5. DISCUSSION
Antiulcer activity:

Peptic ulcer is one of the most common diseases affecting mankind. Despite its unbiased common occurrence in the wide population and several alternative unconventional approaches to its treatment, the etiology of peptic ulcer diseases still continues to remain ambiguous and the recurrence of ulcer on cessation of therapy presents a grave problem. It is well known that peptic ulcer occurs as a result of disturbance of normal equilibrium between mucosal protective factors and aggressive factors. The defense mechanism of the gastrointestinal mucosa against aggressive factors, such as hydrochloric acid, pepsin, bile and NSAIDs mainly consists of functional, humoral and neuronal factors. Mucus secretion, bicarbonate secretion, mucosal microcirculation, cell growth and motility act as functional defensive factors, while prostaglandins and nitric oxide act as humoral defensive factors and capsaicin sensitive sensory neurons (CPSN) act as neuronal defensive factor. Drugs used in peptic ulcer have traditionally been directed mainly against a single luminal damaging agent, hydrochloric acid and drugs like antacids, anticholinergics, histamine H₂-antagonists have flooded the market. Recently, studies have implicated the generation of oxygen derived free radicals and lipid peroxidation in the pathogenesis of peptic ulcers. An ideal antiulcer drug should not only suppress gastric secretion but also have cytoprotective properties.

A variety of methods have been adopted to evaluate the antiulcerogenic effect of a drug against gastro-intestinal lesions. These include gross morphological evaluation, biochemical techniques, computer based morphometric analysis, and a group of miscellaneous methods such as urinary recovery of phenol red, or measurement of tensile strength. No single method can provide detailed information about the mechanisms of antiulcer effects of drugs.
Though the ulcers in most of the experimental models used for ulcer production are acute, non-penetrating, rapidly healing and those which heal with a scar, a large number of drugs are rapidly evaluated with reasonably predictability for their therapeutic usefulness using these models. Rat has been used as an experimental animal in all the ulcerogenic procedures mainly because the lower three-fifth glandular secretory portion of its stomach is analogues to the body of stomach in man, both anatomically and functionally\textsuperscript{244}. Also rats being omnivorous resemble man nutritionally.

In spite of the progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom might provide a useful source of new anti-ulcer compounds for development as pharmaceutical entities or alternatively, as simple dietary adjuncts of existing therapies\textsuperscript{245}. Natural or synthetic compounds with antioxidant potential could produce beneficial effects in peptic ulcer diseases. Plants provide an alternative strategy in the search for new drugs for the treatment of gastrointestinal disorders\textsuperscript{246}. Medicinal plants are used in two different forms (i) as complex mixtures containing broad range of constituents (extracts, tincture, volatile oils, infusions; (ii) as pure chemically defined active principles, the former being very popular in countries with a strong tradition of herbal medicines such as India, China, Germany etc. Since only in a few cases does a drug activity depend upon single component, crude extract and/or its fractions are now gaining more importance. Plant extracts are multicomponent and they are thought to have additive or potentiating effect\textsuperscript{247,248}. Number of medicinal plants and their constituents are reported to possess antiulcer activity. According to one report about 50\% of approved antiulcer drugs are of natural origin\textsuperscript{3}. In the present investigation, root extracts of \textit{C. arundinaceum} are studied for their gastro protective effects in various experimental models.
**Ethanol-induced gastric lesions in rats:**

The antiulcer activity of root of *C. arundinaceum* was evaluated using 50% alcoholic extract (Extract A) in ethanol-induced gastric ulcer model. The extract protected the gastric mucosa from the insults of ethanol with complete suppression of ulcerogenesis. These results led us to separate polysaccharides (Extract P) and saponins (Extract S), the two major fractions of the root. Further, antiulcer study was conducted with these two-fractions (Extract P and Extract S) in the same model. Oral administration of Extract P and Extract S (300 mg kg\(^{-1}\)) inhibited formation of gastric lesions induced by ethanol. When stomach wall was viewed by backlighting, it was apparent that administration of the extracts resulted into a marked increase in the wall thickness when compared with the control group.

The protection afforded by Extract S was found to be more significant than that of Extract P. The results were comparable with the reference standard. The inhibitory effect of Extract S was dose-dependent and the ED\(_{50}\) was found to be 28.21 mg kg\(^{-1}\). Saponins were further screened for antiulcer potential at the dose of 100 mg kg\(^{-1}\) in HCl-ethanol- induced gastric mucosal ulcers in mice, ethanol-induced pylorus ligated gastric mucosal ulcers in rats, ethanol-induced gastric ulcers in N-ethylmaleimide (NEM) pretreated rats and ethanol-induced gastric ulcers in \(\text{NO}\)-nitro-L-arginine methylester (l-NAME) pretreated rats.

Extract S at the dose of 100 mg kg\(^{-1}\) was also evaluated for various biochemical parameters on ethanol-induced gastric ulcer model. It caused significant increment in the gastric wall mucus content (GWMC) as compared to control. Extent of lipid peroxidation was significantly reduced as evident from the low malondialdehyde levels in Extract S pretreated animals than in the group treated with ethanol alone. A significant increment in the levels of preventive antioxidants like SOD and Catalase as well as the chain breaking antioxidant like GSH were also observed.
Discussion

Ethanol causes gastric lesions by its direct action on mucosa, which contributes to the development of the haemorrhagic and necrotic tissue injury$^{249}$. Ethanol and several NSAIDs such as indomethacin irritate the gastric mucosa in both human and animals and may therefore cause injury and bleeding by their direct action and gastric acid participates little in the formation of these lesions$^{150-152}$. The subsequent increase in mucosal permeability together with the release of vasoactive products from mast cells, macrophages and blood cells may lead to vascular injury, necrosis and ulcer formation$^{253}$. It has been suggested that ethanol-induced gastric mucosal injury is due to breakdown of mucous bicarbonate barrier, a decrease in potential difference followed by an increase in back diffusion of hydrogen ions and stasis of blood flow in mucosa. Moreover, it is well known fact that ethanol induced ulcers are not inhibited by antisecretory agents like cimetidine$^{250}$ but are inhibited by agents which enhance mucosal defensive factors$^{207}$ such as sucralfate$^{254}$ and cetraxate$^{255}$. Additionally, reduction in gastric mucus is also a possible cause of the lesion formation induced by ethanol$^{207,256}$. In the present study, Extract S increased alcian blue binding to the mucosa, which represents amount of mucus adherent (undissolved) to the mucosal surface of the stomach$^{257}$. Alcian blue dye is able to bind to negatively charged materials and this increase in bound alcian blue suggests the presence of bound extract or its constituent(s) on the surface of the mucosa. It is believed that visible-mucus adhering to the wall, rather than mucus dissolved in gastric secretion plays a more important role in protection against auto digestion of the gastric mucosa$^{258}$. The results of the present study suggest that the major mechanism of gastric mucosal protection might be reinforcement of resistance of the mucosal barrier and thereby prevention of back diffusion of $H^+$ ions.

There is increasing evidence that the beneficial effects of many mucosal strengthening drugs occur through venues different to the
prostaglandin-mediated mechanisms\textsuperscript{259-263}. This may include alteration in physiochemical parameters of mucus gel and its constituents\textsuperscript{261} and influence on the activities of the enzymes involved in processing and modification of molecules responsible for the maintenance of mucosal integrity\textsuperscript{191,261}. Such molecules are nonprotein sulfhydryl compounds (endogenous glutathione)\textsuperscript{264}, polyamines\textsuperscript{265}, endothelium derived relaxing factor (nitric oxide)\textsuperscript{266,267}, dopamine\textsuperscript{268} and oxygen derived free radicals \textsuperscript{139,259,269,270}. Further, it has been shown that vascular changes seem to be the most pronounced feature in ethanol-induced gastric mucosal injury and severe damage in such injury is associated with extensive lesions of mucosal capillaries, increased vascular permeability and reduction of blood flow in mucosa\textsuperscript{270-272}. Thus it is possible that maintenance of mucosal vasculature and normal blood flow may be the major mechanism of cytoprotection shown by Extract S in the present study.

Prostaglandins are known to protect whereas leukotrienes predispose to gastric ulceration. Prostaglandins are cyclooxygenase derived and leukotrienes are lipoxygenase-derived products of arachidonic acid. Prostaglandin production is calcium independent\textsuperscript{273-275} while leukotrienes production is calcium dependent\textsuperscript{276,277}. Increased vascular permeability leads to massive intracellular accumulation of calcium ions, resulting in gastric injury. Leukotrienes are potent vasoconstrictors and are generated by gastric mucosa particularly after exposure to ethanol\textsuperscript{278,279}. Oxygen derived free radical-induced gastric damage is mediated through lipid peroxidation\textsuperscript{280}. There is an intimate relationship between lipid peroxidation and production of arachidonic acid metabolites. Recent reports show that inhibition of leukotrienes synthesis is accompanied by the reduction in gastric mucosal damage in different experimental models including ethanol-induced gastric ulcer model\textsuperscript{281,282}.
It is widely known that the process of lipid peroxidation is mediated by the interaction of the hydroxyl radicals with the cell membrane; subsequently producing lipid derived free radicals such as conjugated dines lipid peroxidase\textsuperscript{283}. These free radicals are known as extremely reactive products that cause oxidative damage\textsuperscript{283}. Further, gastric lesions caused by ethanol have been attributed to free radical formation and subsequent formation of lipid peroxidation products\textsuperscript{284-286}. The metabolism of ethanol generates superoxide radicals, which in turn may promote lipid peroxidation\textsuperscript{253,287}. The stomach and the upper GI tract are the main sites of ethanol metabolism, and recent studies have implicated ethanol generated free radicals, ethanol-induced lipid peroxidation and depression of glutathion as mechanisms of alcohol-induced gastric injury\textsuperscript{287,288}. Glutathion is an important component of the intracellular protective mechanism against various noxious stimuli including oxidative stress\textsuperscript{289}. In humans a reduction in glutathione and cystein in the gastric body and antrum after ethanol consumption, was observed and glutathione pretreatment significantly reduced the ethanol-induced damage\textsuperscript{289}. In the present study significantly lower level of malondialdehyde was observed with Extract S pretreatment when compared with control group receiving ethanol alone. Reduction in MDA level suggests decreased lipid peroxidation and free radical-induced damage. The significant increment of preventive antioxidants like SOD and Catalase suggests that the generated free radicals are rapidly converted into H\textsubscript{2}O\textsubscript{2} with the help of SOD and that is scavenged immediately by increased activity of Catalase. Glutathione peroxidase catalyses the oxidation of glutathione at the expense of a hydroperoxide, which might be hydrogen peroxide or other species such as a lipid hydroperoxide\textsuperscript{280}. The increased level of chain breaking antioxidant like GSH suggests the free radical scavenging activity of Extract S.
HCl-Ethanol-induced gastric mucosal ulcers in mice:

Oral administration of necrotizing agents like ethanol, HCl and ethanol–HCl stimulates release of prostaglandins by stomach to prevent gastric lesions through adaptive cytoprotection\(^{207,291}\). Prostaglandins are known to play an important role in maintaining mucosal integrity to protect the gastric mucosal integrity\(^{292}\) and to protect the gastric mucosa against various damaging agents\(^{291,293-296}\). This activity is not related to their antisecretory property\(^{297}\). In the present study, ulcer inhibitory action of the Extract S indicated that it might be improving the levels of gastric mucosal defensive factors such as mucus and prostaglandins. Further, it is interesting to note that oxygen derived free radicals not only cause inflammation but may also be involved in the pathogenesis of gastric mucosal damage induced by ethanol–HCl\(^{298}\). In our study, Extract S was found to reduce lipid peroxidation as evident by reduction in malondialdehyde content and thereby stimulating the healing of the acute gastric mucosal injury.

Ethanol-induced pylorus ligated gastric mucosal ulcers in rats:

In the healthy stomach, there is a balance between aggressive factors and the protection afforded by pre-epithelial, epithelial and sub-epithelial mechanisms of gastric mucosa. Secretion of mucus and bicarbonate by surface epithelial cells constitute a mucus-bicarbonate barrier, which is regarded as first line of defense against potential ulcerogens. Although pathogenesis of ulcer is multifactorial, ulcer is considered to be due to derangement of balance between aggressive and defensive factors. Hence, study was undertaken to observe the status of aggressive factors namely acid and pepsin and important defensive factors such as mucin secretion.

Extract S at the dose of 100mg kg\(^{-1}\) caused significant antiulcer activity in ethanol plus pylorus ligated gastric ulcer in rats. Pylorus
ligation is a widely used method for producing experimental gastric ulcers. It is suitable for first line antiulcer screening, as the agents are retained in the stomach and may act by a variety of mechanisms. With the use of this method, reproducibility and high incidence of ulceration has been reported. The main advantage is that one can measure gastric secretory rate, percentage ulceration and ulcer severity in the same animal. A survey of the clinically useful drugs on experimental ulcers suggests that the pylorus-ligated rat method is probably the single test for the prediction of clinical usefulness of an antiulcer drug.

Apart from direct ulcerogenic effect of ethanol, in the stomach digestive effect of accumulated gastric juice in the induction of gastric ulcer is well documented in the pylorus ligation model. Biochemical constituents of the gastric juice like, gastric acid and pepsin are the important factors for the formation of ulcers in pylorus ligated rat. Since Extract S (100 mg kg\(^{-1}\) p.o.) significantly inhibited lesion formation in the glandular stomach and reduced both acid concentration and pepsin activity together with rise in pH values, it is suggested that the extract can suppress gastric damage induced by aggressive factors.

The pH dependent pattern of mucosal injury is best explained by very low activity of gastric pepsin above pH 4.0. Benefits from gastric acid inhibitory therapy are proportional to the degree of elevation of gastric pH. Among the treatment options for acid-related disorders antacids, histamine H\(_2\) receptor antagonists and proton-pump inhibitors confer healing by raising intra-gastric pH. Hence, increase in pH with the use of Extract S in the present study might by serving as an additional factor in providing the protection.

In addition to gastric acid secretion, reflex or neurogenic effects have also been suggested as playing an important role in the formation of
gastric ulcers in the pylorus ligation model\textsuperscript{308,309}. It is a known fact that gastric secretion in shay rats is under vagal control\textsuperscript{310} and the anti-cholinergics significantly reduce it in this model. The effect of Extract S on reduction in gastric acid secretion may involve inhibitory effect on cholinergic system. However, additional study is required to confirm involvement of this mechanism in the gastro-protective activity afforded by extract S.

Increased synthesis of nucleic acids and increased metabolism of carbohydrates and thereby exhaustion of carbohydrates and other compensatory mechanisms could be responsible for ulceration due to pylorus ligation\textsuperscript{311}. It is essential to understand the pathological phenomenon related to ulcer formation at the level of the cell membrane, glycoproteins, mitochondria, nucleic acids, enzymes and proteins and to study selectively each of these factors with regard to time and lesion development\textsuperscript{312}. Moreover, one of the essential criteria to determine the status of mucus resistance / barrier is the state of mucus secretion\textsuperscript{313}.

Certain antiulcer drugs increase the amount of gastric mucus secretion in gastric mucosa\textsuperscript{214,314-317}. This mucus consists of mucin type of glycoproteins, which can be estimated by the ratio of total carbohydrates (TC) (sum of hexoses, hexosamine, fructose, sialic acid) to proteins (PR) in the gastric juice\textsuperscript{318}. These high molecular weight glycoproteins are mainly responsible for viscous and gel forming characteristics of the mucus\textsuperscript{148,319}. Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms, including lessening of stomach wall friction during peristalsis and gastric contractions, improving the buffering of acid gastric juice and by acting as an effective barrier to back diffusion of H\textsuperscript{+} ions\textsuperscript{149,320}.
The total carbohydrate to protein ratio TC/PR ratio reflects the functional integrity of the mucosal barrier and has been accepted as a reliable index of mucosal resistance or mucin secretion\textsuperscript{321}. Low TC/PR ratio indicates damage to the gastric mucosa, resulting in the leakage of plasma proteins into gastric juice\textsuperscript{149,322}. In the present study, Extract S altered both the total carbohydrates and proteins and so the TC/PR ratio. The results of the present study suggest that the major mechanism of gastric mucosal protection might be the antisecretory activity of Extract S on acid and pepsin. In addition, direct cytoprotection may be involved as evident from the increased TC/PR ratio.

Therefore, the mechanisms of the anti-ulcer effect of Extract S could be due to reduction in acid secretory parameters and strengthening of gastric mucosal barrier. In addition, direct cytoprotection may be involved as evident from the increased TC/PR ratio.

**Ethanol-induced gastric mucosal ulcers in N-ethylmaleimide (NEM) pretreated Rats:**

Ethanol-induced gastric damage has been shown to be associated with depletion of endogenous sulfhydryl compounds, especially glutathione\textsuperscript{323,324}. This depletion can be due to oxidation of glutathione by ethanol generated toxic metabolites\textsuperscript{325} or binding of glutathione to acetaldehyde generated through the oxidation of ethanol by the gastric alcohol dehydrogenase activity\textsuperscript{326}. Moreover, part of ethanol could be acted upon by enzyme aldehyde oxidase in the liver, which is known to produce superoxide radical\textsuperscript{327}. Also, depletion of sulfhydryl compounds is associated with increased susceptibility of gastric mucosa to oxidative metabolites\textsuperscript{328}. SH-containing compounds prevent hemorrhagic erosions caused by ethanol\textsuperscript{324}. The common mechanisms involved are reduction in vascular damage\textsuperscript{329} and scavenging of free radicals and thereby preventing membrane damage brought about by lipid peroxidation.
Thus, removal of O$_2$ derived free radicals stimulates the healing of acute gastric mucosal injury.

To investigate the effect of endogenous sulfhydryls in the protective effect of Extract S, sulfhydryl-blockers, like N-ethylmaleimide (NEM) was subcutaneously injected at the dose of 10 mg kg$^{-1}$, 30 min before the administration of the respective treatment orally$^{217}$. Pretreatment with NEM prevented the gastroprotection of sulfhydryl-containing substances$^{323}$. The gastroprotective effect of Extract S at the dose of 100 mg kg$^{-1}$ in ethanol-induced gastric lesions was reduced with NEM pretreatment when compared with the control group. This indicates that preservation of endogenous sulfhydryls may be mechanism involved in the gastroprotection shown by this extract.

**Ethanol-induced Gastric Mucosal Ulcers in N$^\text{G}$-nitro-L-arginine methylester (l-NAME) pretreated Rats:**

NO rapidly undergoes addition, substitution, redox and chain terminating reactions. These reactions serve as the molecular bases for its biological effects in human body. The target molecules of NO are intracellular thiol and metal containing proteins and low molecular weight thiols like glutathione and cysteine etc$^{330}$. NO acts as a "double edge sword" in health and disease. The main physiological role of NO is controlled by type I and III NOS expression via intracellular Ca-calmudulin complex dependent mechanism. Both deficiency and excess of NO are believed to be involved in different pathophysiological states like stroke in brain, ischemia, gastrointestinal dysfunction, achalasia, Hirschprung's disease etc$^{331}$. NO, interactively with prostanoids and sensory neuropeptides, regulates gastric mucosal integrity in rats$^{332}$. NO participates in the gastric defense mechanisms by regulating the gastric mucosal blood flow and gastric mucus secretion$^{333}$. 


To investigate the effect of endogenous NO in the protective effect of Extract S, NO-blocker, like N⁶-nitro-L-arginine methylester (l-NAME) was subcutaneously injected at the dose of 70 mg kg⁻¹, 30 min before the respective oral treatment. The prior administration of l-NAME, an NO-synthase inhibitor completely inhibited the antulcerogenic activity of the Extract S (100 mg kg⁻¹), suggesting that NO participates in the gastroprotection of this extract.

**Indomethacin-induced Gastric Mucosal Ulcers in Rats:**

Use of nonsteroidal antiinflammatory drugs (NSAIDs) is the second most common cause of peptic ulcer diseases. Indomethacin-induced gastric lesions are characterized by multiple erosions and bleeding. Extract S caused dose dependent reduction in ulcerated area and score for intensity in indomethacin-induced gastric ulcer in rats. NSAIDs are known to inhibit the gastric mucosal blood flow via inhibition of prostaglandin synthesis. As already mentioned prostaglandins are known to play an important role in maintaining mucosal integrity and to protect the gastric mucosa against various damaging agents. This activity is not related to their antisecretory property. Further, the potential mechanisms involved in prostaglandin action are multiple, including stimulation of mucus and bicarbonate output, gastric mucosal blood flow, strengthening of gastric mucosal barrier, decreasing gastric motility, increasing the release of endogenous mediators of gastric cytoprotection like sulfhydryl and endothelial growth factor, decreasing release of endogenous mediators of gastric injury-vasoactive amines and leukotriens and stimulation of cellular growth and repair. Moreover, it has been shown that vascular changes seem to be the most pronounced features in ethanol-induced gastric mucosal injury and severe damage in such injury is associated with extensive lesions of mucosal capillaries, increased vascular permeability and reduction of blood flow in mucosa. Thus it is possible that maintenance of mucosal vasculature and normal blood flow may be the one of the probable
mechanisms of cytoprotection shown by the extract S in indomethacin-induced gastric ulcer model.

The reduction in prostaglandin E2 (PGE2) secretion is caused by inhibition of key enzyme cyclooxygenase whereas reduction in mucus production is at least partly due to the damage of epithelial cells and mucus cells. Subsequently substantial luminal free H+ diffuse back to the gastric mucosa leading to formation of gastric lesions.

It is widely accepted that gastric mucus plays an important role in the protection of gastric mucosa and that the reduction in mucus production is one of the causes of ulcerogenic effects of NSAIDs.

Several studies have shown that indomethacin-like substances interfere with cell metabolism due to trapping of drug anion within cells of mucosa, enzyme synthesis, mucopolysaccaride production and increase in the cell turnover. Thus there is disruption of gastric mucosal barrier, which disturbs the continuity of surface epithelial cells and enhances back diffusion of acid into the mucosal tissue. There are many reports stating ability of PGE2 to protect gastric mucosa against indomethacin, ethanol, bile salts, coldstress, or indomethacin induced gastric mucosal damage. The cytoproteective properties of the prostaglandins include prevention of acid back diffusion, increase of mucosal blood flow, gastric mucosal barrier damage and inhibition of inflammatory mediator release from mast cells and of free radical production.

Oxygen-derived free radicals play a key role in the mechanism of NSAIDs-induced acute gastric mucosal injury. The free radicals in the process of the indomethacin-induced injury may come from more than one source. The inhibition of prostaglandins by
indomethacin results in tissue hypoxia and ischaemia, which induce transformation of the native xanthine dehydrogenase into a predominantly oxidase form. During ischaemia, xanthine oxidase uses any available oxygen as an electron acceptor and converts xanthine or hypoxanthine to uric acid, with the production of the superoxide radical.

Reduction of gastric mucosal blood flow allows back diffusion of H\(^+\) ions. This diffusion may lead to several adverse side effects:

(a) Disruption of mucosal vasculature, an action, which results in intramural hemorrhage, in addition to enhancing degree of tissue ischaemia, thereby ensuring continued presence of xanthine oxidase (XOD) and subsequent production of superoxide radicals.

(b) Change in the mucosal pH that can convert the enzyme xanthine dehydrogenase to an oxidase form, thereby generating oxyradicals and

(c) Tissue irritation and microscopic injury, which may result in increased free radical production.

Further, the polymorphonucleocytes (PMNS) infiltrating the vicinity of the aspirin-induced injury may be other source of free radicals. These cells undergo oxidative bursts that generate highly reactive metabolites of oxygen, such as H\(_2\)O\(_2\) and hypochlorite, as well as the superoxide and hydroxy radicals. These highly labile and reactive species have capacity to disrupt cell membranes by lipid peroxidation of membrane constituents. The cellular injury can induce the release of endogenous cytotoxic agents like lysosomal enzymes.

Changes in permeability of mucosa by NSAIDs may result in release of histamine, which produces changes in the vascularity of the mucosa and rupture of the histamine dilated capillaries resulting in the production of gastric damage. Also centrally mediated release of catecholamines from adrenal medulla as well as peripherally released histamine could be responsible for the gastric mucosal damage.
induced by NASIDs\textsuperscript{370,371}. Histamine plays a central role in the gastric mucosa in the gastric acid secretion\textsuperscript{372}. Studies have suggested that the release of histamine is a major regulatory event in stimulation of acid secretion and that increased histamine secretion may be associated with mucosal damage\textsuperscript{373}.

In the present study, Extract S dose dependently suppressed ulcerogenesis in indomethacin treated animals. ED\textsubscript{50} was found to be 28.53 mg kg\textsuperscript{-1}. As discussed earlier, lipid peroxidation and prostaglandin synthesis are related and a very efficient antioxidant protection will slow down prostaglandin synthesis at least until sufficient PGE\textsubscript{2} is formed to maximally activate cyclooxygenase\textsuperscript{374}.

It has been reported that acute mucosal damage produced by NSAIDs can be prevented by elevation of gastric pH of gastric contents at pH > 4.0\textsuperscript{305} and only highly effective gastric acid inhibition reliably reverses NSAIDs-induced injury. Extract S has shown both pH elevatory as well as antisecretory actions in pylorus-ligated rats. These may be contributory factors in gastro protection afforded by Extract S in the present study.

**Cysteamine-induced Duodenal Ulcers in Rats:**
Cysteamine-induced duodenal ulcer is one of the most widely used models for studying duodenal ulcers\textsuperscript{375}. 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 poorly understood human disorder\textsuperscript{376}.

Cysteamine-induced duodenal ulcer usually develops on the region of two vascular networks, one from the pyloric canal of the duodenum and the other from the anal side of duodenum.
In the present study, Extract S showed significant reduction in total lesion area and score for intensity. The pathogenesis of cysteamine-induced duodenal ulcer involves enhanced gastric acid secretion, decreased mucosal resistance, increased duodenal motility, delayed gastric emptying, decreased duodenal bicarbonate secretion in response to acid activity and increased plasma levels of gastrin. It has been suggested that an increase in duodenal activity after cysteamine administration may contribute to ulcer formation.

The increase in duodenal activity by cysteamine may be through interference with the back flow of pancreatic and biliary alkaline secretions throughout the duodenum. In fact, cysteamine is reported to have little effect on basal HCO₃⁻ transport but has been shown to inhibit the ability of the duodenal epithelium to respond to luminal acid with a compensatory rise in alkaline secretion thereby leading to a rise in surface pH. Moreover, the leakage of gastric contents into the duodenum could cause erosion of the duodenum in which mucosal resistance has been lowered by the reduction of bicarbonate secretion caused by cysteamine. Agents or treatments, which inhibit acid secretion or gastric motor activity and neutralize or divert the acid flow, have been shown to prevent the formation of these ulcers. Further, higher concentration of histamine, especially due to increased activity of histidine decarboxylase is shown to be involved in the pathogenesis of duodenal ulcers. Histamine is a key component of gastric function as well as in diseased states such as gastroduodenal ulcers.

In the present study, there was a significant reduction in gastric acid output and pepsin levels in pylorus-ligated rats. This indicates antisecretory activity as one of the mechanisms involved in antiulcer activity exerted by Extract S. It has been postulated that cysteamine-induced duodenal ulcer is associated with gastric acid hypersecretion. Therefore, the efficacy of Extract S in inhibiting ulcer formation could be explained by its potent antisecretory effect.
Reactive oxygen species are known to be involved in cysteamine-induced duodenal ulceration\textsuperscript{392}. Extract S is shown to possess antioxidant potential, which may also be serving as one of the contributory factors in the protection afforded by it.

**Antioxidant activity:**

Fatty acids are important components of biological membranes in which they serve as building blocks for major membrane constituents such as phospholipids, glycolipids and triglycerides. Especially, unsaturated fatty acids impart desirable properties upon the fluidity of membranes. However, these essential unsaturated molecules are most susceptible to oxidative processes\textsuperscript{393-394}. An uncontrolled oxidative activity is accepted as a general mechanism of tissue damage in a variety of pathological conditions like inflammations, carcinogenesis, aging, atherosclerosis, diabetes mellitus and more recently gastroduodenal ulceration\textsuperscript{395-399}. Furthermore, metabolic disruptions caused by diverse toxic agents acting at various sites may in part, be expressed by abnormal levels of reactive oxygen species (ROS)\textsuperscript{400}. ROS exert damaging effects by reacting with nearly every molecule found in living cells, including DNA, proteins, membrane lipids, and carbohydrates. The radicals induce lipid peroxidation, which alters membrane fluidity and permeability resulting in disruption of membrane structure and cell function\textsuperscript{401}. Decreasing damage could be feasible by increasing the normal or diminished antioxidant defenses against free radical reactions leading to increased global antioxidant capacity\textsuperscript{402,403}. Thus agents such as antioxidants that can control states of oxidative stress represent a major line of defense regulating general health status\textsuperscript{404}.

There are several proteins and biomolecules in the living organism, which act as free radical scavengers. Similarly flavonoids, phenols,
glycosides, terpenoids, lignans etc act as natural free radical scavengers\textsuperscript{73-75}.

Reactive oxygen species (ROS) such as superoxide, hydroxyl radical, iron-O\textsubscript{2} complexes, H\textsubscript{2}O\textsubscript{2} and lipid peroxides are generated by several reactions: metabolism of triplet O\textsubscript{2} molecules, one electron reduction of O\textsubscript{2}, catalytic decomposition of H\textsubscript{2}O\textsubscript{2} and lipid hydroperoxides by metal ions; attack of metal or metal-O\textsubscript{2} complexes, light and X-ray irradiation and intake of exogenous radicals\textsuperscript{405}. These radicals react with biological molecules such as DNA, proteins, and phospholipids and eventually damage membranes and other tissues\textsuperscript{406}.

It is an established fact that lipid peroxidation is one of to the reactions set into motion as a consequence of the formation of free radicals in the cells and tissues. The one-electron reduction products of oxygen, superoxide (O\textsubscript{2}\textsuperscript{-}) anion, H\textsubscript{2}O\textsubscript{2}, hydroxyl (OH\textsuperscript{-}) radical actively participate in the initiation of lipid peroxidation\textsuperscript{407}. The formation of carbon-centered free radicals is important step in the peroxidation of unsaturated lipids (free radicals abstract a hydrogen atom from the methylene (-CH\textsubscript{2}-) group). The carbon radical is stabilized by molecular rearrangement to produce conjugated diene, which then reacts with an oxygen molecule to form a peroxy (R-OO\textsuperscript{-}) radical. Peroxy radical can form cyclic peroxide and cyclic endoperoxide. Fragmentation of these peroxides leads to formation of compounds containing carbonyl (>C=O) group, especially aldehydes like 4-OH 2, 3 transnonenal and malondialdehyde (MDA)\textsuperscript{408,409}. 4-OH 2, 3 transnonenal attacks essential sulfhydryl group of many proteins\textsuperscript{409,410} and MDA attacks amino groups of the protein molecule to form intra and intermolecular crosslinks\textsuperscript{409,411}. These aldehydes react with thiobarbituric acid forming thiobarbituric acid reactive substances (TBARS)\textsuperscript{409}. Continued fragmentation of fatty acid side chains to produce aldehydes and hydrocarbons will eventually lead to loss of membrane integrity\textsuperscript{406}. 
In the view of the known involvement of oxygen free radicals in tissue damage, the extract from *Chlorophytum arundinaceum* is tested for its interaction with reactive oxygen species (ROS) in various ROS-generating chemical reactions.

The free radical scavenging activity of Extract S was tested by its ability to bleach the stable DPPH radical. This assay provided the information on the reactive of the test compound with a stable free radical since its odd electron DPPH gives strong absorption band at 516 nm in visible spectroscopy. As this electron becomes paired of in the presence of free radical scavenger, the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. Extract S showed anti radical activity by inhibiting DPPH radical with an EC$_{50}$ value of 0.8 mg/ml.

Superoxide (O$_2^-$) anion scavenging assay performed by monitoring the reduction of yellow dye nitro-blue tetrazolium (NBT) to produce a blue colored formazan, which is directly proportional to the concentration of superoxide (O$_2^-$) anion in the system$^{53}$. NBT in presence of O$_2^-$ is reduced to a tetrazolium, two of which combine together to give a blue dye (monoformazan). In the present study, the inhibitory effect observed with the use of the Extract S suggests its superoxide (O$_2^-$) scavenging potential. The action may depend on hydrogen atom donation leading to the formation of secondary radical species that are resonance stabilized, like many phenolic antioxidants$^{412}$.

Radical chain reactions are generally catalyzed by transition metal ions, mainly iron and copper. Many antioxidants act by binding to metal ions$^{413}$. In the present study, Extract S exhibited a notable and dose dependent inhibition of FeSO$_4$-induced lipid peroxidation, which was indicated by significant decrease in the MDA formation. In conclusion it is postulated that the antioxidant effect of extract S may be, at least in part, due to its inhibitory effect on membrane lipid
Discussion

peroxidation and free radical formation or due to its free radical scavenging activities. These results further confirm antioxidant potential of Extract S as observed in ex-vivo studies in experimental ulcer models.

Effect on Central Nervous System:

Barbiturate Sleeping Time:

Extract S potentiated pentobarbitone-induced hypnosis in normal mice. This may be either due to inherent depressant activity or inhibition of the liver microsomal enzyme system responsible for degradation of pentobarbitone by the extract. No attempt was made in this study to find exact mechanism of prolongation of pentobarbitone hypnosis.

Nociceptive Response:

It is paradoxical to see antiinflammatory, analgesic and antiarthritic effects in a compound possessing antiulcer activity, since anti-inflammatory agents are often ulcerogenic. Antinociceptive drugs are generally classified into centrally or peripherally acting with respect to the site of action. In the present study antinociceptive effect was studied in mice. Writhing test and a tail flick test were performed.

Several chemicals can induce writhing responses in animals\(^4\)\(^{14},\)\(^{15}\). Acetic acid induces writhing response by activating the chemosensitive nociceptors in animals; while tail flick response is considered to be selective for opioid like substances in several animals\(^4\)\(^{16},\)\(^{17}\).

Extract S demonstrated significant reduction in acetic acid-induced writhing. It is worthwhile to note that antihistaminics and anticholinergics can reduce the writhing response\(^4\)\(^{18}\). Considering that abdominal constriction is related to sensitization of nociceptive receptors by prostaglandins\(^3\)\(^{69}\), antinociception exhibited by Extract S could be due to its spasmolytic or antiinflammatory properties.
Prolongation of tail flick response time was observed at higher dose, which is presumed to be acting centrally in modulating nociception, similar to morphine and its congeners\textsuperscript{419}. Aspirin on the other hand did not elevate the pain threshold produced by thermal stimulus\textsuperscript{420,421} (analgesia brought about by aspirin in normal circumstances is due to inhibition of prostaglandin synthetase, which is absent when the thermal stimulus is used\textsuperscript{422}) so Pethidine was used as positive control at the dose of 5 mg kg\textsuperscript{-1} \textsuperscript{423}.

It is difficult to say at this stage about the exact mechanism involved in antinociceptive action exerted by Extract S. These results indicate that the analgesic effect of the extract could be due to presence of more than one component having individually selective central or peripheral antinociceptive action.

In conclusion, the present study suggests that the antiulcer activity of Extract S on ethanol-induced and HCl-ethanol-induced gastric ulcer model can be attributed to its cytoprotective, mucoprotective and free radical scavenging property. The probable mechanisms of protective effect of Extract S on ethanol-induced pylorus ligated gastric ulcer model could be reduction in acid secretory parameters and strengthening of gastric mucosal barrier. The gastroprotective effect of Extract S in ethanol-induced ulcer model was reduced with NEM pretreatment this indicates the preservation of endogenous sulphhydrlys may be one of the mechanisms involved in the gastroprotection shown by this extract. In ethanol-induced gastric ulcer model prior administration of 1-NAME, completely inhibited the antiulcerogenic activity of the Extract S, suggesting that NO participates in the gastroprotection. The antiulcer effect shown by Extract S on indomethacin-induced gastric ulcer was independent of the endogenous prostaglandins. The mechanism involved in protection by Extract S in cysteamine-induced duodenal ulceration can be associated with its antisecretory and antioxidant potentials.