INTRODUCTION

Peptic ulcer represents a major health problem, both in terms of morbidity and mortality. Because of its frequency and worldwide distribution, peptic ulcer continues to be a subject of numerous investigation, both experimental and clinico pathological. In this respect peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology. Quineke (1) was probably the first to use the term "Peptic ulcer" which is an excoriated area of gastroduodenal mucosa caused by digestive action of gastric juice.

DRUG INDUCED ULCER

It is now well accepted that drugs like analgesic, antipyretic, anti-inflammatory, steroid, histamine etc. can induce gastroduodenal ulcers in man and animals.

INDOMETHACIN

In 1962 indomethacin was introduced as a synthetic, non steroidal and inflammatory, analgesic and antipyretic agent for the treatment of rheumatoid arthritis, degenerative joint disorder and other inflammatory conditions. Wanka *et al.* (2) and Kaig *et al.* (3) for the first time reported that indomethacin caused peptic ulcer in human subject. There are also reports (4, 5, 6) suggesting that the patients taking indomethacin sometimes develop gastro intestinal symptoms. Ballabio (7), however, found no evidence of ulcer but only bleeding in patients taking indomethacin as drugs. In animal experiment it was noted (8, 9) that indomethacin caused gastric, duodenal, antral and jejunal ulceration, haemorrhage and perforation in rats and guineapigs.

ASPIRIN

It was demonstrated (10, 11) that parenteral administration of aspirin does not produce gastric haemorrhage in animals or man, while other workers have shown that direct contact of aspirin with gastric mucosa is not necessary for production of gastric bleeding (12, 13, 14). Brodie, Chase (15) and Djahanguiri (16) reported that subcutaneous or intraperitonial injection of aspirin produced 90 – 100% ulcerogenic response in rats. Furthermore, dose dependent response of aspirin by oral or intraperitonial route was also demonstrated (15).

There was observation (15) that aspirin induced gastric ulcers were significantly reduced by antacids, anticholinergic, ganglion blocking agent and vagatomy, but not by central nervous system depressant. Catecholamines, however, were involved in aspirin induced gastric ulceration (17). There were suggestions (18, 19) that hypothalamic pituitary, adrenal system is involved in salicylates induced gastric ulcer. Salicylates mimic in many respect the pharmacological properties of cortisone (20) but the evidence is conflicting regarding the release of cortisone by the action of salicylates on the pituitary adrenal axis. In a comprehensive review of the subject, Smith (21) has concluded that the majority of effects of salicylates are due to their intrinsic properties rather than to secondary endocrine influences.

ANALGIN AND PARACETAMOL

Analgin and paracetamol are generally thought to be safe antipyretic and analgesic having anti inflammatory activity too. Paracetamol was originally used in late 19th century and in the last few years it is widely used as an antipyretic and

analgesic agent. Adverse effect following therapeutic dosages have rarely been recorded. There is, however, a study on 41 cases of acute paracetamol poisoning in which one died of gastrointestinal haemorrhage and acute massive necrosis of the liver (22). Analgin, on the other hand, has been shown to cause a granulocytosis as a severe toxic effect (23, 24).

PHENYLBUTAZONE

A non-steroid, synthetic compound "phenylbutazone" was introduced in 1949 for the treatment of rheumatoid arthritis, muscoloskeletal diseases and allied disorders. Meuer (25) in a survey of 3934 patients treated with phenylbutazone reported 40 proved cases of peptic ulcers, 9 cases of haemorrhage and an additional 424 individuals also developed gastrointestinal symptoms including epi-gastric pain. Others who reported development of peptic ulcer after constant use of phenylbutazone were Leonard (26), Deseze and Levernievx (27) and Kern *et al.* (28)

Kirener (29) told that Phenylbutazone is a good example in which dosages level employed are of utmost importance. Low dosages appear to be well tolerated. A high dosage of the same compound have been observed to produce gastroduodenal ulcer both in experimental animals and in human.

Epigastric pain, nausia, vomiting, acute ulcer, activation of pre existing peptic ulcer or development of peptic or duodenal ulcer are the common features after prolonged use of phenylbutazone (30 – 34).

Experimental studies also showed that mice, rats, guineapigs and dogs were vulnerable to peptic ulcer after oral or parenteral administration of phenylbutazone (35 – 37).

GLUCOCORTICOIDS

Numerous reports are there showing glucocorticoids viz. hydrocortisone, cortisone, prednisolone and ACTH produce gastric ulcer in normal rats (38, 39), the Shay rats (40), dogs (41, 42) and in man (43, 44). Khan *et al.*(45) have reported extension and perforation of the ulcers as well as reulceration of a healed ulcer area in rats receiving cortisone.

Several mechanisms have been put forward to explain the pathogenesis of glucocorticoid induced gastric ulcer (46 – 49). Somewhere it was considered that anticholinergic drug, methapopolamine bromide prevent the steroid induced ulcer, while somebody suggested that the anti polgistic properties of steroid may be

concerned for gastric ulceration. There were also suggestions that diminution of tissue resistance related to the anti inflammatory action of corticosteroid is responsible in the genesis of ulceration. Histamine is also considered involved in corticoid induced gastric ulcer. Levis (50) observed vascular dilatation resulting in localized haemorrhagic thrombosis in corticoid induced gastric ulceration. Sanyal and co-workers (51) reported that anti 5 HT agent (cyproheptadine) fail to prevent prednisolone induced gastric ulcer.

HISTAMINE

Of the many substances that have been used to the experimental production of ulcer in laboratory, histamine is perhaps the only physiological agent. Popielski in 1920 (52) demonstrated that histamine is a powerful stimulator of gastric secretion. Injected intravenously histamine stimulates acid secretion not only from the intact stomach but also from totally denervated gastric pouch. Evidence has been accumulating that it might even be physiological stimulant of the acid secreting parietal cell.

More recently in the investigation of the so called histamine releasor, experimentalist have noted the occurrence of gastric ulceration in the cat (53) and also in some small animals such as rats, mice etc. (54, 55). Ulcerogenesis has been attributed to the endogenous histamine released from the tissue. Similarly, following injection of gastrotoxin and its anaphylaxis due to horse serum, endogenous histamine is held responsible for production of ulcer in the stomach (55).

DIMAPRIT

On the basis of universally recognized hypothesis about the involvement of H2 receptors in the pathogenesis of peptic ulcer, an appropriate rat model has been designed with dimaprit as specific H2 receptor agonist. Dimaprit was administered intraperitonially or through intra venous route to 24 hours fasted rats and the animals were sacrificed four hours after the injection. The drugs for studying their gastroprotective effect were given 30 minutes before dimaprit. The procedure is extremely simple and rapid.Its feasibility and specificity are added advantage. It is very useful for evaluating not only the absolute potency of a drug given by any route but also of other pharmacodynamic parameter, particularly the duration of action which seems to be an important criterion in selecting new potentially H2 antagonistic drugs (56).

Recently, Del Soldato *et al.* (57) have shown that dimaprit also induces duodenal ulcer in guineapigs.

RESERPINE

Reserpine produces severe haemorrhagic ulcer at the glandular part of the stomach which has been attributed to significant degranulation of gastric mast cell and consequent liberation of histamine. These events are thought to be cholinergically mediated (58). The morphological changes in gastric mucosa are very similar to the destructive changes found in the mucosa of human gastric ulcer (59).

SEROTONIN

Serotonin ulcer, one of the chemically induced experimental gastric ulcer, has been described by Wilhelmi (60), Hedinger and Veraguth (61). Since then it has been widely used for the investigation of the etiology of peptic ulcer disease and as a tool in the search for new anti-ulcer drug.

DULCEROZINE

Kurebayashi *et al.* (62) reported that acute perforating duodenal ulcer can be produced in the rat following single oral administration of dulcerozine, a compound structurally related to non-steroidal anti-inflammatory drug such as phenylbutazone and other known to cause gastro intestinal damages in man and in animals. It has been proposed that prolonged gastric hyper secretion might be an important factor contributing to the pathogenesis of duodenal ulcer in this species. The dulcerozine induced duodenal ulcer in rats is a useful model for studying the pathogenesis of duodenal ulcer and testing the anti- ulcer drug from the practical and pathogenic standpoint because

1. The lesions develop are analogous to the clinical disease with respect to location and histology.
2. The factor producing the pathologic changes is similar in man and animals used.
3. The drug treatment and a surgical operation effective in animals could be clinically useful.
4. It is extremely simple to perform the massive production of ulcer and the results are obtained within 18 hours.

CYSTEAMINE

Experimental duodenal ulcer in rats induced by cysteamine hydrochloride was first described by Selya and Szabo (63). The pathogenic mechanism leading to ulceration has not yet been fully explained, but both protective and aggressive factor influencing the resistance of the duodenal mucosa seen to be involved. Gastric emptying is delayed and serum gastrin concentration is increased. Cysteamine induced ulcer resemble duodenal ulcer in man to its location, histopathology and some aspects of pathophysiology. The development of duodenal ulcer in response to cysteamine is inhibited by the anticholinergic agents, antacid, prostaglandin and H2 receptor antagonist. Since multiple dosing is necessary to prevent cysteamine induced ulcers, the usefulness of the model in a screening programme is limited by the large quantity of drug required. Hence, cysteamine has also been used in mice to produce duodenal ulcer which can be used for the evaluation of anti-ulcer drug overcoming the above mentioned drawback seen in rats.

ENDOTOXIN (LIPOPOLYSACCHARIDE)

Administration of endotoxin produce a moderate degree of gastric mucosal damage in rats ((64). The lesions remained confined to the glandular mucosa and consisted of small punctiform lesions, erosions and petechial haemorrhages. The characteristic feature of these lesions was a typical submucosal ecchymosis in the glandular stomach observed in about 30% of the animals. Pretreatment with ranitidine, pirenzepine, proglumide, sucralfate and naloxone provide significant protection(65).

MPTP

Szabo *et al.* (66) have shown that the parkinsonism inducing agents 1-methyl-4-phenyl-1,2,3,6 tetra hydro pyridine (MPTP) given in multiple daily doses induces solitary or doubled ("kissing") duodenal ulcer in a rat in a dose dependent manner. MPTP produces duodenal ulcer by impairing defense in the duodenal bulb. Dopamine agonist like bromocriptine, lergotrile and monoamine oxidase inhibitors like pargyline and deprenyl have been shown to prevent MPTP induced duodenal ulcers in rats.

ACETIC ACID

Acetic acid produces gastric ulcer in rats. This model of ulcer, resembling

human peptic ulcer, may be quite useful for the study of human ulcer and the evaluation of pharmacological agents used for this disease (67).

HYDROCHLORIC ACID

Robert *et al.* (68) observed that 1 ml of 0.6 N hydrochloric acid can produce gastric erosions and ulcers in albino rats which can be inhibited by pretreatment with prostaglandins.

ETHYL ALCOHOL

1 ml of 80% ethyl alcohol when administered orally to 12 hour fasted rats can induce gastric ulcers in rats. The ulcer formation is through the generation of free radicals (69).

SODIUM HYDROXIDE

1 ml of 0.6 N sodium hydroxide can produce gastric lesions in rats (70).

OTHERS

Chemicals like silver nitrate, formalin, nicotine, epinephrine, hypertonic saline etc. can induce gastric ulcers in experimental animals like rats, cats and dogs. The ulcers, though resembled human gastric ulcer grossly and histologically, healed rapidly and completely within 2 – 3 weeks (68, 71-74).

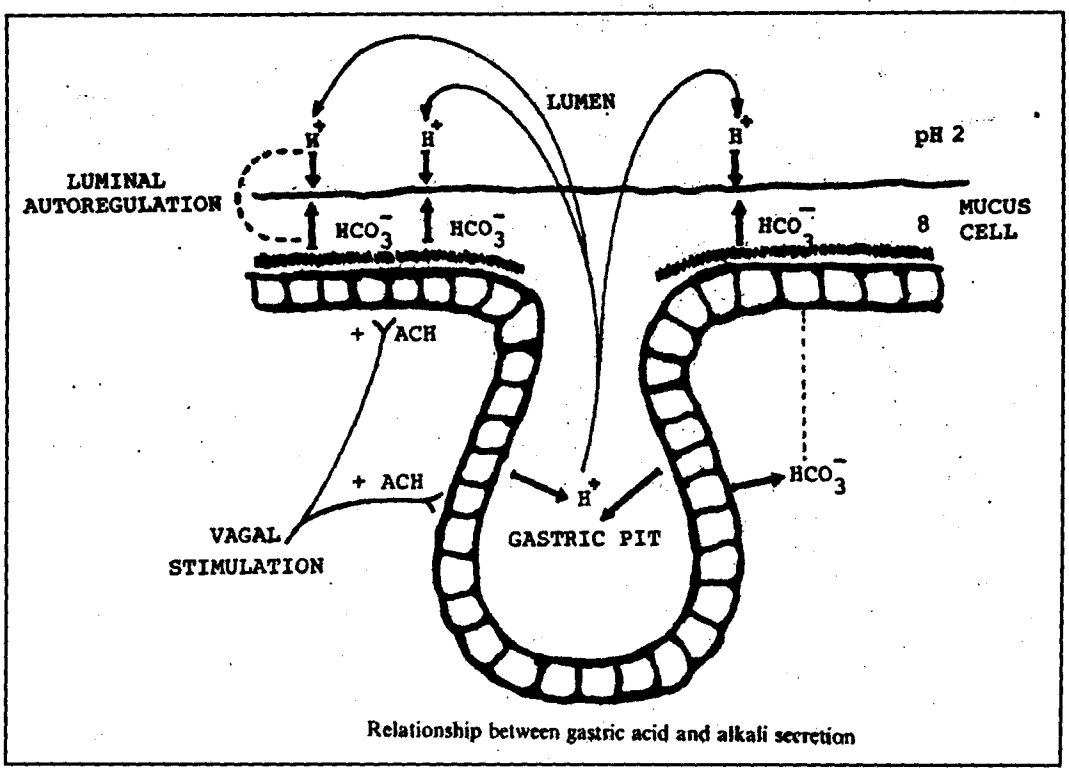
MECHANISM OF DRUG INDUCED ULCERATION

Various factors have been recognized to explain the mechanism of drug induced ulceration.

ACID – PEPSIN

The stomach has a pivotal role in the digestive process, functioning both as a reservoir and a mill by virtue of gastric glands secreting hydrochloric acid and pepsin. Acidification of ingested food initiated the process of digestion by creating optimal conditions for peptic digestions of proteins. Parietal cells of stomach secrete hydrochloric acid while chief cells formed pepsinogen which is activated into pepsin in the acid medium of gastric juice. Both acid and pepsin have proteolytic actions on living tissue and are capable of autodigestion of gastroduodenal mucosa (75).

For several decades, the dictim "no acid – no ulcer" has dominated the pharmacological basis of ulcer therapy, and the drugs used, reduced acid secretion.



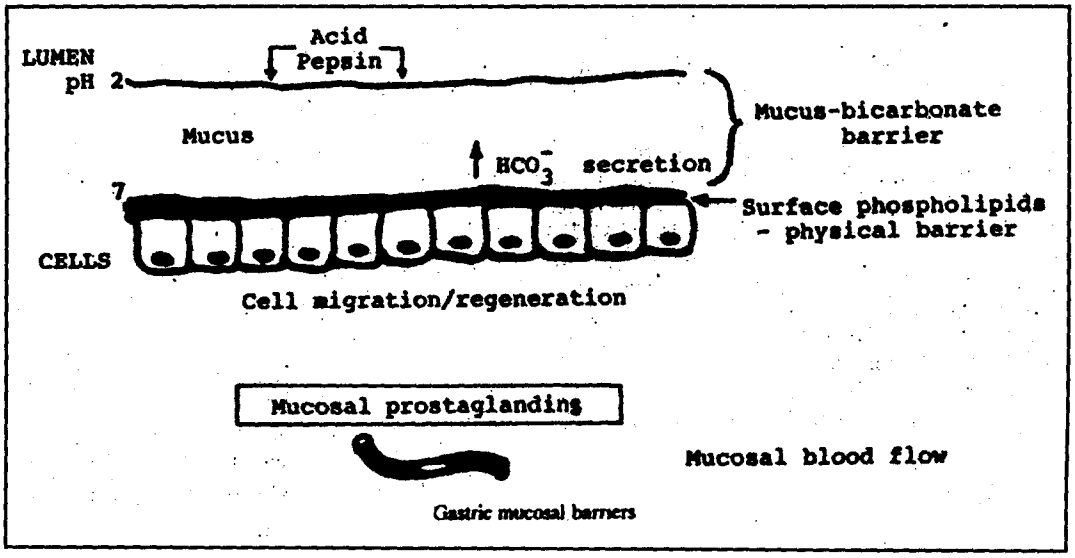
Relationship between gastric acid and alkali secretion

However, it was observed that in 45-75% patients of duodenal ulcer acid secretion was within normal limits, whereas in gastric ulcer patients acid secretion was either normal or subnormal (76). Patients of Zollinger-Ellison syndrome, characterized by abnormally high acid secretion, show minimal incidence of peptic ulceration (77). It was, therefore, apparent that peptic ulcer was not solely induced by the offensive factors of the acid and pepsin . In experimental studies also same picture was found out . Nicoloff (78) showed that ulcerogenic effect of indomethacin in albino rats did not seem to be related to acid hypersecretion. Lynch *et al.* (79) reported an increase, no change or a decrease in gastric secretion and acidity by salicylates depending upon the preparation and species studied. There are reports that aspirin caused decrease in volume and acidity of gastric secretion in rat (80,81). There is also a report that aspirin given by mouth in customary dosage has no effect on hydrochloric acid secretion (82). Similar findings were also noted by other workers in experimental gastro-duodenal ulcerations (83 – 88).

MUCUS SECRETION

Mucus is one of nature's perfections, protecting the gastrointestinal tract from infective, chemical and physical insults. It has been commented that it encloses the gastric juice in the stomach as if it was an impermeable porcelain vase. Mucus is secreted into the gastroduodenal lumen by surface epithelial cells and mucus neck cells (goblet cells) and submucosal Brunner's glands. The secretion has two components, a water insoluble gel adherent to the mucosal surface and soluble mucus in the lumen. The latter can either be secreted directly into the lumen or may be derived from the mucus gel by proteolytic degradation or mechanical shearing during digestion (89).

Mucus consists of about 1% by weight of salts and other dialyzable components, 0.5 – 1% of free proteins and a similar quantum of carbohydrate rich glycoproteins and 95% or more of water. The glycoprotein component of mucus is responsible for the characteristic viscous gel forming property, believed to be important for the functional role of mucus. Native human mucus glycoprotein has a high molecular weight of about two million daltons and is formed by polymerization of four glycoprotein subunits, joined by disulphide bridges. Each subunit consists of a protein core with about 150 side chains, studded along the length of the protein core, projecting out like bristles of a test tube brush. About 25% of the protein core, which is bereft of these carbohydrate side chains, is non-glycosylated, and the disulphide


LUMEN
pH 2
Acid
Pepsin
Mucus
HCO_3^- secretion
Mucus-bicarbonate barrier
7
CELLS
Surface phospholipids - physical barrier
Cell migration/regeneration
Mucosal prostaglandins
Mucosal blood flow
Gastric mucosal barriers

bridges, 78 per molecule, are located in this region. Disulphide bond splitting reagents, like mercaptoethanol and proteolytic enzymes, break the native glycoprotein molecule into its four component subunits which are water soluble and do not have the viscous gel forming characteristics of the parent glycoprotein molecule. The linkage between carbohydrate and protein in the mucus glycoprotein is o-glycosidic and the monosaccharide involved is N-acetyl galactosamine, which is attached to either serine or threonine. Apart from these two, the other predominant amino acid in the protein core is proline which does not permit alpha-helix conformation within the peptide chain, providing the requisite close packing of the carbohydrate chains found in these glycoproteins. The carbohydrate side chains comprise over 80% by weight of the glycoprotein molecule and their presence is compatible with the high degree of hydration essential for the special rheological properties of the molecule. There are approximately 600 carbohydrate side chains per molecule of native glcoprotein and each branched side chain consists of about 15 oligosaccharide units composed of galactose, fucose (methyl pentose), N-acetyl glucosamine, N-acetyl galactosamine and sialic acid, with traces, if any, of mannose and no uronic acid , which distinguish them from serum glycoproteins and proteo glycans, respectively. The carbohydrate chains are often negatively charged due to the presence of ester sulphate and sialic acid, the latter being located in the terminal position. The most important sialic acid is N-acetyl neuraminic acid. Rejection of the neighbourly negatively charged group of glycoproteins results in molecular expansion and increase in viscosity. An interesting feature of mucus glycoprotein is that the structure of the terminal parts of the sugar chain is similar to that determining the specificity of ABO blood groups on the erythrocyte surface (90 – 92).

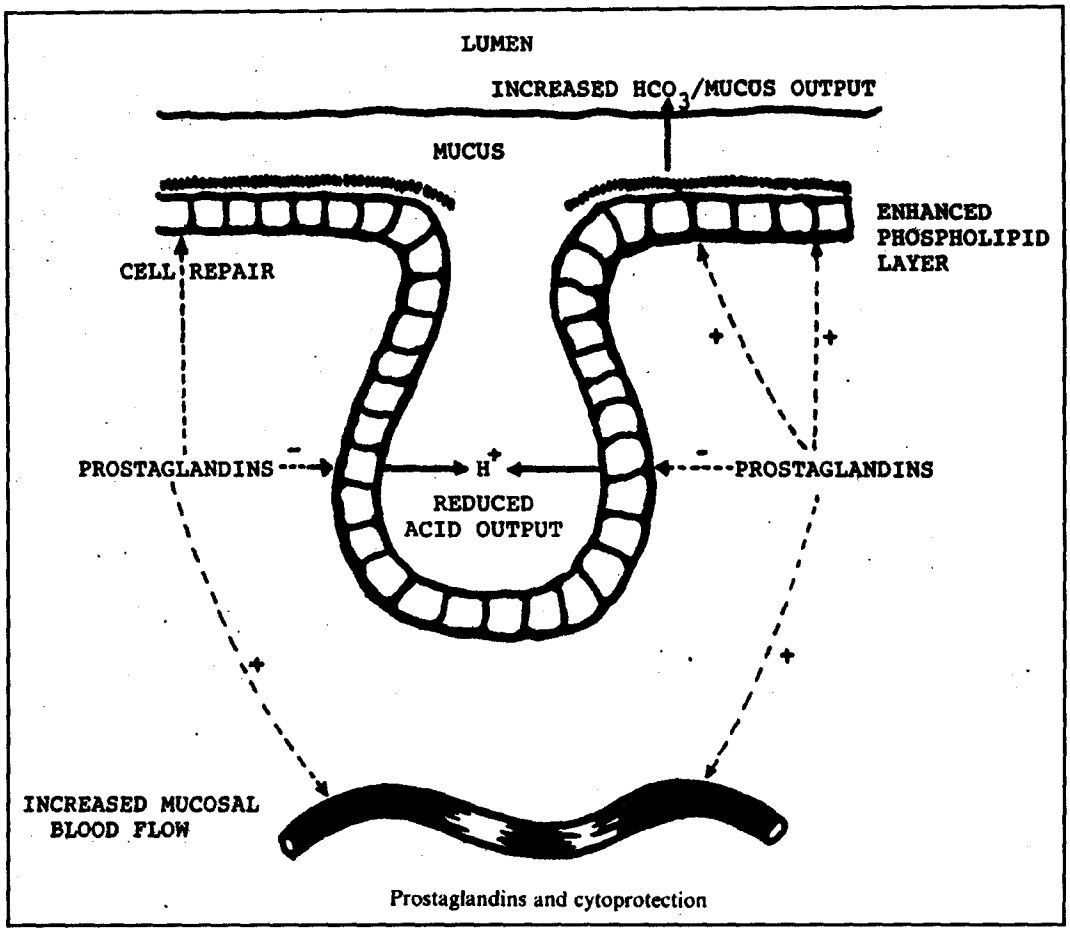
Mucus has several functions which contribute significantly to cytoprotection.

a) *Lubrication and mechanical protection :*

The mucus gel covers the entire surface of the gastroduodenal mucosa with a variable thickness of less that 500 μm, lubricating the mucosa and forming the first line of defense against noxious gastric contents. It exists in a dynamic balance between production on one hand and degradation by pepsin and shear forces on the other (93).

b) *Mixing barrier :*

Mucus is readily permeable to hydrogen ions and provides a mixing barrier at the mucosal surface , preventing the relatively small amounts of bicarbonate ions



Prostaglandins and cytoprotection

from mixing with the bulk of hydrogen ions in the lumen, thus confining the neutralization at the mucosal surface, ensuring a pH gradient across the mucus layer . The glycoprotein molecules appear to retard the diffusion of hydrogen ions, the rate of diffusion being four times slower than that in unstirred water. However, this retardation of hydrogen ion diffusion across the mucus layer is unlikely to be the sole factor protecting the mucosa from acid since this would require the renewal of a mucus layer of at lease 0.4 mm thickness , ten times each second, in order to maintain epithelial surface neutrality (94).

c) Unidirectional flux of hydrogen ions :

The mucus facilitates unidirectional flux of hydrogen ions from the gastric glands into the lumen. The passage of acid secreted by the parietal cells appears to occur through mucus channels which are highly sulphated. These channels, with high negative charge, contributed by sulphate and sialic acid radicals, produced predominantly by the neck mucus cells, behave as cation exchangers. Periodically, instead of an increasing pH gradient across mucus, there is a sudden drop in pH. A concentration gradient of sodium ions appear to be generated across the mucus layer by the continuous activity of Na/K-ATPase at the baso-lateral membrane of mucus cells. Sodium diffusing across this gradient will generate a diffusion potential positive at the cell facing surface of the mucus gel. It is postulated that this potential moves hydrogen ions into the lumen and retards back diffusion (95).

d) Prevention of back diffusion of pepsin and pepsinogen activation :

Since mucus exploits the phenomenon of phase separation, it attenuates back diffusion of macromolecules like pepsin from gastric juice. Mucus also has a function in transporting pepsinogen and preventing its activation into pepsin (96).

e) Repair of superficial mucosal damage :

Following damage of surface cells, there is rapid release of large amounts of mucus and plasma proteins which, together with the cellular debris, form a coating over the destroyed area, providing a favourable micro-environment for repair and restitution (97).

f) Antibacterial activity :

Identification of bacteria in human stomach in gastritis indicates that normal gastric mucus may have antibacterial activity (98).

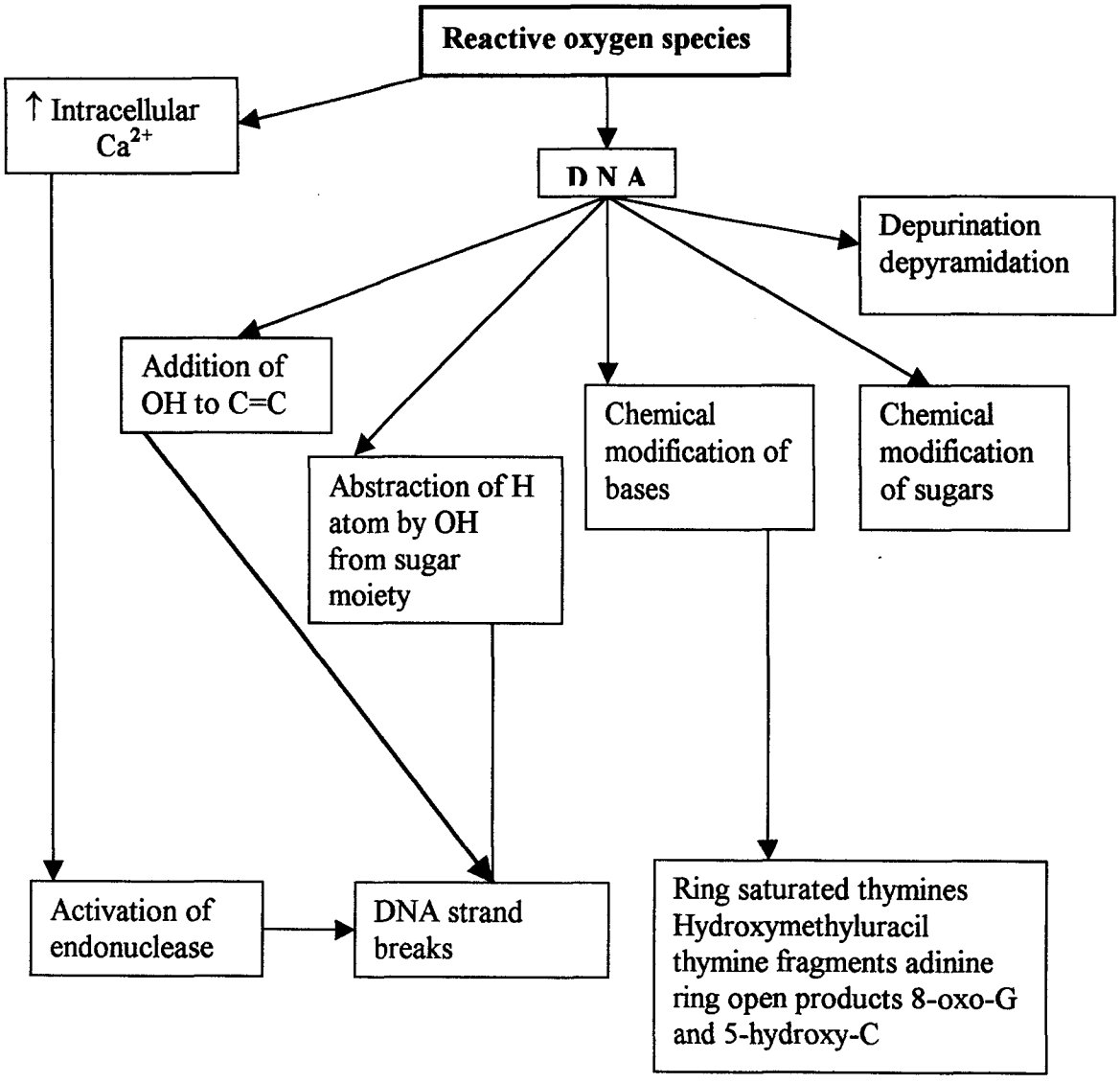
The ability of mucus to afford mucosal protection appears to depend upon its

thickness commonly known as mucus gel thickness. It has been reported that the gel is resistant to hypertonic saline, bile, ethanol and nonsteroidal anti-inflammatory drugs and its secretion is augmented by chemical irritants, carbachol, carbenoxolone, prostaglandins and secretin (99). It has also been suggested that bicarbonate secretion (100), intrinsic barrier properties of epithelial cells (101), mucosal blood flow (102), acid-base balance and acid secretion (103), endogenous prostaglandins (104), *Helicobacter pylori* (105), dietary factors (106), nonsteroidal anti-inflammatory drugs (107) etc. are somehow related with the mucus gel thickness.

Role of gastric mucus both in clinical and experiment ulcers has been studied by several workers. Patients of gastric ulcer have an abnormal mucus gel structure containing less polymeric glycoproteins (108) and certain pepsins, more prevalent in duodenal ulcer patients induce marked digestion of the glycoprotein matrix (109).In experimental system, aspirin has been shown to reduce the rate of synthesis and secretion of mucus (110,111). It seems possible, as Menguy suggested (110), that it is the loss of mucus 'barrier' that permits the back diffusion of hydrochloric acid into the aspirin-damaged gastric mucosa. Davenport (112) showed that aspirin damaged gastric mucosal barrier which allows back diffusion of hydrogen ion. He suggested that when the mucosa is damaged by salicylates histamine is released and capillary permeability increases which is followed by bleeding. While studying the mucus secretion in vagally denervated gastric antral pouch of dog during administration of indomethacin for twenty one days, Menguy showed (110) that indomethacin lowered the rate of secretion of gastric antral mucus and diminished the amount of carbohydrate incorporation into the mucosubstance. Working on phenylbutazone induced gastric ulcer in albino rats, number of workers have given importance to the mucus barrier whose absolute or relative deficiency acts as a factor in the production of ulceration (113 – 116). Zaidi *et al.* observed a steady decrease of mucin in the gastric juice after thirty days of phenylbutazone treatment in guinea pigs (117).

LIPID PEROXIDATION

Role of lipid peroxidation in gastric mucosa has been extensively studied in the pathogenesis of peptic ulcer. It has been found out that lipid peroxidation mediated by active oxygen species plays an important role in the pathogenesis of peptic ulcer (118 – 123). Antioxidants, however, act as anti-ulcer agents by inhibiting lipid peroxidation through decrease of reactive oxygen metabolites (124 – 126).



Mechanism of oxidative damage to DNA

Chemical agents like quercetin, rebamipide, zinc acexamate etc. also exert their anti-ulcer activity by inhibiting lipid peroxidation (127 – 132).

Guth (133 – 134) explained that oxygen derived free radicals, specially the superoxide radical, play an important role in ischaemic gastric mucosal lesions, particularly in presence of hydrochloric acid. Ischaemia is followed by utilization of high energy compounds like adenosine tri phosphate (ATP), resulting in accumulation of adenosine mono phosphate (AMP) in the presence of reduced oxidative phosphorylation. AMP is further catalyzed to hypoxanthine, which tends to accumulate. Ischaemia also converts the enzyme xanthine dehydrogenase to xanthine oxidase form. The latter requires the presence of oxygen for its activity, and when oxygen is available after rapid reperfusion, the enzyme acts on hypoxanthine to produce superoxide radicals, hydrogen peroxide and hydroxyl radicals. These free radicals, each containing an unpaired electron in their outer shell, are highly reactive and are potent oxidizing and/or reducing agents, including cell wall damage and the release of intracellular lysosomal enzymes.

DRUG INDUCED ULCER – ROLE OF NATURAL PRODUCTS

The use of some plant and mineral drugs, including vegetable banana, narikelkhand (coconut) and tamrabhasma (copper preparation), has been advocated in Ayurveda for the therapy of a symptom complex akin to the modern version of peptic ulcer syndrome. Some of these have been subjected to experimental evaluation.

Vegetable banana (*Musa paradisiaca*) has been extensively investigated by Sanyal and his coworkers for nearly three decades. The antiulcerogenic activity of unripe green banana was first reported in 1961 (135) and later confirmed on a variety of experimental models (136 – 137). Though it was initially postulated that the anti-ulcer effect was due to the high serotonin content of green banana (135), it was later realized that the effective dose of banana did not contain sufficient serotonin to justify this postulate (138). Further studies not only confirmed the anti-ulcer activity of banana but also showed that this effect was not due inhibition of acid-pepsin output but was associated with augmentation of gastroduodenal mucosal protective factors (139 – 142). Some of the active principles likely to be responsible for the anti-ulcer action of banana have been identified (143 – 146).

Tamrabhasma inhibits acid-pepsin secretion and also exerts significant mucosal protection in experimental ulcer (147 – 149). Narikelkhand also exerts

significant anti-ulcer effect due to mucosal protection (150).

Anti-ulcer activity of amlaki (*Emblica officinalis* Linn.) (151 -154), black tea extract (155), *Piper longum* Linn. (156), Ul-409, Cauvery-100 and PK-2001 (multiconstituent herbal preparations) (157 – 159), *Symplocos racemosa* (160), *Synclisia scabrida* (161), Shankha Bhasma (162), *Rhamnus triquerta* (163) etc. were also studied and reported in the literature.

From pilot experiments we came to know that Nirmali (*Strychnos potatorum* Linn.) exerts anti-ulcer effect on experimental ulcers as induced by

1. Aspirin and
2. Indomethacin.

Since two experimental models are not sufficient to evaluate the anti-ulcer property of a plant, it was thought worthwhile to study the same in more experimental ulcer models. The present work, thus, was an attempt to evaluate the anti-ulcer activity of Nirmali (*strychnos potatorum* Linn.) and its effect on various biochemical parameters in drug induced experimental ulcer models.

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