5.1 Abstract

Patients with ischemic stroke are more prone to develop peptic ulcer due to disturbed vascular homeostasis. Continuation of current antiplatelet therapy even worsens the condition of the ulcer by increasing gastric bleeding. Peptic ulcer is prevalent in about 4% of the world population, and nearly 10% of people have been affected by a peptic ulcer at some point in their life. Therefore, there is a need for newer efficient and safe anti-ulcer agents. In the present strategy, we have prepared a novel bioactive glass containing 1.3 mol% of barium oxide (BaBG) and evaluated its antiulcer potential in ischemic and other gastro-duodenal ulcer models. Prophylactic effect of BaBG pretreatment was evaluated for 5 days in ischemic, ethanol, aspirin and pyloric ligation-induced gastric ulcer and cysteamine-induced duodenal ulcer models. Repeated treatment of 10 days of BaBG was evaluated in the healing ulcer model of acetic acid. BaBG significantly reduced the ulcerative damage against all the six tested ulcer models. Scanning electron microscope (SEM) images have shown that BaBG forms a physical protective barrier over the gastroduodenal epithelium cell. In the ischemic, pyloric-ligation, ethanol and aspirin models, BaBG showed significantly increased in gastric pH, indicating antacid like activity. BaBG treatment significantly increased vascular endothelium growth factor (VEGF) in ischemic gastric tissue and recovered the gastric blood flow in ischemic ulcer model. Treatment with BaBG significantly enhanced cell proliferation in the pyloric model. Thus, BaBG mediates antiulcer action by improving the gastric blood flow, forming a protective physical barrier against harsh luminal factors, acid neutralization and cell proliferation.

Keywords

Barium containing bioactive glass, Ischemic ulcer, Gastro-duodenal ulcer, Physical protective barrier, Acid neutralization, Cell proliferation.

5.2 Introduction

In gastro-duodenal ulcer, the integrity of the mucosal lining is disturbed (Selmi et al. 2017). However, regular feed with adequate rest and avoidance of ulcerogenic agents such as non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, tobacco and caffeine can prevent the formation of the peptic ulcer (Matsui et al. 2011). NSAIDs are the second most important cause of the peptic ulcer. NSAIDs are responsible for the 15 to 35% of all cases of peptic ulcer disease (Eisner et al. 2017). Treatment of pain, rheumatic, vascular disease and more recently for the prevention of colon cancer and Alzheimer's disease involve the uses of the NSAIDs (Scarpignato et al. 2015; Sostres et al. 2010). Thus, avoidance of such ulcerogenic agents is difficult because they are the first line of the drug in the treatment of the different disease conditions. NSAIDs inhibit, cyclooxygenase enzyme (COX) and suppress prostaglandin (PG) that protect mucosal lining from the acidic medium which leads to gastric mucosal injury (Sostres et al. 2010).

Proton pump inhibitors and histamine type 2 (H_2) receptor blockers class of drugs are widely used for the treatment of gastric ulcer (Sostres et al. 2010). These drugs are useful, but complete healing and prevention require continued use. Long-term use of such agents has a long list of the side effect ranging from atrophic gastritis, dryness of mouth to achlorhydria, encephalopathy, osteodystrophy, arrhythmias, hematopoietic changes and impotence (McQuaid, 2007). Therefore there a need for more safe and effective anti-ulcer agents.

Bioactive glass is silica-based biomaterial which is firstly discovered by Hench and Wilson termed as 45S5 bioglass®. It is known for the ability to bind to bone and muscle tissues. Bioactive glasses are of different types according to their compositions such as 45S5 bioglass® (Na_2O-CaO-SiO_2-P_2O_5), 70S30C (CaO–SiO_2) and mesoporous bioactive glass (CaO-SiO_2-P_2O_5) (Pei et al. 2016; Zhang et al. 2014; Zhang et al. 2016; Zhao et al. 2015; Zhu et al. 2011). The bioactive glass was reported for its hard tissue regeneration potential (McQuaid, 2007). However, there is a paucity of information regarding the soft tissue repair or regeneration potential of bioactive glasses (Ma et al. 2013). Several studies have reported that the 45S5 bioactive glass can bind and regenerate soft tissue such as the skin (Verrier et al. 2004; Zhang et al. 2002). A recent study has demonstrated that bioactive glass dissolution product proliferates ethanol-injured GES-1 human gastric mucosa epithelial cells in 48 and 72 h in vitro after exposure (Ma et al. 2013). Further, another clinical study has reported that the surface reactivity of the bioactive glasses stimulates wound healing of the burn tissue (Wang et al. 2006). When the bioactive glass contacts the body physiological environment, it triggers a surface reaction which includes the ion exchange between hydrogen from the fluid and sodium from a bioactive glass (Brückner et al. 2016; Ogino et al. 1980). This may lead to an increase in gastric pH and can act as a local antacid. Ions like calcium and silicon are leached out to the surrounding area from the bioactive glass which generates a porous silica-rich layer followed by the development of hydroxyl‑carbonate-apatite on the bioactive glass surface (Hench, 1991; Kim et al. 1989; Zhong et al. 2002). Further, this silica-rich layer and the hydroxyl‑carbonate-apatite layer formed on the bioactive glass surface, offer enormous binding sites to permit bioactive glass particles to bind at the wound and to allow cells and proteins to bind at their surfaces. This may offer a protective sheet to ulcer and prevent

further damage by ulcerogens. Bioactive glass can stimulate the expression of genes related to wound healing like CD44 antigen hematopoietic form precursor, vascular endothelial growth factor (VEGF) precursor, fibroblast growth factor receptor-1 precursor, fibronectin receptor beta subunit, and vascular cell adhesion protein-1 precursor (V-CAM 1) (Leach et al. 2006; Xynos et al. 2001). Thus, the release of these precursors at the site of the ulcer may be helpful in the healing of the ulcerated gastric epithelium. Simultaneously, the release of the soluble silicon ions, silica oxide ions and calcium ions, has been reported to stimulate cell proliferation and differentiation (Xynos et al. 2000, 2001). Similarly, another study indicated that bioactive glass promotes angiogenesis and its application in the healing of the soft tissue wounds (Day, 2005; Day et al. 2004; Keshaw et al. 2005). Therefore, the bioactive glass may have potential antiulcer activity due to its effects on acid neutralization, forming a protective physical barrier against ulcerogens and gastric cell proliferation.

Substitution of barium oxide in 45S5 bioactive glass enhanced the bioactivity (Leenakul et al. 2013). Substitution of up to 1.6% mol of barium oxide for SiO_2 content in the 45S5 system caused the shift of exothermic peaks to lower temperatures (Arepalli et al. 2015). As a result, lower energy is required to promote crystallization in the glass. Further barium oxide substitution at 1.2 and 1.6 mol% contents was found to possess a higher rate of dissolution, and hence the maximum pH values were recorded (Arepalli et al. 2015). This enhancement in pH behavior may show an antacid effect. Further higher dissolution leads to the early development of hydroxycarbonate apatite layer on the sample surface with more crystallinity as compared with base 45S5 bioactive glass. The above condition favours forming of the protective physical barrier. From the recent study, it has been found that biocompatibility was improved in all the bioactive glasses due to the presence of barium oxide content and the

sample with barium oxide has a higher potential as biomaterials (Arepalli et al. 2015; Leenakul et al. 2013). Therefore based on the above reports, in the present study, we have selected a 1.3 mol% substitution of barium oxide in the 45S5 bioglass® system for potential enhanced bioactivity.

Thus, in the present study, we have pharmacologically evaluated bioactive glass containing 1.3 mol% of barium oxide for its antiulcer potential in various gastroduodenal ulcer models and possible mechanism of action.

5.3 Materials and methods

5.3.1 Animals

Adult male albino Wistar rats 250-280 g were procured from the central animal house, IMS (Institute of Medical Sciences), Banaras Hindu University, Varanasi; India. The animals were acclimatized for one week at 25 ± 1 °C with a 12-h light-dark cycle and were allowed free access to food (Amrut Laboratory Animal feed, Sangli, India) and water throughout the experiment. All efforts were made to reduce the number of animals used, and all experiments were conducted as per the principles of laboratory animal care (National Research Council US Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011) guidelines. Prior to animal experiments, an approval from animal ethics committee was taken (Ref No. Dean/2015/ CAEC/1421). All surgical procedures were conducted under aseptic conditions.

5.3.2 In-vitro pH behavior of bioactive glass samples in SBF

5.3.2.1 Preparation of SBF

Simulated body fluid (SBF) has inorganic ion concentrations similar to the human body fluid, and therefore the pH behavior of the bioactive materials has been studied in the SBF. SBF

was prepared according to Kokubo's method (Kokubo and Takadama, 2006). The SBF solution was prepared at 37 °C by dissolving the reagent grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 into double distilled water and it was buffered at pH=7.4 with TRIS buffer (trishydroxy methyl aminomethane) and 1 N HCl (Kokubo and Takadama, 2006).

5.3.2.2 pH behavior of bioactive glass samples in SBF

Bioactive glass sample was dispersed in the SBF solution, and pH behavior of the solution was checked to assess the different stages of formation of hydroxyl carbonate apatite (HCA) layer on the surface of the samples. Powdered bioactive glass sample (0.5 g) was soaked in 5 mL of SBF solution at 37 °C for 7 days, and the pH of the leached solution was measured continuously every day at definite intervals of time, using Universal Biomicroprocessor pH meter at room temperature. The ratio of bioactive glass samples and SBF solution was selected based on the previous study (Arepalli et al. 2015). The volume of the SBF was selected by considering the volume of gastric fluid in the rat, which is about 5 ml. Before measuring the pH, the instrument was calibrated with standard buffer solutions of pH 4.0 and 7.0 (Arepalli et al. 2015). The experiment was performed in triplicate.

5.3.3 Treatment protocol

In all the experimental models, the animals were divided into six groups, each group containing six animals (n=6). Group I rats (normal control) and Group II rats (ulcer control) received a suspension of 0.5% carboxy methylcellulose (CMC). Groups III, IV, and V rats received BaBG suspended in 0.5% CMC at dose levels of 0.3, 1.0, and 3 mg/kg, p.o., respectively. Groups VI rats received the standard drug, omeprazole (20 mg/kg, p.o.) suspended in 0.5% CMC. Suspension of 0.5% CMC was prepared and BaBG micro-

particulate powder was suspended at the concentration of 0.06 mg/ml, 0.2 mg/ml and 0.6 mg/ml for 0.3, 1.0 and 3 mg/kg doses respectively. The dose-volume was 5 ml/kg body weight for all the six groups. Both BaBG and omeprazole (Schneeweiss et al. 2006) were administered orally once daily for 5 days for protective ulcer studies and 10 days for ulcer healing study. The dose range (0.3 to 3 mg/kg) and dosing interval (24 h) of BaBG administration in rats was decided based on the previously published literature (Schneeweiss et al. 2006). Fasting of the animals (with free access to water ad libitum) in all the ulcer models was done for 18 h.

5.3.4 Gastric ulcer study

5.3.4.1 Celiac artery occlusion induced ulcer

Drugs were administered for a period of 5 days. On day 5, after the last dose, the gastric ulcer was induced using ischemic ulcer model. The celiac artery was ligated for 30 min. One hr after the reperfusion, the ligature was removed to achieve ischemic-reperfusion condition. Three days after the reperfusion, rats were anaesthetized by pentobarbitone (35 mg/kg, i.p.) followed by evaluation of stomach blood flow. Thereafter, stomachs were dissected out for ulcer damage score and vascular endothelial growth factor (VEGF) study. The volume of gastric juice was measured, and pH was recorded (Wada et al. 1996).

5.3.4.1.1 VEGF estimation

Ischemic gastric mucosal tissue extracts from BaBG and vehicle-treated rats 3 days after celiac artery occlusion (CAO) were dissected on ice. The tissues (150 mg/ml) were homogenized in DMEM and centrifuged for 10 min at 10,000 g at 4 ° C. The tissue extracts were then divided into 200 µl triplicate samples for the VEGF determination (Ma et al. 2000).

5.3.4.1.2 Measurement of gastric blood flow

Gastric blood flow was estimated with laser speckle blood flow imager (Omegazone OZ-2; Omegawave, Tokyo, Japan) as reported earlier (Paliwal et al. 2017). Image pixels were analyzed to produce average perfusion values using the software OZ-2 (LSIv3.3.1 and LIAv3.3.0). The unit of mean blood flow was an arbitrary unit (AU).

5.3.4.2 Ethanol-induced ulcers

Drugs were administered for a period of 5 days. On day 5, after the last dose, the gastric ulcer was induced using absolute ethanol (5 ml/kg). One hour after ethanol administration, all the animals were killed and the stomach was incised along the greater curvature for the examination of the ulcers. The volume of gastric juice was measured, and pH was recorded (Hollander et al. 1985).

5.3.4.3 Aspirin-induced ulcers

Drugs were dosed once daily for 5 consecutive days. After the last dose on day 5, the gastric ulcer was induced with aspirin in a dose of 500 mg/kg. After 4 h of the aspirin administration, all the animals were killed, and stomachs were incised along the greater curvature for the examination of the ulcers. The gastric contents were collected in tubes for estimation of gastric volume and pH (Goel, 1985).

5.3.4.4 Pylorus ligation (PL)-induced ulcers

Drugs were administered for a period of 5 days as described above. On day 5, after the last dose, pylorus ligation was performed following the method as described earlier (Sanyal et al. 1971). The animals were deprived of water during the post-operative period. After 4 h, stomachs were dissected out for ulcer damage score and cell proliferation study. Further gastric contents were collected in tubes for estimation of gastric volume and pH.

5.3.4.5 Acetic acid-induced chronic ulcers

The rats were anaesthetized with pentobarbitone (40 mg/kg, i.p.) (Tripathi et al. 2017). The abdomen was opened and the stomach was visualized. Gastric ulcers were produced by injecting 50% acetic acid (0.06 ml per animal) into the anterior serosal surface of the glandular area of stomach 1 cm away from the pyloric end using the method as described earlier (Han et al. 2017). BaBG was given orally once daily, 4 h after the application of acetic acid and continued 10 days after induction of ulcer. The animals were then killed by cervical dislocation on the 11th day of an experiment to assess the ulcer healing.

5.3.5 Duodenal ulcer study

5.3.5.1 Cysteamine-induced ulcers

Drugs were administered for a period of 5 days. On 5th day of treatment schedule, cysteamine (30 mg/kg s.c., 100 mg/ml) was administered to the animals 1 h after drug treatment. The animals were fasted overnight and killed by cervical dislocation at 10:00 h on the following day. The animals were checked for the ulcers on the anterior and posterior wall of the duodenum near the pyloric end (Sairam et al. 2003).

5.3.6 Gastric volume and pH

Stomachs were isolated immediately from the sacrificed rats. The gastric fluid was collected from the isolated stomach, and its volume was measured. Further, the pH of the collected gastric fluid was recorded using Eutech pH Meter (Sairam et al. 2003).

5.3.7 Gross ulcer index and histopathological analyses

To determine the degree of gross mucosal damage, stomach was cut along the greater curvature followed by washing in ice-cold saline and photographed using a digital camera (Palacios-Espinosa et al. 2014). The sum of the area of all lesions in the glandular portion of

the stomach of each rat was calculated with ImageJ software program and used as the gross damage index (Cristians et al. 2013; Paliwal et al. 2018).

Stomach was immediately fixed with 10% formalin solution for the histology study. Further, the formalin-fixed stomach was cut from the cardia to the pylorus, longitudinally with 5-mm width. Then the tissue specimens were dehydrated and embedded in paraffin. Again the tissue specimens were sectioned by 6 μm, and stained with hematoxylin and eosin and analyzed with an Olympus BH2 light microscope. Images of selected, damaged and intact mucosal sections were captured by a camera mounted on the microscope (Lacy and Ito, 1982; Shakya et al. 2011).

5.3.8 Scanning electron microscope (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) analysis

The stomach and duodenum were fixed in 2.5% glutaraldehyde for overnight at 4 °C. The specimens were then washed in phosphate buffer thrice for 15 min at 4 °C. After washing in wash buffer and dehydration in a graded series of acetone solutions, the specimens were dried in a critical point drying apparatus (Polaron) by liquid carbon dioxide, mounted on aluminium stubs and vacuum coated with gold-palladium (EMITECH SC 7620). Coded specimens were then viewed in a scanning electron microscope (SEM, EVO LS 10, Carl Zeiss, Germany) operated at 10 kV coupled with energy dispersive spectrometry to investigate the elemental analysis of the attached particles (Shakya et al. 2011).

5.3.9 Cell proliferation

DNA and protein content were determined in the gastric mucosal homogenate following the method described earlier (Lowry et al. 1951; Mukhopadhyaya et al. 1987). DNA

concentration was expressed as μg DNA/mg protein, which is an indicator of cell proliferation as reported earlier (Mukhopadhyaya et al. 1987).

5.3.10 Statistical analysis

All the data were presented as Mean ± SEM. Repeated measures of Two-way ANOVA was performed for the data analysis followed by Bonferroni's Post-hoc test. A level of $p < 0.05$ was considered significant in all the data analysis.

5.4 Result

5.4.1 pH assessment

The pH pattern of the bioactive glass sample dispersed in the SBF is shown in Fig. 5.1. The initial increase of the bioactive glass SBF solution pH on day 1 maybe because of the fast release of Na+ and Ca2+ ions from the sample and exchange with H+ or H3O+ ions into the solution. Hydroxyl concentration of the solution increases because of the H+ ions being replaced with cations and responsible for the formation of silanols due to the attack of hydroxyl ions in the silicate glass network. Further precipitation of Ca2+ ions from the solution to form calcium phosphates and carbonates, decreases the pH of the solution on day 4 onward due to decrease of the Na+ and Ca2+ ionic concentration from the sample surface. Similar behavior and changes in pH after in vitro dissolution of the samples for various time periods have been reported (Abebaw et al. 2017; Cerruti et al. 2005; Deliormanlı, 2012; Filho et al. 1996). In addition, the pH of the BaBG increased from day 1 to day 4 and then stabilized from day 4 to day 7; whereas the pH of the 45S5 initially increased and there was a decrease thereafter. Moreover, the pH of the BaBG on day 6 and 7 found to be significantly more compared with the 45S5. This shows that BaBG was able to maintain a higher pH in SBF.

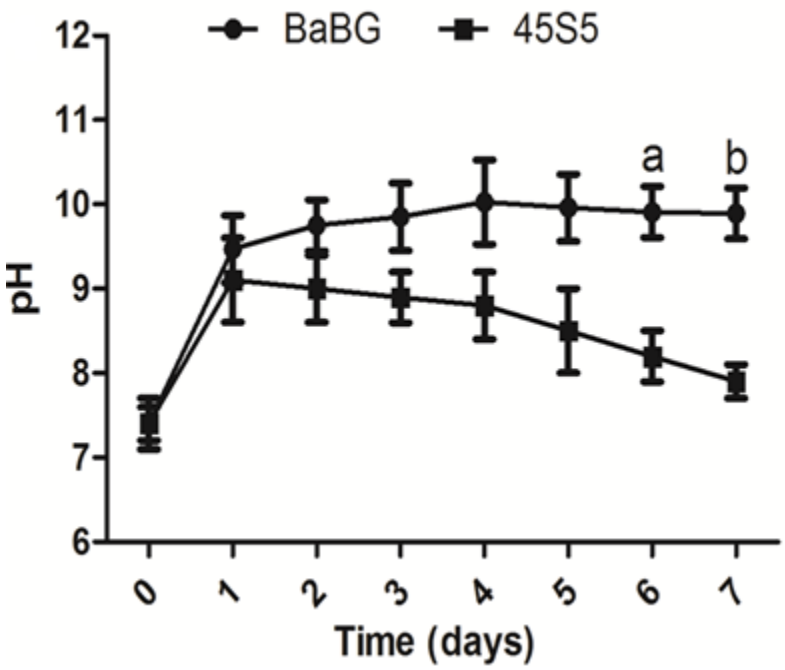

Figure 5.1 pH behavior after immersion of the BaBG and 45S5 samples in SBF. Data are expressed as means ± SEM (n=3). $^{a}p < 0.05$ and $^{b}p < 0.05$ compared with the 45S5 group on day 6 and 7 respectively.

5.4.2 Effect of BaBG on acute gastric lesions induced by caeliac artery occlusion

Caeliac artery occlusion reduced mean gastric blood flow and induced severe gastric damage and this reduction in mean gastric blood flow and gastric damage were attenuated by the BaBG and omeprazole pretreatment as seen in Fig. 5.2 and Fig.5.3 respectively. BaBG pre-treatment significantly reduced gross ulcer indices even at a lowest dose of 0.3 mg/kg in comparison to vehicle group [$F(5, 35)=13.40$; $p < 0.05$]. BaBG at the dose of 3.0 mg/kg significantly enhanced gastric pH. Further, Fig 5.4 shows that VEGF levels, estimated using ELISA assay, were significantly increased in BaBG treated ischemic gastric mucosal tissue compared with vehicle group [$F(5, 29) = 39.81$; $P<0.05$] at 3 days after ischemia and the increases was found to be dose-dependent. Blood flow plays an important role in the protection of the gastric mucosal layer and the healing of damaged mucosa. Statistical

analysis showed that BaBG causes a significant increase in the mean blood flow dose-dependently among groups [F (5, 29) =54.13; P<0.05].

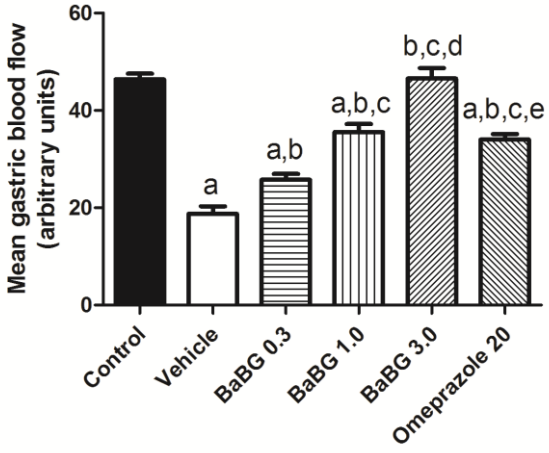

Figure 5.2 Bars represent the effect of BaBG on gastric blood flow. The results are expressed as the mean ± SEM from six animals per group. [a]P<0.05 compared to control group, [b]P<0.05 compared to vehicle, [c]P<0.05 compared to BaBG 0.3, [d]P<0.05 compared to BaBG 1.0 and [e]P<0.05 compared to BaBG 3.0. [One-way ANOVA followed by Student Newman-Keuls post-hoc test].

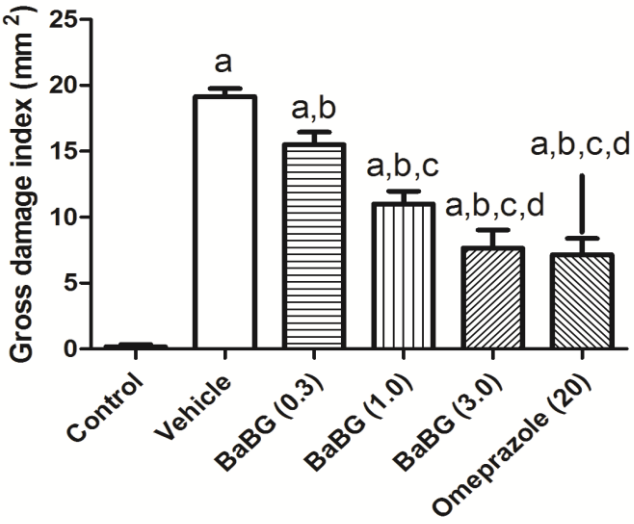

Figure 5.3 Bars represent the effect of BaBG on gross damage index in CAO rats. The results are expressed as the mean ± SEM from six animals per group. [a]P<0.05 compared to control

group, [b]P<0.05 compared to vehicle, [c]P<0.05 compared to BaBG 0.3 and [d]P<0.05 compared to BaBG 1.0. [One-way ANOVA followed by Student Newman-Keuls post-hoc test].

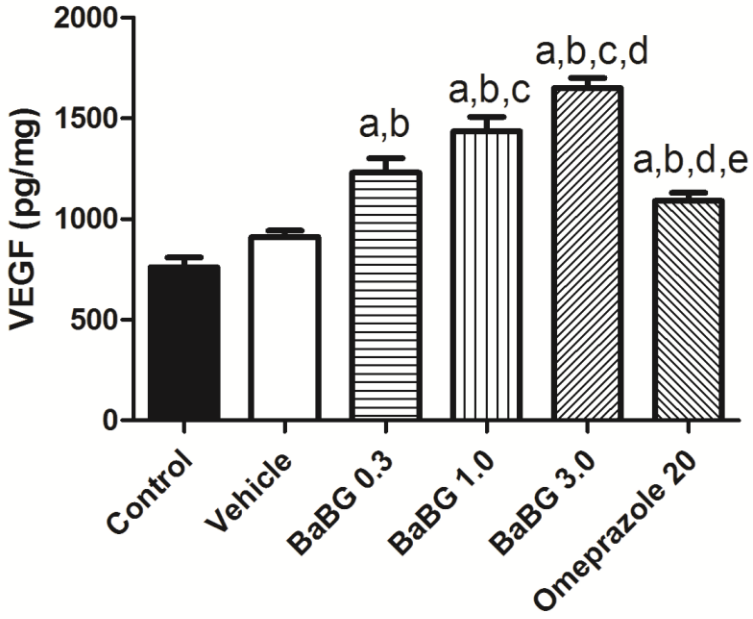

Figure 5.4 Bars represent the effect of BaBG on VEGF level on ischemic gastric mucosal tissue in CAO rats. The results are expressed as the mean ± SEM from five animals per group. [a]P<0.05 compared to control group, [b]P<0.05 compared to vehicle, [c]P<0.05 compared to BaBG 0.3, [d]P<0.05 compared to BaBG 1.0 and [e]P<0.05 compared to BaBG 3.0 [One-way ANOVA followed by Student Newman-Keuls post-hoc test].

5.4.3 Effect of BaBG on acute gastric lesions induced by ethanol

Absolute ethanol-induced severe gastric damage and this gastric damage were attenuated by the BaBG and omeprazole pretreatment as seen in Fig. 5.5. BaBG pretreatment significantly reduced gross ulcer indices at a dose of 3.0 mg/kg in comparison to vehicle group [F (5, 35)=12.80; $p < 0.05$]. The SEM image of the treatment group (3.0 mg/kg; BaBG) shows the formation of a protective layer over the damaged gastric epithelial cell as seen in Fig 5.6C.

Further, in the image (Fig 5.6D) we can observe the binding of BaBG at the erosive part of the stomach. BaBG at the dose of 3.0 mg/kg significantly enhanced gastric pH (Table. 5.2).

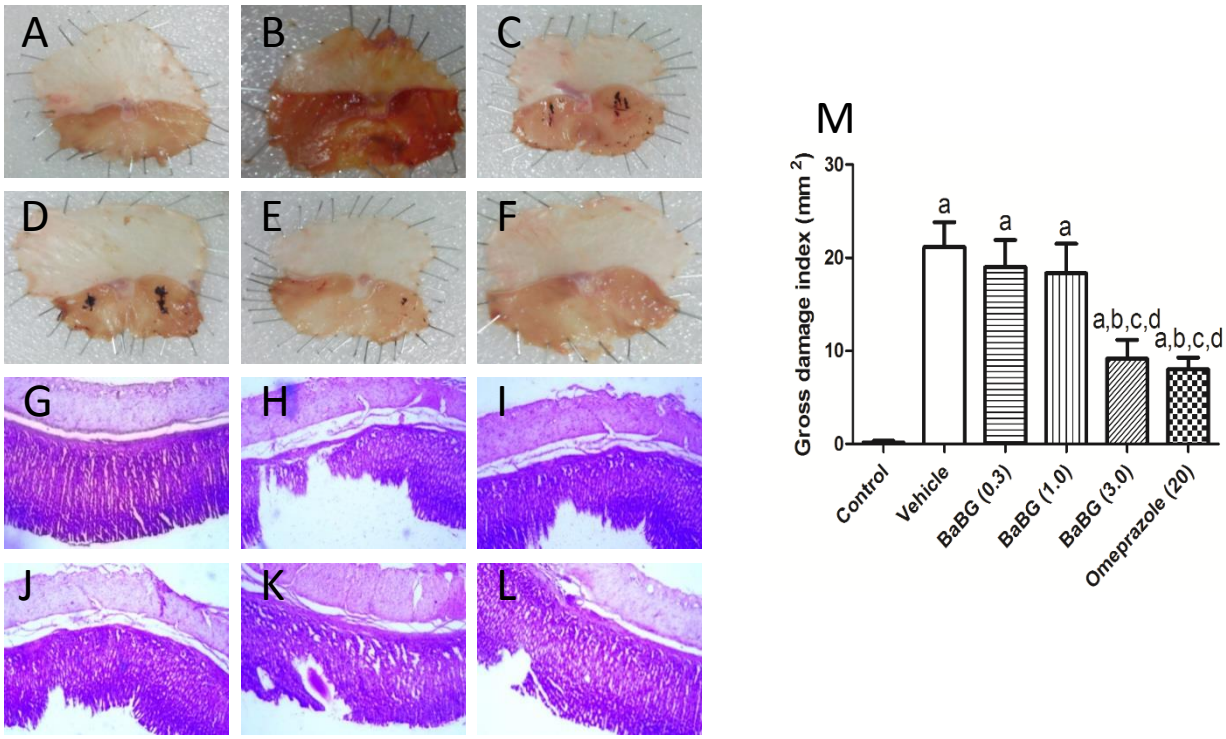

Figure 5.5 Control group rats of ethanol-induced ulcer model have no disruption of the surface epithelium (A & G). Rats in the vehicle group have severe disruption of the surface epithelium, and extensive oedema of the submucosal layer and leukocyte infiltration are present (B & H). Rat in BaBG 0.3 mg/kg has disruption of the surface epithelium, and there is submucosal oedema (C & I). BaBG 1.0 mg/kg rat has moderate disruption of the surface epithelium with oedema of the submucosal layer (D & J). Rats in BaBG 3 mg/kg showed a mild disruption of the surface epithelium (E & K). The gastric mucosa in animals pretreated with omeprazole showed a mild disruption of the surface epithelium (F & L). Macroscopic damage indices were quantified (M). The results are expressed as the mean ± SEM from six animals per group. $^{a}P<0.05$ compared to control group, $^{b}P<0.05$ compared to vehicle, $^{c}P<0.05$ compared to BaBG 0.3 and $^{d}P<0.05$ compared to BaBG 1.0 [One-way ANOVA followed by Student Newman-Keuls post-hoc test].

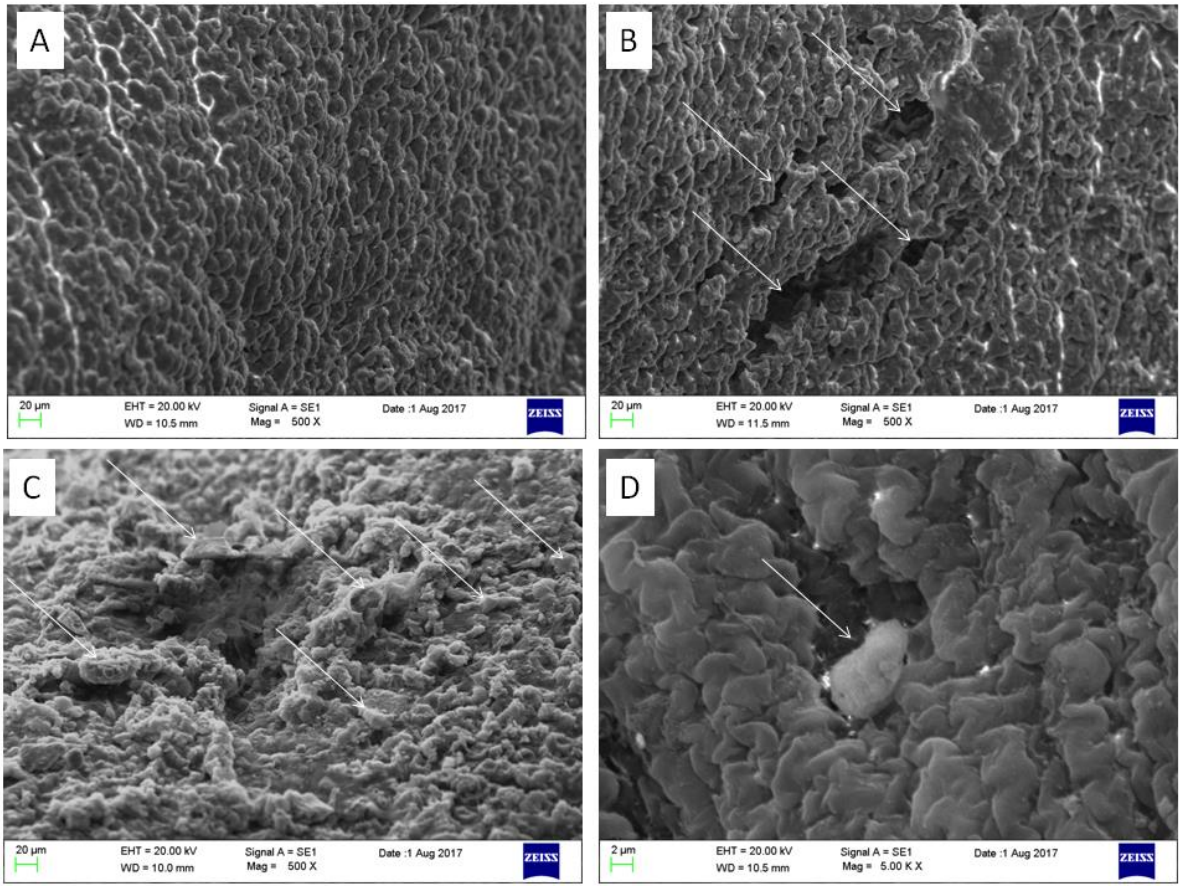

Figure. 5.6 (A-D) shows the SEM images of gastric epithelial cells. (A) shows image of the sham group of the ethanol-induced model with normal gastric epithelial cell. (B) shows image of vehicle group and white arrow indicates severe damage in gastric epithelial cell. (C) shows image of treatment (1.0 mg/kg) group and white arrow indicate the formation of BaBG protective layer over the damaged gastric epithelial cell. Further image (D) clearly shown binding of BaBG at the erosive part of the stomach.

5.4.4 Protective effects of oral gavage of BaBG on pylorus ligation-induced gastric ulcers in rats.

The antiulcerogenic effects of BaBG on pylorus ligation induced ulcer model in rats are shown in Fig. 5.7. The gross ulcer indices reduced significantly after pretreatment with BaBG at a dose of 1.0 and 3.0 mg/kg in comparison to vehicle group [$F(5, 35)=16.88$; $p < 0.05$]. In addition, BaBG at a dose of 1.0 mg/kg and 3.0 mg/kg had a significant effect on DNA content of mucosa of the stomach, indicating cell proliferation (Table 5.1). Further, pH value was also significantly increased in doses of 1.0 and 3.0 mg/kg BaBG and omeprazole (Table 5.2).

Table 5.1 Effect of BaBG on cell proliferation of stomach in pylorus-ligated rats (data are mean ± S.E.M., n=6 in each group)

Treatment (mg/kg x 5 days)	Cell proliferation	
	Protein (µg/100 mg wet tissue)	µg DNA/mg protein
Control	5384.2 ± 562.7	106.5 ± 11.2
Vehicle	5916.6 ± 836.4	098.3 ± 07.8
BaBG 0.3	5937.5 ± 368.6	099.3 ± 09.5
BaBG 1.0	5736.9 ± 594.1	114.0 ± 14.1*
BaBG 3.0	5572.1 ± 824.8	118.2 ± 12.1*
Omeprazole 20.0	5525.6 ± 636.0	103.7 ± 08.3

*P<0.05 compared to the vehicle group

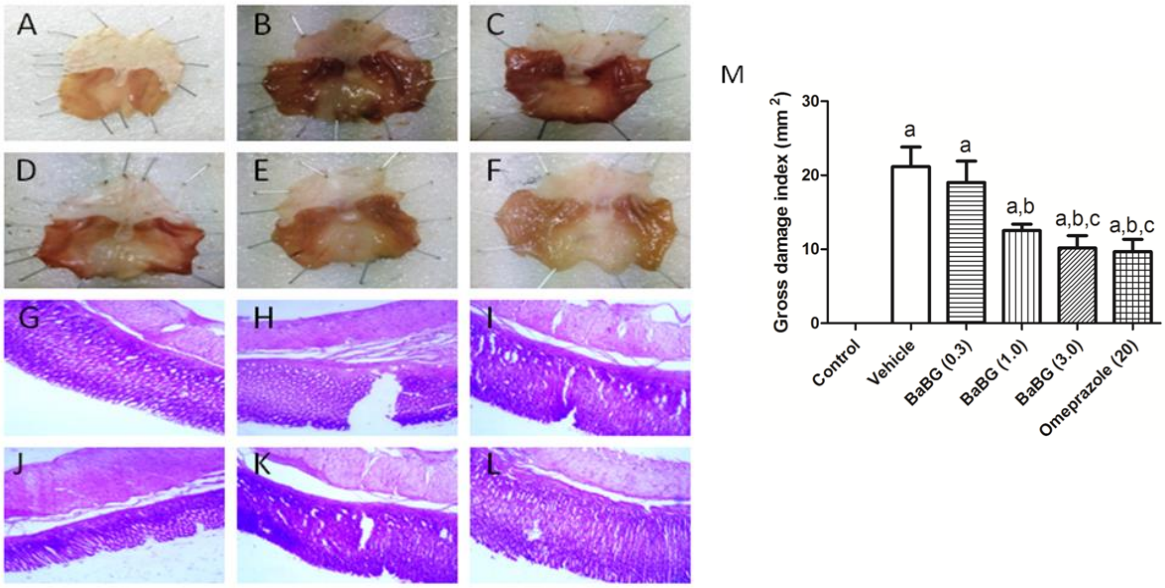

Figure 5.7 Control group rats of pylorus ligation induced ulcer model have no disruption of the surface epithelium (A & G). Rats in the vehicle group have severe disruption of the surface epithelium, and extensive oedema of the submucosal layer and leukocyte infiltration are present (B & H). Rat in BaBG 0.3 mg/kg has disruption of the surface epithelium, and there is submucosal oedema (C & I). BaBG 1.0 mg/kg rat has moderate disruption of the surface epithelium with oedema of the submucosal layer (D & J). Rats in BaBG 3 mg/kg showed a mild disruption of the surface epithelium (E & K). The gastric mucosa in animals pretreated with omeprazole showed a mild disruption of the surface epithelium (F & L). Macroscopic damage indices were quantified (M). The results are expressed as the mean ± SEM from six animals per group. aP<0.05 compared to control group, bP<0.05 compared to vehicle and cP<0.05 compared to BaBG 0.3 [One-way ANOVA followed by Student Newman-Keuls post-hoc test].

5.4.5 Protective effects of oral gavage of BaBG on aspirin-induced gastric ulcers in rats

Nonsteroidal-anti-inflammatory drugs (NSAIDS) such as aspirin have the ability to cause gastroduodenal ulceration. This effect is related to their ability to suppress prostaglandin synthesis and increase acid secretion. In the stomach, prostaglandins play a vital protective role, stimulating the release of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair. Fig. 5.8 presents the effects of BaBG against aspirin (500 mg/kg body weight) induced gastric ulcers in rats. At the dose of 3.0 mg/kg, BaBG significantly reduced the gross ulcer indices compared with the vehicle group [$F(5, 35)=15.79$; $p < 0.05$]. In addition, BaBG significantly increased pH value at 3.0 mg/kg dose (Table 5.2).

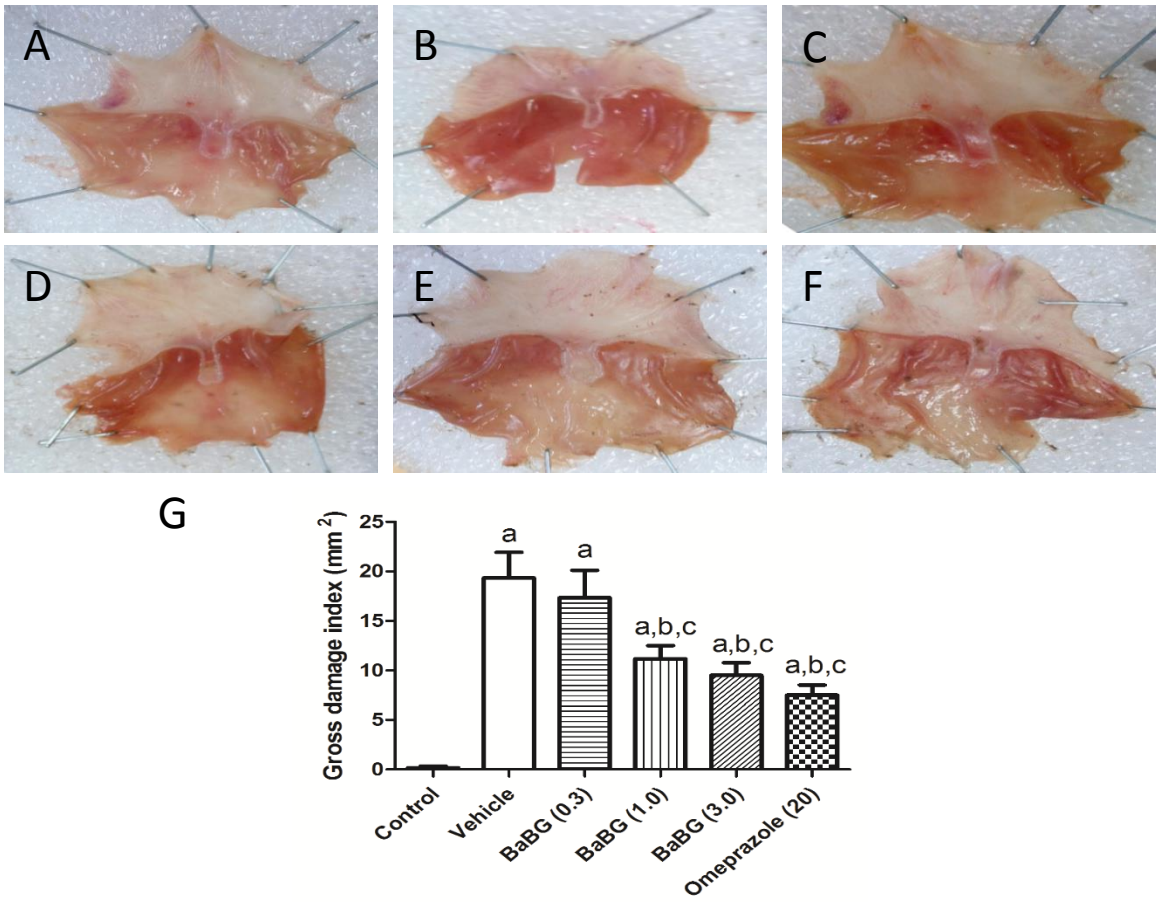

Figure 5.8 Rats in the control group of aspirin-induced gastric ulcers model have no disruption of the surface epithelium (A). Rats in the vehicle group have severe disruption of the surface epithelium (B). Rat in BaBG 0.3 mg/kg has disruption of the surface epithelium (C). BaBG 1.0 mg/kg rat has moderate disruption of the surface epithelium (D). Rats in BaBG 3 mg/kg showed a mild disruption of the surface epithelium (E). The gastric mucosa in animals pretreated with omeprazole showed a mild disruption of the surface epithelium (F). Macroscopic damage indices were quantified (G). The results are expressed as the mean ± SEM from six animals per group. $^{a}P<0.05$ compared to control group, $^{b}P<0.05$ compared to vehicle and $^{c}P<0.05$ compared to BaBG 0.3 [One-way ANOVA followed by Student Newman-Keuls post-hoc test].

Table 5.2 Effect of BaBG on gastric pH in rats

Treatment	**Gastric pH**			
	Ischemic group	**Aspirin group**	**Pylorus group**	**Ethanol group**
Control	2.35 ± 0.17	2.29 ± 0.13	2.45 ± 0.17	2.31 ± 0.21
Vehicle	2.21 ± 0.19	2.52 ± 0.19	2.51 ± 0.16	2.38 ± 0.19
BaBG (0.3)	2.71 ± 0.22	2.55 ± 0.14	2.78 ± 0.18	2.41 ± 0.24
BaBG (1.0)	2.80 ± 0.22	2.78 ± 0.17	3.39 ± 0.21*	2.48 ± 0.25
BaBG (3.0)	3.30 ± 0.21*	3.20 ± 0.19*	3.47 ± 0.23*	3.38 ± 0.26*
Omeprazole (20)	3.52 ± 0.24*	3.60 ± 0.22*	3.52 ± 0.25*	3.61 ± 0.31*

Data expressed as mean ± SEM (N=6). The significant difference was found against vehicle group: * $p < 0.05$, (One-way ANOVA followed by Student Newman-Keuls post-hoc test)

Table 5.3 Effect of BaBG on gastric fluid volume in rats

Treatment	Gastric volume (mL)			
	Ischemic group	Aspirin group	Pylorus group	Ethanol group
Control	0.77 ± 0.09	0.79 ± 0.08	0.84 ± 0.10	0.65 ± 0.07
Vehicle	1.36 ± 0.17	1.45 ± 0.17	2.50 ± 0.21	1.21 ± 0.10
BaBG (0.3)	1.34 ± 0.14	1.35 ± 0.18	2.41 ± 0.28	1.39 ± 0.11
BaBG (1.0)	1.25 ± 0.16	1.21 ± 0.19	2.46 ± 0.19	1.56 ± 0.14
BaBG (3.0)	1.35 ± 0.14	1.41 ± 0.15	2.49 ± 0.25	1.50 ± 0.15
Omeprazole (20)	0.75 ± 0.11*	0.68 ± 0.09*	1.42 ± 0.16*	0.71 ± 0.06*

Data expressed as mean ± SEM (N=6). The significant difference was found against vehicle group: *p < 0.05, (One-way ANOVA followed by Student Newman-Keuls post-hoc test)

5.4.6 Healing effect of BaBG on chronic gastric lesions induced by acetic acid

Fig. 5.9 A–F presents the effects of BaBG against acetic acid [(50% acetic acid) 0.06 ml per animal] induced chronic gastric ulcers in rats. The macroscopic ulcer indices reduced significantly after pretreatment with BaBG at a dose of 3.0 mg/kg in comparison to vehicle group [F (5, 35)=15.26; p < 0.05] (Fig. 7.9).

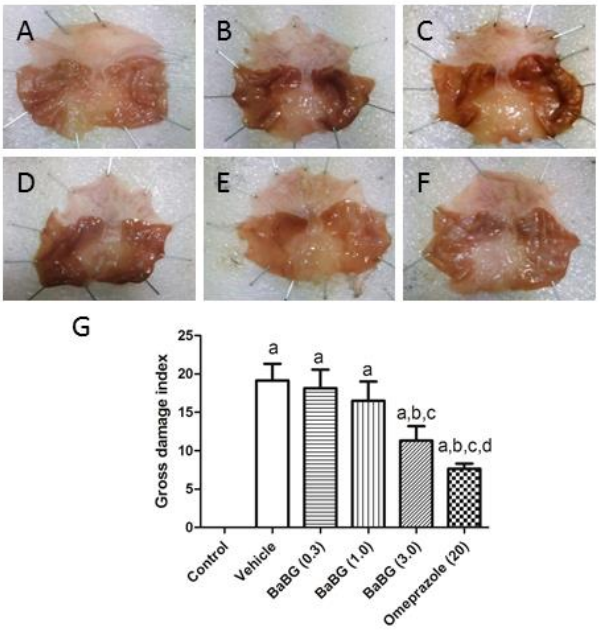

Figure 5.9 Rats in the control group of acetic acid-induced chronic gastric ulcers model have no disruption of the surface epithelium (A). Rats in the vehicle group have severe disruption of the surface epithelium (B). Rat in BaBG 0.3 mg/kg has disruption of the surface epithelium (C). BaBG 1.0 mg/kg rat has moderate disruption of the surface epithelium (D). Rats in BaBG 3 mg/kg showed a mild disruption of the surface epithelium (E). The gastric mucosa in animals pretreated with omeprazole showed a mild disruption of the surface epithelium (F). Macroscopic damage indices in rats were quantified (G). The results are expressed as the mean ± SEM from six animals per group. $^{a}P<0.05$ compared to control group, $^{b}P<0.05$ compared to vehicle, $^{c}P<0.05$ compared to BaBG 0.3 and $^{d}P<0.05$ compared to BaBG 1.0. [One-way ANOVA followed by Student Newman-Keuls post-hoc test].

5.4.7 Effect of BaBG on duodenum lesions induced by cysteamine

BaBG significantly protected the animal against cysteamine induced duodenum ulcers after 5 days of treatment (Fig. 5.10). At a dose of 1.0 and 3.0 mg/kg, BaBG significantly decreased gross ulcer indices [$F(5, 35)=47.60$; $p < 0.05$] The SEM images (Fig. 5.11) and EDS spectra (5.11E) of treatment group (3.0 mg/kg; BaBG) shows binding of BaBG over the damaged duodenal epithelial cell layer. The same image has been captured at different magnifications; Image A was taken at magnification 200×; B was at 500×, C was at 1000×; D was at 5000×. Images A and B at lower magnification shows microvilli of the intestine; while images C and D at higher magnification are showing BaBG particle adsorbed at the surface of the microvilli. Image E shows particle adsorbed at the surface of the intestinal villi have barium and silica peaks that confirm the particles are of BaBG.

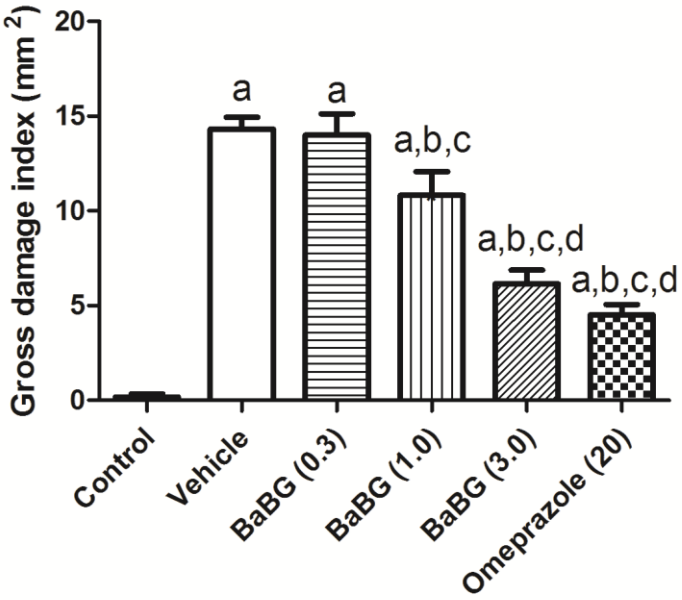

Figure 5.10 Bars represent the macroscopic damage indices in rats. The results are expressed as the mean ± SEM from six animals per group. [a]P<0.05 compared to control group, [b]P<0.05 compared to vehicle, [c]P<0.05 compared to BaBG 0.3 and [d]P<0.05 compared to BaBG 1.0. [One-way ANOVA followed by Student Newman-Keuls post-hoc test].

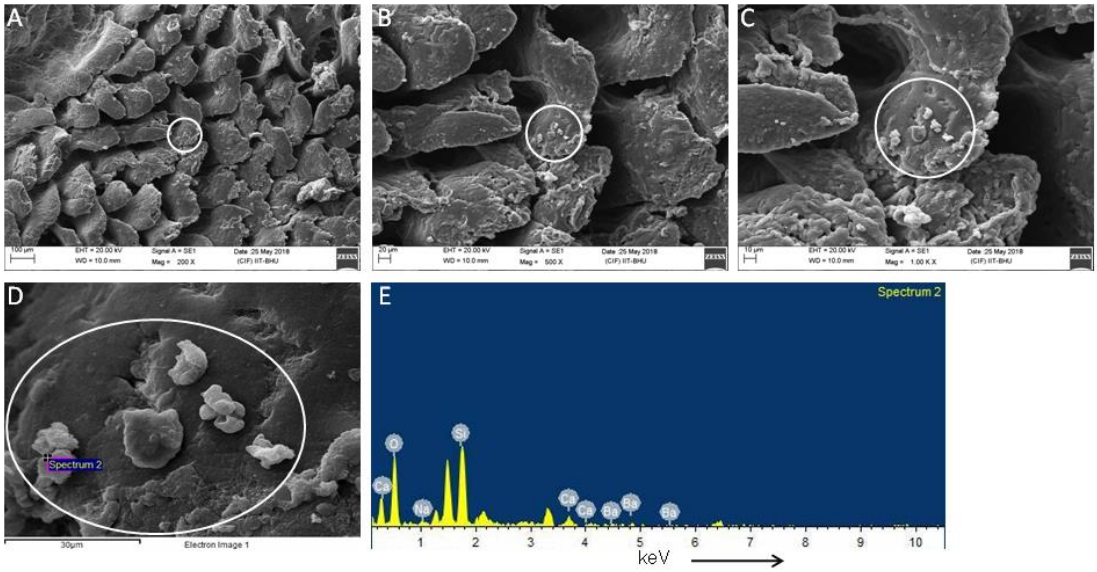

Figure 5.11 SEM images (A-D) and EDS analysis (E) of the intestine of cysteamine-induced duodenal ulcer models at 3 mg/kg BaBG dose confirm the binding of BaBG onto the surface

of the intestine. Duodenum images A, B, C and D were captured at a magnification of 200, 500, 1000 and 5000×. Lower magnification (A and B) shows microvilli of the intestine while the same images at higher magnification (C and D) show BaBG particle adsorbed at the surface of the microvilli. Image E shows particle adsorbed at the surface of the intestinal villi have barium and silica peaks that confirm the particles are of BaBG.

5.5 Discussion

The 1.3 mol% barium oxide in 45S5 bioglass® system shows significant anti-gastric and duodenal ulcer activities in acute as well as chronic ulcer models. BaBG in the dose of 3.0 mg/kg prevented and healed gastric-duodenal ulcers in rats induced by various ulcerogens.

The anti-ulcer activity may be due to antacid-like effect and formation of the protective layer over the gastro-duodenum epithelium cell surface.

Our findings from invitro pH study show that BaBG was able to increase and maintain higher pH; whereas there seems to be a trend to initial increase and then decrease with 45S5 in SBF. This indicates that BaBG may have potentially better antacid-like effect compared to 45S5. Further, we have evaluated the anti-ulcer potential of BaBG in animal models.

Accumulation of gastric juice in the pyloric ligation model leads to the breakdown of the protective mucosal barrier due to autodigestion of the mucosa. In the present study, administration of BaBG at the dose of 1 and 3 mg/kg significantly increased gastric pH in pyloric ligated rats. The increment in gastric pH indicates an acid-neutralizing effect of the BaBG. This was further confirmed by in-vitro pH behaviour of BaBG immersed in SBF and incubated at 37 °C for 1 week. This may be due to the release of Na^+ and Ca^{2+} ions from the bioactive glass and in-exchange for H^+ or H_3O^+ ions into the solution (Abebaw et al. 2017; Deliormanlı, 2012). Further, there was no change in the volume of gastric secretion with BaBG. As BaBG enhanced gastric pH without altering the gastric volume, it may have an

antacid property which protected the stomach from damage by ulcerogens. Hydroxyl concentration of the solution increases because of the H^+ ions being replaced with cations and responsible for the formation of silanols due to attack of hydroxyl ions in the silicate glass network (Cerruti et al. 2005). Silanols can cause cell proliferation and differentiation which facilitates in ulcer healing. Studies on bioactive glass reported the release of the soluble silicon ions, silica oxide ions and calcium ions from bioactive glass can also stimulate cell proliferation and differentiation (Xynos et al. 2000, 2001). In the present study, DNA content in BaBG treated rats was significantly enhanced, indicating mucosal cell proliferation. An earlier report also suggested that 45S5 bioglass® promotes proliferation of injured gastric epithelial cells which is responsible for local ulcer protective properties (Ma et al. 2013).

Intra-gastric administered ethanol leads to severe erosions in the stomach (Ma et al. 2013). The multifactorial pathogenesis of ethanol-induced gastric ulcers includes depletion of the protective mucus content of gastric wall (Jabri et al. 2017). and damage to microvessels of the gastric wall (Doggett and Breslin, 2014). Ethanol-induced gastric haemorrhage and necrosis are mostly due to stasis in gastric blood flow (Chandra et al. 2015; Park et al. 2015). In the present study BaBG significantly enhanced gastric pH in ethanol-induced ulcer rats. Thus, this local antacid effect may be responsible for the anti-ulcer activity of BaBG in ethanol-induced acute gastric ulcer model. Further, BaBG bound with the ulcerated gastric epithelial cell and formed a protective layer over the damaged gastric epithelial cell. SEM images of gastric mucosa further corroborate the above results, which show that BaBG glass particles covered ulcerated lesions on the gastric epithelium. Histopathological studies performed in ethanol and pyloric ligation ulcer models confirmed that the mucosal

epithelium of the 3 mg/kg of BaBG treated rats had normal architecture, fewer haemorrhages as well as less necrosis. Also, the silica rich layer and the hydroxyl‑carbonateapatite layer created on the glass surface allow it to bind at the wound (Zhong and Greenspan, 1998) and protect the mucosal lining from further damage.

NSAID's like aspirin aggravate offensive factors by increasing gastric acid secretion, interfering with the prostaglandin synthesis and back diffusion of hydrogen ions (Ignatius et al. 2013). BaBG enhanced gastric pH significantly in aspirin-induced ulcer rats. In the present study, BaBG treatment has no impact on gastric fluid volume. However, it significantly reversed the ethanol, aspirin and pyloric ligation induced gastric ulcer effect, acting as an effective barrier by increasing gastric pH and significantly decreased gross ulcer index. Protective effect of repeated administration of BaBG was also observed in cysteamine-induced duodenal ulcer. Cysteamine stimulates gastric acid secretion rate and significantly decreases the neutralization of acid in the proximal duodenum, resulting in duodenal ulcers (Adinortey et al. 2013). BaBG significantly decreased the gross ulcer index in the duodenum of the cysteamine induced duodenal ulcer rats indicating the protective effect of the BaBG. The protective effect was confirmed by SEM images of ulcerated duodenum. BaBG at 3 mg/kg was found to bind over the surface of the duodenum epithelium cell in cysteamine induced model indicating the protective effect of BaBG at both acidic as well as a basic environment of the gastro-intestinal tract. EDS confirm the presence of barium and silica in the adsorbed particles. The retention study would have given a better understanding of anti-gastric ulcer activity of BaBG. However, in the present study, the gastric and duodenum tissues from acute gastric ulcer models show BaBG particles 24 h after

administration. Hence it can be inferred that BaBG is retained in stomach and duodenum at least for 24 h.

Acetic acid is reported to produce ulcers by gastric obstruction leading to an increase in acidic gastric juice and is used as a model to evaluate ulcer healing (Li et al. 2016). Previous study studies stated that bioactive glass exchange ions between hydrogen from the fluid and sodium from the bioactive glass when it contact with the physiological environment (Hench, 1991; Kim et al. 1989; Ogino et al. 1980; Zhong et al. 2002), indicating the potential of bioactive glass in healing gastric lining by the hydrochloric acid formation. The above results suggest that administration of BaBG may have resulted in a decrease in gross ulcer index due to repeated acetic acid-induced ulcer in rats. Blood flow plays an essential role in protecting the gastric mucosal layer and the healing of damaged mucosa. BaBG treatment enhanced the gastric pH and attenuated gastric damages induced by caeliac artery occlusion. In addition, BaBG recovered the blood flow and improved microcirculation as evident by enhancing VEGF levels in ischemic gastric mucosal tissue.

BaBG at 3 mg/kg significantly reduced gastric ulcers in all the tested animal models. In the ischemic, pyloric, ethanol and aspirin model, BaBG showed significantly increased in gastric pH; however, no significant effect was observed on gastric volume indicating antacid like activity. BaBG treated rats had normal architecture, fewer haemorrhages as well as less necrosis in ethanol and pyloric ligation ulcer models. BaBG treatment significantly increased cell proliferation in the pyloric model, and it forms a protective layer over gastric as well as duodenum epithelium as confirmed by the SEM images in ethanol and cysteamine models. BaBG significantly decreased gross ulcer index in the duodenum of the cysteamine induced duodenal ulcer rats indicating the protective effect of the BaBG. Further, BaBG healed

gastric ulcers induced by acetic acid. The antiulcer effect of BaBG in both preventing and healing model can be attributed mainly due to its physical protective barrier forming property, acid-neutralizing and cell proliferation mechanism.