for this purpose.

Accordingly, a study was undertaken to assess the immunoregulatory activity of tea decoction in rat and mice models. The results obtained in the anti-inflammatory models in the present study shows that tea possesses pronounced activity against both acute and chronic experimental models of inflammation.

In the model of acute inflammation as exemplified by the carrageenan-induced paw edema, a significant anti-inflammatory action of the test drug was observed. Carrageenan is a sulfated mucopolysaccharide derived from Irish sea moss, *Chondrus*. It was found to be a superior phlogistic agent over other varieties like brewer's yeast, formalin, dextran and egg albumin. Carrageenan-induced rat paw edema is found to be biphasic, the initial phase is due to release of histamine, 5-hydroxytryptamine (5-HT) and kinin in the first hour after the administration of carrageenan and a more pronounced second phase is attributed to the release of prostaglandin, bradykinin, protease and lysosome like substances in 2-3 hrs (Brooks *et al*.).[13] The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents (Smucker *et al*.).[14] It can therefore be deduced that the anti-inflammatory activity may be mediated through inhibition of any of these mediators either alone or in combination.

The higher (20%) and lower (10%) doses of green tea inhibited rat paw volume by 70% and 65% after 4 hrs of carrageenan injection. The results were comparable to indomethacin that reduced the paw volume by 93%. Thus it can be concluded that the anti-inflammatory action of green tea in a carrageenan-induced edema model is by inhibition of histamine, 5-HT, bradykinin and prostaglandin, individually or in combination.[15] In future, to more precisely determine the chemical mediators that are being inhibited by tea, individual mediators can be injected and the study repeated in the same fashion.

In the dextran-induced rat paw edema model, tea showed significant anti-inflamatory activity. Dextran-induced edema is mediated mainly by histamine and 5-HT (Ghosh *et al*.)[16] and dextran is known to be a powerful liberator of histamine (Rowley *et al*.). Green tea (20% and 10%) showed an inhibition of 71.7% and 50%, respectively, in comparison to the standard drug indomethacin that showed an inhibition of 98.3%. All showed statistically significant anti-inflammatory activity. Therefore, it can be said that the tea has got an inhibitory action against either or both of these agents.

In the second phase of the present study some well-accepted experimental animal models have been taken for assessing both the central and peripheral analgesic activity. Although the classification of central and peripheral analgesics is definitely too simplified (Bannwarth *et al*.), it provides a guide for differentiation by pharmacological methods.[17] In the tail immersion method of assessing the central analgesic activity the green tea did not show any significant activity.

In order to distinguish between central and peripheral analgesic action of tea decoction, acetic acid-induced writhing response in mice was used to examine the effect. This method is not only simple and reliable but also affords rapid evaluation of this type of analgesic action. It was found that green tea significantly inhibited acetic acid-induced responses in a dose-dependent manner. It effectively reduced the wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limb in mice due to the nociceptive property of acetic acid. The number of writhes in untreated controls was 55.25 ± 1.26; in comparison to 33.25 ± 4.72 in aspirin-pretreated animals. Inhibition of number of writhes whereas percentage inhibition of writhing produced by 20% green tea was 35.74% (P<0.001) and was close to that produced by 30 mg/kg of aspirin, the standard drug that inhibited 39.81% (P<0.002). This suggests that the tea possesses analgesic property that is peripherally mediated.

Hyperalgesia can be produced by the sensitization of peripheral nociceptors. Sensitization occurs when chemical products, such as bradykinin, 5-HT, histamine and eicosanoids (prostaglandins and leukotrienes) are released near nociceptor terminals after or during tissue inflammation. For example, a noxious stimulus applied to the skin or produced during inflammation may destroy cells near nociceptor. Dying cells then release proteolytic enzymes that react with circulating globulins to from bradykinin. Bradykinin then binds to a receptor on the membrane of the nociceptor and activates a second messenger system, which in turn sensitizes the nerve ending.[18] Bradykinin also provokes the release of neuropeptides such as substance P, neurokinin A and CGRP.[19]

Histamine released from mast cells and 5-HT released

from platelets also can elicit pain but they are 50 times less potent than bradykinin. Prostaglandins sensitize the nerve endings to the effects of bradykinin and other algogens.[20] All of them do sensitize the nerve endings either by opening the ion channels or by activating second messenger. Therefore, it is possible that the peripheral analgesic activity of tea observed was due to its concomitant anti-inflammatory activity.

Taken together, our data indicate that green tea has a potential anti-inflammatory and analgesic action, this corroborates with the current trend of tea being promoted as 'health drink'.[21] Our results support the idea that tea has a beneficial effect. However, such a study would be difficult to undertake in humans as the majority of the population are 'tea consumers'. Hence, we propose that after a 'wash out' period of maybe 2-4 weeks, preliminary studies can be undertaken with normal healthy volunteers.

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