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Gastric ulcer healing activity of Sri Lankan black tea (*Camellia sinensis* L.) in rats

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ABSTRACT

This study examined the gastric ulcer healing potential of black tea (*Camellia sinensis*) using Sri Lankan high grown Dust grade No: 1 black tea in rat acetic acid-induced gastric ulcer model. Three oral doses (84, 167 or 501 mg/ml) of black tea brew (BTB) made according to ISO recommendations were used in the evaluation of gastric ulcer healing activity. The results showed a significant ($P < 0.05$) and dose-dependent gastric ulcer healing activity (in terms of reduction in gastric ulcer area). This effect had a rapid onset (with in 14 days). The gastric ulcer healing activity of BTB was however inferior (by 9 fold) to omeparazole, the reference drug. BTB displayed *in vitro* antioxidant activity (using DPPH assay; by 2985–3923 Trolox equivalents µg/l), and also inhibited *in vitro* nitric oxide production (3–78 %). In addition, BTB had antihistamine activity (by wheal test; by 33.5%) and increased the gastric pH (from 3.3 to 5.9) and impaired the gastric acid output (by 69%). It is concluded that black tea possessed strong, oral gastric ulcer healing activity which is mediated via multiple mechanisms.

Keywords: Black tea, *Camellia sinensis,* Gastric ulcer healing.

INTRODUCTION

Tea is the second most consumed beverage in the world (1). It is made from freshly harvested tender shoots, comprising two or three topmost immature leaves and buds of *Camellia sinensis* L. (Family: Theaceae) plant. 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). Of these, black tea accounts for about 78% of world tea production and about 80% global tea consumption (1).

Recently, we showed experimentally in rats that black tea brew (BTB) of *C. sinensis* has gastroprotive action (2) as is claimed by some Sri Lankan traditional practitioners This gastroprotective action was marked and dose-dependent (2). On the otherhand, regular consumption of green tea brew is shown to promote healing rates of gastric ulcers (3). But, sofar, gastric ulcer healing potential of BTB has not been scientifically investigated and reported. A possibility, however, exists that BTB may also has gastric ulcer healing activity as green tea since black tea contains almost the same phytoconstituents (1,4) and common pharmacological activities such as antioxidant (1,5), diuretic (6), anti-inflammatory (7), or hypoglycaemic (8).

The aim of this study was to investigate the gastric healing potential of black tea. This was tested in rats using Sri Lankan high grown Dust grade No: 1 black tea. The grade of black tea selected and doses used are identical to what has been used previously to show gastroprotective activity (2).

METHODS AND MATERIALS

Experimental Animals

Adult male Wistar rats (200 – 225 g) purchased from the Medical Research Institute, Colombo, Sri Lanka, were used. These animals were housed in the standardized animal house conditions (temperature: 28–31 °C, photoperiod: approximately 12 h natural light per day, relative humidity: 50–55%) at the animal house of the Department of Zoology, University of Colombo. All rats had free access to pelleted food (Master Feed Ltd, Colombo, Sri Lanka) and domestic tap water.

All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care and guidelines (guiding principles in the use of animals in toxicology, adopted by the society of toxicology in 1999), and rules of the Department of Zoology, 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- rotorvane manufacture technique. Tea samples were packed in triple laminated, aluminum foil bags and stored at −20 °C until use.

Preparation of black tea brew (BTB)

BTB was made according to the ISO standards (9): adding 2g of black tea to 100 ml of boiling water and brewing for 5 min. This contains 43.7% (w/w) tea solids in water (8). Based on this data, 501 mg/ml (equivalent to 9 cups, 1 cup = 170 ml) of BTB in 2 ml was made by adding 8g black tea to 20 ml 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.

Evaluation of gastric ulcer healing activity using rat acetic acid-induced gastric lesion model

Thirty rats were fasted for 24 h and randomly assigned into 5 equal groups (n = 6/group). Chronic gastric ulcers were induced in this rats as described by Fernandopulle et al., 1996 (10). Briefly, these rats were anaesthetised with ether and their abdomens were incised using aseptic preoccupations. 0.015 ml of 20% acetic acid solution was injected into the submucosal layer of stomach of each of these rats at the junction of the fundus an antram. The abdomens were then sutured. Polymycin antibiotic cream (Astron Ltd., Ratmalana, Sri Lanka) was applied and the animals were allowed to regain consciousness. Form day 1 of surgery to day 14, the rats were orally treated daily (11.00–12.00 h) with BTB or water in the following manner: rats in group 1 with 2 ml of water; group 2 with 84 mg/ml of BTB; group 3 with 167 mg/ml of BTB; group 4 with 501 mg/ml and group 5 with 20 mg/kg of omeparazole (Cadila Healthcare Ltd, Ahmedabad, India). The animals were sacrificed on day 15 with ether and their stomachs removed. Each stomach was instilled with 5ml of 10% formalin solution and split along the greater curvature. The outline of the induced gastric ulcer was taken on to a transparency sheet and the area was determined using a graph paper.

Serotonin-induced acute gastric lesions

Twelve rats were randomly divided into two equal groups (n = 6/ group) and fasted for 24 h with free access to water. The rats in one group were orally administered with the 501 mg/ml of BTB and the other with 2 ml of water. Thirty minutes later, 20 mg/ kg dose of serotonin (Fluka Chemicals, Buchs, Switzerland) was injected subcutaneously to each of these rats (11). Eighteen hours later, the animals were sacrificed, their stomachs were removed, opened along the greater curvature and, the number, length and area of macroscopic haemorrhagic lesions were recorded.

Measurement of the gastric juice volume, pH, acidity and acid output of gastric secretion

Twelve rats were randomly distributed into two equal groups (n = 6/ group). One group was orally administered with the 501 mg/ml of BTB and the other with 2 ml of water. One hour later, the rats were anaesthetised with ether, their upper abdominal regions were opened with a mid ventral incision using aseptic precautions. The pylori were ligated using silk ligatures, taking care not to interfere with the blood supply to the stomachs. The animals were then sutured, polymycin antibiotic cream applied, allowed to regain consciousness and kept for 4 h without access to water. The animals were then killed with ether, the abdomens opened and an another ligature was placed around the oesophagus closed to the diaphgram. The gastric contents were aspirated using a plastic syringe, and their volumes were recorded. These were centrifuged at 1500 rpm for 15 min, and the supernatants were removed. The pH of the supernatants was measured using a pH meter (TOH Electronics, Tokyo, Japan). Acidity (using phenol red as the indicator) of supernatants was determined by titrating against 0.1 N NaOH as described by Varley (1962) (12). The basal acid output was calculated

as the product of acidity and volume of gastric juice and expressed in terms of μ Eq/ 100 g b.w. (13).

Evaluation of antihistamine activity

Eighteen rats were randomly assigned into two equal groups (n = 9/ group). The left posterior lateral side of their skin was cleanly shaved under aseptic conditions. One group was orally treated with the 501 mg/ml of BTB and the other with 2 ml of water. After 1 h, 50 μl of 200 μg/ ml of histamine (Fluka Chemicals, Buchs, Switzerland) 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 (14).

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 (5). Briefly, 3 concentrations of BTB (84, 167, 501 mg/ml) were made, and 750 μl of these samples were added to 750 μl of DPPH solution (in triplicate) and incubated at 30 °C for 5 min. The 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}

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 °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) (15). The peritoneal cells were plated in 96 well tissue culture plates at 1×10^6 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 °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 °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 $NaNO_2$. (15).

Statistical analysis

Data are expressed as means ± standard error of mean (SEM). Statistical comparisons were made using Mann-Whitney U test. Regression analysis were done using Spearman co-relation test. $P \leq 0.05$ was considered as statistically significant.

RESULTS

Acetic acid-induced gastric ulcers

Serosal application of acetic acid induced prominent circular dark purple coloured gastric ulcers in the gastric mucosa of each rat. The results obtained are summarized in Table 1. As shown, BTB treatment significantly ($P < 0.05$) impaired the area of the gastric ulcer formed (84 mg/ml by 29%; 167 mg/ml by 51% and 501 mg/ml by

Table 1: Effect of oral treatment of black tea brew of Camellia sinensis on gastric ulcers in rats (Mean ± SEM: ranges are given in parenthesis)

Treatment	Gastric Ulcer area (mm^2)	% Inhibition
Control	20.83 ± 0.07	–
Water	(3–28)-	
Black tea brew		
84 mg/ml	14.82 ±0.10* (5–15)	29
167 mg/ml	10.20 ±0.25* (3–12)	51
501 mg/ml	4.16 ±0.32* (0–8)	80
Reference drug		
Omeparazole (20mg/kg)	0.41± 0.01* (0.1–0.6)	98

*P< 0.05 compared to control (Mann-Whitney U- test)

Table 2: Effect of oral treatment of 501 mg/ml of black tea brew of Camellia sinensis on serotonin-induced gastric ulcers in rats (Mean ± SEM)

Treatment	Number of lesions	Total length of a lesions (mm)	Total area of a lesions (mm^2)
Control water	2.40 ± 0.40	18.00 ± 3.03	31.80 ± 4.04
Black tea brew (501 mg/ml)	0.80 ± 0.20*	4.40 ± 1.21*	5.60 ± 1.63*

*P< 0.05 compared to control (Mann-Whitney U- test)

80%) and reference drug omeparazole (by 98 %). This effect was dose-dependent (r^2= 0.85; P < 0.05).

Serotonin-induced acute gastric lesions

As shown in Table 2, the 501 mg/ml of BTB significantly (P < 0.05) and markedly impaired the number (by 67%), the length (by 75%) and the area (by 80%) of gastric lesions in the mucosa induced by subcutaneous administration of serotonin.

Evaluation of the gastric juice volume, pH, acidity and acid output of gastric secretion

As shown in Table 3, 501 mg/ml of BTB significantly (p < 0.05) and profoundly reduced the gastric juice volume (by 67%) and acid output (by 69%), and increased the pH of gastric juice (by 76.8%).

Evaluation of antihistamine activity

501 mg/ml of BTB significantly (P < 0.05) reduced (by 33.5%) the area of the wheal formed following subcutaneous injection of histamine (control vs treatment: 48.77 ± 1.12 vs 32.44 ± 0.59 mm^2).

Evaluation of the antioxidant activity (DPPH assay)

As shown in Table 4, the BTB exhibited dose-dependent (r^2 = 0.83, p < 0.05) antioxidant activity *in vitro*.

Evaluation of nitric oxide production by peritoneal cells

As shown in Table 5, the BTB dose-dependently (r^2 = 0.79; P < 0.05) inhibited the *in vitro* nitric oxide production by the peritoneal cells.

Table 3: Effect of oral treatment of 501 mg/ml of black tea brew of Camellia sinensis on some parameters of gastric content in pyloric ligated rats (Mean ±SEM)

Parameter	Control group (Water)	Treatment group (501 mg/ml)
Gastric volume (ml/100g bw)	3.25±0.57	1.05±0.26*
pH	3.33±0.09	5.89±0.03*
Basal acid out put (µEq/100g bw)	120.0±23.6	37.15±1.04*

**P< 0.05 compared to control (Mann-Whitney U- test)*

Table 4: In vitro antioxidant activity of Sri Lanka black tea brew of Camellia sinensis, as determine by DPPH assay (mean ± SEM)

Tea sample	Antioxident activity (Trolox equivalents µg/l)
Black Tea Brew	
Low concentration (84 mg/ml)	2985 ± 6.0
Mid concentration (167 mg/ml)	3572 ± 86.5
High concentration (501 mg/ml)	3923 ± 6.5

Table 5: In vitro Nitric oxide activity of Sri Lanka black tea brew of Camellia sinensis as determine by nitric oxide assay

Black tea brew 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

DISCUSSION

This study examined the gastric ulcer healing potential of black tea using rat acetic-induced gastric lesion model and Sri Lankan high grown Dust grade No: 1 black tea. The ulcers induced in this model resemble human gastric ulcers and is used widely to evaluate potential gastric ulcer healing drugs (16). The results show that BTB has marked oral gastric ulcer healing activity (in terms of reduction in gastric ulcer area) in acetic acid-induced gastric ulcer model. This stimulatory activity of gastric healing was dose-dependent and had a rapid on set (within 7 days). The presence of a dose response relationship suggests that gastric ulcer healing effect is genuine and treatment related and not due to spontaneous healing: both gastric and duodenal ulcers heal spontaneously (18). However, gastric ulcer healing activity of BTB was inferior to omeparazole, the reference drug used (by nine fold).

Promotion of gastric ulcer healing activity by black tea appears to be mediated by several mechanisms. It is well known that gastric ulcer healing rates are positively related to the degree of gastric acid inhibition and removal of acid favours gastric healing (17), and that H_2 receptor antagonists, antacids and proton pump inhibitors promotes gastric ulcer healing (18). BTB is this study inhibited gastric acid output and elevated the gastric pH. Obviously, in this study, this mechanism is likely to play a vital role in triggering gastric ulcer promotion action of black tea. BTB induced impairment of gastric acid output is likely to be mediated via H_2 receptors as it had marked antihistamine activity (in terms of wheal test). BTB is a rich source of flavonoids (1,4) and flavonoides impair histamine secretion (19) and gastric acid release (19). In addition, gastric acid inhibition could results from proton pump inhibition as BTB contains catechins, quercetin and rutin (1, 4) which are potent inhibitors of this pump (19, 20). It is possible that BTB-induced acceleration of healing of gastric ulcer may be due

protection of basic fibroblast growth factors from acid since basic fibroblast growth factors are considered to be responsible for endothelial regeneration in healing of gastric ulcers (23).

Active oxygen species are linked with the pathogenesis of gastric ulcers (21) and antioxidant are known to offer gastroprotection (19). BTB has been shown to possess marked antioxidative (1, 5) and lipid peroxidation inhibition action (1). In this study, *in vitro* antioxidant activity of BTB made from Dust grade No: 1 black tea was demonstrated (using DPPH assay). Tea is one of the most potent antioxidants (1). Thus, it is reasonable to attribute gastric ulcer healing activity of BTB to its antioxidative activity which is mediated via its flavonoids (1).

One of the mechanisms by which gastric ulcer healing drugs act is via site-protective mechanisms (eg: sucralfate, omeparazole) by acting as a protective barrier that prevents back diffusion of hydrogen ions into the ulcer (17). BTB may act via this mechanism as it is shown to increase gastric mucus production (in terms of alcian blue technique) (2) and thickness of gastric mucus layer (by histological studies) (2). Moreover, BTB has negatively charged (2) phyto constituents (4) which could complex to positively charged partially denatured proteins at the base of the ulcer there by forming a protective barrier at the ulcer site.

Another potential mechanism of gastric ulcer healing activity of BTB may be attributed to improvement of blood supply. In this study, BTB impaired serotonin-induced gastric lesions. This is indicative of improvement of gastric mucosal microcirculation (22). Reduction of pepsin level can also cause acceleration in gastric ulcer healing (18). But, BTB has no such activity (2).

Nitric oxide is known to elicit gastric antiulcerogenic effects (24) and nitric oxide inhibitors like L-NAME provokes formation of gastric ulcers (25). But, unexpectedly in this study although BTB impaired nitric oxide production *in vitro* it induced marked gastric ulcer haling activity. We cannot offer a valid explanation for this observation unless nitric oxide inhibition activity of BTB is measured *in vivo*.

There are other potential mechanisms which can induce accelerated tissue repair and gastric healing process in the ulcerated mucosa which resulted from serosal application of acetic acid in this study. But, we have no evidence (direct or indirect) in support of such mechanisms in this study.

It is beneficial for gastric ulcer healing agent to have simultaneous gastroprotective activity and antinociceptive action (26). In this regard, it is interesting to note that BTB has both of gastroprotective (2) and antinociceptive (27) actions when taken orally.

In conclusion, this study demonstrates, for the fist time in rats, gastric ulcer healing activity of black tea when taken orally.

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