Role of Reduced Glutathione and Nitric Oxide in the Black Tea Extract-Mediated Protection Against Ulcerogen-Induced Changes in Motility and Gastric Emptying in Rats

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ABSTRACT—The aim of the present study was to investigate the underlying mechanism of the role of hot water extract of black tea [Camellia sinensis (L). O. Kuntze Theaceae] in normalizing the changes in intestinal transit and gastric emptying induced by various ulcerogenic agents in experimental rats. Intestinal transit as well as gastric emptying were significantly reduced in rats treated with glutathione (GSH) depleting agents, diethyl maleate (DEM), indoacetamide (IDA) and N-ethyl maleimide (NEM). Prior oral administration of black tea extract (BTE) at 20 ml/kg of a 10% solution, i.g. once a day for 7 days significantly increased the intestinal transit and gastric emptying with restoration of serum GSH level. Singular administration of succimer (60 mg/kg, i.g.), the standard sulfhydryl containing antiulcer agent used as a reference drug, was also effective. Increase in intestinal transit caused by BTE was reversed both by N-omega-nitro-L-arginine methyl ester (L-NAME) (25 mg/kg, i.p.) and N-omega-monomethyl-L-arginine (L-NMMA) (25 mg/kg, i.p.), but not with N-omega-nitro-D-arginine methyl ester (D-NAME) (25 mg/kg, i.p.). Furthermore, restoration of intestinal nitric oxide synthase (NOS) activity was found to be associated with BTE treatment. These results provide evidence that nitric oxide may play a role in BTE-mediated improvement of intestinal motility changes and gastric emptying induced by DEM, IDA and NEM.

Keywords: Camellia sinensis, Reduced glutathione, Ulcerogen, Nitric oxide, Gastric motility

The role of gastrointestinal motility in the pathogenesis of gastric lesions has been suggested by many investigators (1 – 3). Tissue injury produced by noxious agents may result in the accumulation of toxic free radicals in mucosal cells (4). Endogenous thiol such as reduced glutathione (GSH) are able to bind reactive free radicals (5) and prevent the lesion formation produced by various ulcerogens (6). GSH is also important for maintenance of mucosal integrity, since depletion of GSH from the gastric mucosa by electrophilic compounds induces macroscopic mucosal ulceration (7, 8). Recent advances in tea research have revealed diverse pharmacotherapeutic effects including hypocholesterolemic (9), hypoglycemic (10), anticarcinogenic (11) and antiatherosclerotic (12) effects. Although the composition of manufactured tea may vary according to numerous factors like climate, manufacturing process, botanical variations, etc., the major soluble solid components of tea are polyphenols. They are present as catechins in green tea, whereas in black tea, they are mainly present as thearubigins and theaflavins. We have recently demonstrated the involvement of GSH in the antiulcer effect of black tea (13). Nitric oxide (NO), a free radical produced by the enzyme nitric oxide synthase (NOS) from the terminal guanido nitrogen of L-arginine, has recently been identified as one of the neurotransmitters of the nonadrenergic, noncholinergic (NANC) neurons in the stomach and the small intestine (14 – 16). Neuronal NO is an important inhibitory regulator of gastrointestinal motility (17). At the level of the stomach, NO is involved in NANC relaxation of the fundus (18) and pylorus (19). Inhibition of NOS delays gastric emptying of liquids in rats (20) and solids in dogs (21). The aim of the present study was to investigate the protective role of black tea, if any, on gastrointestinal motility and to monitor GSH and NO status in the cytoprotection afforded by black tea against reduced intestinal transit and gastric emptying induced by GSH-depleting ulcerogens.

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MATERIALS AND METHODS

All experiments were done with CTC (curl, tear and crush) BOP (broken orange pickoe) grade black clonal tea processed in October, 1991 and supplied by Tocklai Experimental Station, Jorhat, Assam, India (collection No. 01091). A voucher specimen has been kept at the Tea Research Association, Calcutta, India. The processing of black tea was carried out in the miniature factory of Tocklai Experimental Station, Jorhat, Assam, India as described earlier (13).

Preparation of hot water extract of black tea

Ten grams of processed black tea was soaked in 100 ml of boiling distilled water for 5 min and filtered. The filtrate was designated as black tea extract (BTE). In our estimate, almost 2.8 – 3.0 g of dried material is contained in 100 ml of filtered hot water extract of black tea.

Chemicals

Diethylmaleate (DEM), indoacetamide (IDA), N-ethyl maleimide (NEM), succimer, N-omega-nitro-L-arginine methyl ester (L-NAME), N-omega-monomethyl-L-arginine (L-NMMA), N-omega-nitro-ω-arginine methyl ester (o-NAME), S',S-dithiodiis-(2-nitrobenzoic acid) (DTNB), NADPH, charcoal, gum acacia, phenol red, methyl cellulose, dithiothreitol (DTT), EDTA, GTP, phenylmethyl-sulfonyl fluoride (PMSF) and trichloroacetic acid were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals used were of analytical grade.

Animals and experimental design

The experiments were carried out with outbred albino rats (Sprague Dawley strain; IICB Colony, Calcutta, India) of either sex weighing 150 – 200 g. There were two groups of control animals. The normal control group received distilled water (20 ml/kg body wt. per day) intragastrically once daily at around 11 a.m. for 7 days. The other group, the BTE control group, received BTE (20 ml/kg body wt. per day) intragastrically once daily at around 11 a.m. for 7 days. Experiments were performed on day 8 and food was withdrawn for 18 h before all experiments, but the animal had free access to drinking water. Rats were treated subcutaneously with DEM (1 ml/kg) or IDA (10 mg/kg) or NEM (5 mg/kg). In some experiments, rats were pretreated intragastrically with succimer (60 mg/kg) administered 30 min before DEM, IDA or NEM treatment. Rats were sacrificed 1 h after DEM, IDA or NEM treatment. The small intestine and colon were isolated and washed, and the mucosa was scraped. The mucosal scrapings were assayed for NOS activity.

Determination of small intestinal transit (22)

Rats were starved for 18 h with free access to water. One hour after the subcutaneous administration of DEM, IDA or NEM, rats were treated intragastrically with 10 ml/kg of an aqueous suspension of 10% charcoal and 5% gum acacia. Control as well as treated rats were killed by exsanguination 30 min after administration of the charcoal meal. The intestine of each rat was removed, and the distance travelled by the charcoal from the pylorus was measured and expressed as the percentage of total intestinal length from the pylorus to the caecum. In another set of experiments, rats were treated intragastrically with succimer (60 mg/kg) 30 min before DEM, IDA or NEM administration.

In some groups, rats were treated intraperitoneally with l-NAME (25 mg/kg), l-NMMA (25 mg/kg) or D-NAME (25 mg/kg) concomitantly with the subcutaneous (s.c.) administration of DEM, IDA or NEM.

Determination of gastric emptying (23)

For the study of gastric emptying of liquids, the meal consisted of 1.5 ml/rat of a solution of 50 mg phenol red in 100 ml of 1.5% methyl cellulose. It was constantly stirred and held at 37°C. The meal was administered by gavage, and the animals were sacrificed 30 min after administration of the meal. After laparotomy, the stomach and the small bowel were quickly ligated at the lower esophageal, pyloric and the ileocaecal sphincter and removed. Within the 30 min after its administration, the front of the phenol red solution had not yet reached the ileocaecal sphincter. The stomach and the small bowel (including the contents) were separately homogenized in 100 ml of 0.1 M NaOH. For the assay of phenol red, 5 ml of the homogenized mixture was centrifuged (2,600 × g) for 10 min, and then 2 ml of the supernatant was added to 0.2 ml of 20% trichloroacetic acid in order to precipitate the proteins. After vortexing and centrifugation (30 min, 2,600 × g), 1 ml of the supernatant was added to 0.5 ml of 1 M NaOH to develop the maximum intensity of the color. The solutions were colorimetrically assayed at 560 nm with a spectrophotometer (model 200 GL; Digispec, Bombay, India). Gastric emptying (%) was calculated according to the following formula:

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100 \times \left(1 - \frac{\text{Amount of phenol red recovered in stomach}}{\text{Total amount of phenol red recovered}}\right)
\]

Drug treatments for gastric emptying experiments were similar to that described for intestinal transit.

Assay of GSH

Whole blood collected by cardiac puncture into syringes containing 100 µl 0.05 M disodium EDTA was processed by the addition of 4 vol of ice-cold 5% (w/v) metaphosphoric acid. Acid extract of blood obtained by centrifu-
tion at 10,000 × g for 5 min was assayed for GSH by reacting it with DTNB according to Tietze (24). The absorbance was measured at 412 nm.

**Determination of NOS activity**

Mucosal scrapings (100 mg) were homogenized for 30 s at 4°C in 0.9 ml ice-cold 50 mM Tris-HCl, pH 7.4 containing 0.1 mM EDTA, 0.1 mM EGTA, 0.5 mM DTT and 1 mM PMSF. Homogenates were centrifuged at 20,000 g for 60 min at 4°C and the supernatant was used as the source of NOS. NOS activity was determined by measuring the decrease in absorbance at 340 nm for 3 min continuously as NADPH was consumed during the conversion of l-arginine to l-citrulline by NOS according to Sherman et al. (25).

**Statistical analysis**

All values are expressed as the mean ± S.E.M. Mean values of the measurements made were examined for significant differences by one way analysis of variance (ANOVA). Differences in mean values with P<0.05 were considered significant.

**RESULTS**

**Intestinal transit**

The distance travelled by charcoal at 30 min was 64.4 ± 1.7% (n = 8) of the intestinal length in control rats. Prior administration of ulcerogens like DEM, IDA and NEM results in a significant decrease in the distance travelled by the marker (Table 1). Daily administration of BTE for 7 days had no significant effect on the intestinal propulsion of charcoal in the control rats, but significantly increased (P<0.001) intestinal transit in all three ulcerogen-treated rats. Similar to BTE, singular dose succimer pretreatment also significantly increased the charcoal transit in all three ulcerogen-treated rats. Since NO is known to have an important role as a mediator of intestinal motility, it was thought worthwhile to study the effect of NOS inhibitors on the intestinal transit induced by ulcerogens. Reduced intestinal transit induced by various ulcerogens was found to be unaffected by pretreatment with L-NAME and L-NMMA (Table 2). However, when these NOS inhibitors were administered in BTE-pretreated animals, the protection afforded by BTE in increasing the charcoal transit in ulcerogen-treated rats was reversed (Table 2). The intestinal transit in these animals was close to those in the ulcerogen-treated groups. Moreover, when l-arginine (300 mg/kg, i.p.) was coadministered with L-NAME and L-NMMA, the intestinal transit was unaltered in BTE-treated and ulcerogen-treated groups (Table 2).

**Table 1. Effect of BTE and succimer on intestinal transit in ulcerogen treated rats**

| Treatment       | No. of animals | Dose | Transit (%) ± S.E.M. |
|-----------------|----------------|------|----------------------|
| None            | 10             |      | 64.4 ± 1.7           |
| BTE             | 9              | 20 ml/kg, i.g., daily for 7 days | 69.1 ± 2.4 |
| Succimer        | 8              | 60 mg/kg, i.g. | 61.5 ± 2.2 |
| DEM             | 6              | 1 mg/kg, s.c. | 29.6 ± 1.7* |
| BTE + DEM       | 6              |      | 53.7 ± 2.4*          |
| Succimer + DEM  | 6              |      | 46.8 ± 2.1*          |
| IDA             | 6              | 10 mg/kg, s.c. | 36.9 ± 1.9* |
| BTE + IDA       | 6              |      | 57.7 ± 2.5*          |
| Succimer + IDA  | 6              |      | 47.3 ± 2.2*          |
| NEM             | 6              | 5 mg/kg, s.c. | 26.4 ± 1.7* |
| BTE + NEM       | 6              |      | 49.8 ± 2.4*          |
| Succimer + NEM  | 6              |      | 42.6 ± 1.8*          |

Results are each a mean ± S.E.M. and expressed as the percentage of the intestinal length travelled by the charcoal meal. *Significant decrease as compared to the control (P<0.001). †Significant increase as compared to ulcerogen-treated rats (P<0.01).

**Table 2. Effect of NOS inhibitors on intestinal transit in ulcerogen-treated animals**

| Treatment       | No. of animals | Transit (%) ± S.E.M. |
|-----------------|----------------|----------------------|
| None            | 10             | 64.4 ± 1.7           |
| DEM             | 6              | 29.6 ± 1.7           |
| BTE + DEM       | 6              | 53.7 ± 2.4           |
| DEM + l-NAME    | 5              | 32.3 ± 1.6           |
| DEM + l-NMMA    | 5              | 27.7 ± 1.6           |
| BTE + DEM + l-NAME | 5     | 36.2 ± 1.7*         |
| BTE + DEM + l-NMMA | 5     | 38.5 ± 2.0*         |
| BTE + DEM + l-NMMA + Arg | 4  | 48.8 ± 2.3          |
| IDA             | 6              | 36.9 ± 1.9           |
| BTE + IDA       | 6              | 57.7 ± 2.5           |
| IDA + l-NAME    | 5              | 33.3 ± 1.7           |
| IDA + l-NMMA    | 5              | 37.8 ± 1.8           |
| BTE + IDA + l-NAME | 5     | 43.7 ± 1.9*         |
| BTE + IDA + l-NMMA | 5     | 45.6 ± 1.8*         |
| BTE + IDA + l-NMMA + Arg | 4  | 55.5 ± 2.4          |
| NEM             | 6              | 26.4 ± 1.7           |
| BTE + NEM       | 6              | 49.8 ± 2.4           |
| NEM + l-NAME    | 5              | 28.2 ± 1.4           |
| NEM + l-NMMA    | 5              | 29.4 ± 1.2           |
| BTE + NEM + l-NAME | 5     | 35.3 ± 1.4*         |
| BTE + NEM + l-NMMA | 5     | 37.4 ± 1.5*         |
| NEM + BTE + l-NMMA + Arg | 4  | 45.8 ± 2.0          |

Results are each a mean ± S.E.M. and expressed as the percentage of the intestinal length travelled by the charcoal meal. *Significant decrease as compared to the ulcerogen + BTE group (P<0.01).
Gastric emptying

The gastric emptying of the liquid meal after i.p. injection of saline was 51.2 ± 1.6% (n = 8) in control rats (Table 3). Treatment with the ulcerogens (DEM, IDA and NEM) significantly reduced the gastric emptying (P<0.001) compared to the control (Table 3). Although BTE pretreatment did not significantly influence gastric emptying per se, it prevented the inhibitory effect of the ulcerogens (Table 3). Succimer also could prevent the inhibitory effect of ulcerogens (Table 3). In order to ascertain the role of NO in the protective effect of BTE, pretreatment with BTE was examined. Pretreatment with l-NAME (25 mg/kg, i.p.) gave significant inhibition (P<0.01) of BTE-mediated protection, but had no effect in ulcerogen-treated rats (Table 4). The inhibitory effect was mimicked by l-NMMA, but d-NAME had no effect. Furthermore, L-arginine (300 mg/kg, i.p.) could completely neutralize the inhibitory effects of both L-NAME and l-NMMA when coadministered (Table 4).

Serum GSH

Since the ulcerogens used in this study are mainly GSH-depleting agents, serum GSH levels were estimated in various treated groups and are shown in Fig. 1. All the three ulcerogens (DEM, IDA and NEM) reduced the GSH level in serum. However, BTE pretreatment was found to increase GSH levels significantly in the various ulcerogen-treated rats (P<0.001). The level of GSH in serum was 1.15 ± 0.03 μM in the untreated group and 0.62 ± 0.01, 0.55 ± 0.02 and 0.43 ± 0.01 μM in DEM-, IDA- and NEM-treated groups, respectively. In BTE pretreated rats subjected to DEM, IDA and NEM treatment, the GSH levels in serum were 0.92 ± 0.02, 0.84 ± 0.02 and 0.81 ± 0.03 μM, respectively. There were significant differences between the normal untreated group and the ulcerogen-treated groups (P<0.001) and between the ulcerogen-treated group and BTE group (P<0.01).

NOS activity

The effect of various ulcerogens on small intestinal NOS activity is presented in Fig. 2. All three ulcerogens (DEM, IDA and NEM) decreased NOS activity significantly (P<0.001). Pretreatment with BTE prevented the decrease of NOS activity induced by DEM, IDA and NEM. BTE, when administered by itself, could not effect any change of NOS activity in controls. Succimer, used as a positive control, also elevated NOS activity in DEM-, IDA- and NEM-treated animals.

Table 3. Effect of BTE and succimer on gastric emptying in ulcerogen-treated rats

| Treatment     | No. of animals | Gastric emptying % ± S.E.M. |
|---------------|----------------|-----------------------------|
| None          | 8              | 51.2 ± 1.6                  |
| BTE           | 6              | 53.5 ± 2.2                  |
| Succimer      | 6              | 48.8 ± 2.1                  |
| DEM           | 5              | 26.6 ± 1.7                  |
| BTE + DEM     | 5              | 42.1 ± 2.1                  |
| Succimer + DEM| 5              | 37.8 ± 1.9                  |
| IDA           | 6              | 30.5 ± 1.4                  |
| BTE + IDA     | 5              | 44.2 ± 1.7                  |
| Succimer + IDA| 5              | 39.6 ± 1.9                  |
| NEM           | 6              | 23.7 ± 1.7                  |
| BTE + NEM     | 5              | 37.8 ± 1.5                  |
| Succimer + NEM| 5              | 34.3 ± 1.7                  |

Table 4. Effect of NOS inhibitors on gastric emptying in ulcerogen treated animals

| Treatment     | No. of animals | Gastric emptying % ± S.E.M. |
|---------------|----------------|-----------------------------|
| None          | 8              | 51.2 ± 1.6                  |
| DEM           | 5              | 26.6 ± 1.7                  |
| BTE + DEM     | 5              | 42.1 ± 2.1                  |
| DEM + l-NAME  | 5              | 28.4 ± 2.0                  |
| DEM + l-NMMA  | 5              | 29.2 ± 1.7                  |
| BTE + DEM + l-NAME | 4 | 33.4 ± 1.8^a |
| BTE + DEM + l-NMMA | 4 | 35.1 ± 1.7^a |
| BTE + DEM + d-NAME | 4 | 40.8 ± 1.8      |
| BTE + DEM + l-NAME + Arg | 4 | 43.4 ± 1.8      |
| BTE + DEM + l-NMMA + Arg | 4 | 41.5 ± 1.9      |
| IDA           | 6              | 30.5 ± 1.4                  |
| BTE + IDA     | 5              | 44.2 ± 1.7                  |
| IDA + l-NAME  | 4              | 28.8 ± 1.6                  |
| IDA + l-NMMA  | 4              | 27.4 ± 1.5                  |
| BTE + IDA + l-NAME | 4 | 33.6 ± 1.6^a |
| BTE + IDA + l-NMMA | 4 | 31.4 ± 1.8^a |
| BTE + IDA + d-NAME | 4 | 46.3 ± 2.4      |
| BTE + IDA + l-NAME + Arg | 4 | 43.8 ± 2.0      |
| BTE + IDA + l-NMMA + Arg | 4 | 42.3 ± 2.2      |
| NEM           | 6              | 23.7 ± 1.7                  |
| BTE + NEM     | 5              | 37.8 ± 1.5                  |
| NEM + l-NAME  | 4              | 25.3 ± 1.4                  |
| NEM + l-NMMA  | 4              | 26.4 ± 1.2                  |
| BTE + NEM + l-NAME | 4 | 33.6 ± 1.6^a |
| BTE + NEM + l-NMMA | 4 | 34.1 ± 1.7^a |
| BTE + NEM + d-NMMA | 4 | 39.4 ± 1.7      |
| BTE + NEM + l-NAME + Arg | 4 | 37.2 ± 1.9      |
| BTE + NEM + l-NMMA + Arg | 4 | 39.1 ± 2.1      |

Results are each a mean ± S.E.M. and doses of drugs are as described in Table 2. Significance as compared to the ulcerogen + BTE group (P<0.01).
DISCUSSION

Although the cause of gastric ulcer is poorly understood, the main physiological events are suggested to be dismotility, prostaglandin formation, alteration in gastric mucosal barrier and blood flow, and impaired gastric secretions. We reported earlier the prominent antiulcer activity of BTE and the involvement of gastric mucosal defense mechanisms, primarily GSH in the antiulcer activity of BTE (13, 26). The present study deals with the protective role of BTE in reversing the gastrointestinal motility changes induced by various ulcerogens and possible involvement of both GSH and NO in mediating the reversal. The ulcerogens (DEM, IDA and NEM) used in this study are known to produce acute ulceration by depleting gastric GSH (13). Succimer was used as the reference sulfhydryl antiulcer drug (27). The marked reduction in both intestinal transit and gastric emptying observed in all the three ulcerogen-treated rats support the suggestion that gastrointestinal motility is involved in the pathogenesis of gastric lesions (28, 29). In an earlier report from this laboratory, single administration of a 3% solution of BTE was found to increase gastric transit (30). The failure of BTE in the present study to increase gastric transit may be due to the higher concentration; i.e., 10% solution of BTE administered chronically (once daily for 7 days). It was observed that BTE pretreatment alone could reverse the reduced intestinal transit and gastric emptying associated with DEM, IDA and NEM treatment, a property also shared by succimer. Another major feature of BTE induced reversal of the effects of ulcerogens is the significant increase in serum GSH level. The GSH level in serum is significantly less in rats treated with DEM, IDA and NEM as compared to

![Graph 1](image1)

**Fig. 1.** Effect of BTE on reduced glutathione concentration in serum in ulcerogen-treated rats. Values are each a mean ± S.E.M. (n = 5 or 6). *, compared with normal control (P<0.001); †, compared with the respective ulcerogen-treated groups (P<0.01).

![Graph 2](image2)

**Fig. 2.** Effect of BTE on intestinal NOS activity in ulcerogen-treated rats. Values are each a mean ± S.E.M. (n = 5 or 6). *, compared with the normal control (P<0.001); †, compared with the respective ulcerogen-treated groups (P<0.01).
control rats. This suggests that GSH may have a role to play in the reduced gastrointestinal motility induced by these agents. The observation that BTE could reverse the serum GSH level to a great extent provides evidence for the involvement of GSH in the protective effect of BTE. Such a conclusion is further strengthened by our earlier observation that BTE could increase both the gastric mucosal GSH and GSH peroxidase activity in ulcerogen-treated animals (13).

Since the NO pathway has been implicated in physiologic changes of intestinal motility and since BTE pretreatment afforded the reversal of reduced gastrointestinal motility induced by DEM, IDA and NEM, we tested the hypothesis that endogenous NO may be involved in the protective effect of BTE. All three ulcerogens induced a decrease in intestinal NOS activity which in the present study was found to correlate with their effect on intestinal transit. Although BTE by itself had no effect on intestinal NOS activity, it effectively prevented its inhibition by DEM, IDA and NEM. The reduction in the extent of ulcerogen-induced inhibition of NOS activity by BTE was accompanied by normalization of the intestinal transit and gastric emptying. We have also shown that L-NAME and L-NMMA, reversible inhibitors of NO synthesis from L-arginine, prevented BTE-mediated reversal of delayed intestinal transit and gastric emptying induced by the ulcerogens. The NO synthesis inhibitors used in the present study are usually considered to inhibit specifically the enzymes that generate NO in peripheral tissues (31) and in the central nervous system (32). In an earlier study, it was reported that L-NMMA increased gastric transit significantly (30). The failure of L-NMMA and L-NAME to reverse the ulcerogen induced decrease in gastric transit in the present study may be due to the higher dose; i.e., 25 mg/kg in our study as against 500 μg/kg used in the previous study. Since maximal inhibition of NOS activity by the NOS inhibitors has been found at the dose of 25 mg/kg (33), this dose was used in the present study. The effect of L-NAME was stereospecific since D-NAME was not effective. L-Arginine could counteract the inhibitory effect of L-NAME on the intestinal motility. Taken together, these results suggest that BTE-mediated reversal of reduced intestinal transit and gastric emptying in rats could involve the L-arginine: NO pathway. This was further substantiated by the studies on NOS in which the enzyme activity was found to be increased in BTE-pretreated and ulcerogen-treated rats. Knowledge regarding the source of NOS in intestinal mucosa is still not clear. However, a recent report of abundant myenteric NOS-containing neurons issuing long descending projections to the smooth muscle and myenteric ganglia in both small and large intestine is suggestive of the major role of neuronal NOS on gut motility (34). It may be mentioned that NO is known to enhance the frequency and amplitude of spontaneous electrical activity and to increase the rate of contraction in the rabbit colon in vitro (35). In addition, NO is responsible for the increase in intestinal transit induced by castor oil in vivo (36). These findings support the concept that neuronal NO could be involved in BTE-mediated increase of intestinal transit. However, endothelial NOS may also play an important role in the maintenance of mucosal integrity. Endothelium-derived NO is rapidly destroyed by an O2 generating system such as xanthine/xanthine oxidase (37). BTE, having catechin-like polyphenols with antioxidant property, may therefore exhibit the antiulcer action by scavanging free radicals generated in the injured mucosa or by inhibiting inactivation of endothelium-derived NO by O2.

Epigallocatechin gallate, a constituent of green tea was reported to inhibit inducible NOS gene expression and enzyme activity in murine macrophages (38). In addition, it has been reported that topical application of tumor promoter reduces the production of NOS by epidermis and that pretreatment with green tea extract can reverse this inhibition (39). In the present study, the increased NOS activity associated with BTE pretreatment in ulcerogen-treated rats may be the result of an increase in the GSH level in serum and gastric mucosa. GSH, present in high concentrations in mammalian cells, has been suggested to play a possible role in NO synthesis, as a reducing cofactor for NO production (40) or more likely, by preventing early inactivation of NOS by radical intermediates or NO itself (41). The overall results presented here suggest that besides GSH involvement, NO also has an important role to play in the protective mechanism of BTE on gastrointestinal motility induced by DEM, IDA and NEM.

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36 Mascolo N, Izzo AA, Autore G, Barbota F and Capasso F: Nitric
33 Mascolo N, Izzo AA, Barbato F and Capasso F: Inhibitors of
34 Ekblad E, Alm P and Sundler F: Distribution, origin and projec-
37 Rubanyi GM and Vanhoutte PM: Oxygen-derived free radicals,
38 Chan MM, Fong D, Ho CT and Huang HI: Inhibition of induc-
39 Ahmad N, Srivastava RC, Agarwal R and Mukhtar H: Nitric
27 Szabo S, Trier JS and Fraukel PW: Sulphydryl compounds may
28 Garrick T, Buack S and Bass P: Gastric motility is a major factor
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