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RESEARCH ARTICLE

Development and evaluation of a gastro-retentive delivery system for improved antiulcer activity of ginger extract (*Zingiber officinale*)

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Abstract

Aim was to develop and optimize multiunit gastro-retentive floating beads (FBs) intended for localized and prolonged release of ginger for treating gastric ulcers. Protective effect of ginger extract (GE) against ulcer is well documented, but therapeutic use is compromised due to poor bioavailability and physicochemical properties. GE was only slightly soluble (3.19 ± 0.38 mg/ml) in simulated gastric fluid (SGF; pH 1.2). The solubility decreased in water to 0.69 ± 0.03 mg/ml and further by 26% in the presence of calcium carbonate (0.5% w/v). We prepared FBs of GE using calcium carbonate and sodium alginate in different proportions. Beads were evaluated for diameter, buoyancy, entrapment, and porosity. *In vitro* dissolution showed a Fickian release with a cumulative release of >80% at 24 h. Preclinical evaluation was done in cold-restraint stress induced gastric ulcers, in albino rats, in terms of (i) ulcer index, hemorrhagic streaks (l), mucus content, (ii) oxido-nitrosative stress, and (iii) histopathology. GE loaded FBs (200 mg/kg) were significantly better than free GE and better/equivalent to cimetidine (10 mg/kg). The system was evaluated for therapeutic effect (curative), i.e. after the induction of ulcers. Most of the natural phytochemical or antioxidants show pretreatment effectiveness. We, however, developed and established GE FBs for sustained curative effect.

Keywords: Ginger extract, gastro-retentive drug delivery system, curative effect, cold-restraint stress, sustained release

Introduction

Ginger (*Zingiber officinale* Roscoe, *Zingiberacae*) is not only widely used around the world in foods as a spice but is also among the top five antioxidant foods. The major pungent phenolic constituent of ginger is 6-gingerol which possesses many pharmacological activities. Ginger extracts (GEs) predominantly containing gingerols or shogaols are reported to posses many pharmacological activities including PGE_2 inhibition (Goto et al., 1990; Scott, 2006; Pan et al., 2008).

More recently, cytoprotective effect of GE on gastric mucosa has been reported (Bhandari et al., 2009; Siddaraju et al., 2009). Pungent component of GE at a dose of 100 mg/kg significantly inhibited the gastric lesion upon pretreatment (Johji et al., 1988). The active phenolic constituent of ginger, mainly gingerol and zingerone, play a major role in inhibiting parietal cell H^+, K^+-ATPase. Activity of this enzyme plays a very crucial role in proton pump and promotes gastric acid secretion; latter is responsible for a variety of ulcers irrespective of the root cause (Sachs et al., 1978). GE also shows protective effect against *Helicobacter pylori* induced ulcers (Siddaraju and Dharmesh, 2007).

However, the remarkable pharmacological activities of ginger or 6-gingerol are compromised due to its poor pharmacokinetic properties. 6-gingerol is cleared very rapidly from plasma with a short terminal half-life of 7.23 min and a total body clearance of 16.8 ml/min/kg after intravenous administration (Ding et al., 1991). After oral administration, 6-gingerol is rapidly

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absorbed into the plasma and the maximal concentration (4.23μg/ ml) is reached after 10 min of oral dosing (Jiang et al., 2008).

Considering a rapid absorption and disposition of 6-gingerol, it will be desirable to design a slow release system for achieving a localized effect in stomach. The real challenge in the development of such a controlled drug delivery system is not just to sustain the drug release but also to prolong residence time of the dosage form in the stomach. There are several approaches to increase gastric residence time: (i) a bioadhesive delivery system that adheres to mucosal surface (Akiyama and Nagahara, 1999), (ii) a swellable delivery system, which increases in size after swelling to retard passage through the pylorus (Curatolo and Lo, 1995), and (iii) a density-controlled delivery system, which either floats or sinks in gastric fluid (Moes, 1993; Wong et al., 1993). Among these, floating drug delivery system (FDDS) is of particular interest for those drugs that act locally and are primarily absorbed in the stomach. On the basis of above, incorporation of GE in to FDDS was considered to be an appropriate option for treatment of gastric ulcers. Further, multiple unit FDDS are uniformly distributed within the gastric content such that the inter- and intra-subject variabilities in drug absorption and chances of dose dumping are reduced (Bechgaard and Nielson, 1978; Vervaet et al., 1995).

Involvement of reactive oxygen species (ROS) is implicated as a major cause of various types of gastric ulcers, inducing lesions caused by stress (Konturek et al., 2006), alcohol consumption (Talley et al., 2009), and use of nonsteroidal anti-inflammatory drugs (García Rodríguez and Jick, 1994; Deng et al., 2007). Several reports present in the literature discuss the role of antioxidants in gastric ulcers (Yoshikawa et al., 1993; Repetto and Llesuy, 2002).

Aim of the present study was to establish therapeutic antiulcer potential of gastro-retentive floating beads (FBs) of GE. Such a system will restrain GE within the gel polymer mesh work (alginate-HPMC) so that GE will diffuse out slowly from the beads. Further, the floating nature of beads will prolong the gastric residence time. A slow release coupled with extended stay will improve therapeutics of GE which is otherwise quickly absorbed into and lost from systemic circulation due to fast elimination. Unabsorbed GE will pass from stomach to the intestine and finally in to the feces without eliciting the desired physiological effect at the site because of limited contact. Furthermore, slightly soluble nature of free GE (as shown in the present study) in gastric juices also limits its therapeutic activity. Emerging research continues to provide evidence that diets including plant-based phytochemical antioxidants can reduce and/or delay the onset of various oxidative, stress-induced pathologies including chronic inflammatory diseases.

Literature indicates the protective role of these dietary antioxidants, in preclinical studies, where these agents are administered as a pretreatment, i.e. administration is started prior to the induction of oxidative stress or more specifically the disease. However, the development and establishment of these agents as therapeutics for treatment post-induction of the disease/ pathological state would be a worthwhile contribution. Securing a suitable place for these agents in the physician's armamentarium can be gained by pharmaceutical couturing of these agents, and this is the intent of the current study.

The present study describes development of an effective FDDS for GE and provides evidences for the potential ulcer-preventive ability of GE. The formulation was evaluated in terms of its protective effect against gastric ulcers, post-induction, in terms of ulcer index, extent of mucus secretion, oxidative and nitrosative stress, and ulcer-induced histopathological changes.

Materials and methods

Materials

GE (16% gingerol and 6% zingiberene) was procured as a gift sample from Nisarga biotech, Satara, India. It was claimed to be prepared under supercritical CO_2 extraction at 300 Bar and 39°C; content of gingerol was 16% as determined by high-performance liquid chromatography analysis method. Sodium alginate, hydroxypropylmethyl cellulose (HPMC), calcium carbonate, calcium chloride, methanol and acetic acid were purchased from S.D. Fine Chem. Ltd., Mumbai, India, and were of analytical reagent (AR) grade. All other chemicals or reagents used in the study were of AR or guranteed reagent (GR) grade.

Validation of spectrophotometric method of analysis for GE in different solvents

Various standards (10, 20, 40, 60, 80, 100 μg/ml) were prepared from a 100 μg/ml stock solution of GE in methanol, mixture of triple distilled water-methanol (90:10 v/v) and mixture of simulated gastric fluid (SGF pH 1.2)-methanol (90:10 v/v). These standards were subsequently used to prepare a calibration curve (λ_{max} 281 nm) of GE. The method was validated with respect to linearity, accuracy, and precision.

Preparation of FBs

Calcium alginate beads were prepared by orifice ionic gelation method (Choi et al., 2002). Briefly solution of GE was prepared by dissolving 3 g of GE in 5 ml PEG 400. The solution was dispersed in sodium alginate solution (3% w/v) containing HPMC (alginate: HPMC = 9:1 w/w). The gas forming agent calcium carbonate was added to the solution in weight ratio ranging from 0.25:1 to 0.75:1 (gas forming agent: alginate w/w). The mixture was degassed under bath sonicator (20–30 min) to remove any entrapped air. The resulting solution was dropped through a 26 G syringe needle into 1% w/v calcium chloride solution containing 10% v/v acetic acid. The solution containing suspended beads was allowed to stir for 1 h to improve mechanical strength and for completion

of the reaction to produce gas at room temperature. Carbonate salts are insoluble at neutral pH while its cation (calcium ion) is released in the presence of acid. The formed beads were separated, washed initially with alcohol, subsequently with distilled water and freeze-dried overnight using freeze dryer maintained at a temperature of –40°C. Product was lyophilized further for 6 h at –70°C. Table 1 lists the formulation variables for the prepared GE loaded calcium alginate FBs.

Characterization and evaluation of GE FBs

Determination of diameter

The diameter of calcium alginate beads was determined using particle size analyzer (Malvern instruments limited, Malvern, UK).

Determination of drug entrapment efficiency (DEE) of FBs

Accurately weighed GE beads (50 mg) were crushed in a mortar and 15 ml methanol was added to them. The mixture was transferred to a tube mixed thoroughly on a vortex and centrifuged. Suitable dilutions were prepared, in methanol, and the samples were analyzed spectrophotometrically against methanol as a blank. DEE was calculated according to the following equation.

$$\%\,\text{DEE} = \frac{\text{Actual drug content in beads}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

Determination of buoyancy

The floating ability of the beads was determined using USP type II dissolution test apparatus. Fifty beads were placed in the vessel containing 500 ml of SGF dissolution media maintained at 37 ± 0.5°C and stirred at 100 rpm (Ishak et al., 2007). The number of beads settling down after 24 h was measured by visual observation, and the percentage of beads which remain floating was determined.

Determination of porosity and bulk density

Beads were filled in a 10-ml graduated measuring cylinder up to the 10-ml mark. Then the cylinder was tapped for around 500 times and the volume noted subsequently. Initial volume or the bulk volume was 10 ml in all the cases and the final volume indicated tap volume of the beads. The Porosity and bulk density of the developed beads were calculated according to the following equation(s) (Martin et al., 2005).

$$\text{Porosity}\,(\epsilon) = \frac{Vb - V}{Vb} \times 100 \quad (2)$$

$$v = Vb - Vp \quad (3)$$

$$\text{Bulk density}\,(\delta) = M / Vb \quad (4)$$

Vb = Bulk volume of the particles

V_p = True volume of the particles

V = Void volume of the particles (spaces of the particles)

M = Mass of 10 ml FBs

Surface characterization by scanning electron microscopy

The external and internal morphology of the freeze-dried calcium alginate FBs were studied by scanning electron microscopy (SEM). Samples were coated with gold film under vacuum to modify in conducting materials and investigated. The internal structure of the beads was examined by cutting them in a half with a steel blade.

In vitro dissolution cum release study

Solubility studies

Prior to putting up the dissolution studies of beads, solubility of GE was determined in water and in simulated gastric fluid (SGF pH 1.2; USP, 2004), at 37 ± 2°C. Changes in solubility of GE in water in the presence of various formulation components viz. sodium alginate, calcium carbonate, poly ethylene glycol 400 (PEG 400), HPMC, calcium chloride, and a mixture of all these ingredients together, in the proportion used for the formulation were also determined.

In vitro dissolution cum release study

Dissolution of the FBs equivalent to 84.55 mg of GE were performed using the USP type II dissolution test apparatus at 100 rpm and 37 ± 0.2°C temperature. The drug release study was carried out in 900 ml of SGF (pH 1.2). Five milliliter aliquots of the solution were withdrawn at predetermined time intervals, replaced by fresh dissolution media. The samples were analyzed for GE content spectro photometrically at 281 nm, against dissolution medium taken as blank. Drug release data obtained during *in vitro* dissolution study were analyzed using ZOREL software with in-built provision for applying the correction factor for volume and drug losses during sampling (Singh et al., 1997; Singh and Singh, 1998).

Table 1. *In vitro* study of different batches of ginger extract FBs ($n = 3$).

Batch	Polymer: $CaCO_3$	Particle size (μm)	DEE* (%)	Buoyancy (%)	Porosity	Bulk density (mg/ml)
B_1	1:0.25	1054 ± 27.40	64.14 ± 1.78	39.33 ± 1.76	80.00 ± 2.00	250.33 ± 3.06
B_2	1:0.50	1087 ± 2.52	84.55 ± 3.43	77.33 ± 2.31	86.67 ± 1.53	241.67 ± 2.08
B_3	1:0.75	1110 ± 10.50	75.40 ± 2.33	82.00 ± 1.54	93.00 ± 2.00	230.33 ± 2.52

*$n = 5$.

In vivo evaluation in cold-restraint stress-induced gastric ulcer

Induction of ulcers

Female wistar rats, not more than 250 g, bred in the Central animal house, Panjab University, Chandigarh, India, were used. Animals caged together and kept under natural light/dark cycle, were given food and water *ad libitum*. They were deprived of food but were allowed free access to water, 24 h before the start of experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee, Punjab University, Chandigarh, India. Animals were divided into nine groups; each group consisting of five animals. Group I comprised of naive control (24 h fasted animals). Remaining 40 animals were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 3 h, at temperature of 4 ± 1°C (Kasugai et al., 2007). This was done to generate a cold-restraint stress (CRS). Latter is reported to lead to gastric ulcers and the main course for the same is oxidative stress (Goel et al., 2004). Further, the animals were divided into group II to group IX. Groups II and III CRS control groups, groups IV and V received cimetidine (10 mg/kg) orally, groups VI and VII received free GE (200 mg/kg) orally, and groups VIII and IX received GE FBs (equivalent to 200 mg/kg of GE) orally. All animals were sacrificed by cervical dislocation and severity of ulceration was judged based on the scale (Narayan et al., 2004) at two different time points of 2 h and 10 h post CRS and administration of a suitable treatment. Groups II, IV, VI and VIII constituted the 2-h data points while groups III, V, VII and IX were sacrificed 10 h after CRS induction and suitable treatment. Ulcer index was calculated by adding the total number of ulcers plus severity of ulcers. The sum of the respective lengths of various hemorrhagic streaks (l) was also measured and used as another parameter for assessing the extent of ulcers.

Histopathological examination

For histopathological examination of gastric lesions, stomachs were opened along the greater curvature, and quickly fixed in a buffered formalin solution.

Estimation of gastric wall mucus

The glandular segments of respective stomachs were scraped with a blunt spatula, weighed and incubated in tubes containing 0.1% alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h (Bilgin et al., 2008). The alcian blue binding extract was centrifuged and the absorbance of supernatant was measured at 598 nm. The quantity of alcian blue extracted (μg/g of glandular tissue) was then calculated from the molecular extinction coefficient calculated suitably for alcian blue.

Biochemical analysis of stomach homogenates

Removed stomachs were rinsed with ice cold saline and weighted. A 10% w/v of stomach homogenate was prepared in 0.1 M phosphate buffer saline (pH 7.4) which was further used for lipid peroxidation (El-sokkarya et al., 2007), catalase (Cuppett et al., 2005), superoxide dismutase assay (Schinella et al., 2000), protein estimation (Gornall et al., 1949) and measurement of nitrosative stress (Radenovic et al., 2003).

Statistical analysis

The raw data obtained from *in vitro* study is expressed as mean ± SD (standard deviation). Data obtained from *in vitro* dissolution cum release studies were analyzed using the ZOREL software (Singh and Singh, 1998).

The *in vivo* results are expressed as mean ± SEM (standard error of mean). The intergroup variation was measured by one-way analysis of variance (ANOVA) followed by Dunnett test. Statistical significance was considered at $P < 0.05$.

Results

Validation of spectrophotometric method of analysis for GE

Linearity range for GE in all the solvent systems was found to be 10–100 μg/ml ($r^2 = 0.999$). Accuracy and precision of the UV spectrophotometric method of analysis for GE was assessed and the obtained values were within the statistical limits (Table 2).

Particle size

Average particle size of GE FBs was invariably between 1.0 and 1.1 mm (Figure 1; Table 1). Vacillating the ratio of calcium carbonate to alginate did not result in any significant difference in particle size. Arithmetic increase in particle size with an increase in the amount of gas-forming agent was, however, observed as is apparent from the values in Table 1. Further, the particle size for 0.75:1 ratio was significantly larger ($P < 0.05$) than those for 0.25:1.

Buoyancy

Floating ability of the prepared beads was evaluated in SGF pH 1.2. An increase in buoyancy from 39.33 ± 1.76 to 82.00 ± 1.54 was observed, when weight ratio of calcium carbonate to alginate was increased from 0.25:1 to 0.75:1

Table 2. Spectrophotometric validation of ginger extract in different solvents.

Media/solvent system	$E^{1\%}_{1\,cm}$	% Accuracy	Precision (%RSD*)
TDW-methanol mixture (90:10, v/v)	90.07	102.78	2.61
SGF-methanol mixture (90:10, v/v)	40.00	102.35	3.34
Methanol	90.02	98.99	1.79

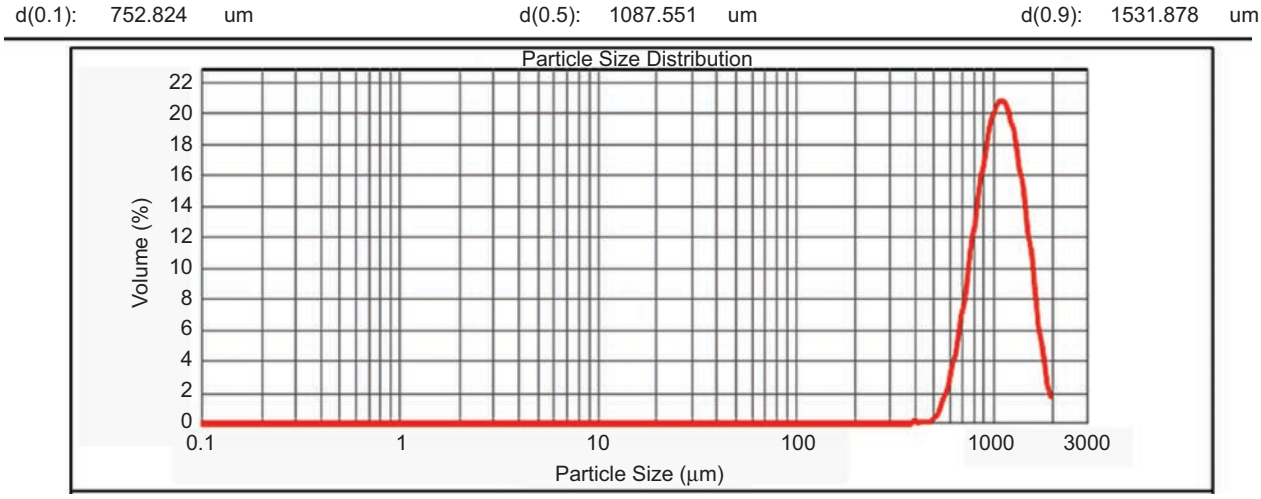

Figure 1. Particle size distribution of GE loaded FBs (0.5:1: calcium carbonate: sodium alginate).

(Table 1). Floating ability is directly related to the gas content of the polymer matrix.

Drug Entrapment efficiency

Entrapment efficiency for various GE formulations was found to vary from 64.14% to 84.55% (Table 1), and the 0.50:1 formulation showed significantly higher entrapment efficiency ($P < 0.05$).

Porosity and bulk density

An increase in porosity of GE FBs was observed from 80% to 93% when weight ratio of calcium carbonate to alginate was increased from 0.25:1 to 0.75:1 (Table 1). Bulk density of the samples decreased accordingly.

Surface and cross sectional characterization by SEM

The surface and cross sectional SEM picture of GE loaded FBs (B_2) are shown in the Figure 2. GE loaded FBs show circular shape with wrinkled surface due to the released carbon-dioxide from the surface of beads. The cross sectional views of the beads show many closed channels or pores and the number or frequency of observed pore appears to be directly related to the amount of incorporated calcium carbonate. 0.25:1.00 GE loaded FBs show a lesser frequency of closed channels/pores as compared to the 0.50:1.00 and 0.75:1.00 (Figure 2).

In vitro dissolution cum release study

GE shows a very slight aqueous solubility (0.69 mg/ml) which increases by more than 4 times in acidic pH of SGF (Table 3). Since there was an increase in solubility at acidic pH and apprehending the affect of alkaline calcium carbonate, we evaluated the effect of components used in the preparation of FBs on the solubility of GE.

As expected the solubility showed a decrease in calcium carbonate and also in calcium chloride; however, an improved solubility was observed with polyethylene glycol, sodium alginate and HPMC. Very interestingly, the mixture of all the ingredients in a proportion in which they are used for the formation of FBs resulted in a remarkable increase in solubility, pH of the mixture was 5.79.

It may be noted from Figure 3 that free GE dissolves in SGF very slowly and only 59.76 ± 2.14% of the total added was solubilized at 5 h (the gastric contents are emptied at 2–5 h). When GE was added to the dissolution medium, it remained floating on the surface and part of it also stuck to the paddle. To overcome discrepancy in obtained results because of these factors, we reevaluated free GE, one after dissolving it in a minimum quantity of methanol and adding to the dissolution media (all of it dissolved in no time) and second by using a dialysis bag tied to the paddle. Latter procedure indicated a significantly slower dissolution/release of free GE (Figure 3).

The value of release exponent(s), calculated for GE containing FBs, using ZOREL came out to be less than 1 indicating zero order sustained release but value varied from 0.360 to 0.467 (Table 4), i.e. the system behaved in a Fickian or a non-Ficikian manner depending upon the nature of the FBs. Release of GE from the B_1 FBs was less than 50% at the end of 24 h, whereas the other two FBs showed a $T_{50\%}$ release at 5.03 h and 9.02 h, respectively for B_2 and B_3 FBs. The overall release rate (9.52 ± 15.02 mg/h) and cumulative percentage release (80.71 ± 1.046) at the end of 24 h was, however, the highest for B_2 FBs (Figure 3 Table 4).

Considering a high entrapment and release obtained with 0.50:1.00 FBs, latter were used for the *in vivo* studies.

In vivo studies

A significant ulcer index of >17.0 scores was observed in the CRS groups (group II and group III). Treatment of rats with cimetidine, free GE and GE FBs significantly reduced the number as well as the extent of gastric mucosal ulcers (Figure 4A and 4B). FBs are expected to stay in stomach for a much longer period of time and produce a more efficient local effect as compared to when free GE or

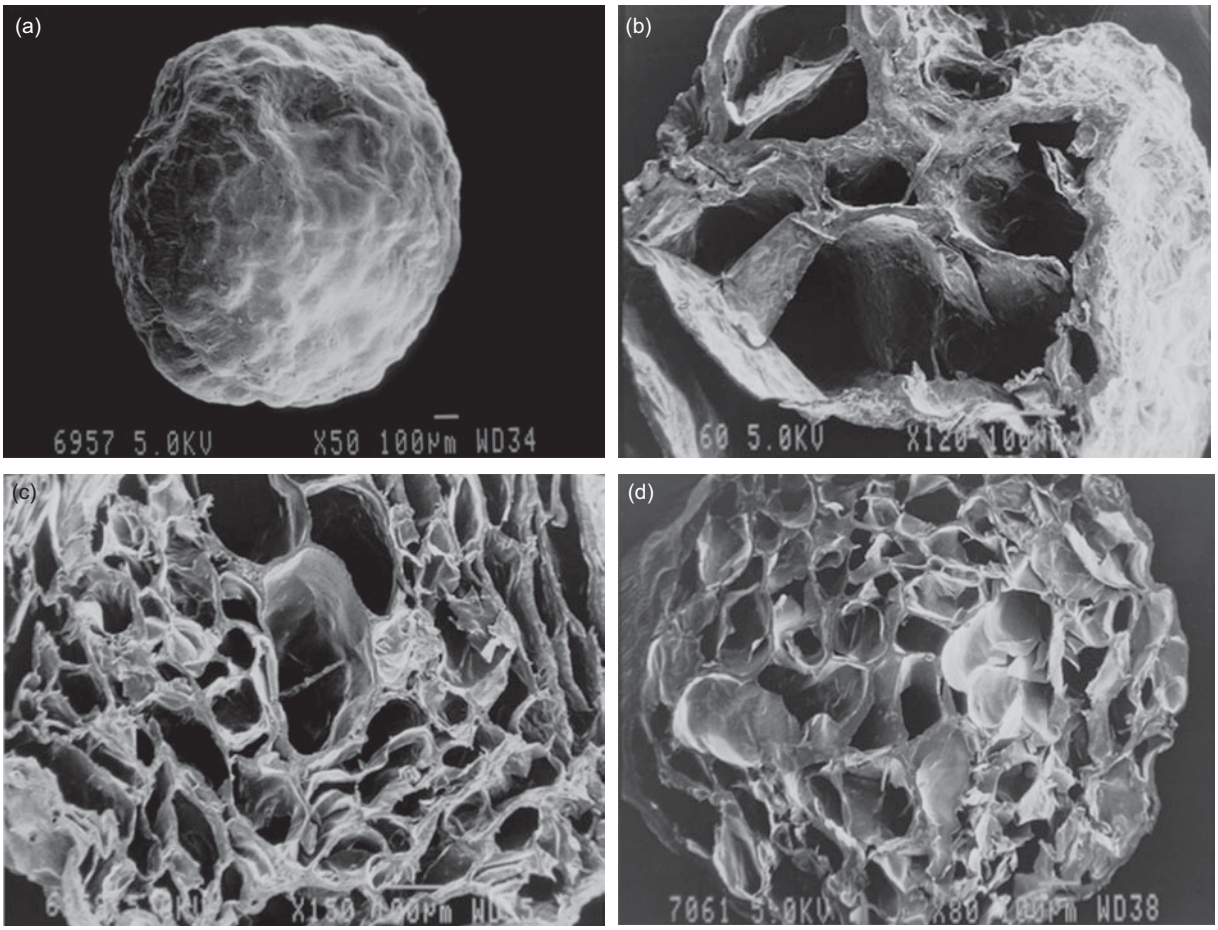

Figure 2. Surface and cross sectional characterization by scanning electron microscopy (SEM). (A) Whole ginger beads (0.50:1); (B) Cross sectional ginger beads (0.25:1); (C) Cross sectional ginger beads (0.50:1); (D) Cross sectional ginger beads (0.75:1).

cimetidine is administered as such. Intact FBs (20–22% of the total administered) could be observed in the rat stomach even at the end of the study (10 h), when the animals were sacrificed (Figure 5); however, when free ginger is administered, it passed out of the stomach and subsequently from the entire gastrointestinal tract without complete solubilization. The latter is indicated by its poor solubility of 0.69 ± 0.03 mg/ml after 24 h at 37°C. As per dissolution studies (Figure 3), only 0.06 mg/ml of GE dissolved in 5 h, so free GE will exhibit effects coinciding with not more than 1.5–3.0 mg (considering human stomach volume 25–50 ml) even if we increase the dose to any extent. We assessed the effect of FBs, free GE and cimetidine at 2 h and 10 h post administration to the animals.

Histopathological examination

Histopathological study of gastric mucosa was performed after staining with hematoxylin and eosin. Figure 6A shows normal healthy mucosa of naive control animal with the identity of glands being maintained. CRS induced group show mucosal hyperemia and hemorrhagic lesions with edema covering the total glandular area of the stomach, evidently indicating acute ulceration (Figure 6B). In addition, gastric mucosal damage with dilation and exfoliation of gastric epithelial cells, disruption of mucosal layer, and the glands losing their identity was also observed. Even though posttreatment with free GE at the end of 10 h did reduce the extent of lesions, but more than half of the mucosa was still damaged (Figure 6C). Cimetidine treatment showed better recovery of gastric mucosa as compared to free GE treated group, with most part of the mucosa regaining its identity (Figure 6D). GE-FBs treated group showed better recovery than cimetidine and free GE treated group with gastric mucosa having regained its complete identity and to maintaining its architecture (Figure 6E).

Mucus content determination

It has been observed that exposure of rat stomach to cold stress leads to a significant decrease in its mucus content. CRS induction reduced the mucus content by 46%. Treatment with the free GE and cimetidine significantly ($P < 0.05$) suppressed or restored the decreased mucus levels. GE FBs showed significantly ($P < 0.05$) better effect as compared to the cimetidine treated and free GE treated groups (Figure 7). This is due to local and sustained effect produced by GE FBs in rat stomach.

Biochemical analysis

We determined LPO, catalase, SOD and nitrite levels of the GE, cimetidine and GE-FBs treated and CRS induced rat stomach homogenates, as the markers of oxidative stress

and compared the values obtained with naive control values. Results suggest that ROS could be one of the important parameters in the pathogenesis of stress-induced mucosal damage. A significant reduction ($P<0.05$) in the oxidative stress was observed for free GE; cimetidine and GE-FBs treated groups when compared with CRS group. The results obtained indicate that the FBs of GE exert a significantly better gastro-protective effect than when it is used as free GE ($P<0.05$). Further, the effect was similar (LPO and catalase) or significantly ($P<0.05$) better (SOD and nitrite) than the cimetidine treated groups (Figure 8A, 8B, 8C and 8D). The most interesting observation is the fact that after 10 h of treatment GE FBs could completely attenuate the induced oxidative stress such that all the values (except nitrite levels) approached the values for naive control rats establishing the effectiveness of the therapy to control or revert the induced mucosal damage to normal. Significantly, higher nitrite levels in CRS induced group suggest the involvement in the pathogenesis of stress-induced ulcers. Similar increase in the serum nitrite levels has been reported by Demirbilek et al. in CRS rats (Demirbilek et al., 2004).

Table 3. Solubility studies of ginger extract in different medium ($n=4$).

Medium	Solubility (mg/ml)
Simulated gastric fluid (pH 1.2)	3.19 ± 0.38
Water	0.69 ± 0.03
Sodium alginate (3% w/v)	0.84 ± 0.026
Calcium carbonate (0.5% w/v)	0.51 ± 0.02
Poly ethylene glycol (5% v/v)	0.95 ± 0.03
Calcium chloride (1% w/v)	0.47 ± 0.02
HPMC (0.33% w/v)	0.93 ± 0.03
Mixture of all these ingredients	2.63 ± 0.66

Discussion

Result of the present study demonstrated, for the first time, a therapeutic effect of GE, a natural antioxidant against gastric ulcers. Most of the natural phytochemicals or antioxidants show pretreatment effectiveness (Rao et al., 2008; Meyre-Silva et al., 2009); however, presently we establish GE FBs for a sustained curative action.

Free GE has the following limitations: (i) slight solubility in gastric juices which will decrease further as it passes to higher pH regions of duodenum and ileum; (ii) any medicament and material shows a limited transit time of <2–4 h in the stomach; (iii) whatever part is solubilized will be immediately absorbed, because GE shows fast absorption, such that local therapeutic action cannot be elicited sufficiently; (iv) not much systemic effect is expected as it is reported to show a very fast elimination (Jiang et al., 2008).

Considering the above-pointed limitations of GE above study was planned with two principle objectives. First, development of GE loaded FBs for post-induction protective effect against gastric ulcers and the second objective was to assess the effects of formulation variables on bead characteristics, entrapment and release. The study indicated that 0.50:1 w/w ratio of calcium carbonate and sodium alginate yields beads with a significantly better DEE and *in vitro* release profile as compared to the other two formulations (B_1 and B_3). The better entrapment efficiency observed for 0.50:1 formulation in comparison to the one with lower concentration of calcium carbonate could be due to internal ionotropic gelation effect of divalent Ca^{2+} of calcium carbonate on alginate resulting in stronger gels, such that the developed beads show significant

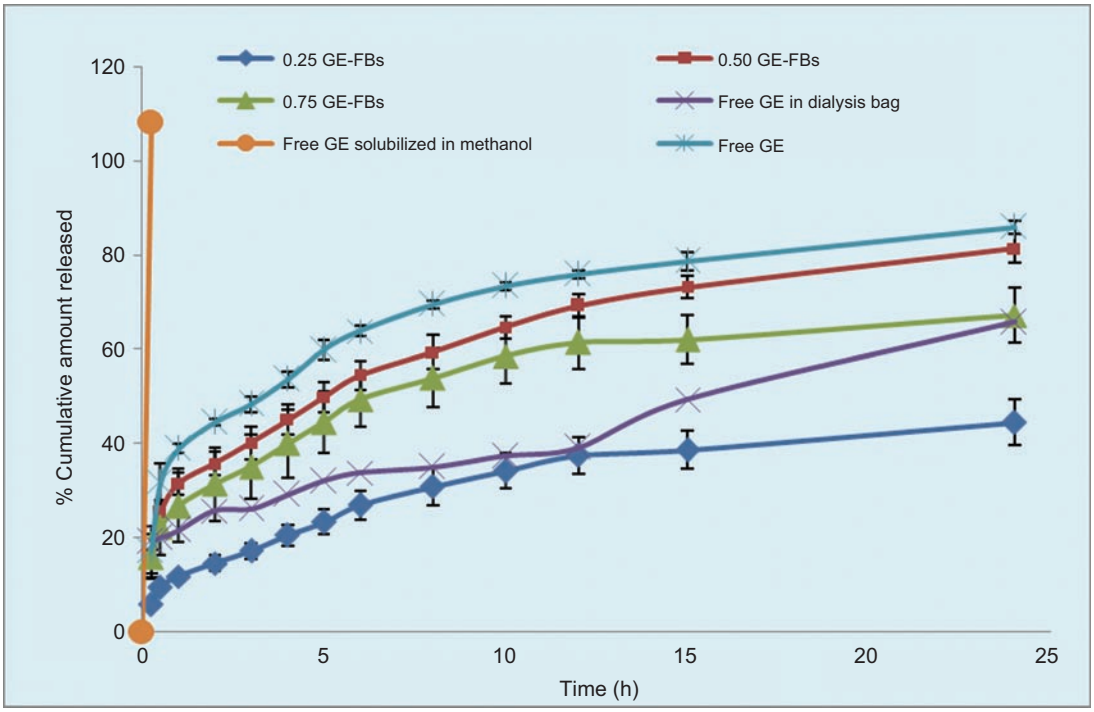

Figure 3. Comparative dissolution profile of GE loaded FBs ($n=4$).

Table 4. Drug release parameters of various batches prepared as per experimental design ($n=4$).

Formulation code	Kinetic constant (K)	Release exponent (n)	Overall rate of drug release (mg/h)	$T_{50\%}$ (h)	% Cumulative amount
B_1	0.10	0.467	3.748 ± 4.84	6.991*	41.39 ± 1.77
B_2	0.28	0.360	9.517 ± 15.02	5.027	80.71 ± 1.05
B_3	0.22	0.377	7.306 ± 11.89	9.018	64.43 ± 0.91

*$T_{25\%}$.

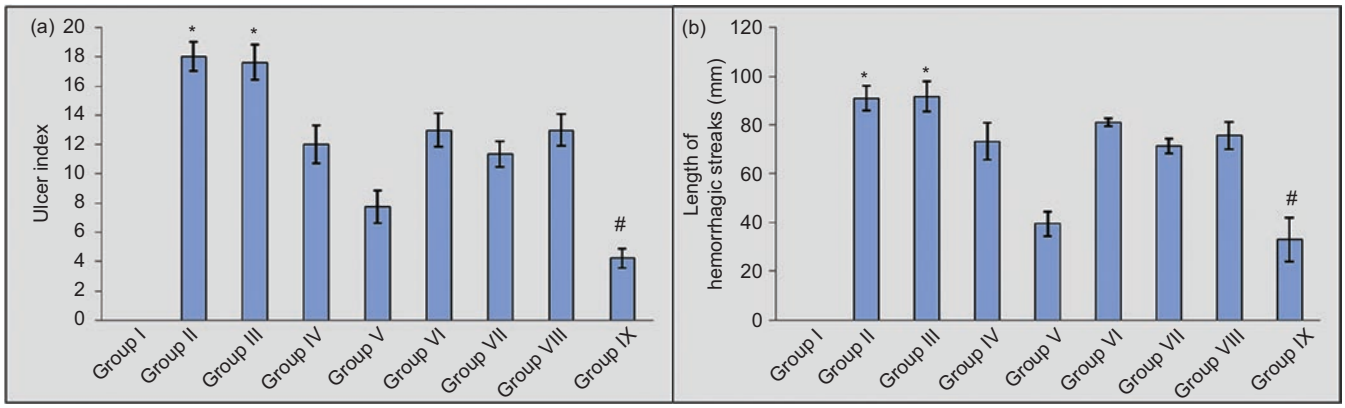

Figure 4. Effect of treatment with formulations in terms of (A) ulcer index and (B) hemorrhagic streaks. *$P<0.05$ as compared to group I; #$P<0.05$ as compared to group V.

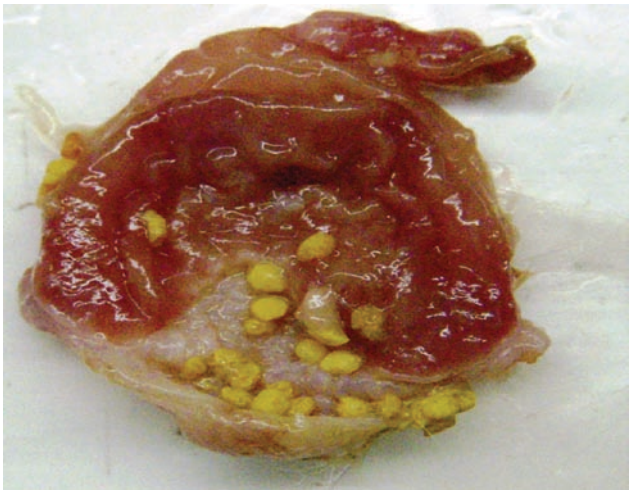

Figure 5. Rat stomach containing floating beads when animals were sacrificed at the end of 10-h study.

entrapment (Schinella et al., 2000). As the concentration of gas-forming agent (calcium carbonate) increase (from 0.25:1.00 to 0.50:1.00) entrapment efficiency increases from 64.00% to 84.55% for B_2; however, a high proportion of gas-forming agent (B_3) can make the beads highly porous and fragile (Choi et al., 2002) due to which the beads are unable to retain the drug efficiently. Thus, when concentration of calcium carbonate was increased from 0.50:1.00 to 0.75:1.00 (75.40%), a decrease in entrapment efficiency was observed.

The solubility data do indicate an increase in solubility at gastric pH, but GE is still only slightly soluble. Further improvement in solubility was observed with polyethylene glycol, sodium alginate and HPMC, which could be due to the polymeric nature of these ingredients such that GE is trapped within their interstitial spaces. Additionally, the co-solvent and surfactant nature of PEG 400 cannot be ignored. However, just enhancing the solubility may not result in desired local effects as it will pass over to the subsequent parts of the gastrointestinal tract and may not form an intimate contact with the gastric mucosa for a significant effect. The latter may, however, be achieved more effectively by incorporating it in to FBs. It may thus be assumed that GE is restrained within the mileu of the alginate-HPMC gel beads in a soluble form (as shown in Table 3; an almost 100% increase in solubility than that in SGF) and will be released slowly from within the beads by diffusion. This increase in solubility is also probably responsible for high entrapment (84.55%) of GE within the FBs.

In vitro release exponent(s), indicate that the system behaved in a Fickian or a non-Ficikian manner depending upon the nature of the FBs. Batch B_2 and B_3 showed diffusion controlled release which could be due to the increment of calcium carbonate concentration resulting in a higher incidence of diffusion channels with in these beads (as confirmed in the SEM pictures also; Figure 2) whereas, batch B_1 showed non-Fickian release indicating that GE is majorly being released due to the swelling of alginate-HPMC gel. Latter showed only 25% release from within the beads up to 7 h.

The porosity and floating properties of the beads, as expected, increased with increase in the gas content of the polymer matrix. This could be due to the increasing quantity of the gas forming agent ($CaCO_3$) used in their formulation, which would result in an increment in pore size and number of pores/area of the formulated FBs, as is apparent from SEM pictures of cross sections of respective

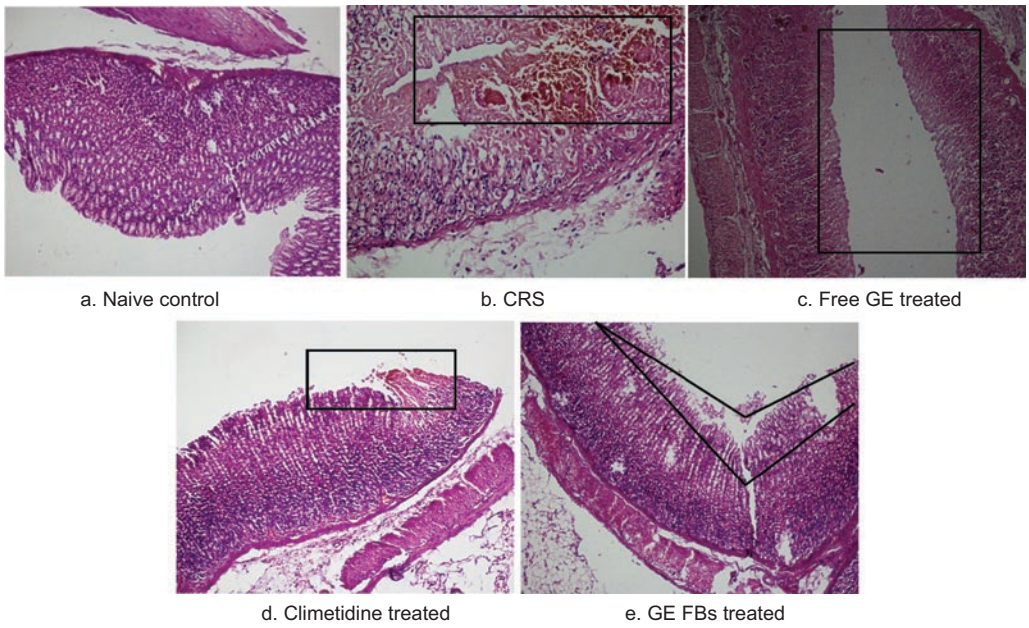

Figure 6. Histological micrographs of rat stomachs at the end of 10-h study. (A) Naive control, (B) CRS, (C) GE treated, (D) Cimetidine treated, (E) GE-FBs treated.

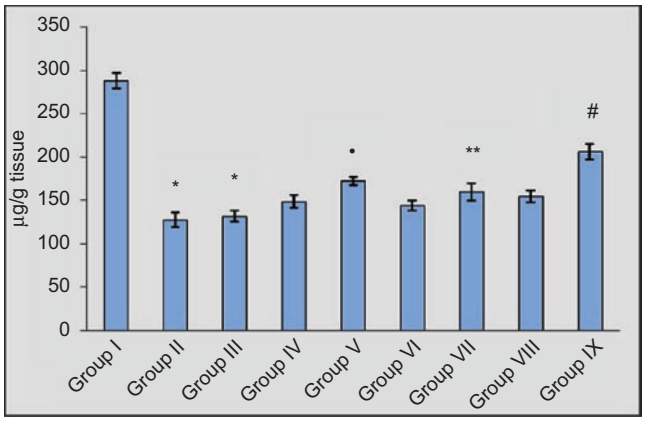

Figure 7. Effect of treatment with formulations in terms of mucosal content. *$P < 0.05$ as compared to group I; **$P < 0.05$ as compared to group IV and V; #$P < 0.05$ as compared to group V; •$P < 0.05$ as compared to group VII.

FBs. Chemical reaction between calcium carbonate and acetic acid results in the release of CO_2. During the formation of beads calcium carbonate effervesces releasing carbon-dioxide which is entrapped in the gel network (HPMC-alginate), producing a formulation that remains buoyant for prolonged periods. So higher the calcium carbonate quantity, more CO_2 will be produced such that more and/or larger pores will be formed.

CRS induces gastric mucosal damages and is responsible for the production of free radicals leading to lipid peroxidation, which damage cells and cell membranes. Possible reasons assigned to CRS induced damage are as follows: (i) lipid peroxidation, oxidation of some critical cellular proteins, and depletion of antioxidants indicating production of ROS during gastric ulceration; (ii) activation of SOD which in turn favors endogenous accumulation of hydrogen peroxide; (iii) generation of oxygen ion at an enhanced rate during stress, as evidenced by increased SOD activity; (iv) transition metal ions play an important role in the generation of stress-ulcer; and (v) hydroxyl ion is generated at a higher rate from oxygen ion and hydrogen peroxide through the metal-catalyzed Haber-Weiss reaction and accounts for the major oxidative damage in stress-induced gastric ulceration (Banerjee et al., 1997).

Gastric mucus is an important protective factor for the gastric mucosa. Moreover, mucus also acts as an antioxidant and thus can reduce the mucosal damage mediated by oxygen free radicals.

A decrease in mucus content is responsible for damages of gastric mucosa, CRS decreases the mucus content in the stomach (Repetto and Llesuy, 2002) as observed by us presently and increases prostaglandin levels (Ye et al., 2003).

The changes in the LPO, catalase, SOD, nitrite and mucus content levels induced by stress were attenuated to normal values by GE FBs. Latter could last significantly for even up to 10 h within the stomach (Figure 5), confirming the prolonged stay of FBs. In addition to being a floating system, presence of HPMC may also provide mucoadhesive property to the beads (Ahmed et al., 2010) such that they are retained on the gastric mucosa for longer times. However, no remnants of free GE could be found in rat stomachs upon surgery. Histopathological studies also indicate the maintenance of cellular integrity and architecture of the gastric mucosa upon treatment with GE FBs.

Conclusion

Gastro-retentive dosage form is becoming popular to retain drug agents in the stomach for prolonged period. Presently, we prepared FBs of GE by orifice ionic gelation method. The percentage drug entrapment and drug content of the formulated beads were found to be satisfactory

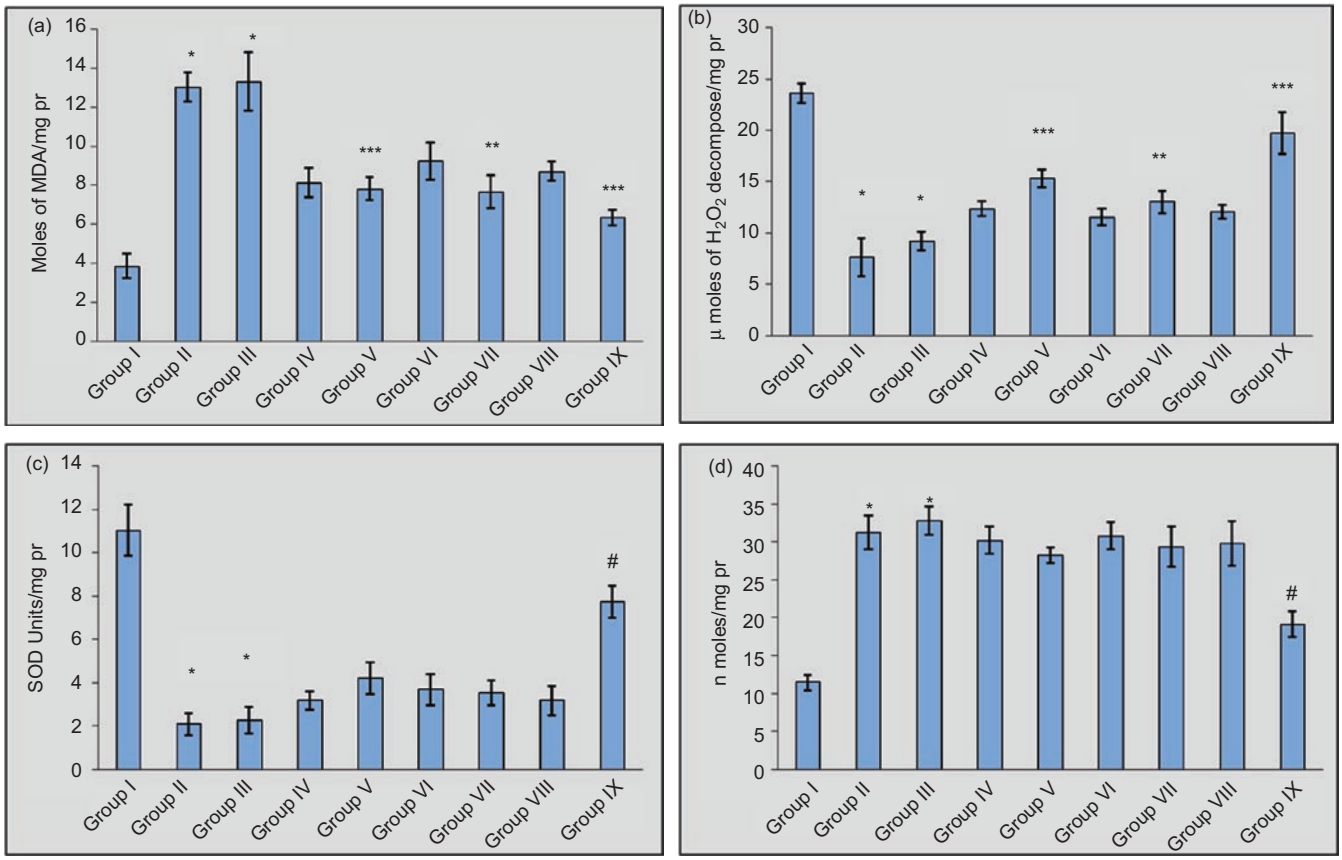

Figure 8. Effect of treatment with formulations in terms of LPO; SOD; catalase and nitrite levels. (a) Effect of treatment with formulations in terms of LPO.*P<0.05 as compared to Group I; **p<0.05 as compared to Group IV and V; ***P<0.05 as compared to Group VII. (b) Effect of treatment with formulations in terms of catalase. *P<0.05 as compared to Group I; **p<0.05 as compared to Group II and III; ***P<0.05 as compared to Group VII. (c) Effect of treatment with formulations in terms of SOD.*P<0.05 as compared to Group I; #P<0.05 as compared to Group V. (d) Effect of treatment with formulations in terms of nitrite levels.*P<0.05 as compared to Group I; #P<0.05 as compared to Group V

by this method. More than 75% of the prepared sodium alginate beads of GE remain floating even at the end of 24 h in SGF and a sustained drug release was observed from the beads for a prolonged period of time; latter is expected to improve the therapeutic efficacy of GE used for the diseases associated with the stomach. Release pattern of the beads was found to be Fickian with the release of GE being controlled by diffusion, through the water filled channels in the beads. *In vivo* studies confirm suitability and effectiveness of GE FBs for the treatment of ulcers post-induction. In the present study, FBs of GE was found to be more interesting and promising candidates for the treatment of induced ulcers. Further, it can provide a suitable platform for spatial delivery of other herbal antioxidants also.

Declaration of interest

The authors declare no conflict of interest.

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