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DISCUSSION

The interaction between membrane proteins and lipids of parietal cells is far from being understood. Alteration in membrane fluidity have been correlated with changes in many membrane function. A conformational change in transmembrane protein or lateral mobility of membrane lipid, may alter enzyme activity and ionic transport. Furosemide is an anionic surface acting agent and disturb the phospholipid bilayer in erythrocyte cells\textsuperscript{102} and regulate the membrane bound ATPase activity\textsuperscript{103}. The secretagogue mediated acid secretion from parietal cells and interaction of phospholipid bilayer is still a subject of study.

The current study on furosemide was to evaluate the efficacy of furosemide as an antiulcer drug. The effectiveness of any antiulcer drug is essentially dependent on its capability to reduce acid secretion and /or strengthen the defensive mechanism. Common cause of acute ulceration today apart from Helicobacter pylori infection, is the extensive use of non-steroidal antiinflammatory drugs such as aspirin, brufen, indomethacin combiflam etc and excessive consumption of alcohol. Experimental result reveal that in furosemide untreated ulcerated control group (induction of indomethacin at a dose of 5 mg /kg body weight for 3 days), there is an extensive damage of epithelial layer and tubular glands including lamina-propria resulting in an acute ulceration (Figure IA) but in furosemide pretreated ulcerated group, these is only a slight damage to epithelial layer (Figure IB).
The very reasons these non steroidal anti-inflammatory drug causes mucosal damage is the basis of their mechanism for relieving pain. They inhibit the synthesis of prostaglandin which act as signalling molecule in the mediation of pain by sensory nerves through out the body. Because of the inhibition of the synthesis of prostaglandin, the mucus secretion and bicarbonate secretion are also inhibited\(^{51,52}\). Furosemide pretreatment in non steroidal anti-inflammatory drug induced ulcer model, may confer cytoprotection through mucosal defense mechanism. However, a detail reference on mucosal defense mechanism has been discussed in the later part of this discussion.

Oral administration of furosemide at a dose of 2mg/kg body weight day for 7 consecutive days appears to be most effective and significantly resist the alcohol induced gastric mucosal lesions (Figure II). Furosemide pretreatment not only reduces the ulcer index but also reduces the mean incidence of ulceration. The histological findings also support above observation with an impression that in control group, mucus membrane undergoes desquamation of the epithelial layer to an extent of severe hemorrhages (Figure IIA) but in furosemide pretreated group there is only a partial destruction of epithelial layer (Figure IIB). If appears from all the above mentioned results, furosemide dose 2mg/kg body weight is most effective out of the arbitrary dose, 1mg, 2mg and 3 mg /kg body weight chosen. Hence for the detail study, the furosemide dose selected was 2mg/kg body wt.

Dinoso et al\(^{104}\) markedly observed a decrease in the mucus layer and mucin content of lining epithelial cells after exposure of the gastric mucosa to different
concentration of ethanol. Gastric lesions caused by ethanol may be due to the damage of gastric mucosal barrier. Moreover, many reports suggested that gastric lesions caused by ethanol are due to free radical generation which result in lipid peroxidation.\textsuperscript{105,106,107,108} though Pihan et al reported that ethanol induced lesion are only slightly affected by antioxidants or superoxide dismutase.\textsuperscript{109} This indicates a need for detail study on the gastric cytoprotective activity of furosemide against ethanol induced gastric lesion, over this controversial free radical mechanism and/or if any, other mechanism involved. A detail study on free radical mechanism is discussed in the latter part of this discussion. But irrespective of the mechanism involved our results show furosemide has a gastric cytoprotective potentiality against experimentally induced gastric mucosal lesions eg; alcohol and non steroidal anti-inflammatory drug (indomethacin) induced ulcer.

Efficacy of any antiulcer drug depend upon its ability to reduce aggressive factors and/or increase defensive factors. Stomach's defensive mechanism is made of mucus and bicarbonate level. Mucin plays an important role in protecting the surface epithelium from physical as well as chemical trauma.\textsuperscript{110,111} It confer its action due to its viscoelastic properties. Biochemical analysis of gastric juices (collected using pyloric ligation model) and gastric tissue reveal that furosemide not only reduces the acid secretion but enhances the mucus and hexosamine content both in gastric tissue and gastric secretion. (Table I) The increased hexosamine content is an indirect measure of mucus output and mucus layer thickness is an important index of protective function.\textsuperscript{112} These studies give evidences in support of the furosemide's cytoprotective potentiality possibly by
strengthening its defensive mechanism. Thus support our assumption that furosemide may have a role in protecting from gastric lesions.

Besides cytoprotective effect, therapeutic effect of furosemide in the context of ulcer treatment needs to be evaluated.

Under the review of literature the mechanism of ulcer healing is discussed that there are certain line of repairs like restitution, epithelial cell growth and acute wound healing. Ongoing epithelial cell replication is essential to maintain mucosal integrity. These are normal functions of stomach. When the defense and epithelial repair mechanism fail to keep pace with ongoing cell injury, acute wound in the basement membrane occur. Healing or repair of these wound is studied in healing ulcer model.

Histopathological observation gives a lot of information about the gradual recovery during the process of healing of experimental gastric mucosal lesions in rats with or without furosemide (2 mg/kg body weight) treatment. Analysis of histological event occurring during recovery shows that in untreated ulcerated group after 7 days of ulcer induction, there is no remarkable sign of healing is observed (Figure IIC), whereas in furosemide treated groups after 7 days of ulcer induction markable healing is observed, including repair of lamina propria has started (Figure IID). After 14 days of ulcer induction in untreated ulcerated group only partial and incomplete repair of lamina propria is observed (Figure IIE) whereas by this time lamina propria is completely repaired in furosemide treated group (Figure IIF). There is complete reepithelisation

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after 21 days in furosemide treated group (Figure III G) so it appear more like the normal tissue whereas in untreated ulcerated group complete reepithelisation requires more days (Figure III H).

These histopathological findings clearly shows the therapeutic or healing effect of furosemide. The oral administration of furosemide at a dose of 2 mg/kg body weight /day for 21 days completely heal the mucosal lesions induced by indomethacin in experimental ulcer healing model. However, these observation need, to be supported by other biochemical parameter.

During ulcer formation there is extensive damage of tissue or cell loss due to which the DNA and protein content of the gastric tissue decreases. Measurement of DNA and protein content of gastric tissue during healing experiment is a good index of healing process. Results of Table II supports biochemically that furosemide induces ulcer healing in which the DNA and protein content of furosemide treated ulcer group reaches to the control level i.e. normal value with in 21 days of ulcer induction. However, in untreated ulcer it is only 78% of the control. This suggest that furosemide may helps in epithelial cell regeneration in damaged gastric mucosa. However, this action of furosemide is not found in normal tissue and the mechanism of underlaying ulcerated tissue regeneration in presence of furosemide is not understood.

Histamine plays a critical role in the regulation of gastric acid secretion through the activation of parietal cell H2 -receptor. There are evidences suggesting that histamine

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is the final messenger acting on the parietal cell surface even for acetylcholine and gastrin. Anderson et al reported that furosemide prevents the release of histamine from lung. Our experimental result shows that furosemide significantly prevent the release of histamine in gastric tissue (Table –III) which support the earlier finding. The action of furosemide on histamine release is explained by its action on histamine secreting mast cell. Histological observation of mast cell clearly indicate that furosemide stabilises the mast cell hence preventing the release of histamine. In our experiments, it was observed that the number of stabilised mast cell in furosemide treated group (figure IVC) is more in comparison to normal group (figure IVA) and ulcerated group (figure IVB).

This finding gives a possible explanation that by preventing the release of histamine from the mast cell, furosemide may help in preventing the gastric mucosal lesion by inhibiting the release of aggressive factors as well as promote the healing process by an indirect way. However, this is not the case in ulcerated rat (without furosemide treatment) as in these group repair mechanism fails to keep pace with the ongoing injury due to the release of histamine and ultimately, increased acid secretion.

Mucosal prostaglandins play an important role in normal mucosal defense apart from its antisecretory effect. Their actions are diverse and depend in part on the type of prostaglandin. Cyclooxygenase inhibitors such as aspirin and non-steroidal antiinflammatory drug (NSAID) produce a spectrum of mucosal injuries in the stomach. Part of the cause of these ulceration is decrease in prostaglandin-mediated mucosal defense. The prostaglandin actions that are decreased by NSAID inhibition include
normally increase mucus secretion, increased bicarbonate secretion, inhibition of gastric acid secretion\textsuperscript{114,115}. NSAIDs may thereby compromise mucosal mechanisms that prevent the development of gastroduodenal ulceration\textsuperscript{116,117}. Weber and Scherer\textsuperscript{66} in 1976 reported that furosemide stimulate the release of arachidonic acid a preccursor of prostaglandin (C\textsubscript{20}: 4) and through this it increases the formation of PG endoperoxides and or PGE\textsubscript{2}. It is also reported that after administration of the drug, the level of C\textsubscript{20}: 4 remained at elevated level. Result of our experiment (Table V) shows that the level of prostaglandin increases significantly on treatment with furosemide. The level of prostaglandin in furosemide treated ulcerated rat is much more in comparison to ulcerated rat. Our result corroborate with the findings of Weber and Scherer. From this finding it may be suggested that furosemide induced cytoprotection of mucosal layer is through the endogenous release of prostaglandin and thus by increasing the level of mucus and hexosamine (Table 1).

There is a growing body of evidences that reactive oxygen metabolites such as superoxide radicals (O\textsubscript{2}\textsuperscript{-}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and hydroxyl radical (OH\textsuperscript{-}) mediate some of the cellular injury associated with gastric ulceration\textsuperscript{118,119,120}. \textit{Helicobacter pylori} releases a chemotactic factors for neutrophils and monocytes, possibly explaining the almost universal presence of a mucosal inflammatory cell infiltrate in \textit{Helicobacter pylori} infection\textsuperscript{121,122,123}. Subsequent elaboration of cytokines or reactive oxygen metabolities may account for at least a portion of the mucosal injury. Despite the continuous exposure to potentially harmful oxidant which are generated spontaneously in the body, the healthy surface epithelium appears to be unaffected by the presence of these reactive species.
This suggests that the gastrointestinal mucosa is endowed with efficient mechanisms for the protection and/or removal of these oxidants. Potential mechanisms include intracellular scavengers, i.e., superoxide dismutase, catalase, glutathione, and total sulfhydryl group. The study on the effect of furosemide on these intracellular scavengers becomes very necessary to rationalize the cytoprotective as well as therapeutic (healing) use of furosemide as an antiulcer drug.

During the process of tissue degeneration with the reactive oxygen species, malondialdehyde is formed as one of the end products of lipid peroxidation. The decrease in the level of malondialdehyde (Table VI) in furosemide-treated gastric mucosa compared to untreated ulcerated group suggests furosemide's ability to protect gastric mucosa against the ulcerative agents, preventing cell damage mediated through the free radical generation.

Furosemide increases the activity of superoxide dismutase in ulcerated rat treated with furosemide but in untreated ulcerated rat the activity of superoxide dismutase reduces significantly (Table VII). Protecting effect of superoxide dismutase against gastric injury has been established by Terano et al. Protecting effect of superoxide dismutase against gastric injury has been established by Terano et al. and it has been reported that diminish leukocyte adherence in microvessels protect the gastric mucosa against neutrophil damage. Reduction of lipid peroxidation i.e., the level of malondialdehyde may be explained with the increased level of superoxide dismutase on furosemide treatment. But in contradictory, catalase activity remains normal even when treated with furosemide.
Reduced thiols have long been reported to be essential for recycling of other oxidant like vitamin E and vitamin C\textsuperscript{127}. Perturbation of glutathione status of a biological system has been reported to lead to serious consequences\textsuperscript{128}. Administration of thiol compounds such as glutathione, cysteine and methionine are known to protect against oxidative stress in human and animals\textsuperscript{129, 130}. Furthermore, excessive peroxidation can cause increase glutathione consumption\textsuperscript{131}. Indomethacin induced depletion of gastric glutathione is counteracted by maintenance of normal glutathione tissue concentration which inhibit gastric mucosal injury possibly through scavenging indomethacin generated metabolites\textsuperscript{132}.

It is interesting to note from our observation that though furosemide reduces MDA level and increases SOD activity but fail to alter other free radical scavenging endogeneous components i.e. catalase and glutathione, a major non protein thiol compound (total tissue sulphhydryl group, Table IX) level. Furosemide being an anionic surface acting agent, causes the aberration on the surface of the gastric parietal cell (Figure XB and XC), results changes in the composition of membrane bound phospholipids. The reduced level of MDA on furosemide treatment suggest an alternative pathway of gastric cytoprotection or healing ulcer other than scavenging free radical system however, enhanced SOD level is still uncleared and unexplained.
The current investigation demonstrates that furosemide has got cytoprotective as well as therapeutic potentiality as it enhances the defensive factors thus preventing ulcer formation by the release of mucus and hexosamine, a result of endogenous synthesis of prolaglandin or by stabilising the mast cell causing lowering of the aggressive factors.

To study the role of furosemide in H⁺ transport, classical frog chamber model is adopted and experimental result clearly shows the effect of furosemide on H⁺ transport. Furosemide significantly inhibit histamine stimulated H⁺ transport which reaches to basal level within hours. The action of furosemide is found to be from either side of the gastric membrane i.e., both the nutrient (Figure VII) and secretory side (Figure VI). Furosemide at the same time increases the K⁺ flux through the gastric mucosa. Nandi et al. have also demonstrated the influx of K⁺ of gastric superficial epithelial cells (SEC) and that may count for Na⁺/K⁺/Cl⁻ contransport system of the cells. Furosemide being a mild carbonic anhydrase inhibitor (Table IV) may be involved in gastric cell’s Na⁺/K⁺/Cl⁻ co-transport system. The inhibitory effect of furosemide on H⁺-transport can be rapidly reverse by replacing Na⁺ with equimolar K⁺ ion concentration (Figure VIII) in secretory solution. This suggest that effect on furosemide may occur at or near the secretory membrane and as furosemide is a mild surface acting agent, it may interfere with one of the affinity site for K⁺ ion that is essential for H⁺ transport. Scanning Electron Microscopic photographs supports the above findings. Furosemide causes aberration on the gastric mucosal cell mainly parietal cell. With increase in furosemide concentration aberration becomes more intense (Figure XB and Figure XC) All these findings thus, suggest that action of
furosemide is on the surface of gastric mucosal surface and alter the membrane integrity and as consequences changes the membrane function.

There is much evidence suggesting that secretory membrane located H⁺,K⁺-ATPase is involved in gastric H⁺ transport by the acid secreting cells of fundic mucosa of stomach. It exchanges K⁺ for H⁺ in an electroneutral fashion. From frog chambered study in our investigation, it was found that furosemide inhibit H⁺ transport at 1mM concentration maximally. To specify this action the effect of furosemide on isolated gastric microsomal H⁺,K⁺-ATPase becomes necessary. Experimental result of present study (Table XI) give information that furosemide inhibit H⁺,K⁺-ATPase activity in dose dependent manner which is maximum (50%) at 1mM concentration. It is also found that at 1mM concentration furosemide compete with ATP for active catalytic site of H⁺,K⁺-ATPase (Figure V). It is possible because furosemide share structural similarity with ATP. Experiments and results shown in Table XI suggest that furosemide interact with the gastric microsome but not involved in the enzyme catalysed reaction mechanism which supports the observation from Scanning Electron Microscopic study.

This investigation gives emphasis on the basic and fundamental searches in detail regarding the effect of furosemide in peptic ulcer. Gastric microsomal membrane integrity thus, plays an important role either in the pathogenesis or in the gastric cytoprotective as well as healing effect of gastric cells. This thesis is structured to be as consistent and systematic as possible. Every effort has been made to make it
comprehensive and update. However, due to speedy developments in the field of peptic ulcer some area need to be further updated.

Peptic ulcer is a multifactoral disease and as its pathogenesis is not complete so its therapeutic intervention in future is difficult to predict. There are few area like eradication of *Helicobacter pylori*, epidermal growth factor, modulation of immune system, use of mitogenic and angiogenic substance and recombinant DNA technology, release of hormones, receptor bindings, isotopic study and membrane interaction in which the role of furosemide need to be established. 

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