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Anti-inflammatory activity of Sri Lankan black tea (*Camellia sinensis* L.) in rats

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ABSTRACT

This study examined the anti-inflammatory potential of Sri Lankan black tea (*Camellia sinensis* L. Family: Theaceae) using both acute (carrageenan-induced paw oedema) and chronic (formaldehyde-induced paw oedema and cotton pellet granuloma test) rat inflammatory models. Three dose of black tea brew (BTB) [84 mg/ml, equivalent to 1.5 cups; 168 mg/ml, equivalent to 3 cups; and 501 mg/ml, equivalent to 9 cups] were made using high grown unblend Dust grade No: 1 black tea samples and was orally administed to rats (n = 6-9/ dose/ test). The results showed that Sri Lankan BTB possesses marked and significant ($P < 0.05$) oral anti-inflammatory activity against both acute and chronic inflammation. This anti-inflammatory activity was dose-dependent in the carrageenan-induced paw oedema test and cotton pellet granuloma test. Further, in the carrageenan paw oedema model, the anti-inflammatory activity of BTB was almost identical to green tea brew of both Chinese and Japanese types. Further, the BTB had significant antihistamine activity (in terms of wheal test) phagocytic cell migration inhibitory activity (in terms carrageenan-induced leucocyte peritoneal infiltration test), nitric oxide production inhibitory activity, antioxidant activity (DPPH method) and prostaglandin synthesis inhibition activity (in terms of rat enteropooling test). It is concluded that Sri Lankan black tea has marked anti-inflammatory potential against both acute and chronic inflammation which is mediated via multiple mechanisms.

KEY WORDS: *Camellia sinensis*; black tea; anti-inflammatory activity; anti-inflammation

INTRODUCTION

Tea which is made from the topmost immature leaves and the buds of the perennial evergreen shrub, *Camellia sinensis* (L) O. Kuntz (Family: *Theaceae*) is the most widely consumed drink in the world besides water (1). Depending on the manufacturing technique there are three main types of teas. Black (fully aerated or fermented), green (unaerated or unfermented) and oolong (partially aerated or semifermented) (1). Tea and health have been inextricably linked. There is an increasing interest on the role of tea in maintaining health and treating disease. Many health benefits of tea are now scientifically shown (1,2). One such potential health benefit attributed to tea, particularly to the green type, is anti-inflammatory activity (2). This is an important and an useful bioactivity of tea because inflammation is a common medical condition for which available drug therapies are poor (3): current anti-inflammatory therapies rely heavily on non steroidal anti-inflammatory drugs, steroids and board spectrum immunosuppressives, an unacceptable position that is increasingly leading to the characterization and use of biologicals and neutracuticals. Tea falls within the latter category and receiving considerable attention as an anti-inflammatory agent. However, the anti-inflammatory activity of Sri Lankan black tea has not been tested and reported although Sri Lanka is the second largest

producer and exporter of tea (4). This is worth examining since several factors such as the country of origin, the geological background of soil, the elevation of the tea plantation, the collecting season, technological processes during tea production and brewing conditions affects the final chemical composition of tea brew (5,6,7) and hence its pharmacological effects.

Therefore, the study reported herein was initiated to examine the anti-inflammatory potential of Sri Lankan black tea in rats using high grown Dust grade No: 1 black tea. The Dust grade was selected, as it is the most widely consumed type of tea by Sri Lankans.

MATERIALS AND METHODS

Experimental animals

Healthy adult Wistar male rats (weight: 200 – 250 g), and male mice of ICR strain (weight: 35-40 g) purchased from the Medical Research Institute Boralla, Sri Lanka were used. The animals were kept under standardized animal house conditions (photoperiod: approximately 12h natural light per day; temperature 28-30 ^{0}C; relative humidity; 50-55%) with free access to tap water and pelleted food (Master Feed Ltd., Colombo, Sri Lanka). All animals experiments were conducted in accordance with the internationally accepted laboratory use and care, and guidelines and rules of the Faculty of Science, University of Colombo, for animal experimentations.

Manufacture of tea samples

The black tea belonging to the grade of Dust No: 1 was manufactured at St. Coombs estate tea factory of the Tea Research Institute, Talawakelle, Sri Lanka, with its own green leaves (1382 m above mean sea level) using the orthodox- rotovane manufacture technique. The Chinese type of green tea has been manufactured at Gowarakelle estate (1280 m above mean sea level), Bandarawela, Sri Lanka by subjecting the shoots to heat by steaming and bypassing the typical fermentation and drying processes. The Japanese type of green tea was manufactured at the Idulgushinna estate (1885 m above mean sea level), Bandarawela, Sri Lanka by dropping the green shoots on to a heated pan and then bypassing fermentation and drying processes. Tea samples were packed in triple laminated, aluminum foil bags, (1 kg each) and stored at –20 ^{0}C until use.

Preparation of tea brews- Black tea brew (BTB) and green tea brew (GTB) were made according to the ISO standards (8): adding 2 g of respective tea samples to 100 ml of boiling water and brewing for 5 min [yield (w/w) for BTB: 43.7%; GTB (Chinese type): 49.5% (Japanese type): 46.6%]. Based on this data 501 mg/ml (equivalent to 9 cups, 1 cup = 170 ml) of BTB, 610 mg/ml (equivalent to 9 cups)of Chinese type GTB and 580 mg/ml (equivalent to 9 cups) of Japanese type of GTB in 2 ml were made by adding respectively 8 g black tea and 6 g of green tea (both types) to 20 ml of boiling water and brewing for 5 min. 167 mg/ml (equivalent to 3 cups) and 84 mg/ml (equivalent to 1.5 cups) concentrations of BTB were then made by diluting appropriately with boiling water. The doses of BTB and GTB selected were identical to what has been used previously for investigation of bioactivities of Sri Lankan tea (9).

Effect on carrageenan-induced paw oedema

Sixty three male rats were selected and randomly divided into seven groups (n = 9/ group). The rats were orally treated in the following manner; group 1: 2ml of water, group 2: 84 mg/ ml of BTB, group 3: 167 mg/ ml of BTB, group 4: 501 mg/ ml of BTB, group 5: 610 mg/ ml of Chinese type of GTB, and group 6: 580 mg/ ml of Japanese type GTB, group 7: indomethacin (State Pharmaceutical Corporation, Colombo, Sri Lanka) (4 mg/kg). After 1 h, 0.05 ml of 1% carrageenan (Sigma Chemicals Company, St' Louis, Mo, USA) suspension was injected subcutaneously into the plantar surface of the left hind paw of each of these rats under mild ether anesthesia. The volumes of the carrageenan injected paws of these rats were measured 1 h prior to the injection of carrageenan and at hourly interval for 6 h after the injection using a plethysmometer (Letica Scientific Instruments, Barcelona, Spain) (10).

Effect on formaldehyde-induced paw oedema

Twenty four male rats were randomly assigned into four equal groups (n = 6/group). The rats were orally treated in the following manner; group 1: 2ml of water, group 2: 84 mg/ ml of BTB, group 3: 167 mg/ ml of BTB, and group 4: 501 mg/ ml of BTB for 7 consecutive days. On days 1 and 3 of the treatment, all these rats were injected with 0.1 ml of 2% formaldehyde (Sigma Chemicals Company, St' Louis, Mo, USA) in normal saline into the plantar surface of the left hind paw under mild ether anaesthesia. The paw volumes of these rats were measured prior to the injection of formaldehyde, at 4 h after the injection on day 1 and at 1 h of oral treatment of BTB from days 2-7. On day 3 of the treatment, the paw volume was measured before the injection of formaldehyde (10).

Effect on cotton pellet grannuloma **-**Twenty four

male rats were randomly assigned into four equal groups (n = 6/group). An autoclaved cotton pellet (5 mg) was implanted subcutaneously, on each rat above the scapula region, under ether anesthesia using aseptic precautions. These rats were then orally treated in the following manner; group 1: 2ml of water, group 2: 84 mg/ ml of BTB, group 3: 167 mg/ ml of BTB, group 4: 501 mg/ ml of BTB for 7 consecutive days starting from the day of cotton pellet implantation. On day 8, these animals were anaesthetised and the cotton pellets along with granulomas were removed and dried in an oven at 60 ^{0}C until a constant weight was obtained (11).

Evaluation of antihistamine activity

Eighteen rats were randomly assigned into two equal group (n = 9/ group). The left posterior lateral side of their skins were clearly shaved under aseptic conditions. One group was orally treated with 501 mg/ml of BTB and the others with 2 ml water. After 1 h, 50 µl of 200 µg/ ml of histamine (Fluka, Buchs, Switzarland) in normal saline was subcutaneously injected to the shaved area of the skin and the area of the wheal formed was determined after 1.5 min (12).

Evaluation of carrageenan-induced migration of phagocytes to peritoneal fluid

Twelve mice were randomly assigned into two equal group (n = 6/ group). One group was orally treated with 501 mg/ml of BTB and the other with 2 ml of water. After 1 h, carregeenan was injected into the peritoneal cavity under ether anesthesia. Four hours later, 10 ml of sterile 1X phosphate buffered saline (PBS) was injected into the peritoneal cavity of each of these mice. After 5 min, 5-8 ml of peritoneal fluid was drained using 18 G cannula and was centrifuged at 3000 rpm for 10 min at 4 ^{0}C. The supernatant was removed and the peritoneal cells were resuspended in 1 ml of 1 X PBS. A 50-µl aliquot of the cell suspension was mixed with 10 µl of 1% Neutral Red to visualize the phagocytic cells. Phagocytic/macrophage cell counts were made using a haemocytometer (Fison Scientific Equiments, Loughborough, UK) (13).

Evaluation of nitric oxide production by peritoneal cells

Twelve rats were randomly assigned into two equal group (n = 6/ group). One group was orally treated with 501 mg/ml of BTB and the other with 2 ml of water. After 1 h, 0.05 ml of carrageenan was injected into the peritoneal cavity of each of these rats under ether anesthesia. Two hours later, 40 ml of sterile 1X PBS was injected into their peritoneal cavities. After 5 min, 30-40 ml of peritoneal fluid was drained using 18 G cannula, and centrifuged at 150 X g for 10 min at 4 ^{0}C. The supernatant was removed and the peritoneal cells were resuspended in 1 ml of 1 X PBS. Assay for nitric oxide production was performed as described by Nacife et. al., 2004 (13). The peritoneal cells were plated in 96 well tissue culture plates at 1x 10^6 cells/ml in RPMI 1640 medium (GIBCO BRL, Life Technologies) supplemented with 1% bovine serum albumin (Sigma Chemicals Company, St' Louis, Mo, USA). From each animal, cells were plated in triplicate and incubated at 37 ^{0}C in 5% CO_2 incubator (MCO 175, Sanyo electric. Co. Ltd. Tokyo, Japan). After 24 hours, the culture supernatant was aspirated from each well, centrifuged at 15000 X g for 5 min and the clear supernatant was then assessed for production of nitric oxide. For quantification of nitric oxide, 100 µl of culture supernatant was mixed with an equal volume of Griess reagent (mixture of equal proportion 1% sulphanilamide in 5% phosphoric acid and 0.1% n-(1-naphthyl) ethylenediamine hydrochloride in DW), incubated at 25 ^{0}C for 15 min and optical density was read at 540 nm in a ELISA plate reader (ELX 800, Bio-Tek Instruments INC, USA). The nitric oxide concentration was calculated using calibration curve between 0.7-100 µM $NaNO_2$. (13).

Effect on membrane stabilization

This activity was evaluated using heat-induced haemolysis of rat erythrocytes *in vitro* as described by Ratnassoriya et. al. 2002 (10). Vials containing 20 µl fresh rat blood in 1 ml of phosphate buffered saline were treated in triplicate with the BTB so that the final concentrations of the tea brew in the vials were 2.5, 5, 10 and 20 mg/ml. Fifteen microliter of saline was used as the control. The vials were then incubated for 15 min at 37 ^{0}C followed by 54 ^{0}C for 25 min, centrifuged at 3200 x g for 2 min and the absorbance of the supernatant was measured at 540 nm using a spectrophotometer (Jasco V560, Jasco Corporation, Tokyo, Japan). The percent inhibition of heamolysis with respect to the controls was calculated.

Evaluation of the antioxidant activity (DPPH assay)

This was done using 750 µl of freshly prepared 20ppm of 1-1-diphenyl-2-picrylbhydrazyl (DPPH) solution as described in detail by Abeywickrama et al., 2005 (14). Briefly, 3 concentrations of BTB (84, 167, 501 mg/ml) and one concentration of GTB, both Chinese (610 mg/ml) and Japanese (580 mg/ml) types were made,

and 750 µl of these samples were added to 750 µl of DPPH solution (in triplicate) and incubated at 30 ^{0}C for 5 min. Absorbance was then measured at 517 nm using a spectrophotometer. The percentage of the DPPH radical scavenged by the tea extracts was calculated, and the antioxidant activity was expressed as the Trolox equivalent in µgl^{-1}

Effect on small intestinal secretion

Intestinal secretion was indirectly evaluated by the enteropooling assay described by vitali et al. 2005 (15). Briefly, 18 mice were randomly divided into three groups (n = 6/group). Mice in group 1 were orally treated with 0.2 ml of water, group 2 with 0.2 ml water and group 3 with 501 mg/ml of BTB. Forty minuits later, mice in groups 2 and 3 were orally administed with 0.2 ml of castor oil. After 30 min, all the mice were killed with ether and their small intestines were removed and weighed. The weights were then expressed as mg/20g body weight. The difference in the intestinal weight between the normal control and caster oil treated control was considered as the caster oil-induced accumulation of intestinal fluid.

Statistical analysis

Data are given as means ± SEM. Statistical comparisons was made using the Mann-Whitney U-test. $P \leq 0.05$ was considered as indicating significance.

RESULTS

Effect on carrageenan-induced paw oedema

The results are summarized in Table 1. As shown, the low dose of BTB did not significantly ($P > 0.05$) impair paw oedema. On the other hand, compared to control, both mid dose (by 6-39%) and high dose (by 54-76%) of BTB significantly ($P < 0.05$) inhibited the paw oedema at each time point measured. This effect was dose-dependent (1st h: $r^2 = 0.96$, $P < 0.05$; 2nd h : $r^2 = 0.99$, $P < 0.05$; 3rd h : $r^2 = 0.95$, $P < 0.05$; 4th h : $r^2 = 0.92$, $P < 0.05$; 5th h : $r^2 = 0.85$, $P < 0.05$; and 6th h : $r^2 = 0.86$, $P < 0.05$). The high dose of GTB, both Chinese (by 54-78%) and Japanese (by 53-77%) types significantly ($P < 0.05$) suppressed the paw oedema at all time points with similar magnitudes as the high dose of BTB.

Effect on formaldehyde-induced paw oedema

As shown in Table 2, all the three doses of BTB tested (except on day 1 with low dose and on day 3 with mid dose) significantly ($P < 0.05$) reduced the paw oedema induced by the two formaldehyde injections (low dose by 38-86%; mid dose by 18-44%; and high dose by 31-58%). This effect was however not dose-dependent.

Effect on cotton pellet granuloma - As shown in Table 3, all the three doses of BTB significantly ($P< 0.05$) and markedly (by 93-98%) reduced the weight of the granuloma formed enclosing the implanted cotton pellet.

Evaluation of antihistamine activity

As shown in Table 4, the high dose of BTB significantly ($P < 0.05$) reduced (by 33.4 %) the area of the wheal formed after injection of histamine.

Migration of phagocytes to peritoneal fluid

As shown in Table 5, the high dose of BTB significantly ($P < 0.05$) and profoundly inhibited the number of phagocytic cells infiltrating in to the peritoneal cavity induced by peritoneal injection of carrageenan to mice.

Nitric oxide production

As shown in Table 6, the BTB dose-dependently ($r^2 = 0.79$; $P < 0.05$) inhibited the *in vitro* nitric oxide production by the peritoneal cells.

Plasma membrane stabilization activity

In the rat heat-induced haemolysis test, the tested concentrations of BTB failed to significantly ($P > 0.05$) inhibit haemolysis (Table 7).

Antioxidant activity (DPPH assay)

As shown in Table 8, BTB at tested concentrations, exhibited dose dependent ($r^2 = 0.78$; $P < 0.05$) *in vitro* antioxidant activity.

Small intestinal secretion

As shown in Table 9, oral administration of castor oil significantly ($P < 0.05$) increased the intestinal fluid secretion compared with the normal control. On the other hand, the high dose of BTB significantly ($P < 0.05$) inhibited the castor oil-induced intestinal secretion.

DISCUSSION

This study examined the anti-inflammatory potential of Sri Lankan black tea (Dust grade No: 1) using both acute (carrageenan-induced paw oedema) and chronic (formaldehyde-induced paw oedema and cotton pellet granuloma tests) animal inflammatory models. These models are widely accepted as sensitive and reliable phologistic tools for investigating potential anti-inflammatory agents. The results showed, for the first time, that Sri Lankan black tea possesses marked oral anti-inflammatory activity against both acute and chronic inflammation. The anti-inflammatory activity can be attributed to theaflavins, thearubigins and other polyphenols present in BTB (6, 16, 17). Many

molecular targets that lead to inflammation have been shown to be affected by tea (18). This anti-inflammatory activity was dose-dependent in the carrageenan-induced paw oedema test and the cotton pellet granuloma test. Further, in the carrageenan model (both BTB and GTB were tested only in this model) the anti-inflammatory activity of BTB was almost identical to green tea brew (both Chinese and Japanese types). This is an interesting finding because it is generally presumed that anti-inflammatory potential of green tea is superior to that of black tea and it is also suggested that green tea may have a higher benefit in treating inflammatory disorders (18, 19). The reason for the equipotancy of anti-inflammatory activity between Sri Lankan black tea and green tea brews is unknown at present but could be related to its phytochemicals composition (1, 6). In this regard, it is of interest to note that black tea polyphenols have antioxidant activity comparable to green tea polyphenolic catechin (1) and many biological activities of black tea may be practically related to its antioxidant properties (19).

In the carrageenan-induced paw oedema test the development of oedema (inflammatory response) is a biphasic event with a maintence phase in between (2-3 h): initial non phagocytic exudative inflammatory phase lasting upto 2h and a delayed phagocytic inflammatory phase from 3-5 h (20). The initial phase is primarily mediated by histamine, serotonin and increase in prostaglandin synthesis in the surroundings of the damaged tissue while the late phase is mediated by leukotrienes, mobilized phagocytic cells, polymorphonuclear cells, monocytes, macrophages, prostaglandins produced by tissue macrophages, oxygen free radicals, nitric oxide, proteolytic enzymes and platelet activating factor (20, 21, 22, 23). The oedema maintained between the initial and the late phase (2-3 h) is due to kinin-like substances, especially bradykinin (20, 21). BTB impaired all these phases simultaneously. Curtailment of the initial phase by BTB can be attributed at least, in part, to its antihistamine activity: BTB exhibited marked antihistamine activity, when evaluated by the wheal test. It could also result from inhibition of prostaglandin synthesis. Black tea is known to inhibit cyclooxygenase activity (24) and reduction of intestinal weight in the castor oil experiment (enteropooling assay) in the present investigation also suggests prostaglandin synthesis inhibition (25). On the other hand, this impairment of the initial phase of inflammation is unlikely to be due to inhibition of serotonin since theanine is black tea has been shown to raise serotonin level in various important brain regions (26). Curtailment of the maintenance phase suggests that BTB had inhibited kinin synthesis and or release.

Inhibition of the late phase by BTB can be mediated by several mechanisms. BTB showed marked and dose-dependant antioxidant activity. Tea is one of the most potent natural antioxidants (1). Obviously, this antioxidant action of BTB can be linked to its anti-inflammatory action in the late phase. BTB inhibited the nitric oxide production. Further, black tea polyphenols are known to inhibit expression of nitric oxide synthese (24). Inhibition of nitric oxide is likely to be another mechanism via BTB induced anti-inflammation in the late phase of the carrageenan induced paw oedema test. Nitric oxide is implicated with inflammation (23).

Anti-inflammatory activity of BTB could have also mediated from inhibition of phagocytic cell migration as evident from the *in vivo* peritoneal phagocytic infiltration assay, where the number of peritoneal phagocytic cells were reduced following oral administration of BTB. Phagocytic cell migration is a vital event in inflammatory pathway (27). Cytokines play a pivotal role in inflammation (27). Constituents in tea are known to suppress gene expression of cytokines like tumor necrosis factor (28), interleukin 1α (29) and it is possible that such a mechanism operates in this study as well in inhibiting inflammation. Membrane stabilization effect is an another potential mechanism inducing anti-inflammatory activity (27). However, BTB did not show membrane stabilization activity in rat heat-induced haemolysis test indicating that the release of lysosomal enzymes may not have been inhibited in BTB induced anti-inflammatory action: lysosomes play a vital role in the inflammatory reaction by releasing their enzymes (27).

In addition to these specific mechanisms, several other nonspecific mechanisms may account for the simultaneous and almost equal inhibition of early and late phases of the carrageenan-induced paw oedema induced by BTB. Diuresis is one such mechanism (30). Sri Lankan BTB has been shown to have diuretic activity (31) and this mechanism is likely to be operative in this study. Opioid agonists have acute anti-inflammatory action (32) and BTB has been shown to act via opioid mechanisms in inducing antinociception action in rats (33). A possibility thus

Table 1: The effect of oral treatment of black tea brew and green tea brew of Camellia sinensis on carrageenan-induced paw oedema in rats (mean ± SEM)

Treatment	Dose	Inflammation (increased paw volume) ml					
		1 h	2 h	3 h	4 h	5 h	6 h
Control	2 ml water	0.55 ± 0. 01	0.94 ± 0. 02	1.11 ± 0.01	1.26 ± 0.01	1.14 ± 0.09	1.17 ± 0.02
Black tea brew							
Low	84 mg/ml	0.53 ± 0.02	0.93 ±0.02	1.10 ± 0.02	1.23 ± 0.05	1.16 ± 0.03	1.08 ± 0.02
Mid	167 mg/ml	0.41 ± 0.03*	0.59 ± 0.03*	0.87 ± 0.02*	1.05 ± 0.02*	1.07 ± 0.02*	0.99 ± 0.02*
High	501 mg/ml	0.16 ± 0.03*	0.23 ± 0.02*	0.36 ± 0.02*	0.51 ± 0.03*	0.52 ± 0.02*	0.49 ± 0.01*
Green tea brew							
Chinese type	580 mg/ml	0.13 ± 0.03*	0.21 ± 0.02*	0.34 ± 0.03*	0.47 ± 0.07*	0.52 ± 0.06*	0.51 ± 0.01*
Japanese type	610 mg/ml	0.15 ± 0.03*	0.23 ± 0.03*	0.36 ± 0.03*	0.50 ± 0.07*	0.54 ± 0.05*	0.53 ± 0.01*

*As compared to control *P< 0.05*

Table 2: The effect of oral treatment of black tea brew of Camellia sinensis on formaldehyde-induced paw oedema in rats (mean ± SEM)

Treatment	Dose	Inflammation (increased paw volume) ml							
		4 h	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	2 ml water	0.43 ± 0.001	0.35 ± 0.002	0.33 ± 0.003	0.14 ± 0.002	0.32 ± 0.001	0.32 ± 0.004	0.33 ± 0.002	0.30 ± 0.002
Black tea brew									
Low	84 mg/ml	0.38 ± 0.002*	0.36 ± 0.002	0.13 ± 0.002*	0.02 ± 0.001*	0.20 ± 0.007*	0.20 ± 0.002*	0.18 ± 0.002*	0.17 ± 0.001*
Mid	167 mg/ml	0.24 ± 0.001*	0.32 ± 0.001*	0.27 ± 0.001*	0.15 ± 0.001	0.24 ± 0.002*	0.21 ± 0.008*	0.20 ± 0.006*	0.18 ± 0.002*
High	501 mg/ml	0.29 ± 0.002*	0.32 ± 0.002*	0.14 ± 0.004*	0.07 ± 0.001*	0.22 ± 0.004*	0.20 ± 0.003*	0.19 ± 0.005*	0.18 ± 0.008*

As compared to control * P < 0.05

Table 3: The effect of oral treatment of black tea brew on cotton pellet granuloma in rats (mean ± SEM)

Treatment	**Granuloma (mg)**	**% of inhibition**
Control		
Water (2 ml)	3.52 ± 0.24	-
Black tea brew		
Low dose (84 mg/ml)	0.26 ± 0.008*	92.61
Mid dose (167 mg/ml)	0.23 ± 0.030*	93.46
High dose (501 mg/ml)	0.08 ± 0.006*	97.72

*As compared to control *P < 0.05*

Table 4: Anti-histaminic activity of high dose (501 mg/ml) at black tea brew following oral administration to rats (mean ± SEM)

Treatment	Area of wheal (mm^2)
Control	
Water (2 ml)	48.77±1.12
Black tea brew	
High dose (501 mg/ml)	32.44±0.59*

As compared to control *P < 0.05

Table 5: The effect of oral treatment of high dose of (501 mg/ml) black tea brew on carrageenan-induced peritonitis in mice (mean ± SEM)

Treatment	Leukocytes (x 10^5 ml^{-1})	Leukocytes Inhibition (%)
Control (2ml of water)	6.68 ± 0.13	-
High dose of BTB (501 mg/ml)	3.57 ± 0.06*	46.62

As compared to control *P < 0.05

Table 6: In vitro nitric oxide activity of Sri Lanka black tea brew as determined by Nitric Oxide assay (mean ± SEM)

BTB Concentration (µg/ml)	% Inhibition
Distilled water	-
1000	74.98
500	77.76
250	31.91
125	29.13
62.5	2.73
31.2	23.57
15.6	19.40
7.8	29.13

BTB = Black tea brew

Table 7: Effect of Dust grade No: 1 black tea brew on membrane stabilization of rat erythrocytes in vitro (mean ± SEM)

Concentration (mg/ml)	% Inhibition
PBS	51.3 ± 0.20
2.5	50.5 ± 0.20
5	52.3 ± 0.20
10	50.3 ± 0.21
20	51.5 ± 0.25

PBS = phosphate buffered saline

Table 8: In vitro antioxidant activity of Sri Lanka black tea brew as determined by DPPH assay (mean ± SEM)

Tea sample	Antioxident activity (Trolox equivalents µg/l)
Black tea brew	
Low concentration (83 mg/ml)	2985 ± 6.0
Mid concentration (167 mg/ml)	3572 ±86.5
High concentration (501 mg/ml)	3923 ±6.5

DPPH = 1-1-diphenyl-2-picrylbhydrazyl

Table 9: Effect of oral administration of 501 mg/ml black tea brew on castor oil-induced enteropooling in mice (mean ± SEM)

Treatment	Small intestine weight (mg/20g)	Castor oil-induced intestinal fluid accumulation (mg)
Normal control (water)	829.4 ± 2.3	-
Castor oil control (0.2 ml castor oil + water)	1337.2 ± 2.8[a]	507.8
501 mg/ml of BTB (0.2 ml castor oil + 501 mg/ ml BTB)	1029.3 ± 3.5[a b]	199.9

[a] *P < 0.05 compared to normal control ,* [b] *P < 0.05 compared to castor oil control*

exits that BTB may have acted through opioid mechanisms in producing anti-inflammatory action. Phospholipase A_2 inhibitors suppress both phases of the inflammatory response in the carrageenan-induced paw oedema model (34) as evident in this study. A similar mode of action is possible with BTB. BTB is rich in flavonoids (1, 6), which are powerful inhibitors of phospholipase A_2 (35, 36). Alternatively, such a response may results from BTB-induced release of glycocorticoids (37). The impairment of granuloma formation in cotton pellet test provides indirect evidence in favour of this glucocorticoid related mechanisms (37).

BTB induced anti-inflammatory activity when evaluated in both formaldehyde-induced paw oedema model and cotton pellet granuloma test. This indicates that BTB is effective against the establishment of chronic inflammation which happens at the later stage of acute inflammation (27). Further, showing anti-inflammatory action in formaldehyde-induced paw oedema model and cotton plate granuloma test is claimed to reflect genuine anti-inflammatory action (30, 38). All the BTB induced specific mechanisms responsible for acute anti-inflammatory actions mentioned earlier can play key roles in counteracting this chronic inflammation as well. Since anti-inflammatory action of BTB was not tested in adjuvant-induced arthritis model, it is not known whether it is effective against rheumatoid arthritis which is worth examining. Non steroidal anti-inflammatory drugs which are widely used in inflammatory conditions produce gastric lesions (27). BTB dose not induce gastric lesions and infact is gastroprotective (33). This is an added advantage to the anti-inflammatory action of BTB.

In conclusion, this study demonstrates, for the first time, promising oral anti-inflammatory activity of Sri Lankan black tea which is equally effective as the green tea. This is an important finding which can have health benefits.

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