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Antidiarrhoeal activity of Sri Lankan Dust grade Black Tea (*Camellia sinensis* L.) in mice

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ABSTRACT
This study examined the antidiarrhoeal potential of Sri Lankan black tea (*Camellia sinensis*) in mice using high grown unblend Dust grade No: 1 tea. Different concentrations of black tea brew (BTB) [84 mg/ ml (equivalent to 1.5 cups), 167 mg/ ml (equivalent to 3 cups), 501 mg/ ml (equivalent to 9 cups) or 1336 mg/ ml (equivalent to 24 cups)], or a high concentration (equivalent to 9 cups) of green tea brew (GTB) of Chinese and Japanese types or reference drug, loperamide (10 mg/ kg) were orally administered to different groups of mice (N = 9-12/group) and were subjected to two antidiarrhoeal tests: normal defecation test and castor oil-induced diarrhoea test. The results show that BTB of Sri Lankan Dust grade tea dose-dependently and markedly decreased the number of faecal boluses produced in the normal defecation test and improved the severity of the diarroheal condition in the castor oil-induced diarrhoea test. However, the antidiarrhoeal effect of BTB was superior to Japanese type of GTB and inferior to loperamide. BTB also prolonged the gastrointestinal transit time, impaired intestinal fluid secretion, increased intestinal fluid absorption and reduced *in vitro* nitric oxide production. It is concluded that Sri Lankan black tea possesses marked antidiarrhoeal activity supporting the folkloric claim that Sri Lankan black tea is a good remedy for acute non specific diarrhoea.
Key words: Black tea; *Camellia sinensis*; antidiarrhoea; diarrhoea; Sri Lankan tea

INTRODUCTION

Tea, which is manufactured from the topmost immature leaves and the buds of *Camellia sinensis* (L.). O. Kuntz (Family: Theaceae) plant (1) is the most popular beverage of the world today. Depending on the manufacturing process there are three major types of tea: black (fully aerated or fully fermented), green (unaerated or unfermented) and oolong (partially aerated or semifermented) (1). According to Sri Lankan folkloric medicine black tea brew is a good remedy for acute nonspecific diarrhoea. However, in Sri Lankan traditional and Ayurvedic medicine black tea is not indicated for the treatment of diarrhoea (2). In a recent study conducted in India, it has been shown that black tea brew made from Indian grade of BOP (Broken Orange Pekoe) black tea possesses marked antidiarrhoeal activity (3). However, this finding does not necessarily mean that Sri Lankan black tea also possesses antidiarrhoeal activity since final composition of tea brew and hence its pharmacological properties are known to vary with several factors such as country of origin, geographical background of soil, the cultivating method, the collection season, the age of the leaves, grades of tea and brewing conditions such as time and temperature (4, 5, 6).

Therefore, this study was undertaken to investigate whether Sri Lankan black tea possesses antidiarrhoeal activity. This was tested in mice using unblend Sri Lankan high grown Dust grade No: 1 black tea. The Dust grade was selected as it is the most popular and widely consumed type of black tea in Sri Lanka.

MATERIALS AND METHODS

Experimental animals

Healthy adult ICR mice (25-30 g) and rats (180 -200 g) purchased from Medical Research Institute, Colombo, Sri Lanka were used. These animals kept under standardized animal house conditions (temperature: 28-31 ^{0}C, photoperiod: approximately 12 hours of natural light per day, relative humidity 50-55%) at the animal house of the Department of Zoology, University of Colombo. All animals had free access to pelleted

food (Ceylon Grain Elevators, Colombo, Sri Lanka) and domestic tap water. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care (based on Helsinki convention), and guidelines and rules of the Faculty of Science, University of Colombo, for animal experimentations.

Manufacture of black 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 was manufactured at Gowerakelle Estate (1280 m above mean sea level), Bandarawela, 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 Idalgashinna Estate (1885 m above mean sea level), Bandarawela, 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 $^{\circ}$C until use.

Preparation of Black tea brew (BTB) and Green tea brew (GTB)

Black tea brew (BTB) and green tea brew (GTB) were made according to the ISO standards (7): 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 0.5 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.

Effect of BTB on normal defaecation in mice

Seventy two mice were randomly divided into 8 equal groups (N = 9/group) and orally treated in the following manner; group1 with 0.5 ml of water; group 2 with low dose of BTB (84 mg/ ml)) ; group 3 with mid dose of BTB (167 mg/ ml) ; group 4 with high dose of BTB (501 mg/ ml) ; group 5 with supraphysiological dose of BTB (1336 mg/ml) ; group 6 with high dose of Chinese type of GTB (610 mg/ml) ; group 7 with high dose of Japanese type of GTB (580 mg/ml). After treatment these animals were placed individually in cages with clean filter paper at the bottom. The numbers of faecal boluses expelled during 1st and 2nd hour were counted (3).

Effect of BTB on castor oil-induced diarrhoea in mice

Ninty six mice were randomly assigned in to 8 equal groups (n = 12/group) and fasted for 12 h before the experiment. These animals were orally treated in the following manner: group1 with 0.5 ml of water; group 2 with low dose of BTB (84 mg/ ml); group 3 with mid dose of BTB (167 mg/ml); group 4 with high dose of BTB (501 mg/ ml); group 5 with supraphysiological dose of BTB (1336 mg/ ml); group 6 with high dose of Chinese type of GTB (610 mg/ ml); group 7 with high dose of Japanese type of GTB (580 mg/ ml);and group 8 with 10 mg/kg of loperamide, reference antidiarrhoeal drug. After treatment, these animals were placed individually in cages with clean filter paper at the bottom. One hour later, each mouse was challenged orally with 0.5 ml of castor oil. One hour later, the mice were scored for copious (++), mild (+) or (0) lack of diarrhoea at hourly intervals for 8 hours. The diarrhoea score was calculated by taking the sum of the number of '+' mice and twice the number of '++' mice. Thus, for a group of 12 mice, the maximum score indicating severe diarrhoea is 24. A score of zero indicated no diarrhoea (3).

Effect of BTB on gastrointestinal transit time

Eightyfour mice fasted for 24 h and randomly assigned into seven equal groups (n = 12/group). These mice were treated in the following manner; group1 with 0.5 ml of water; group 2 with low dose of BTB (84 mg/ ml); group 3 with mid dose of BTB (167 mg/ ml); group 4 with high dose of BTB (501 mg/ ml); group 5 with supraphysiological dose of BTB (1336 mg/ ml); group 6 with high dose of Chinese type of GTB (610 mg/ ml); group 7 with high dose of Japanese type of GTB (580 mg /ml). One hour post treatment, 0.1 ml of 1% charcoal suspension was orally administered to each of these mice and the faecal boluses expelled were collected. Each faecal bolus was pressed on a white sheet of paper to examine the presence of charcoal dust. The time for the appearance of the 1st faecal bolus with charcoal dust was recorded (3).

Effect of BTB on small intestinal secretion

Intestinal secretion was indirectly evaluated by the enteropooling assay (8). 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 group3 with 501 mg/ml

(high dose) of BTB. Forty minuets 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 castor oil treated control was considered as the castor oil-induced accumulation of intestinal fluid.

Effect of BTB on intestinal fluid absorption

This test was performed as described by Ogata et al., (9). Briefly, 12 rats were randomly assigned in to 2 equal groups (n = 6/group) and were anaesthetized with ketamine hydrochloride (14 mg/kg, i.m.). In each rat a silicone tube was inserted and tied at the oral end of jejunum and at the anal end of the ileum. The entire small intestine was rinsed cautiously with 20 ml saline and then with 10 ml air (to remove the fluid) using a syringe. Subsequently, either 3ml of high dose of BTB (501 mg/ml) or 3 ml of saline was injected in to the intestinal loop between the two tubes, and the abdomen was closed. One hour later, the volume of the injected fluid remaining in the intestinal loop was measured to determine the net amount of fluid absorbed.

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 the high dose (501 mg/ml) of BTB and the other with 1 ml/kg water. After 1 h, 0.05 ml of carrageenan was injected into the peritoneal cavity of each of these rats under ether anaesthesia. 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 (10). 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 distilled water), 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 $NaNO_2$. (10).

Statistical analysis

Data are expressed as means ± standard deviation (SD). Multiple comparisons were made using one way non-parametric ANOVA followed by Turkey's test. On the other hand single comparisons were made using Mann-Whitney U- test and G-test as appropriate. $P<0.05$ was considered as statistically significant.

RESULTS

Effect of BTB on normal defaecation in mice

As shown in Table 1, high dose (by 64%) and supraphysiological dose of BTB (by 61%), and high dose of Chinese type GTB (by 54%) and high dose of Japanese type of GTB (by 29%) significantly ($P < 0.05$) inhibited the number of faecal boluses expelled during the 1st hour of post-treatment. During the 2nd hour of post treatment all doses of BTB tested significantly ($P < 0.05$) impaired the number of faecal boluses produced (low dose by 82%; mid dose by 74%; high dose by 86% and supraphysiological dose by 89%). This effect was dose-dependent ($r^2 = 0.72$; $P < 0.05$). Likewise, both high doses of Chinese and Japanese types of GTB significantly ($P < 0.05$) reduced the number of faecal boluses expelled.

Effect of BTB on castor oil-induced diarrhoea

The results obtained are depicted in Table 2. In control mice castor oil induced copious diarrhoea through the study period with a diarrhoeal score ranging from 8-23. The peak diarrhoea was evident at 2nd and 3rd hours. As shown, all doses of BTB, Chinese and Japanese types of GTB and loperamide significantly ($P < 0.05$) impaired the diarrhoea score from 1st hour of treatment upto 8th hour of treatment (except the low dose of BTB at the 7th hour of treatment). The antidiarrhoeal effect of loperamide was much more pronounced than tea brews and induced zero diarrhoea score from 2nd hour onwards. This antidiarrhoeal effect of BTB was dose-dependent (1st h; $r^2 = 0.60$, $P < 0.05$: 2nd h; $r^2 = 0.89$, $P < 0.05$: 3rd h; $r^2 = 0.69$, $P < 0.05$: 4th h; $r^2 = 0.69$, $P < 0.05$: 5th h; $r^2 = 0.77$, $P < 0.05$: 6th h; $r^2 = 0.51$, $P < 0.05$: 7th h; $r^2 = 0.68$, $P < 0.05$: 8th h; $r^2 = 0.88$, $P < 0.05$).

Table 1: Effect of oral treatment of black tea brew and green tea brew of Camellia sinensis on antidiarrhoeal activity in mice (mean ± SD)

Treatment	Dose	Number of faecal boluses	
		1st hour	2nd hour
Control	0.5 ml water	8.40 ± 1.55	9.23 ± 1.30
Black tea brew			
Low dose	84 mg/ml	7.66 ± 0.42	1.63 ± 0.13*
Mid dose	167 mg/ml	8.33 ± 0.60	2.41 ± 0.18*
High dose	501 mg/ml	3.06 ± 0.02*	1.33 ± 0.12*
Supraphysiological dose	1336 mg/ml	3.25 ±0.18*	1.00 ±0.07*
Green tea brew			
Chinese type	610 mg/ml	3.83 ± 0.17*	0.41 ± 0.05*
Japanese type	580 mg/ml	6.00 ± 0.35*	1.66 ± 0.11*

compared to control * P< 0.05

Table 2: Effect of oral treatment of black tea brew, green tea brew of Camellia sinensis and loperamide on castor oil induced diarrhoea in mice (mean ± SD)

Treatment	**N**	**Diarrhoea score (maximum score 24)**							
		1 hour	**2 hour**	**3 hour**	**4 hour**	**5 hour**	**6 hour**	**7 hour**	**8 hour**
Control (Water)	12	14	23	23	14	17	11	8	11
Black tea brew									
Low dose (84 mg/ml)	12	5*	11	6	7	7	5	8	5
Mid dose (167 mg/ml)	12	5*	7	6	3	4	3	3	4
High dose (501 mg/ml)	12	2*	2	1	1	1	2	1	1
Supraphysiological dose (1336 mg/ml)	12	3*	2	2	2	2	3	2	1
Green tea brew									
Chinese type (610 mg/ml)	12	2*	2	2	1	1	1	2	3
Japanese type (580 mg/ml)	12	1*	2	3	1	1	2	2	2
Loperamide (10 mg/kg)	12	1*	0*	0*	0*	0*	0*	0*	0*

As compared to control * P< 0.05 (G-test)

Table 3: Effect of oral treatment of black tea brew and green tea brew of Camellia sinensis on movement of charcoal meal in mice (mean ± SD)

Treatment	Dose	Number of animals	Time for the appease of the 1st faecal bolus with charcoal dust (min)
Control	0.5 ml water	12	50.40 ± 2.15
Black tea brew			
Low dose	84 mg/ml	12	51.25 ± 1.53
Mid dose	167 mg/ml	12	52.50 ± 1.89
High dose	501 mg/ml	12	147.33 ± 1.74*
Supraphysiological dose	1336 mg/ml	12	76.25 ± 3.63*
Green tea brew			
Chinese type	610 mg/ml	12	83.83 ± 4.15*
Japanese type	580 mg/ml	12	123.30 ± 2.71*

As compared to control * P< 0.05

Table 4: Effect of oral treatment of black tea brew (501 mg/ml) of Camellia sinensis on castor oil-induced enteropooling in mice (mean ± SD)

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 Black tea brew (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 (Mann-Whitney U-Test)

Table 5: Effect of oral treatment of black tea brew (501 mg/ml) of Camellia sinensis on intestinal fluid absorption in rats (mean ± SD)

Treatment	Intestinal fluid absorption (ml)
Control (2ml water)	0.76 ± 0.03
High dose of Black tea brew (501 mg/ml)	2.13 ± 0.04*

As compared to control * $P< 0.05$

Table 6: Effect of black tea brew on nitric oxide production by rat peritoneal cells in vitro

Black tea brew Concentration (µg/ml)	% Inhibition of nitric oxide production
Distilled water	-
1000.0	74.98
500.0	77.76
250.0	31.91
125.0	29.13
62.5	2.73
31.2	23.57
15.6	19.40
7.8	29.13

Effect of BTB on gastrointestinal transit time

The results are summarized in Table 3. As shown, high (by 192%) and supraphysiological (by 51%) doses of BTB and, high dose of Chinese (by 66%) and Japanese (by 145%) types of GTB significantly ($P < 0.05$) prolonged the time taken for the appearance of the 1st faecal bolus with charcoal dust indicating a delay in gastrointestinal transit time.

Effect of BTB on small intestinal secretion

The results in the enteropooling assay are summarized in Table 4. As shown, oral administration of castor oil significantly ($P < 0.05$) increased the intestinal fluid secretion, compared with the normal control. BTB on the other hand, significantly ($P < 0.05$) inhibited the castor oil induced intestinal secretion.

Effect of BTB on intestinal fluid absorption

As shown in Table 5, high dose of BTB markedly and significantly ($P < 0.05$) increased (by 180%) the intestinal fluid absorption.

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 peritoneal cells.

DISCUSSION

This study examined the antidiarrhoeal potential of Sri Lankan black tea in mice using high grown unblend Dust grade No: 1 tea. This was tested using two widely used rodent models of diarrhoea: castor oil-induced diarrhoea test and normal defecation test (3). The results show, for the first time, that Sri Lankan black tea possesses promising oral antidiarrhoeal activity especially at high doses. This is an interesting and therapeutically important finding which also provides scientific evidence in support of the claim made in Sri Lankan folklore that Sri Lankan black tea is effective against acute nonspecific diarrhoeas (2). Further, the

antidiarrhoeal potential of the BTB was superior to Japanese type of GTB (when evaluated in the normal defeacation test) and inferior to the reference antidiarrhoeal drug, loperamide (evaluated in the castor oil-induced diarrhoea test).

Antidiarrhoeal effect of BTB had a rapid onset (with in 1 h) and a fairly long duration of action (upto 8 h). Further, the antidiarrhoeal action of BTB was dose-dependent indicating phytoconstituents/s mediated genuine and specific action. BTB contains a variety of phytoconstituents (1, 5) of which flavonoids are shown to exhibit antidiarrhoeal potentials (11). Alkaloids too are known to induce antidiarrhoeal activity (11) and BTB contains appreciable amount of alkaloid caffeine (1, 5). Therefore, the antidiarrhoeal of BTB could be attributed to flavonoids and caffeine.

Sri Lankan Dust grade tea appears to mediate its antidiarrhoeal action by multiple mechanisms. In this study, BTB inhibited normal defaecation (in terms of number of faecal boluses expelled) and delayed the gastrointestinal transit time (as judged by the charcoal meal test) of mice. This suggests that BTB acts on all parts of the gastrointestinal tract (3,12). It is now known that prostaglandins are involved in castor oil-induced diarrhoea (13) and prostaglandin synthesis inhibitors impair castor oil-induced diarrhoea (13). BTB inhibited castor oil-induced diarrhoea in this study. Thus, it is possible that BTB produced its antidiarrhoeal activity by inhibiting gastrointestinal prostaglandin synthesis. Indeed, inhibition of expression of COX-2 has been reported with black tea extracts (14) and queracetin which is present in BTB (5) is claimed to inhibit cyclooxygenase activity (11). Furthermore, inhibition of castor oil-induced increase in fluid accumulation in small intestine in the present study also suggest an impairment of prostaglandins biosynthesis (12). Nitric oxide is implicated with castor oil-induced diarrhoea (15) and nitric oxide synthetase inhibitors suppress castor oil-induced diarrhoea (3). *In vitro* suppression of nitric oxide formation by BTB in the present study suggests that this mechanism may have also contributed to the antidiarrhoeal action of BTB. Opioids show antidiarrhoeal activity (16). We have previously shown that Sri Lankan Dust grade tea has opioid activity (17). Such an action of BTB in the present study can contribute to its antidiarrhoeal mechanism. Stimulation of intestinal water resorption can suppress diarrhoea (16). In the present study, BTB enhanced intestinal water resorption indicating that this mechanism may also have been involved in its antidiarrhoeal action. Inhibition of intestinal secretions can produce an antidiarrhoeal action (3). BTB suppressed intestinal secretions in this study indicating the operation of this mechanism. Flavonoids inhibit intestinal secretion (3) and flavonoids are present in BTB. In this study, BTB increased the gastrointestinal transit time which is yet another mechanism to induce antidiarrhoeal action. Such an action could results from impairment of peristaltic activity possibly by the polyphenols present in the extract (1, 5): polyphenols inhibits peristalsis (9).A direct relaxant action on gastrointestinal smooth muscles is also capable of precipitating an antidiarrhoeal action (16). Several flavonoids are known to possess spasmolytic effects (18) and black tea is rich in flavonoids (1, 5, 6). Thus, this mechanism could also account for the antidiarrhoeal action of BTB in this study. Inhibition of electrolyte secretion to gut lumen is yet another mechanism to produce antidiarrhoeal action (16). Although we have no experimental evidence to support the operation of this mechanism in this study its existence cannot be completely ruled out as alkaloids, tannins and flavonoids inhibits electrolyte secretion (11) and these phytoconstituents are present in BTB (1, 5).

In conclusion, this study, for the first time, scientifically demonstrates antidiarrhoeal activity of Sri Lankan Dust grade black tea. This antidiarrhoeal action is mediated by multiple mechanisms. Further, this finding lends support to the folkloric claim that Sri Lankan black tea is a good remedy for acute non specific diarrhoea.

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