DISCUSSION
6. **DISCUSSION**

Since last two decades, a number of drugs have been introduced for the treatment of duodenal and gastric ulcers. Recent researches have made significant contribution in our understanding of the mechanism of mucosal protection and the role of mucus in the recovery of the upper gastrointestinal tract from acute damage. The problems encountered in the experimental evaluation of anti-ulcer drugs result in part from the lack of complete understanding of the physiological and biochemical mechanisms involved in the formation of ulcers. The discovery of novel and more effective anti-ulcer drugs have been accompanied by the introduction of a large number of newer experimental methods to evaluate the anti-ulcer activity of drugs effective in different types of ulcers and elucidate their possible mechanism of action.

Some of these methods produce a consistently high incidence of rapidly discernible ulcers in the area of gastrointestinal tract. It is considered that the appearance, complications, development and healing of certain experimentally induced ulcers are similar to the human clinical ulcers (Brodie, 1968). The main differences, however, are in the location and chronicity of ulcers.

The location of an ulcer is itself a great source of information about the cause or mechanism underlying peptic ulcer. According to the report of Oi et al. (1971), there
is a clear structural influence on the development of peptic ulcer. The majority of experimentally induced ulcers occur in the stomach while clinically the duodenum is the major site.

Gastric ulcer models show ulcers in the region of fundic gland area whereas duodenal ulcer models show ulcers in the region of fundopyloric mucosal boundary, pyloric gland area and in the wall of duodenum. Most experimental ulcers are acute, penetrating, rapidly healing and nonscarring lesions while clinical ulcer is a chronic and penetrating lesion which heals with a scar.

In spite of these limitations, it is possible to evaluate the therapeutic agents rapidly and with reasonable predictability for their clinical usefulness, using the experimentally induced gastric and duodenal ulcer models. Although various experimental models are available for evaluating anti-ulcer activity and for studying the ulcer healing processes, a single model for studying the genesis of ulcers as well as understanding exact mechanism of anti-ulcer effect is not available. Therefore a number of procedures are generally employed to evaluate the anti-ulcer activity of various agents.
CYSTEAMINE INDUCED DUODENAL ULCERS

Cysteamine induced ulcer is one of the most widely used model for studying duodenal ulcers. The chemically induced acute and chronic duodenal ulcers (especially those produced by cysteamine) because of their close resemblance to human duodenal ulceration, may be used as animal models to study the pathogenesis of this complex and poorly understood human disorder (Szabo et al., 1982).

In the present study, verapamil showed significant reduction in total lesion area and score for intensity at the dose of 32 mg/kg. Diltiazem showed statistical insignificant reduction in the total lesion area and score for intensity at lower doses. It has shown aggravation of duodenal ulcers especially at higher doses as indicated by the results. Diltiazem treated group showed higher mortality in 60 mg/kg dose as compared to the control rats.

Nifedipine treatment was done by using two separate groups viz. nifedipine solution in DMSO solvent and nifedipine suspension in 1% CMC solution. This method was especially adopted to check any effect of vehicle (DMSO) on experimental ulcers. Nifedipine solution showed statistically insignificant reduction in total lesion area and score for intensity at the 32 mg/kg dose, whereas its suspension form showed significantly aggravating effect upon ulcers in lower doses which was not observed in previous group i.e. nifedipine
solution treated group. Thus probably nifedipine aggravates ulcer at lower doses which goes parallel with the report of Glavin (1988) who reported ulcer worsening effect of nifedipine at 16 mg/kg and slight protective effect at 32 mg/kg against ethanol induced ulcer.

In the present study also, nifedipine solution and suspension forms showed protective (statistically insignificant) effect against duodenal ulcers at 32 mg/kg dose. These results indicate that in solution form of nifedipine, it is DMSO which prevents ulcer aggravating effect of nifedipine at lower doses as evident from its insignificant reduction in total lesion area and score for intensity. DMSO is found to be scavenger for elimination of free radicals which are responsible for ulcer relapse (Richter, 1990; Salim, 1990). Thus it can be concluded from the results that, DMSO effect is predominant at lower doses of nifedipine but higher doses of nifedipine by themselves show protective effect towards ulceration.

It is clear from the results of the present study that only verapamil is effective against duodenal ulcers at higher dose range compared to the other two calcium channel blockers used in this study.

The pathophysiology of cysteamine ulcers appears different from that of pylorus ligation model. Cysteamine ulcers are considered to be due to a long lasting hypersecretion of
gastric acid (Szabo et al., 1977; Kirkegaard et al., 1980) which may be partly due to decrease in buffering capacity of the duodenum (Szabo et al., 1982) or due to increased plasma levels of gastrin (Lichtenberger et al., 1977). Infact, hyper-secretion of acid disturbed gastroduodenal motility, hyper-gastrinaemia and decreased mucosal resistance have all been implicated in the pathogenesis of cysteamine induced duodenal ulcers (Ischii et al., 1976; Lichtenberger, 1977a). Briden and coworkers (1984) suggested that the specific duodenal ulcerogen cysteamine has little effect on basal bicarbonate transport but inhibits the ability of the duodenal epithelium to respond to luminal acid with a compensatory rise in alkaline secretion thereby leading to a fall in surface pH.

In a preliminary study of Sainath et al. (1985), verapamil orally in a dose of 15 mg/kg, protected rats against duodenal ulceration induced by cysteamine. However, mechanism for anti-ulcer activity against duodenal ulceration by verapamil is not made clear. Szabo and coworkers (1982) reported that acetylcholine, histamine, dopamine and GABA-sensitive passage of acidic gastric juice into the duodenum following delayed gastric emptying seems to be a key element in the pathogenesis of this disease model. This sequence of events appears to involve both peripheral and central (eg. brain) elements.
CCBs have been found to possess inhibitory effect on histamine, gastrin, carbachol and cAMP induced stimulation of gastric acid secretion (Soll, 1981; Kirkegaard et al., 1982; Sewing and Hannemann, 1983). They also inhibit contractility of gastric smooth muscle as well as basal and stimulated gastric acid secretion (American College of Gastroenterology's Committee Report, 1984).

In in vitro studies, verapamil reduces histamine and pentagastrin stimulated secretion from guinea-pig duodenic mucosa (Kirkegaard et al., 1982) and isolated whole stomach of mouse (Szelenyi, 1980). However, effect of verapamil on histamine or pentagastrin stimulated acid secretion is controversial. Controversy also exists in the clinical reports. Verapamil reduces gastrin or pentagastrin stimulated secretion in man (Kirkegaard et al., 1982; Sonnenberg et al., 1984). However, other workers did not confirm that effect of verapamil on acid secretion stimulated by histamine (Sonnenberg, 1984) or pentagastrin (Aadland and Berstad, 1983) or by bethanechol (Sonnenberg et al., 1984).

It is evident from above reports that histamine is a critical component of gastric function as well as in disease states such as gastroduodenal ulcer. Prevention of degranulation of mast cells by verapamil might be responsible for preventing histamine and 5-HT release from the stomach mast.
cells. That would also lead to less $H_1$-histamine and 5-HT receptor mediated smooth muscle contraction (Ogle et al., 1985).

Along with the histamine action, delayed gastric emptying by CCBs may enhance the protective activity against duodenal ulcer model which goes parallel with the report of Brage et al. (1986) that pretreatment with the CCBs like verapamil, diltiazem, cinnarizine delays gastric emptying in rats. Even lowered gastric motility after verapamil could therefore, result from interference with calcium utilization needed for gastric smooth muscle contraction.

In the present study, verapamil showed significant reduction in volume of gastric secretion in aspirin treated pylorus ligated rats. It also reduced total acidity at higher dose. Based upon these effects and above discussion, verapamil may also interfere with the histaminergic mechanisms involved in the secretion of gastric acid and induction of gastric and duodenal ulceration in different experimental models. It is effective in preventing the mast cells degranulation on the gastric and duodenal mucosa and thus reduce the gastric acid secretion and the contractility of stomach wall which in turn may reduce gastric motility and thus decrease mucosal folding in the g.i. tract of animals. However, further studies are necessary to confirm the gastroprotective effect of CCBs and elucidate their exact mode of action.
PYLORUS LIGATED, ASPIRIN-INDUCED AND ASPIRIN PLUS PYLORUS LIGATED GASTRIC ULCERS

In the present study, pylorus ligation, aspirin-induced gastric lesions and aspirin plus pylorus ligation models have been used for investigating the effect of CCBs on gastric ulcers.

Pylorus ligation (PL) is a widely used method for producing experimental gastric ulcers. It is suitable for first line anti-ulcer screening as the agents are retained in the stomach and may act by variety of mechanisms. This method has got high rate and reproducible occurrence of ulceration (Ishii, 1970). The main advantage is that one can study the possible measurement of gastric secretory rate, % ulceration and ulcer severity in the same animal. It can be performed with ease and it is known to produce uniformly high incidence of perforating lesions. A survey of the clinically useful drugs on experimental ulcers suggests that the pylorus-ligated rat is probably the single test for the prediction of clinical usefulness of an anti-ulcer drug (Brodie, 1968).

The gastric mucosal damage induced by NSAIDs as a part of their toxic effects on the gastrointestinal tract form a basis for the use of aspirin model in the evaluation of various drugs against the gastric ulcers. This is the
most extensively used experimental model for the study involving NSAIDs-induced ulcers. When administered in high or multiple doses, many agents (e.g. reserpine, NSAIDs) can be used as (chemical) stressors. The gastric ulcers induced by such agents are due to stress response and the agents are similar to physical stressors like restraint, cold, severe illness, shock, forced exercise etc. (Szabo, 1980).

Besides the above models, aspirin plus pylorus ligation technique was employed in the present study for the evaluation of CCBs against gastric secretion as well as gastric ulcers because aspirin alone does not allow to study the effect of drugs on gastric acid secretion.

The superiority of this model lies in its suitability for evaluation of different physiological and biochemical alterations both in the glandular as well as nonglandular or membraneous regions of the stomach wall (Mozsik et al., 1970). Moreover, the gastric ulcers appear rapidly in the stomach wall by this technique.

In the present study, the three CCBs have been shown to possess anti-ulcer activity in all the above mentioned models of gastric ulceration as evident from the significant decrease in ulcer index.
In case of PL model and aspirin treated PL group of animals, verapamil has shown reduction in volume of gastric secretion in a dose dependent manner. However, this reduction was found to be significant only in aspirin treated pylorus ligated rats. Whereas diltiazem was effective in reducing the volume of gastric contents in all the dose levels in both these models. Nifedipine was effective to some extent only at maximum dose used i.e. 40 mg/kg.

Reduction in the free acidity and total acidity was observed following higher doses of verapamil and diltiazem in these models. However, nifedipine has shown opposite effect to that of verapamil and diltiazem i.e. it has significantly raised total acidity in lower doses in aspirin treated pylorus ligated rats. Furthermore, the reduction in free acidity and total acidity was not found to be consistent in both the models of gastric ulcers.

In addition to these secretory parameters, pepsin activity of gastric juice was also estimated. Verapamil and diltiazem failed to cause reduction in pepsin activity. However, 70% of total reduction in this parameter was observed in the presence of nifedipine at 40 mg/kg dose. Contrary to the observations that pepsin and acid secretion are usually affected by drugs in the same way (Konturek, 1980; Esplugues et al., 1982),
CCBs showed alteration in acid secretion without affecting the pepsin activity in the present study. On the basis of this observation, it appears that Ca\textsuperscript{2+} is involved only in acid secretory activity of gastrointestinal tract but not in the pepsin activity. This fact is supported by earlier report of Brage et al. (1986). They have shown that both verapamil and diltiazem reduce the volume of gastric secretion and total acid output in pylorus ligated rats without affecting pepsin output thus suggesting that Ca\textsuperscript{2+} is not involved in the physiological secretion of pepsin.

Kale and Sharma (1989), in their study with verapamil have suggested that reduction of acid secretion contributes little to anti-ulcer action of CCBs because anti-ulcer effect was seen even at doses which did not decrease acid secretion. Other reports also support this view as the neutralization of total acid has not been found to be effective in preventing glandular ulceration by stress (Dai and Ogle, 1974; Cho and Ogle, 1979). Recently a dihydropyridine, nitrendipine has been shown to possess significant gastroprotective effect against stress induced lesions in rats at all doses such as 8, 16, and 32 mg/kg (Ulak et al., 1991). However, the authors have not suggested the mechanism of this protective effect. It has been demonstrated that gastric acid inhibition by metiamide (Bugajski et al., 1976; Cho and Ogle, 1979) or cimetidine (Okabe et al., 1977 and 1977a) is not wholly
responsible for preventing glandular stress ulcers since non acid inhibiting doses of these histaminergic blockers also confer protection. The anti-ulcer effect has been attributed to a cytoprotective property possessed by these agents.

Koo et al. (1986) reported inhibition of gastric acid accumulation by verapamil in pylorus ligated rats only in low doses. However, using rat isolated perfused stomach, Canfield et al. (1985) failed to observe any effect of verapamil on gastric secretion.

Incidently Glavin (1988) observed reduction of ethanol induced ulcers with verapamil at 16 mg/kg dose to some extent. Absence of such effect was observable at 8 and 32 mg/kg dose. Nifedipine worsened ulcers at 16 mg/kg dose but slight protective effect was observed at 32 mg/kg dose. However, both drugs protected stress ulcers at 32 mg/kg dose. At this stage, it is not possible to assign any reason to these seemingly inconsistent effects of the CCBs under study. That nifedipine attenuates gastric acid secretion in man is evident from a clinical report (Caldara et al., 1985).

In the PL model, it has been proposed that the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulcers (Brodie, 1966). Reduced gastric blood flow (St. John, 1975; Kauffman, 1981; Miller, 1983) and subsequent mitochondrial damage (Harding and Morris, 1976) may be responsible for aspirin induced ulcers too.
Thus the gastroprotective effect of CCBs as evident from the significant reduction of ulcer index in all the models used, appears partly to be due to the reduction in the volume of gastric content and the free and total acid concentration of gastric juice because we could not see uniformly significant reduction in these parameters with all three drugs. Furthermore, CCBs possess vasodilator property which leads to increase in gastric blood circulation (Fleckenstein, 1971) and effect may add to its gastroprotective effect.

Along with the gastric acid secretion, reflex or neurogenic effect has also been proposed to play some role in the formation of gastric ulcers in pylorus ligation model (Anichkov and Zavodskaya, 1968). It is a known fact that gastric secretion in Shay rats is under vagal control (Brodie, 1966) and the anti-cholinergics significantly reduce it in this model. However, a possible central effect of verapamil interfering with any ulcerogenic action in triggering the vagal outflow cannot be ruled out, since vagal overactivity appears to contribute substantially to any stress ulcer formation (Ogle et al., 1985). Decreased centrally mediated nerve impulses by CCBs may also result into reduction in lesions. Snyder and Reynolds (1985) have also identified central binding sites for calcium antagonists.
The greater role for gastrin in vagal stimulated relative to basal secretion, in which Ach and histamine could have a more important contribution to make (Grossman, 1981), may be an explanation for ulcer development. CCBs like verapamil may reduce gastrin or pentagastrin stimulated secretion in man (Kirkegaard et al., 1982; Sonnenberg et al., 1984). Other workers have also observed reduction in gastrin, histamine and carbachol and cAMP stimulated gastric acid secretion with CCBs (Sewing and Hannemann, 1983; McColl et al., 1987).

Davenport (1967) reported that aspirin changes the permeability of gastric mucosa, permitting a rapid back diffusion of gastric acid which has two effects (i) a release of histamine which produces changes in the vascularity of the mucosa and (ii) rupture of the histamine dilated capillaries resulting in the production of gastric lesions. As mentioned above, CCBs may inhibit histamine actions. It is also made clear in the discussion on duodenal ulcers that CCBs might be inhibiting histamine-induced gastric secretions. However, Brodie and Chase (1969) have ruled out the involvement of histamine as a common mediator in the genesis of gastric mucosal damage induced by different drugs including aspirin.

Biogenic amines have been considered to be involved in the gastric mucosal damage induced by a number of anti-inflammatory and other drugs. Bhargava et al. (1973) and Daas et al.
(1977) have shown that the centrally mediated release of catecholamines from the adrenal medulla as well as peripherally released histamine in rats could be responsible for the gastric mucosal damage induced by oxyphenbutazone and aspirin. Djahanguiri (1969) and Hemmati et al. (1973) have also established role of sympathetic nervous system in the production of aspirin induced ulceration in rats. It is possible that CCBs may exert protection against aspirin induced damage by antagonizing the injurious effects of biogenic amines like histamine and 5-HT on the capillaries of gastric mucosa or by decreasing formation or release of histamine in some of them. Further it is reported that verapamil and D-600 also inhibit 5-HT$_2$ receptor binding (Taylor and Defeudis, 1985).

Inhibition of 5-HT receptors by CCBs has already been discussed under the cysteamine model. 5-HT$_2$ receptors are responsible for contraction of gastrointestinal smooth muscle, increasing tone and facilitating peristalsis (Katzung and Chatterjee, 1989). Thus verapamil like drugs may reduce gastric motility by inhibiting 5-HT action. The decrease in gastric motility and inhibition of smooth muscle contraction by CCBs have been reported by Garrick et al. (1986).

It is clear from above discussion that CCBs produce their gastroprotective effect by different mechanisms. The mechanism of their protective effect may not be solely through inhibiting the concentration of free and total acids as a number of other
mechanisms are also responsible for the development of gastric mucosal damage in different models of gastric ulceration used in this study. Such mechanisms involved in ulcer formation are increased metabolism of carbohydrates, increased synthesis of nucleic acids and also the exhaustion of carbohydrates and other compensatory mechanism responsible for ulceration due to pylorus ligation (Mozsik et al., 1969). As such it is essential to recognize the pathological phenomenon related to ulcer formation at the level of the cell membrane, glycoprotein, mitochondria, nucleic acids, enzymes and proteins and to study selectively each of these factors with regard to time and lesion development (Mozsik et al., 1971). Moreover, one of the essential criteria to determine the status of mucosal resistance/barrier is the state of mucus secretion (Blum, 1985).

Based upon these reports, CCBs were also studied for evaluating their effect on insoluble and soluble mucosubstances in aspirin plus pylorus ligation model. All the three CCBs under study have shown rise in gastric wall mucus content at higher dose levels in the aspirin treated PL rats. The loss of visible or insoluble mucus observed in aspirin treated rats (Rainsford, 1975) is prevented by these drugs.

Alongwith the insoluble mucus, soluble (dissolved) mucosubstances were also estimated in the present study. All the three drugs significantly raised total carbohydrates of gastric juice at different dose levels viz. verapamil at 4, 8, and 40 mg/kg,
diltiazem at 60 mg/kg and nifedipine at 16 and 40 mg/kg dose. However, they have not uniformly affected individual carbohydrates like total hexose, hexosamine, fucose and sialic acid of gastric juice. According to potency, verapamil was found to be the most effective than the other two.

The protein content of gastric juice was also determined in presence of all three drugs. Pretreatment with CCBs caused significant fall in protein content of gastric juice at higher dose levels (verapamil, 16 and 40 mg/kg; diltiazem, 30 and 60 mg/kg; nifedipine, 40 mg/kg). Based upon the results of TC and PR content, the TC/PR ratio was estimated which was significantly raised in presence of these drugs viz. verapamil (4, 8 and 40 mg/kg; diltiazem (10, 30 and 60 mg/kg) and nifedipine (40 mg/kg). The effect of an agent on mucin activity is reflected by its effect on TC/PR ratio (Sanyal et al., 1983). In the present study, CCBs have been found to produce higher mucin activity that may explain the anti-ulcer action of these drugs.

Our findings from aspirin treated pylorus ligated rats go parallel with the reports of Ogle et al. (1985, 1985a). They have shown in their study that reduced acid secretion by verapamil could contribute to the anti-ulcer effect by permitting more stomach wall mucus to remain intact. Anti-ulcer action
of verapamil in stress ulcer model is significantly associated with the prevention of stomach wall mucus loss (Koo et al., 1986a). It is believed that visible mucus adhering to the wall, rather than the mucus dissolved in gastric secretion plays a more important role in protection against autodigestion of the gastric mucosa (Hollander, 1954; Williams and Turnberg, 1981). The quantity of visible mucus adhering to the mucosa was therefore measured along with dissolved mucosubstances of gastric juice in the present study.

It is likely that mucus secretion is inhibited by NSAIDs as observed in several studies (Mengny and Masters, 1965; Menguy and Desbaillets, 1967, 1967a; Johnsson and Lindquist, 1971). Mucus biosynthesis as monitored by incorporation of radioactivity into adherent mucus is also inhibited by aspirin (Menguy and Masters, 1965; Kent and Allen, 1967).

The salicylates have relatively potent inhibitory effects on protein biosynthesis in vitro (Kent and Allen, 1968; Rainsford and Smith, 1969; Spohn and McCall, 1979). Many studies have demonstrated that NSAIDs impair glycoprotein synthesis in animals (Kent and Allen, 1968; Perry, 1968; Lukie and Forstner, 1972 and 1972a; Park et al., 1975; Goodman et al., 1977). It is finally suggested that at cellular level, NSAIDs impair cell metabolism, glycoprotein and protein synthesis and increase cell turnover. Various studies have confirmed that aspirin
like substances interfere with several functions like PG synthesis (Pfeiffer and Lewadowski, 1972), energy metabolism due to trapping of drug anion within cells of the mucosa (Rainsford and Brune, 1976; Gerkens et al., 1977), enzyme synthesis and mucopolysaccharide production leading to reduced protective activity of the mucus (Rainsford et al., 1968; Pfeiffer, 1981) and disruption of gastric mucosal barrier leading to back diffusion of H\(^+\) ion (Ivey et al., 1972; Ivey, 1973; Lin et al., 1975).

Thus one of the mechanisms of anti-ulcer activity of CCBs against gastric ulcers observed in the present study appears to be the enhancement of mucopolysaccharide production as evident from the higher values of visible mucus content of stomach observed in the drug treated animals when compared with the control group.

Inhibition of mucus synthesis by NSAIDs appears to be due to the inhibition of most if not all known enzymes involved in the mucus biosynthesis in the g.i. tract. Acetyl-COA synthetase, L-glutamine-D-Fructose-6-Phosphate-amino transferase, UDP-N-acetyl glucosamine-UDP-N-acetyl galactosamine epimerase, UDP-dehydrogenase, UDP-glucoronyltransferase and the transferase enzymes involved in the incorporation of N-acetylglucosamine, glucosamine and other hexoses or hexosamines are particularly affected by salicylates and possibly by other
NSAIDs. The addition of sulfate moiety to form acidic sulfoglycoproteins appears to be inhibited by NSAIDs (Kent and Allen, 1968; Ezer and Szporny, 1970; Rainsford, 1978; Waldron-Edward et al., 1978).

Salicylates inhibit sulfotransferase reaction (Rainsford, 1978), whereas indirect inhibitory effect on this enzyme system could be affected through depletion of ATP by NSAIDs (Spenny and Bhown, 1977; Schrager and Oates, 1978). Besides, release of intracellular lysosomal enzymes which occurs in mucosal cell damage by salicylates could cause hydrolysis of presynthesized mucus glycoproteins (Fouad and Waldron-Edward, 1979). The mucosal lesions which appear due to accelerated stimulation as a result of changes in enzyme activity and mucosal circulation may also be due to changes in the rate of regeneration of the mucosal epithelium (Rasanen, 1971).

The ability of CCBs to reverse the reduction in mucin activity and the mucus content induced by aspirin appears to be through either protection of one or more of the enzyme systems from the inhibitory effect of aspirin, or inhibition of the lysosomal enzymes. No attempt has been made in the present study to measure the enzyme activity. It will be interesting to study the effect of CCBs on such enzyme systems to know more about the mechanism of protective effect of drugs against aspirin-induced ulcers.
As evident from various reports, it is clear that drugs or agents responsible for causing ulcers or preventing ulcers do act on the adherent mucus at two levels, firstly on the biosynthesis of the mucus which requires viable cells and secondly on the thickness of the secreted gel cover.

The mechanisms involved in the causation of chemically-induced gastric lesions are not fully understood at this juncture. Based on the effects of chemicals that cause gastric mucosal lesions and the protective effects of drugs on such lesions, several mechanisms have been considered to be involved in the genesis of these lesions (Kauffman, 1981; Miller, 1983). They include (a) increased gastric acid secretion, (b) inhibition of gastric mucosal prostaglandin synthesis, (c) disruption of gastric mucosal barrier, (d) reduction of gastric mucosal blood flow, and (e) inhibition of gastric mucus and bicarbonate secretion.

CCBs inhibit the entry of calcium into cells and/or its mobilization from intracellular stores with resultant inhibition of calcium effects. As for the mechanisms of their protective effects against chemically induced, gastric lesions, several direct and indirect mechanisms may be involved. One possibility would involve gastric acid-secretion. Ca$^{2+}$ is known to play a critical role in the stimulation-secretion coupling in gastric oxyntic cells and to play an obligatory role in stimulation of
gastric acid secretion by histamine, gastrin, carbachol and cAMP, the effects that can be blocked by CCBs (Kasbekar and Chugani, 1976; Soil, 1981; Kirkegaard et al., 1982; Sewing and Hannemann, 1983). Thus CCBs may protect against gastric lesions by antagonizing the effect of calcium in gastric acid secretion. Our observations also support this contention.

Another possible mechanism may be the prevention of myocellular necrosis (Fleckenstein et al., 1985). In heart, Ca$^{2+}$ overload has been reported to cause myocellular necrosis by reducing myocardial blood flow and causing mitochondrial damage. Similar mechanism may be operative in the g.i. tract.

A third mechanism may involve an effect on arachidonic acid metabolism. The oxidative metabolism of arachidonic acid is known to proceed via two pathways, the cyclo-oxygenase pathway which result in the generation of prostaglandins and the lipoxygenase pathway which generates leukotrienes and hydroxy acids (Samuelsson, 1983; Higgs et al., 1985). There is an evidence that CCBs inhibit lipoxygenase pathway (Levine, 1983). Thus CCBs may protect against chemically induced gastric lesions either by diverting arachidonic acid metabolism towards the synthesis of prostaglandins which are known to be cytoprotective against chemically induced gastric lesions (Miller, 1983), or by decreasing leukotriene formation by inhibiting the lipoxygenase pathway. In recent reports, leukotrienes are
proposed to play a major role in the pathophysiology of tissue injury and trauma (Denzlinger et al., 1985) and to potentiate ethanol induced gastric lesions in rats (Roger et al., 1986; Konturek et al., 1988).

However, Ulak and coworkers (1991) confirmed in their study of nitrendipine that gastroprotective effect of nitrendipine is not likely to be mediated by PG pathway against stress induced lesion formation. This fact is supported by the data showing that the cyclo-oxygenase inhibitor sodium meclofenamate and the non-cyclooxygenase inhibitor salicylic acid failed to block nitrendipine induced protection against stress lesions (Ezer, 1985; Glavin, 1989; Kauffman, 1989).

Another possible mechanism of CCBs as anti-ulcer drugs is protection of gastromucosal damage against glutathione depletion. The depletion of glutathione has been proposed to play a role in genesis of gastric mucosal damage induced by fasting, stress, ethanol and HCl (Boyd et al., 1981; Ghanayem et al., 1985; Parmar et al., 1988). On the other hand sulfhydryls mediate prostaglandin induced cytoprotection and sulfhydryl drugs have been shown to possess significant cytoprotective activity (Szabo et al., 1981). It is also evident that glutathione depletion in hepatocytes result in an increase in cytosolic free calcium concentration (Bellomo et al., 1983). Therefore, glutathione depletion in the gastric
mucosa may cause gastric mucosal lesions indirectly by
triggering an increase in the intracellular calcium concentra­
tion in oxyntic cells. This calcium-induced damage may be
prevented by calcium channel blockers. Thus it is possible
that through calcium antagonist activity, the CCBs may counteract
the ulcerogenic effect of glutathione depletion. However, this
mechanism should be considered to be operative only if it is
confirmed that ulcerogenic drugs used in this study also cause
glutathione depletion. None of the above suggested mechanisms
have been confirmed, and further research is required to explain
the protective effects of CCBs against chemically induced
gastric lesions.

In the present study, CCBs have been found to protect
against gastric mucosal damage caused by different methods.
They increase both soluble and insoluble mucosubstances and
decrease the acid secretory parameters. CCBs seem to increase
not only mucosal cellular mucus but also to secrete more
dissolved mucus in the gastric juice as evidenced by its
effect on gastric juice carbohydrate content and TC/PR ratio.
The presence of dissolved mucosubstances in the gastric juice
seems to be a reliable index of an effective mucosal barrier
(Sanyal et al., 1983). They also reduce protein content of
gastric juice. The mechanism for decrease in protein content
appears to be at least partly, due to prevention of leakage of
proteins from the serum to gastric juice (Grossman, 1978;
Wallance and Whittle, 1986). The rise in glycoprotein content
of gastric mucosa may also be associated with rise in mucus content of stomach wall. These high molecular weight glycoproteins are mainly responsible for the viscous and gel forming characteristics of the mucus (Horowitz, 1977; Allen, 1981).

The results pertaining to carbohydrate and protein contents (causing rise in TC/PR ratio) and increase in visible mucus content of gastric mucosa indicate that overall activity of calcium channel blockers is to strengthen the mucosal barrier of gastric mucosa and thus produce a protective effect against gastric ulcerations of varied origin included in this experimental study.