Evaluation of the anti-inflammatory and analgesic effects of green tea 
(*Camellia sinensis*) in mice

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**ABSTRACT**

**PURPOSE:** To evaluate the anti-inflammatory and analgesic effects of green tea (*Camellia sinensis*) in mice.

**METHODS:** The anti-inflammatory effect of alcoholic extracts of green tea (AE) was evaluated in a cell migration assay with four groups of six Swiss mice receiving 0.07g/Kg or 0.14g/Kg EA (treatment groups), saline (negative control) or 10mg/Kg indomethacin (positive control) by gavage. One hour later 300 µg carrageenan was administered intraperitoneally or subcutaneously. The analgesic effect was evaluated using four groups of six animals receiving 0.07g/Kg or 0.14g/Kg EA, saline or 10mg/Kg indomethacin subcutaneously, followed 30 minutes later by 1% acetic acid.

**RESULTS:** When administered subcutaneously at either dose (0.07g/Kg and 0.14g/Kg), AE inhibited carrageenan-induced cell migration (p<0.05). However, when administered by gavage, only the latter (0.14 g/Kg) was efficient (p<0.05). AE at both doses (0.07g/Kg and 0.14g/Kg) inhibited abdominal contortions (p<0.05), but the effect was not dose-dependent.

**CONCLUSION:** Green tea was shown to have analgesic and anti-inflammatory properties and may constitute a natural treatment option in chronic inflammatory disorders.

**Key words:** Camellia sinensis. Catechin. Anti-inflammatory Agents. Analgesics. Mice.
**Introduction**

After water, tea is one of the most popular beverages in the world. A significant part of this tea is prepared with leaves of the shrub *Camellia sinensis* in the form of green tea, oolong or black tea, especially appreciated in China and Japan. Studies conducted in these countries show that 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 hypoglycemic and anticancer properties.

Green tea is made with fresh leaves which are boiled to avoid fermentation, resulting in a dry and stable product. Catechin, represented by epicatechin, epicatechin 3-gallate (ECG), 3-epigallocatechin and epigallocatechin 3-gallate (EGCG), are the most important flavonoids in tea. These colorless, water-soluble compounds contribute to the characteristic bitterness and adstringence of tea.

Among other benefits, green tea is generally held to be anti-inflammatory. 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. In chronic inflammation, thrombi may be formed due to lipid peroxidation causing vascular occlusion, or neoplasms may develop.

Diets rich in antioxidant compounds have been proposed to minimize inflammatory stimuli, atheromatous plaque formation and risk of malignancy. According to Ryu and Chung, green tea displays antioxidant (free radical scavengers) and metal-chelating 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.

In view of the wide range of pharmacological properties of catechin, the purpose of the present study was to evaluate the anti-inflammatory and analgesic effects of green tea (*Camellia sinensis*) in mice.

**Methods**

The study was previously approved by the Institutional Animal Care and Use Committee of the University of Fortaleza (8008/2009). Forty-eight male Swiss mice weighing 25-35 grams were used in the study. The animals were supplied by the experimental animal facility of the Health Sciences Center at the University of Fortaleza, distributed in groups of six animals each and accommodated in cages (30x17x15cm) in a controlled environment (circadian cycle, 25°C, water and Fri-Ribe® rat chow *ad libitum* throughout the experiment).

**Preparation of alcoholic extract of leaves of *Camellia sinensis***

Leaves (95g) of *C. sinensis* (Amor à Vida Produtos Naturais®) were ground and macerated with 400 mL absolute ethanol at room temperature for five days. The extract was then filtered and the maceration process was repeated with the residue. The solvent of the extract was evaporated by heating in a water bath at 60°C until obtaining a final volume of 25 mL. During the entire procedure, the extract was shielded from direct light exposure. Finally, 70 mL distilled water was added to the extract to make 95 mL solution with a drug concentration of 1 g/mL. The fraction was stored in an amber vial at 4°C until the time of use.

The highest dose of extract administered in the study (0.14 g/kg) was based on the consumption of 1 liter green tea (prepared with 10g *C. sinensis* leaves) by an individual weighing 70 Kg.

**Evaluation of inhibition of inflammation**

Cell migration to the peritoneal cavity was evaluated as described by Spiller *et al.*. Inflammation was induced in all animals by intraperitoneal injection of 300 µL solution containing 300 mg carrageenan diluted in 0.9 % NaCl at 1:1.

**Method 1: oral application of green tea extract:**

Treatment group 1 (n=12): Two groups of six animals each received, respectively, 0.07g/Kg and 0.14g/Kg green tea extract by gavage one hour before carrageenan administration.

Negative control group (n=6): The animals received saline solution by gavage one hour before carrageenan administration.

Positive control group (n=6): The animals received indomethacin solution (10mg/kg) by gavage one hour before carrageenan administration.

**Method 2: subcutaneous application of green tea extract:**

Treatment group 1 (n=12): Two groups of six animals each received, respectively, 0.07g/Kg and 0.14g/Kg green tea extract subcutaneously one hour before carrageenan administration.

Negative control group (n=6): The animals received saline solution subcutaneously one hour before carrageenan administration.
Positive control group (n=6): The animals received indomethacin solution (10mg/kg) subcutaneously one hour before carrageenan administration.

Four hours after carrageenan administration, the animals were euthanized by cervical dislocation, 10 mL saline solution containing 0.1% heparin was injected intraperitoneally and the abdomen was shaken lightly to homogenize the migratory cells. Then, by way of laparotomy, 5 mL peritoneal fluid was retrieved with a plastic Pasteur pipette for migratory cell count.

**Total cell count**

Aliquots of 20 µL samples were added to 380 µL Turk solution. Subsequently, 20 µL of the resulting solution was placed in a Newbauer chamber for total cell count under light microscopy.

**Evaluation of analgesic effect**

To evaluate the analgesic effect, 1% acetic acid (0.1mL/10g) diluted to 1% in distilled water was injected in four groups of six animals each. Abdominal contortions were counted during 20 min, beginning 10 min after acetic acid administration.

Treatment group (n=12): Two groups of six animals each received, respectively, 0.07g/Kg and 0.14g/Kg green tea extract subcutaneously 30 min before acetic acid administration.

Negative control group (n=6): The animals received 0.9% saline solution subcutaneously 30 min before acetic acid administration.

Positive control group (n=6): The animals received indomethacin solution (10mg/kg) diluted in 5% sodium bicarbonate (1:1) subcutaneously 30 min before acetic acid administration.

**Statistical analysis**

The data were submitted to variance analysis followed by the Student-Newman-Keuls test, using the software GraphPad Prism. Mean values ± standard deviation for each group were compared. The level of statistical significance was set at 5% (p<0.05).

**Results**

Figures 1 and 2 show the results of the inhibition of inflammation following the administration, by gavage or subcutaneous injection, respectively, of 0.07g/Kg and 0.14g/Kg alcoholic green tea extract to Swiss mice one hour prior to administration of carrageenan. When the extract was administered by gavage, cell migration was only inhibited at 0.14/Kg (p<0.05), but when the extract was injected subcutaneously, inhibition was observed at both dosage levels (0.07g/Kg and 0.14g/Kg, p<0.05) when compared to the negative controls (saline). Despite the observation of inhibited cell migration in both experiments, the number of migratory cells did not differ significantly between the groups.

**FIGURE 1** - Inhibition of inflammatory cell migration in the peritoneum of Swiss mice inoculated with alcoholic green tea (*Camellia sinensis*) extract by gavage. Data submitted to variance analysis followed by the Student-Newman-Keuls test.

**FIGURE 2** - Inhibition of inflammatory cell migration in the peritoneum of Swiss mice inoculated subcutaneously with alcoholic green tea (*Camellia sinensis*) extract. Data submitted to variance analysis followed by the Student-Newman-Keuls test.

**FIGURE 3** shows the effect of alcoholic green tea extract and indomethacin on 1% acetic acid-induced abdominal contortions in Swiss mice. At both dosages (0.07 and 0.14g/kg), the extract significantly inhibited contortions (p<0.05), though not as strongly as indomethacin (p<0.01).
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Indomethacin, used in our study as positive control, is a powerful non-hormonal anti-inflammatory substance capable of inhibiting cyclooxygenase and reducing the levels of prostaglandins A and E. This anti-inflammatory action was clearly observed in the evaluation of the analgesic effect of indomethacin and alcoholic green tea extracts on Swiss mice with acetic acid-induced abdominal contortions. When administered subcutaneously, the extract reduced contortions significantly ($p<0.05$) regardless of the concentration (0.07g/Kg and 0.14g/Kg), but indomethacin was more efficient than either concentration ($p<0.01$), suggesting that in this model, and with this route of administration, the analgesic action of green tea is not dose-dependent.

Found in high concentrations in green tea, epigallocatechin 3-gallate (EGCG) has been the object of much research. It has a selective inhibitory effect on cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) in human chondrocytes. Prostaglandins synthesized from COX-2 reduce the pain threshold during inflammation and help improve cell permeability during cell migration. iNOS synthesizes nitric oxide in endothelial cells, promoting significant vasodilation during inflammation. The combined effect of these catechin is likely responsible for the inhibition of cell migration and analgesia observed in the present study, since the inhibition of COX-2 and iNOS is known to reduce vascular permeability and vasodilation, respectively.

However, other mechanisms have been proposed to explain the anti-inflammatory effect of green tea. In a study of the effect of polyphenols from green tea in an experimental rat arthritis model, Kim et al. found that green tea reduced IL-17 synthesis and increased IL-10 synthesis. IL-17 is synthesized by Th17 lymphocytes during events favoring chronic inflammation. The reduction in IL-17 synthesis can inhibit several processes required for the development of rheumatoid arthritis, such as Th1 lymphocyte activation, macrophage pro-inflammatory cytokine release, and autoantibody synthesis.

Conclusion

Green tea was shown to have analgesic and anti-inflammatory properties and may constitute a natural treatment option in chronic inflammatory disorders.

References


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