

Healing Properties of Some Indian Medicinal Plants against Indomethacin-Induced Gastric Ulceration of Rats

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Summary The healing activity of the ethanol extracts of *Piper betel*, *Emblica officinalis*, *Terminalia bellerica*, and *Terminalia chebula* against the indomethacin-induced stomach ulceration has been studied and compared with that of misoprostol. Compared to autohealing, all the drugs accelerated the healing process, albeit to different extents. The relative healing activities of the extracts was *P. betel*>*E. officinalis*>*T. bellerica*~*T. chebula*, that correlated well with their *in vivo* antioxidant and mucin augmenting activities. The excellent healing activity of the extracts of *P. betel* and *E. officinalis* indicated a major role of mucin protection and regeneration in the healing of nonsteroidal anti-inflammatory drugs mediated stomach ulceration.

Key Words: antioxidant, Indian medicinal plants, indomethacin, mucin, stomach ulcer

Introduction

Gastrointestinal toxicity associated with nonsteroidal anti-inflammatory drugs (NSAIDs) may be as high as 4–8% per year, despite the recent pharmaceutical advances [1]. For elderly NSAID users, fatal complications are close to 1 per 1,000 person-years of NSAID use, and higher for those with additional risk factors, such as prior history of ulcer disease. Various synthetic anti-ulcer drugs are presently available and some of these like misoprostol are specifically used to cure the NSAID induced gastric ulcer. However, each of these drugs confers simpler to severe side effects [2], warranting a search for non-toxic and inexpensive antiulcer medication [3]. Investigation on dietary plants that are also valued in the traditional systems of medicine might provide

efficient formulation for prolonged use. To this end, we have evaluated the healing effect of different parts of five plants viz. *Piper betel*, *Emblica officinalis*, *Terminalia bellerica*, and *Terminalia chebula* on indomethacin-induced acute experimental gastric ulcerated rats and compare the activity with that of the synthetic anti-ulcer drug, misoprostol.

All the chosen plants are valued in the Indian traditional system of medicine, ayurveda for their multiple health benefits. Ayurveda, the name made up of two Sanskrit words “Ayu” (life) and “Veda” (knowledge) is a holistic system of medicine developed in India over 5,000 years ago that uses a constitutional model to guide people for better health. The historical evidence of Ayurveda can be found in the ancient books of wisdom known as the Vedas, written over 6,000 years ago. It looks each individual as a unique makeup of the three doshas (bad effects) derived from viz. Vata (air), Pitta (metabolism) and Kapha (fluid) and suggests guidelines to reduce these. Thus, besides addressing specific health concerns, it also suggests proper lifestyle for health sustenance.

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The leaves of *P. betel* with a strong pungent and aromatic flavour are widely consumed as a mouth freshener and credited with wound healing, digestive and pancreatic lipase stimulant activities in the traditional medicine. Its utility against various diseases can be traced in the ancient vedic literature, Atharved as early as 3000–2500 BC. Likewise, the fruits of *E. officinalis*, *T. chebula* and *T. bellerica* are essential ingredients of most of the Ayurvedic “rasayana” (rejuvenation and longevity tonics) drugs and credited with a host of health benefits. These are used individually as well as in combination in equal proportions, the mixture being known as triphala [‘tri’ (three in Sanskrit) and ‘phal’ (fruits in Hindi and Sanskrit)]. Triphala is credited with several medicinal properties. Some of the medicinal attributes of these plants ratified worldwide by modern research and pertinent to the present studies are mentioned in the following.

The extracts of *P. betel* leaves are reported to provide cytoprotection against gastric lesions [4], and show digestive [5], and antioxidative [6, 7] properties. The antioxidant [8, 9], and anti-ulcerogenic activities [10–12] of *E. officinalis* have been confirmed. Besides its use by the tribal folks as traditional remedies from several ailments such as fever, cough, diarrhea, skin diseases and oral thrush, other beneficial effects of *T. bellerica* are also reported [13, 14]. *T. chebula* is used as an expectorant, aphrodisiac, tonic and diuretic, and has been found effective for wound healing [15] and as an antioxidant [16].

Materials and Methods

Chemicals and reagents

The plant parts viz. *P. betel* (leaves), *E. officinalis* (fruits), *T. chebula* (fruits), and *T. bellerica* (fruits) were collected from the local market and identified by the Botanical Survey of India, Indian Botanical Garden, West Bengal. 2-Thiobarbituric acid (TBA), Tris, ethylenediamine tetraacetic acid (EDTA) and ethanol were procured from E. Merck (Mumbai, India), while trichloroacetic acid (TCA) was from Thomas Baker, Mumbai, India. Alcian blue, indomethacin, dimethylaminobenzaldehyde, epinephrine, acetyl acetone and bovine serum albumin (BSA) were procured from Sigma Chemicals (St. Louis, MO). Other reagents used were H₂O₂ (35%, Lancaster, Morecambe, England), and perchloric acid, K₂HPO₄, KH₂PO₄, KOH and HCl (all from SRL, Mumbai, India). Stock solutions of EDTA and H₂O₂ were prepared in triply distilled deaerated water just prior to use. Stock solutions (1% w/v) of TBA were prepared in 50 mM NaOH solution and used within a week.

Preparation of the plant extracts

Fresh leaves of *P. betel* and sun-dried fruits of *E. officinalis*, *T. chebula* and *T. bellerica* (each 250 g) were individually extracted with 1 l of 95% ethanol at 22°C for 4 days with

intermittent shaking, and using fresh batch of solvent each day. In each case, the supernatant was decanted and filtered through a nylon mesh. The supernatants were concentrated in vacuo and finally lyophilized to obtain the respective extracts that were stored in a vacuum dessicator. These were designated as PBE (*P. betel*, 2.2% w/w), EOE (*E. officinalis*, 10.1% w/w), TCE (*T. chebula*, 10.6% w/w) and TBE (*T. bellerica*, 11.2% w/w) respectively.

Preparation of the drug

The doses of the extracts (designated as drugs) used for the studies were PBE (120 mg), EOE (100 mg), TBE and TCE (each 200 mg)/kg body weight of rats. Each of the extracts was macerated with a mortar pestle in double distilled water containing gum acacia (2% w/w) to provide the drug. The drugs (1 ml per day) were administered by oral intubation using a feeding cannula.

Experimental protocol for ulceration and healing

The rats were bred at Dr. B.C. Roy Post Graduate Institute of Basic Medical Sciences, Kolkata, India and BARC Laboratory Animal House Facility, Mumbai, India. These were procured after obtaining clearance from the respective Animal Ethics Committee of the two centres and were handled following international Animal Ethics Committee guidelines. Male Sprague-Dawley rats (weighing 180–200 g) were reared on a standard laboratory diet (Ralston Purina, Chicago, IL) and given tap water. They were kept in a room at where temperature (24 ± 2°C), humidity (65–70%), and day/night cycle (12 h/12 h) were controlled. A total of 40 rats were randomly divided into 8 groups (5 animals in each group) and each set was replicated three times. Group I rats serving as the normal control received only the vehicle oral dose of gum acacia in distilled water (1 ml per rat). Ulceration in the groups II–VIII rats was induced with indomethacin (30 mg/kg body weight, oral intubation) dissolved in distilled water. Rats were deprived of food but had free access to tap water 24 h before ulcer induction. Group II rats serving as the ulcerated control received only indomethacin, and were sacrificed 4 h after indomethacin administration. Group III rats were given the vehicle (1 ml per rat) only for seven days. Rats in the groups IV–VII received the drugs (PBE, EOE, TCE and TBE) respectively, while group VIII rats received misoprostol (1.43 µg/kg body weight) once daily by oral intubation starting from four hour after the indomethacin administration. After seven days, the rats of groups I, and III–VIII were sacrificed by cervical dislocation followed by cutting off the abdominal aortic artery.

Quantification of ulceration

The areas of mucosal damage were calculated in square millimeters and expressed as a percentage of the surface of

the glandular stomach according to a reported procedure [17].

Preparation of tissue homogenate

After noting the wet weight, the stomach tissue was homogenized in a 50 mM phosphate saline buffer (PBS) pH 7.2 under cold condition, using a glass-teflon homogenizing tube. The homogenate was centrifuged at 2500 rpm for 10 min, the supernatant was carefully removed from the pellet and used for the biochemical analyses.

Quantification of lipid, protein and DNA damages during ulceration and healing

The lipid peroxidation products were estimated [18] in terms of malondialdehyde (MDA) formation. The amounts of protein carbonyls and DNA contents were estimated following reported methods [19, 20].

Assessment of enzymatic activities

The specific activity of catalase (CAT) in the tissue was estimated according to a reported method [21]. The tissue homogenate (20 μ l) was added to a H₂O₂-phosphate buffer mixture (3 ml), maintaining the optical density against buffer at 240 nm to 0.500 ± 0.010 ($d = 1$ cm). The rate of change of optical density at 240 nm with time was recorded for the calculation of the catalase activity.

Following a reported method [22], the tissue superoxide dismutase (SOD) activity was measured. This involves assaying the SOD-mediated inhibition of epinephrine auto-oxidation at an alkaline medium. For this, the absorbances of the samples at 480 nm were noted at an interval of 30 s. The enzyme activity was measured in arbitrary units, considering 50% inhibition as 1 unit of enzyme activity.

Mucin assay

Following a reported method [23], the free mucin in the gastric tissues was estimated by measuring the amount of Alcian blue (Ab) bound to mucin. Briefly, the gastric tissues were incubated with a 1% buffered solution of Ab in aqueous 3% acetic acid (0.5 ml) at 37°C for 30 min. After centrifuging the solution, the concentration of Ab in it was measured from the absorbance at 615 nm.

Hexosamine assay

The hexosamine concentrations in gastric tissues were assayed according to a reported method [24] with a minor modification. The gastric tissues were hydrolyzed in acidic medium, the hydrolysate neutralized with 3N NaOH (litmas) and diluted to 10 ml with double distilled water. To an aliquot (1 ml) was added acetyl acetone solution (1 ml, prepared by dissolving 1 ml of acetyl acetone in 50 ml 0.5 N sodium carbonate), the solution mixed well, and heated on a boiling water bath for 15 min avoiding evaporation. After cooling, ethanol (5 ml, 95%) was added to the mixture

followed by Ehrlich's reagent (1 ml, prepared by dissolving 0.8 g *para*-dimethylaminobenzaldehyde in 30 ml methanol and 30 ml conc. HCl). The mixture was diluted to 10 ml with 95% ethanol, allowed to stand for 30 min, and its absorbance at 530 nm was read.

Estimation of the total phenolics and flavonoids contents in the plant extracts

Following our reported method [25], the amounts of total phenolics and flavonoids in the extracts were determined. Each test extract (100 μ l) was mixed with 1:10 Folin-Ciocalteu's reagent (500 μ l) followed by addition of aqueous Na₂CO₃ (400 μ l, 7.5%). After incubating the reaction mixture at 24°C for 2 h, the absorbance at 765 nm was recorded. Gallic acid monohydrate was used as the standard. The total phenolic contents of EOE, TCE and TBE are expressed in terms of mg gallic acid equivalent (GAE)/g dry weight of the plant extract.

Each extracts (100 μ g) was added to 0.4 ml distilled water followed by NaNO₂ (0.03 ml, 5%). After 5 min at 25°C, AlCl₃. 6H₂O (0.03 ml, 10%) was added followed by aqueous NaOH (0.2 ml, 1 M) after 6 min. The mixture was diluted with water to 1 ml and the absorbance at 510 nm was read. Epicatechin was used as the standard and the total flavonoid contents of EOE, TCE and TBE are expressed as mg epicatechin equivalents (ECE)/g dry weight of the extract.

Statistical analyses

All the results were expressed as mean \pm SE. Student's *t* test at $p < 0.05$ was used to assess statistical significance in various groups of animals.

Results

Effect of the drugs on gastric ulcer healing in rats

Oral administration of the plant extracts at different doses, e.g. 50–250 mg/kg body weight for 7–10 days, accelerated the rate of healing of gastric lesion induced by indomethacin (data not shown). It was found that a seven day post-ulcerative treatment with the individual drugs at the designated doses was optimal for effective ulcer healing. Hence all subsequent experiments were carried out with the same protocol. The dose of the positive control, misoprostol (1.43 μ g/kg body weight) was decided based on its recommended dose for humans.

Treatment of rats with indomethacin produced typical acute lesions in the gastric mucosa resulting in substantial ulcer index. This was reduced by 36.2% for the group III rats due to autohealing. Compared to the zero day ulcerated group (group II), treatment with PBE and EOE reduced the ulcer index by 78.3, and 85.7% respectively. TCE and TBE were significantly less active with 48.5 and 43.6% healing capacities. In comparison, misoprostol (1.43 μ g/kg body

weight) gave 85.4% reduction in the ulcer index. The results are summarized in Table 1. The ulcer healing data of all the drug-treated groups were significantly different from those of the zero day and seven day untreated experimental control rats (groups II and III) ($p < 0.05$). However, the results of groups IV and V as well as of groups VI and VII rats were not significantly different.

The gastric tissue morphologies of the rats of groups I–V and VIII were also observed macroscopically (Fig. 1a–f). This revealed that compared to the vehicle treated rats, oral administration of indomethacin (30 mg/kg) produced acute lesions in the gastric mucosa within 4 h with number of blood clots in the ulcer spots and perforations (*cf.* Fig. 1a, b). The seven day ulcerated (untreated experimental control)

Table 1. The healing capacity of the drugs on ulcerated rats^a

Samples	Dose of drug	Ulcer index	% Protection ^b
Unulcerated control	—	0	—
Ulcerated control ^b	—	26.71 ± 1.6	0
Ulcerated untreated control		17.62 ± 1.2	36.18 ± 1.4
Ulcerated PBE treated	120 mg/kg body weight	5.84 ± 0.3	78.28 ± 2.8
Ulcerated EOE treated	100 mg/kg body weight	3.81 ± 0.5	85.73 ± 3.7
Ulcerated TCE treated	200 mg/kg body weight	13.81 ± 1.7	48.50 ± 3.8
Ulcerated TBE treated	200 mg/kg body weight	14.31 ± 1.4	43.63 ± 2.1
Ulcerated misoprostol treated	1.43 µg/kg body weight	3.95 ± 0.3	85.40 ± 3.5

^aStomach ulceration in rats was induced by oral administration of indomethacin (30 mg/kg body weight). The doses of the drugs are as mentioned in the Materials and Methods section. The ulcer indices values are mean ± SEM and were compared statistically by one-way ANOVA. *Significant at $p < 0.05$ as compared to the zero day and seven day untreated experimental control rats (groups II and III). The values of groups IV, V and VIII were significantly different ($p < 0.05$) from those of groups VI and VII. However, there was no significant difference in the values of groups IV, V and VIII as well as of groups VI and VII. ^bConsidering an ulcer index of 100 for the ulcerated, untreated rats; ^cThe ulcer indices were measured 4 h after indomethacin administration. For other samples the measurement was carried out after 7 days.

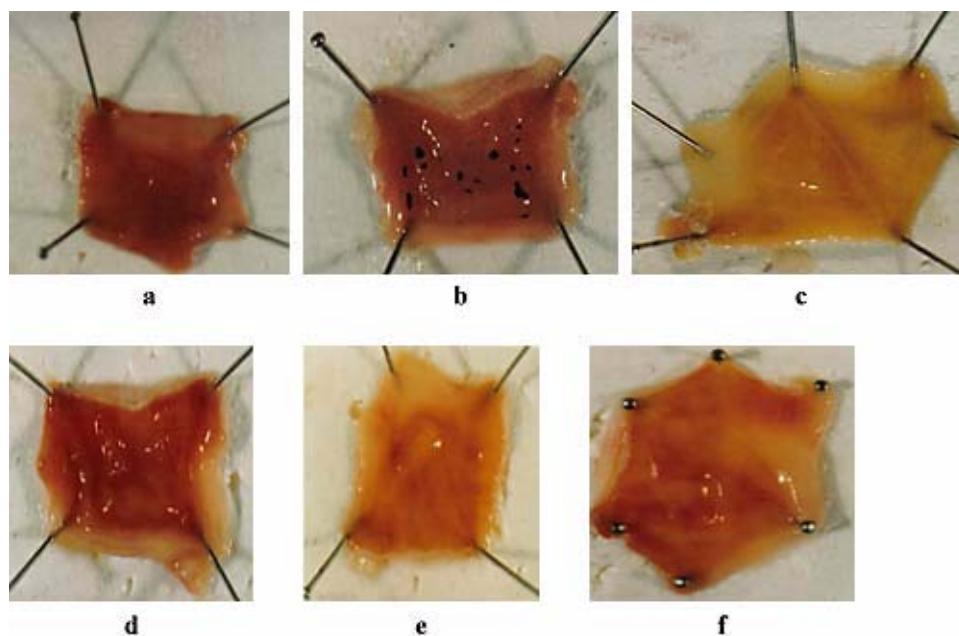


Fig. 1. Macroscopic assessment of the healing of acute gastric mucosal injury induced by indomethacin in rats and its prevention by PBE, EOE and misoprostol. Section of rat stomachs obtained from a: normal control rats; b: ulcerated untreated control rats 4 h after indomethacin administration; c: ulcerated untreated control rats 7 day after indomethacin administration; d: ulcerated rats treated with PBE for 7 days; e: ulcerated rats treated with PBE for 7 days; f: ulcerated rats treated with misoprostol for 7 days.

stomach showed lesser spots but the tissues were hyaline in nature (Fig. 1c). In comparison, stomachs of the PBE and EOE-treated rats were healthy almost without any ulcer spots (Fig. 1d, e). The stomachs of the rats treated with PBE were equivalent to those of the normal control rats (group I) (*cf.* Fig. 1d, a). In comparison, stomachs of the rats treated with misoprostol showed less ulcer spots but the tissues remained hyaline in nature (Fig. 1f).

Effect of the drugs on lipid peroxidation and DNA and protein contents in gastric tissues

The effects of indomethacin intake alone, and following administration of the drugs on the extent of lipid peroxidation (measured in terms of MDA), protein oxidation (measured in terms of proteins carbonyls), and DNA damage in the gastric tissues of rats are shown in Table 2. Indomethacin administration markedly stimulated lipid peroxidation in gastric tissues, and the MDA content was elevated by about 252% compared to the un ulcerated control rats. This was reduced by 25.7% after seven days due to autohealing for the untreated control rats (group III), although the MDA content remained significantly high (187%) compared to that in the normal rats. Treatment with PBE and EOE reduced it by 54.9 and 53.7% respectively, while TCE and TBE brought them to even less than normal value (10.0 nmol/mg prot.). Thus, amongst the test drugs, TBE and TCE could inhibit lipid peroxidation most efficiently. The effect of misoprostol was similar to that of EOE. The data for the treatment with the drugs are significant compared to those of rats of groups II and III ($p < 0.05$).

Compared to the normal rats, the indomethacin-induced ulceration led to extensive protein oxidation as revealed from 329% increase in the protein carbonyls content, which was reduced by 45.3% due to autohealing. Compared to ulcerated rats, treatment with PBE, EOE, TCE and misoprostol reduced it by 65.1, 60.0, 60.7 and 62.4% respectively, while the effect of TBE was much less (~54.8%). Thus, the relative protective activities of the drugs against protein oxidation was PBE>TCE~EOE>TBE. The data for the treatment with the drugs are significant compared to those of the groups II and III rats ($p < 0.05$). The data with the groups IV–VI were significantly different compared to that of the group VII rats ($p < 0.05$). However, the data of the groups IV–VI and VIII rats were not significantly different ($p < 0.05$).

The tissue DNA concentration was significantly (57.1%) reduced by indomethacin administration. During autohealing, the DNA level increased to 61.5% of the normal value. Treatment with EOE, TCE and misoprostol brought back the tissue DNA level to near normalcy (~1.7–1.8 for the treated groups vs 1.82 for group I rats), while surprisingly the PBE treatment increased the DNA level by 196% compared to the normal rats. The augmentation of DNA by the drugs was significant ($p < 0.05$) as compared to the ulcerated controls (group II). The DNA level for the TBE treated rats was only 67% of the un ulcerated rats, which was similar to that observed with autohealing.

Effect of the drugs on the SOD and CAT levels in gastric tissues

The effect of indomethacin intake alone, and following

Table 2. The effect of the drugs on lipid, protein, DNA, SOD and CAT levels of ulcerated gastric tissue of rats^a

Samples	MDA (nmol/mg prot.)	Carbonyl (μ g/mg prot.)	Total DNA (mg/g tissue)	SOD (U/min/mg prot.)	CAT (U/min/mg prot.)
Unulcerated control	9.96 \pm 0.1	5.26 \pm 1.34	1.82 \pm 0.18	22.2 \pm 2.34	21.2 \pm 1.12
Ulcerated control ^b	25.09 \pm 1.4	17.29 \pm 1.8	0.78 \pm 0.10	5.76 \pm 2.32	9.02 \pm 2.54
Ulcerated control	18.64 \pm 3.2	9.46 \pm 2.15	1.12 \pm 0.21	11.58 \pm 1.32	14.2 \pm 2.84
Treated with PBE	11.32 \pm 2.1	6.03 \pm 1.5	3.56 \pm 0.05	21.14 \pm 1.24	19.04 \pm 2.15
Treated with EOE	11.62 \pm 1.2	6.93 \pm 1.6	1.65 \pm 0.6	18.54 \pm 2.5	19.32 \pm 1.9
Treated with TCE	9.24 \pm 1.6	6.80 \pm 1.8	1.78 \pm 0.50	21.32 \pm 3.1	21.61 \pm 2.2
Treated with TBE	9.60 \pm 2.1	7.82 \pm 1.8	1.22 \pm 0.31	22.52 \pm 2.3	18.61 \pm 2.3
Treated with misoprostol	11.63 \pm 1.5	6.54 \pm 1.1	1.68 \pm 0.23	16.71 \pm 2.6	15.84 \pm 2.1

^aStomach ulceration in rats was induced by oral administration of indomethacin (30 mg/kg body weight). The doses of the drugs are as mentioned in the Materials and Methods section. ^bThe assays were carried out 4 h after indomethacin administration. For other samples these were done after 7 days. The values are mean \pm SEM and were compared statistically by one-way ANOVA. *Significant at $p < 0.05$ as compared to the zero day and seven day untreated experimental control rats (groups II and III). The values for protein carbonyl with the groups IV–VI and group VIII were significantly different compared to that of the group VII rats ($p < 0.05$), without being significantly different among each other. The values for SOD and catalase with the groups IV–VII were significantly different compared to that of the group VIII rats ($p < 0.05$). For SOD, the values with the groups IV, VI and VII were significantly different compared to that of the group V rats ($p < 0.05$), without being significantly different among each other. For catalase, the values with the groups IV, V and VII were significantly different compared to that of the group VI rats ($p < 0.05$), without being significantly different among each other.

administration of the drugs on the levels of SOD and CAT in gastric tissues of rats are also presented in Table 2. The activities of both the enzymes were reduced following indomethacin administration. The SOD activity decreased by 74%, while that of CAT was reduced by about 57.5% compared to those in normal control rats. Autohealing restored the SOD and CAT levels to 52 and 67% of the normal values. Treatment with PBE, TCE and TBE restored the SOD level almost to normalcy. EOE was also effective restoring the activity by 83.3% of the normal value. Thus, all the drugs were effective in protecting oxidative damage to SOD with a relative protective order of TBE>TCE~PBE>EOE. All these data were significant ($p<0.05$).

With CAT, TCE was very effective in restoring the enzyme level to near normalcy. In comparison, PBE, EOE and TBE restored the CAT level to 89.8, 91.0 and 87.7% of the control value respectively. In this case TCE was most effective, while the other extracts also showed impressive CAT augmenting abilities. The effect of misoprostol was less, increasing the SOD and CAT levels up to 75.2 and 74.5% of the normal values. The data for the SOD and CAT levels for the drug-treated rats were significantly different ($p<0.05$) from those of the untreated zero and seven day animals (groups II and III).

Effect of drugs on hexosamine and mucin contents of gastric mucosa

Indomethacin administration to rats decreased defensive mucin secretion significantly as indicated by decrease in the contents of Alcian blue-binding protein (43.5% decrease, $p<0.05$) and mucosal glycoproteins (76.4% decrease, $p<0.05$) in the ulcerated rats of group II compared to those in unulcerated rats (group I). Treatment with PBE and EOE enhanced tissue mucin levels to those in normal unulcerated rats, while the mucosal glycoprotein content was also increased to 65 and 61% of the normal value respectively. Surprisingly, treatment with TCE or TBE had no effect on the mucin level. Further, although TBE marginally improved the hexosamine level to ~34% of the normal value, TCE did not augment it. The results on the levels of mucin and hexosamine are summarized in Fig. 2 and Fig. 3 respectively. The augmentation of mucin levels by PBE and EOE were significant ($p<0.05$) as compared to untreated ulcerated controls (group II). Misoprostol also increased the mucin secretion and mucosal glycoproteins in the ulcerated rats to 94 and 90% of the normal values.

Discussion

Currently the NSAIDs are preferred drugs for various diseases. However, these are known to generate oxygen free radicals that are known to play a role in the pathogenesis of mucosal injury [26]. The antioxidants are advocated to offer

effective protection against induction and progression of gastric ulcer. The reported medicinal attributes and antioxidant property of the chosen plants triggered us to assess their possible protective effects against indomethacin-induced gastric lesions in rats. Although the gastrocytoprotective properties of PBE and EOE have been reported by us [4, 10] and others [11, 12], these do not automatically guarantee their healing potency for the treatment of acute gastric ulcer. The reported [12] ulcer-healing activity of *E. officinalis* was based on a very limited study. Further, the study was conducted with an extract prepared in toxic methanol, and did not include any NSAID as the causative agent. The present detailed study used the pharmaceutically acceptable solvent, ethanol for preparing the plant extracts. It is also worth noting that compared to methanol, ethanol is a better extractant for the antioxidant phenolics.

Measurement of the ulcer indices of the rats demonstrated that treatment with the plant extracts led to faster ulcer-healing compared to the untreated rats. The healing potency of the drugs was different, with a relative order of PBE>EOE>>TCE~TBE. This was also corroborated by the macroscopic gastric tissue morphology.

Tissue damage is always associated with lipid peroxidation, loss of DNA content and impairment of protein synthesis [27] due to excess generation of free radicals. These free radicals also damage the cellular antioxidant enzymes such as CAT, SOD and others, leading to aggravated tissue damage during stomach ulceration [28]. Our results revealed that the indomethacin-induced stomach ulceration was accompanied with a severe oxidative stress in the gastric tissues causing damages to key biomolecules such as lipids, proteins and DNA. This was apparent from the stimulated lipid and protein oxidation leading to increased accumulation of MDA and protein carbonyls, as well as reduction in the tissue DNA contents. The gastric activities of SOD and CAT were also decreased notably following indomethacin intake. Treatment with the drugs could bring these parameters towards normal levels, than observed in natural recovery. Suppression of most of the biochemical adverse effects by the drugs might decrease the ulcer progression and promote healing of gastric lesions induced by acute intake of indomethacin.

Although the phenolic contents of PBE and EOE were less, they were rich in flavonoids accounting for their superior ulcer-healing activities via the antioxidant action. In contrast, the flavonoid contents of TCE and TBE were significantly lower compared to their phenolics contents (Table 3). Apparently, the phenolics in these are present in more complex forms that are not easily bioavailable due to high polarity. Consequently, both these plant-preparations showed significantly less ulcer-healing property.

Ulcer-healing is a complex process involving a combination of wound retraction and re-epithelialization [29]. Release of

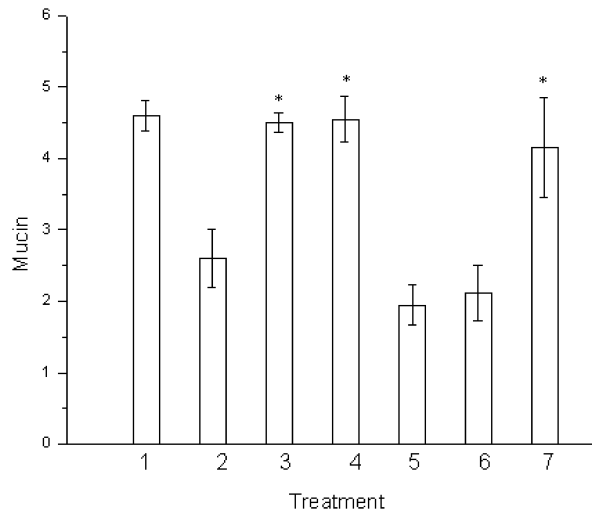


Fig. 2. The effect of drugs on mucin level of ulcerated rats. Stomach ulceration in rats was induced by oral administration of indomethacin (30 mg/kg body weight). The drugs at the doses mentioned in the Materials and methods section were administered 4 h after indomethacin administration. The mucin assay was carried out after 7 days for all the samples except for the ulcerated control. The assay for the ulcerated control was carried out 4 h after indomethacin administration. 1: normal control; 2: ulcerated control; 3: ulcerated PBE treated; 4: ulcerated EOE treated; 5: ulcerated TCE treated; 6: ulcerated TBE treated; 7: ulcerated misoprostol treated. The values are mean \pm SEM and were compared statistically by one-way ANOVA. *Significant at $p < 0.05$ as compared to the zero day experimental control rats (group II). The values with the groups III, IV and VII were significantly different from that of the groups V–VI ($p < 0.05$) without being significantly different from each other.

performed mucus also plays a role in promoting epithelial recovery after acute injury by forming a mucoid cap beneath which re-epithelialization occurs [30]. Besides providing significant buffering capacity for the neutralization of luminal acid, the mucus can offer protection against the endogenous aggressors like, acid, pepsin and oxidants produced in the gastric lumen, as well as against exogenous damaging agents, such as NSAIDs.

Thus, besides antioxidant action that protects the mucus layer and arrests ulcer progression, drugs that increase the synthesis and secretion of gastric mucus would accelerate gastric ulcer healing. In this study, the decreased mucin secretion in the indomethacin-administered rats indicated reduced ability of the mucosal membrane to protect the mucosa against hemorrhagic ulcer from physical damage and back diffusion of hydrogen ions. The reduction in the content of hexosamine, the major glycoprotein of gastric mucosa further proved the decreased ability of the gastric mucosa to withstand the offensive onslaught. Treatment

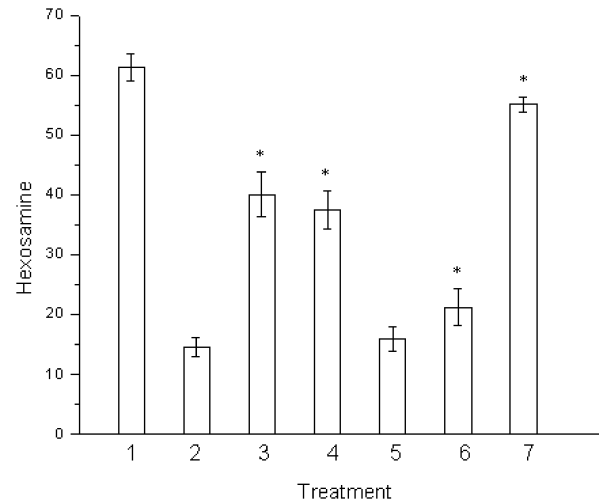


Fig. 3. The effect of drugs on hexosamine levels of ulcerated rats. Stomach ulceration in rats was induced by oral administration of indomethacin (30 mg/kg body weight). The drugs at the doses mentioned in the Materials and methods section were administered 4 h after indomethacin administration. The hexosamine assay was carried out after 7 days for all the samples except for the ulcerated control. The assay for the ulcerated control was carried out 4 h after indomethacin administration. 1: normal control; 2: ulcerated control; 3: ulcerated PBE treated; 4: ulcerated EOE treated; 5: ulcerated TCE treated; 6: ulcerated TBE treated; 7: ulcerated misoprostol treated. The values are mean \pm SEM and were compared statistically by one-way ANOVA. *Significant at $p < 0.05$ as compared to the zero day experimental control rats (group II). The values with the groups III, IV and VII were significantly different from that of the groups V–VI ($p < 0.05$). The values with the group III and group IV were not significantly different from each other, but were significantly different from that of group VII.

Table 3. The total phenolic and flavonoid contents of the plant extracts^a

Plant extract	Phenolic content (mg GA equivalents/g)	Flavonoid content (mg CA equivalents/g)
PBE	32.0 \pm 2.8	35.0 \pm 2.1
EOE	20.0 \pm 3.6	57.6 \pm 3.0
TCE	277.0 \pm 21.5	16.5 \pm 1.9
TBE	424.5 \pm 20.3	64.3 \pm 2.8

^aThe values are mean \pm SEM ($n = 4$)

with the drugs, PBE, EOE and misoprostol significantly accelerated the ulcer healing process, which is associated with an increase in the mucin and hexosamine levels in the gastric mucosa. This indicated that enhancement of the mucus modulation by these drugs play a significant role in their ulcer healing potency. The relative order of augmentation of

both mucin and hexosamine by these drugs correlated well with their ulcer healing capacities. In contrast, the auto healing observed with the untreated control and healing shown by the treatment with TCE and TBE can possibly be attributed to other mechanisms.

Mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals [31]. Apparently, the free radicals scavenging property of drugs might be contributing in protecting the oxidative damage to gastric mucosa that accelerates healing of gastric ulcers. An increase in mucus production usually assist the healing process by protecting the ulcer crater against irritant stomach secretions (HCl and pepsin) [32] thereby enhancing the rate of the local healing process.

Given that some drugs can show mild-to-severe side effects even after short-term intake, we also evaluated the possible toxic effects of the drugs upto a dose of 25 mg/kg body weight with both mice and rats. There was no observable physical sign change, and the animals had normal food and water as well as stool during the experimental period. These findings suggested that the drugs given at the current dose do not have any potential side effects in the animals.

Conclusions

The biochemical data obtained with the tested drugs correlated grossly with their ulcer-healing capacities. The anomaly observed especially with TCE and TBE might be due to the complexity of the ulcer-healing process. Apparently, the supremacy of PBE and EOE over the other plant extracts in healing gastric ulceration can be attributed to their higher capacity to augment the stomach mucin level. Overall, the present study established that the chosen plant extracts especially PBE and EOE can heal indomethacin-induced stomach ulceration in rats by their antioxidant action and ability to form mucus. Earlier, our group has also reported the cytoprotective activity of the above plant against stomach ulceration. These results, taken together along with their non-toxicity established the potential of PBE and EOE as potent anti-ulcerogenic drugs. Comparison of their efficacy with that of misoprostol further confirmed the findings.

Ulceration due to NSAID is also believed to occur because of non-selective inhibition of cyclooxygenase leading to reduction in prostaglandins which, in turn, inhibits the release of mucus. The relationship between prostaglandins and leukotrienes is an important factor in gastric ulcers, and many phenolic compounds are known to modulate the arachidonic acid [33]. Thus, it would be of interest to study the effects of the above plant extracts on the prostaglandin-dependent pathway of healing gastric ulcer. Investigation in this regard is currently in progress in our laboratory and the results will be reported latter.

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