

Black tea (*Camellia sinensis*) decoction shows immunomodulatory properties on an experimental animal model and in human peripheral mononuclear cells

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ABSTRACT

Background: Black tea (*Camellia sinensis*) has been used as a daily beverage for time immemorial. Immunomodulatory effects of tea are recognized as it stimulates the proliferation of cultured human peripheral blood mononuclear cells. Anti-inflammatory effects of tea have also been depicted in the available literature. Therefore, we designed this study to examine the potential immunomodulatory and anti-inflammatory activities of black tea in a rat model and in human peripheral mononuclear cells. **Aims:** The purpose of the study was to determine (1) evaluation of anti-inflammatory effects of black tea on rats, (2) evaluation of immunomodulator effects of black tea on rats, and (3) evaluation of immunomodulator effects of black tea on human peripheral mononuclear cells. **Materials and Methods:** Black tea decoction (10% and 20%) was prepared. Acute anti-inflammatory activity of tea decoction was evaluated using carrageenan and dextran whereas chronic anti-inflammatory (Immunomodulatory) effects were evaluated in a complete Freund's adjuvant-induced arthritis model. Immunostimulatory role was evaluated in cultured human (*in vitro*) peripheral mononuclear cells (T-lymphocytes) by using methyl thioazolyl tetrazolium (MTT) and Trypan blue assay. **Study Design:** An experimental study was designed. **Results:** Black tea decoction (10% and 20%) strength has shown significant anti-inflammatory effects (64.8% and 77% reduction, respectively), on carrageenan-induced acute inflammatory models (rat paw edema) which can be comparable with the standard drug indomethacin (89.1%). In a chronic anti-inflammatory model, black tea decoction (10% and 20%) has shown significant suppressive effects on rat paw edema (38.56% and 69.53%) observed on 21st day. Lymphoproliferative action of tea was evaluated on human peripheral mononuclear cells using an MTT assay where the number of living cells were expressed in terms of optical density at 570 nm. An experiment has shown that black tea increases the maximum number of T-lymphocytes at 72 h with a maximum strength of 20%. Maximum number of viable cells (T-lymphocytes) was observed with black tea at 20% strength at 72 h. The results were expressed as mean \pm SD, and the significance was evaluated by Student's *t*-test *versus* control, with $P < 0.05$ implying significance. **Conclusions:** Taken together, our data indicate that black tea has potential anti-inflammatory and immunomodulatory action and this corroborates with the current trend of tea being promoted as a 'health drink'.

Key words: Anti-inflammatory, black tea, immunomodulation

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INTRODUCTION

The search for anti-inflammatory agents with analgesic effects coupled with minimal side effects led the attention

of Indian workers towards another treasure of remedies—indigenous system of medicine. The importance and utility of medicinal plants in the treatment of any chronic disease is well perceived as they have the additional advantage of being cheap, and may be used for a prolonged period. Chopra pioneered the usefulness of many indigenous drugs in inflammatory conditions.^[1] Thereafter, various medicinal plants have been selected for scientific testing and screening of anti-inflammatory action.

Tea (*Camellia sinensis*) has been used as a daily beverage for several years. The active components of tea responsible for such biological effects are known to be catechins (known as polyphenols), which constitute seven forms including epigallocatechingallate (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 leukocytes 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. However, the immunomodulatory effects of tea are slowly being recognized as it is now known that EGCg stimulates the production of Interleukin 1 α , IL1 β , TNF- α in cultured human peripheral blood mononuclear cells (PBMCs). It protects against UV radiation induced immunosuppression and tolerance induction by reducing IL-10 production and increasing IL-12 production in epidermal and dermal cells. However, detailed immunomodulatory effects of tea on immune cells are currently an area of active research.

This study was undertaken to examine the potential immune-modulator and anti-inflammatory activity of black tea in the rat model and in human peripheral mononuclear cells.

Objectives

1. Evaluation of anti-inflammatory effects of black tea on rats.
2. Evaluation of immune-modulator effects of black tea on rats.
3. Evaluation of immune-modulator effects of black tea on *in vitro* human peripheral T-lymphocytes.

MATERIALS AND METHODS

Animals

The entire study was carried out in a post-graduate teaching institute in eastern India, as a part of post-graduate dissertation using Sprague–Dawley adult rats of either sex, weighing between 150 and 200 g. 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-h alternate light and darkness cycle. The protocol was approved and carried out after the permission of Institutional Animal Ethics Committee.

Preparation of the plant product

Black tea leaves were commercially obtained from P and A Arse, Rajdhani Apt. BIK-I Rg Barua Road, Ganeshguri, Guwahati 781 006. For the preparation of tea extract, tea leaves (100 gm) were extracted with ethyl acetate using soxhlet assembly. The extract was concentrated in a rotary flash evaporator under reduced pressure to semisolid mass. Black tea decoction (10% and 20%) was prepared by soaking 10 and 20 g of black tea in 100 ml boiled water separately, soaked for 2 min and thereafter filtered. This filtrate was designated as black tea decoction. The dose of this decoction orally administered to each rat was 0.1 ml/10 g of body weight. An initial pilot study suggested that 20% of this preparation has given best results. Therefore, we have decided to set forward our study with 10% and 20% black tea decoction.

Models for evaluation of anti-inflammatory activity

In all models, the animals were grouped as follows: [Table 1]

Carrageenan-induced rat paw edema

We followed the method adopted by Winter *et al.*, subsequently modified by Ghosh *et al.*^[2,3] Forty-eight 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 18 h before starting of the experiment. Animals of groups 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 subplantar 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.^[4] The paw volume was measured initially at 0 h and subsequently at 1, 2, and 3rd hour after the carrageenan injection. The mean increase in the paw volume was measured hourly and at every time point, the percentage inhibition of edema was calculated.^[5]

Mean increase in paw volume of Mean increase in paw volume of

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

Statistical analysis

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

Dextran-induced rat paw edema

In this model, dextran was used as the phlogistic agent and the methodology of Rowley *et al.*^[6] was used with slight modifications.^[3] 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 were 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 the right hind paw of each rat (Vogel, 1997a). The paw volume was recorded by a plethysmometer initially at 0 h and subsequently hourly at 1, 2, and 3 h after the dextran injection. The percentage inhibition of edema at every time point was calculated as per Vetrichelvan and Jegadeesan.^[5]

Chronic or immunological induced inflammation

Adjuvant-induced arthritis

The method of adjuvant arthritis in rats as described by Pearson *et al.* exhibits many similarities to human rheumatoid arthritis and was accordingly followed as a model of chronic or immunologically induced inflammation.^[7] Male Sprague-Dawley rats with an initial body weight of 130–150 g were used. Sixty rats were taken and divided into five groups of 12 rats each. The groups were treated as follows: [Table 2].

Table 1:

Group (n = 12)	Treatment
Group I	Control
Group II	Standard drug, indomethacin (10 mg/kg)
Group III	10% Black tea decoction
Group IV	20% Black tea decoction

On day 1, 0.1 ml of complete Freund's adjuvant was injected into the subplantar region of the left hind paw of each rat. (Each milliliter of complete Freund's adjuvant contains 1 mg *Mycobacterium tuberculosis* (H37RA, ATCC 25177), heat killed and dried 0.85 ml mineral oil and 0.15 ml Mannide monooleate). Dosing with a test compound or the standard was started on the same day and continued daily orally for 12 days. Paw volumes of both legs and body weight were recorded on the day of injection. On day 5, the volume of the injected paw was measured again, indicating the primary lesions and the influence of therapeutic agents on this paw. The severity of the adjuvant-induced disease was followed by measurement of the non-injected paw (secondary lesions) with a plethysmometer. On day 21, the body weight was determined again and the severity of the secondary lesions was evaluated visually and graded according to the following scheme.

Arthritic index^[8] [Table 3]

Evaluation

- For primary lesions:* the percent inhibition of paw volume of the injected left paw over control was measured at day 5.
- For secondary lesions:* The percent inhibition of paw volume of the non-injected right paw over control was measured at day 21.
- An arthritic index was calculated as the sum of the scores as indicated above for each animal. The average of the treated animals was compared with the control group.

Lymphoproliferative (Blastogenesis) assay with tea decoction

Separation of peripheral blood mononuclear cells from human blood

Human blood was collected in heparin and layered gently upon Ficoll Hypaque solution, the ratio of blood: Ficoll being 5:3. The suspension was centrifuged at 1000 rpm for 45 min. At the interphase of plasma and Ficoll, a buffy coat/ring of cells was identified. This layer was collected in 10 ml PBS and centrifuged at 2000 rpm for 10 min. This procedure was repeated thrice to remove any contaminating Ficoll. The cell pellet was finally resuspended in a 1.0 ml medium (RPMI 1640) supplemented with 10% fetal calf serum and pencillin/streptomycin. The cells were then diluted 1:50 and counted in a hemocytometer. The cell

Table 2:

Group (n = 12)	Treatment
Group I	Control
Group II	Standard drug, Indomethacin (10 mg/kg)
Group III	Standard drug, Dexamethasone (0.1 mg/kg)
Group III	10% black tea decoction
Group IV	20% black tea decoction

count was then adjusted to 1×10^5 cells per ml. The viability of cells was judged with the trypan blue exclusion test.

Quantification of peripheral blood mononuclear cells

The stock solution of cell is diluted with PBS, and 10 μ l of cell suspension is taken on a hemocytometer for a cell counter. Two microliters of 0.2% trypan blue solution is added to it and number of viable and non-viable cells are counted, based on the principal that trypan blue enters only non-viable cells.

Methyl thioazolyl tetrazolium assay

The methyl thioazolyl tetrazolium (MTT) was weighed and then dissolved in 50 μ l DMSO. Then, the RPMI medium was added so as to make a final solution of 10 mg/ml. Ten microliters of MTT was then added to each well; i.e., 100 μ l was present per well. It was then incubated for 4 h at 37 °C in the presence of 5% CO₂ in a carbon dioxide incubator. After 4 h, the plates were gently tilted and the supernatant was removed, and placed in a microcentrifuge tube. To the remaining sample in the well, 100 μ l of DMSO was added. The tubes were subjected to centrifugation at 2000 rpm for 10 min. After centrifugation, the supernatant was discarded and 100 μ l of solution DMSO was added to the centrifuge tube and transferred to the well. The reading of each well was then taken in an ELISA reader

at OD—570 nm.

RESULTS

Black tea decoction (10% and 20%) has shown significant anti-inflammatory effects on carrageenan and dextran-induced acute inflammatory models which can be comparable with the standard drug indomethacin [Tables 4 and 5]. In chronic anti-inflammatory model, black tea decoction (10% and 20%) has shown significant suppression of rat paw edema on 21st day [Tables 6 and 7]. Lymphoproliferative action of tea is evaluated by using [Figure 1] the MTT assay where the number of living cells are expressed in terms of optical density at 570 nm. Different concentrations of tea ranging from 20% to 1% were used. In controls, tea was not added. Subsequently with lower concentrations, lesser degree of proliferation was observed. Two time points, i.e. 72 and 96 h were selected to decide the optimum assay duration. An experiment has shown that tea increases maximum number of T-lymphocytes at 72 h with a maximum strength of 20% [Figure 1]. Lymphoproliferative action of tea was also evaluated by using the Trypan blue exclusion test at 72 and 96 h where the viability of cells (Lymphocytes) were judged. Maximum number of viable cells was observed

Table 3: Arthritic index

Site of lesion	Nature of lesion	Score
Ears	Absence of nodules and redness	0
	Presence of nodules and redness	1
Nose	No swelling of connective tissue	0
	Intense swelling of connective tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
Forepaw	Absence of inflammation	0
	Inflammation of at least one joint	1
Hind paw	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

Table 5: Effects of black tea decoction on dextran-induced rat paw edema

Gr (n = 12)	Dose	Edema volume (ml) (% inhibition)		
		First hour	Second hour	Third hour
Control	—	0.54 \pm 0.05	0.58 \pm 0.05	0.59 \pm 0.05
Indomethacin	10 mg/kg	0.39 \pm 0.12 (27.7%)	0.35 \pm 0.04 (39.65%)	0.05 \pm 0.03 (91.5%)*
Black tea (10%)	1 ml (p.o.)	0.4 \pm 0.1 (25.9%)	0.32 \pm 0.14 (44.8%)	0.16 \pm 0.08 (64.8%)*
Black tea (20%)	1 ml (p.o.)	0.54 \pm 0.07 (0%)	0.51 \pm 0.09 (20.6%)	0.09 \pm 0.08 (85%)*

p.o., per orally. *P < 0.001.

Table 4: Effects of black tea decoction on carrageenan-induced rat paw edema

Gr (n = 12)	Dose	Edema volume (ml) (% inhibition)		
		First hour	Second hour	Third hour
Control	—	0.32 \pm 0.03	0.32 \pm 0.05	0.37 \pm 0.08
Indomethacin	10 mg/kg	0.2 \pm 0.04 (34.27%)	0.15 \pm 0.07 (53.12%)	0.04 \pm 0.04 (89.1%)*
Black tea (10%)	1 ml (p.o.)	0.23 \pm 0.06 (28.12%)	0.2 \pm 0.05 (37.5%)	0.13 \pm 0.05 (64.8%)*
Black tea (20%)	1 ml (p.o.)	0.22 \pm 0.07 (31.25%)	0.14 \pm 0.07 (56.25%)	0.08 \pm 0.08 (78.4%)*

p.o., per orally. *P < 0.001

Table 6: Effects of black tea decoction on Freund's adjuvant induced arthritis in rats

Gr (n = 12)	Dose	Edema volume (ml) (% inhibition)		
		Fifth day	Thirteenth day	Twenty-first day
Control	—	1.37 \pm 0.40	1.36 \pm 0.3	1.3 \pm 0.1
Indomethacin	1 mg/kg	0.70 \pm 0.16 (48.9%)**	0.35 \pm 0.04 (39.65%)	0.98 \pm 0.13 (24%)
Dexamethasone	0.1 mg/kg	0.37 \pm 0.1 (73%)	0.35 \pm 0.12	0.2 \pm 0.1 (84.6%)*
Black tea (10%)	1 ml (p.o.)	1.07 \pm 0.3 (21.90%)	0.32 \pm 0.14 (37.5%)	0.8 \pm 0.08 (38.46%)*
Black tea (20%)	1 ml (p.o.)	0.75 \pm 0.19 (45.26%)**	0.2 \pm 0.07 (56.25%)	0.5 \pm 0.1 (61.53%)*

p.o., per orally. *P < 0.001; **P < 0.05.

with tea at 20% strength at 72 h. All other strengths of tea showed almost equal efficacy at 72 and 96 h.

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*, explored usefulness of indigenous drugs in inflammatory arthritic conditions. In our laboratory, Nag *et al*, and many other workers have screened active principles of a number of plants for this purpose.^[1,9] Accordingly, a study was undertaken to assess the immunomodulatory activity of tea decoction in the rat model. The results obtained in the anti-inflammatory models in this study shows that tea possesses pronounced activity against the acute experimental model 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 [Table 4]. Carrageenan is a sulfated mucopolysaccharide derived from Irish sea moss, *Chondrus*. It was found to be a superior phlogistic agent over other varieties such as 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 bradykinin 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 h.^[10,11] The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents.^[12] 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. Thus, it can be concluded that the anti-inflammatory action of black tea in a carrageenan-induced edema model is by inhibition of histamine, 5-HT, bradykinin, and prostaglandin, individually or in combination. 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-inflammatory activity. Dextran-induced edema is mediated mainly by histamine and 5-HT and dextran is known to be a powerful liberator of histamine.^[6,13] Black tea (20% and 10%) showed an inhibition of 82% and 64.8% [Table 5], respectively, in comparison to the standard drug indomethacin that showed an inhibition of 91.89%. A similar kind of study by Ratnasooriya and Fernando showed statistically significant anti-inflammatory activity of black tea by multiple mechanisms.^[14] In our study also black tea had shown statistically significant anti-inflammatory effects like indomethacin.

Histamine released from mast cells and 5-HT released from platelets also can elicit pain, but they are 50 times less potent than bradykinin.^[15] Prostaglandins sensitize the nerve endings to the effects of bradykinin and other algogens.^[16] In the immunologically mediated chronic

Table 7: Effect of black tea on the arthritic index in a model of Freund's adjuvant induced arthritis

Treatment groups (n = 12)	Dose	Arthritic index at twenty-first day
Control	–	4 ± 0
Indomethacin	1 mg/kg p.o.	3.25 ± 0.5*
Dexamethasone	0.7 mg/kg	1.25 ± 0.58**
Black tea (10%)	1ml (p.o.)	2.75 ± 0.96*
Black tea (20%)	1ml (p.o.)	2.5 ± 0.58**

*P < 0.001, **P < 0.05.

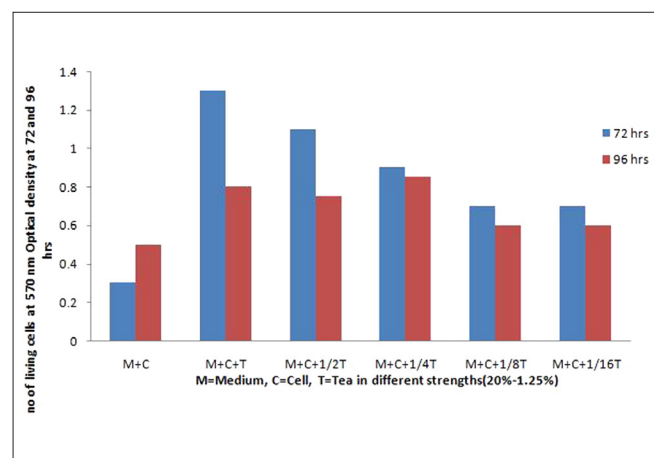


Figure 1: Evaluation of lymphoproliferative action of black tea using MTT assay

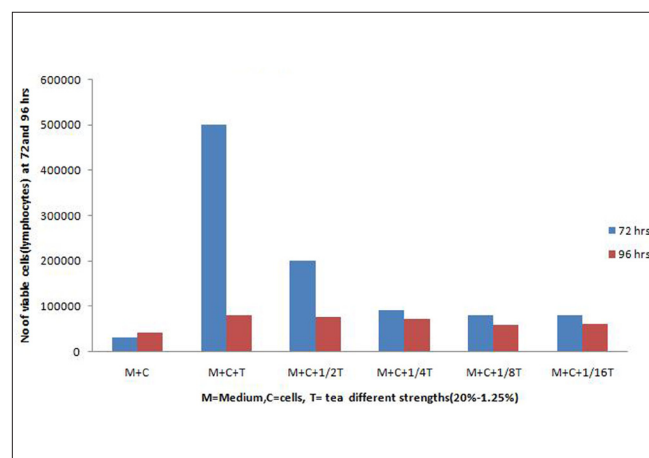


Figure 2: Evaluation of lymphoproliferative action of black tea using the Trypan blue exclusion test

inflammatory model of Freund's adjuvant-induced arthritis, considered as the best available model of rheumatoid arthritis, tea showed a profound degree of anti-inflammatory activity in both the primary and secondary phase.^[17] This procedure has been proposed by several authors to differentiate between anti-inflammatory and immunosuppressive activity.^[18] It has been proposed that those anti-inflammatory compounds capable of inhibiting secondary lesions can be considered as immunosuppressive agents.^[19] In this model of immunologically mediated chronic synovial inflammation and arthritis, macrophages play a central role. After activation, they are capable of synthesizing mediators such as PGE₂ and cytokines such as TNF- α and IL-1. In turn, these synthetic products induce the production of variety of enzymes which initiate cartilage and bone destruction.^[20] Indomethacin and black tea 20% produced a significant inhibition of 51.2% and 45.26%, respectively, on 5th day, which indicates its action in the primary phase [Table 6]. Dexamethasone showed most significant inhibition on 5th and 21st day [Tables 6 and 7]. Black tea (10%, 20%) decoction at above-mentioned doses showed a maximum inhibition of 38.56%, and 69.53%, respectively, and was statistically significant [Table 6]. Therefore, tea definitely has some effects on the immunological and systemic secondary phase of adjuvant arthritis.

Finally, to examine another aspect of its immunomodulatory action, a lymphoproliferative assay was undertaken with black tea decoction. Stock solution was made to 20%. The degree of proliferation was measured by two approaches, namely the MTT assay and Trypan blue dye exclusion assay [Figures 1 and 2]. MTT is methyl thiazolyl tetrazolium, a yellow compound, which in the presence of mitochondrial dehydrogenase is converted into formazan which is purple in color. As only living cells produce this enzyme, by performing this assay, the amount of living cells may be assessed. Hence, the greater the amount of enzyme, the larger will be the amount of formazan produced, more will be the optical density (O.D.). This represents a greater number of living cells. Different concentrations of tea ranging from 20% to 1% were used. As is evident from Figure 1, maximum proliferation was observed at the maximum concentration used, i.e. 20%. Subsequently with lower concentrations, the degree of proliferation was less. From this experiment, we can firmly conclude that tea has stimulatory effects on T lymphocytes, and in 72h gave the best time kinetics. Due to economic constraints, we could not use a mitogen in this study.

The results obtained by the MTT assay were corroborated with a cell count assay using Trypan blue, based on the known principle that Trypan blue enters only nonviable, i.e. dead cells. The cell count assay showed a similar profile

in which maximum proliferation was observed at 72 h [Figure 2] using the highest concentration of tea. Black tea (20%) decoction displayed maximum immunomodulatory (immunostimulatory) properties in our *in vitro* system.^[21]

Taken together, our data indicate that black tea has potential acute and chronic anti-inflammatory action. This corroborates with the current trend of tea being promoted as 'health drink'. Our results support the idea that tea has a beneficial effect. Significant antiarthritic activity was observed with regular administration of black tea 10% and 20% in the Freund's adjuvant-induced model of arthritis. Roy *et al.*, stated that chronic treatment with Black tea (in arthritic rats) resulted in a decrease of paw diameter and tissue lipid peroxidation, along with a restoration of GSH, catalase, and superoxide dismutase levels.^[22] Ratnasooriya and Fernando showed that black tea possessed strong, oral gastric ulcer healing activity which is mediated *via* multiple mechanisms.^[23]

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 4 weeks, preliminary studies can be undertaken with normal healthy volunteers.

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