Discussion
DISCUSSION

Majority of drugs used in the treatment of peptic ulcer disease falls into two broad therapeutic categories, one that counteract the effect of gastric acid and other, which exert a cytoprotective effect on gastro-duodenal mucosa. Gastric hypersecretion appears to be the primary causative event at one end of the disease, however mucosal membrane also becomes equally important as it protects against the aggressive factors and may directly resist injury.

Gastric ulcers are typified by a reduced basal and stimulated acid output. Some amount of acid is, however, always required as peptic ulcer disease rarely develops in patients of achlorhydria. Herbal drugs are beneficial and far more protective to the mucosal membrane. If normal integrity of mucosa is maintained, the blood flow removes the acid that has diffused across a compromised mucosa. Perhaps increasing the mucosal blood flow (Robert 1979, Gasill et al., 1982), stimulating the secretion of mucosa and bicarbonates (Hogan et al., 1994). The other causes of peptic ulcer disease include H. pylori infections, NSAIDs, cigarette smoking, diet and psychological stress.

Selection of experimental models for evaluation of anti-ulcer drugs needs adequate reasoning and consideration. Keeping in view the complex multifactorial process involved in the pathogenesis of gastric and duodenal ulcers, it is difficult to have a single model for studying the genesis of ulcers as well as understanding the mechanism of anti-ulcer activity of drugs. Methods for the evaluation of gastric and duodenal anti-ulcer agents have been viewed by Parmar and Desai, 1993. Selection of the model should be based on simplicity, reproducibility and reasonable amount of predictability. However presently available anti-ulcer drugs in the market do not produced response in all the models. H₂ antagonist has less/no effect on ethanol induced gastric ulcers, as the model is based on the mechanism of cytoprotection (Robert 1979, Konturek et al., 1989). A number of procedures, which operate through different mechanisms of ulcerogenesis, are generally employed to evaluate the anti-ulcer activity of various agents. In the present investigations, rat have been used as experimental animals. The rat stomach can be divided into two parts, the rumen and the
pylorus. The rumen is upper two-fifth nonsecretory portion which is translucent and thinner than the glandular portion. The lower three-fifth glandular secretory portion of the stomach. The later portion is analogous to the body of the stomach in man anatomically and functionally (Shay et al, 1945). Further, the rats being omnivorous, resembles man nutritionally.

In the rats, the gastric ulcers were induced by pylorus ligation technique, absolute ethanol, 0.6 M HCl and nonsteroidal anti-inflammatory agents (NSIADs) like indomethacin. Duodenal ulcerations were induced in rats by cysteamine. The choice of the methods was based on their reproducibility, validity and predictive value as evident from the available literature. The methods incorporated in this study have been extensively utilized for inducing gastric and duodenal ulcerations and fulfill these criteria.

The Shay model (Shay et al., 1945) is simple, reproducible and highly predictable model for evaluation of anti-ulcer drugs. It utilizes neither exogenous ulcerogens and is not influenced by exogenous interfering factors. This method has been used to test the efficacy of anti-ulcer agents for future drug development and human use (Dedieu-Chaufour et al., 1994). Pylorus ligation has been also used because the gastric ulcers appear rapidly in the stomach wall and different physiological and biochemical alterations found both in the glandular and non-glandular regions of the stomach wall (e.g. HCl, pepsin, gastric juice, carbohydrates and proteins) can be evaluated simultaneously. Robert et al., (1979) introduced the concept of gastric cytoprotection, a mechanism other than the inhibition or neutralization of gastric acid secretion. The most commonly used model employed to evaluated the cytoprotective effect of a drug is absolute ethanol induced acute gastric haemorrhagic lesion as it is an acid independent injury and antisecretory drugs like H2 antagonist and anti-cholinergics do not produce any protection (Robert 1979, Konturek et al., 1989). NSAIDs produce a spectrum of injury to the gastro-duodenal mucosa, which involves tissue destructive free radicals, denaturation of mucus glycoproteins and mucus and parietal cell, inhibition of PG cyclo-oxygenase and mast cell degranulation etc (Rainsford and Brune, 1978; Siegel et al., 1979; Sullivan and Parker, 1974).
Results of this study establish a cytoprotective action of AETP, EAETP, AFAETP, AELR and AEBR as they were found effective against both the models viz. ethanol and 0.6 M HCl used for producing cytodestructive damage in the gastric mucosa of rats. Cytoprotection by drugs has been considered to be due to the generation of prostaglandins by anti-ulcer drugs when used in their non-antisecretory doses (Robert et al., 1979). However, the above extracts which demonstrated cytoprotection in absolute ethanol and HCl models did not show any significant decrease in gastric volume, total acid and pepsin activity leading to hypothesis that these extracts do not effects on the aggressive mechanisms. However, the AELR extract in 20 mg/kg was found to decrease in the gastric volume, total acid and pepsin activity. Although it reduced the total acid output, failed to cause significant reduction in pepsin output. These results are contrary to the general convention that alteration in acid secretion by drugs is always associated with an alteration in pepsin secretion (Konturek 1989; Espluges et al., 1982).

There are a number of models for producing gastric ulcers or erosions in the rat and many are used for investigating of the etiology of gastric ulcer and evaluation of anti-ulcer agents. On the other hand, it is a well know fact that rats are unusually resistant to induction of duodenal ulcers. Chemicals like cysteamine HCl, propionitrile, dulcerozine, dimaprit, etc., are presently used for the production of experimental duodenal ulcer. Cysteamine induced duodenal ulcers in rats were first described by Selye and Szabo (1973).

This chemically induced ulcer resembles duodenal ulcer in man in its location, histopathology and some aspects of pathophysiology. The duodenal Brunner’s glands produce mucus, HCO$_3^-$ and epidermal growth factor (EGF), which are important factors in the defense mechanisms of duodenal mucosa (Griffith and Harkins, 1956).

The pathogenesis of the cysteamine induced duodenal ulcers in rat comprises of several components such as gastric acid hypersecretion (Ishii et al., 1976; Kirkegaard et al., 1980) due to release of gastrin (Lichtenberger and Szabo, 1977), decreased gastric emptying time (Lichtenberger et al 1977, Poulsen et al., 1982)
and inhibition of the secretion of bicarbonate, mucus and epidermal growth factor (EGF) from the duodenal Brunner's glands (Poulsen et al., 1982, Kirkegaard et al., 1981).

The cysteamine ulcers are considered to be due to an inhibition of alkaline mucus secretion and also due to a long lasting hypersecretion of gastric acid from the Brunner's gland in the proximal duodenum (Szabo et al., 1977, Kirkegard 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). In fact hypersecretion of acid, which disturbs gastro-duodenal motility, hypergastrinaemia and decrease mucosal resistance have all been implicated in the pathogenesis of cysteamine induced duodenal ulcers (Ischii et al., 1976). The ulcerogenic dose of cysteamine interferes with the natural defense mechanism of the duodenal mucosa by reducing mucosal blood supply and mucus glycoprotein over a prolong period. Briden and co-workers (1984) suggested that in the specific duodenal ulcerogen, cysteamine has little effect on basal bicarbonate transport but it 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.

Over all protective effect of AETP, EAETP, AELR and AEBR against cysteamine induced duodenal ulcers may be due to the strengthening of duodenal mucosa (Garner, 1988) or by other mechanisms like increased gastric and duodenal alkaline secretion (Rees et al., 1982) or by increased luminal prostaglandin levels (Konturek et al., 1987).

In the pylorus ligation 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). Thus gastroprotective effect of AETP, EAETP, AFEATP, AELR, AFAELR and AEBR may markedly alter the gastric mucosal microcirculation, increase the gastric mucosal blood flow and decrease the gastric mucosal ischemia induced by the ulcerogenic procedure and hence produce their anti-ulcer action.
The above discussion describes peptic ulcer disease as a disease associated with an increased in gastric acid secretion. Robert (1979) introduced the concept of cytoprotection, the protection against gastric mucosal injury by a mechanism other than inhibition or neutralization of gastric acid. Cytoprotection refers to the mechanism involving increase in mucus secretion, increase in the bicarbonate secretion, strengthening of gastric mucosal barrier, increase in mucosal blood flow, decrease in gastric motility, increased release of endogenous mediators like prostaglandins, sulfhydryls etc and scavenging of free radicals etc. Cytoprotection does not refer to any effect on total acidity, gastric juice volume and pepsin secretion. Increase in gastric volume in rats may be due to cytoprotective effect of AETP, EAETP, AFAETP, AELR and AEBR and scavenging of free radicals etc. However, we have not studied the influence on prostaglandins and bicarbonates. The extracts were not able to neutralize the effect on gastric secretion.

The essential criterion, which determines the status of the mucosal defense barrier, is the quality and quantity of gastric mucus secretion (Sanyal et al., 1983). Increase 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 and improving the buffering of acid gastric juice. Here the total carbohydrates represent the sum of total hexoses, hexosamine, fucose and sialic acid. Increased protein content of the gastric juice has been suggested to represent exfoliation and shedding of gastric mucosal cells induced by the ulcerogenic agent. Gastric mucosal carbohydrate to protein ratio (TC/PC) has been accepted as a reliable index of mucosal resistance (Sanyal et al, 1983). Gastric mucus secreted into gastro-duodenal lumen by surface epithelial cells and mucus neck cells (Goblet cells) and submucosal Brunner's glands, has two compartments, namely, 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 (Goel and Bhattacharya, 1991). Mucus consists of about 1% by weight of salt and other dialyzable components, 0.5% - 1% of free protein and a similar quantum of carbohydrate with glycoproteins. The glycoprotein content of mucus, by virtue of
its characteristic viscous gel forming property, is believed to be vital for the functional role of mucus.

Results on the studies on the effects of various extracts on total carbohydrates (TC) and protein content (PC) of gastric juice in pylorus ligated rats suggests that AETP and EAETP may be involved in strengthening of the gastric mucosal barrier. Increased TC/PC ratio with AETP and EAETP in the gastric juice of pylorus ligated rats points out specifically to the possibility of strengthening of gastric mucosal barrier as one of the mechanisms involved in their anti-ulcer activity. The higher mucin activity is due to the increase in total hexoses, hexosamine, sialic acid and fucose components of the gastric juice. Pretreatment with AETP and EAETP. The hexosamine and sialic acid content increased significantly. However, the hexosamine content was not increased significantly with AETP 5mg/kg. Sialic acid significantly increased with AETP pretreatment at all doses. AELR and AEBR did not change significantly the carbohydrate or protein content and hence TC/PC ratio. However the protein content was increased with AELR. Hexosamine content was increased with AEBR pretreatment. TC/PC ratio increased with AETP and EAETP leading to the strengthening of the mucosal barrier (Sanyal et al.,1983). Overall there was a decrease in the mucoproteins attributing to an unfavorable TC/PC ratio with AETP. Although the hexosamine content significantly increased. AELR (20 mg/kg dose) significantly increase the sailic acid and protein content and decreased the hexosamine, fucose and total hexose contents in the gastric juice. Omeprazole (8 mg/kg) produced significant increase in TC/PC ratio as compared to control group.

Prostaglandins were the first endogenous compounds implicated in gastric cytoprotection (Robert et al., 1979). The importance of endogenous prostaglandins in mucosal defense mechanism is evident from the observation that NSAIDs damage gastric mucosa. Since prostaglandins increase mucosal blood flow (Gaskill et al., 1982) this property has been suggested to be responsible for their gastroprotective effect. However, various other mechanisms have also been postulated like dilution of noxious agent by prostaglandins stimulated mucus secretion (Johanson and Kollberg, 1979), stimulation of basal
bicarbonate secretion (Smeeton et al., 1983), increase in the concentration of surface active phospholipids (Lichtenberger et al., 1985), stimulation of cyclic AMP (Simon and Kather, 1979), stabilization of lysosomes (Ferguson et al., 1973), decrease in gastric motility and dissolution of gastric mucosal folds (Merseraeu and Hinchey, 1982), and maintenance of mucosal sulphydryl groups (Szabo et al., 1973). Prostaglandins probably also have a repair function by stimulating rapid resolution of disrupted surface epithelium (Hawkey and Rampton, 1985). It has been shown that prior exposure of gastric mucosa to mild irritants protect it from damage by more noxious agents. This 'adaptive cytoprotection' is mediated by prostaglandins (Robert et al 1983). The ulcerogenic effects of prostaglandin depletion by synthesis inhibitors like indomethacin are known and the exogenously administered prostaglandins protect the gastric mucosa from various varieties of noxious stimuli (Spadara et al., 1987; Del Soldato and Varin, 1983).

Recently much attention has been focused on oxygen derived free radicals, which play an important role in various diseases. Oxygen derived free radicals have been shown to be involved in cataract, atherosclerosis, rheumatism, arthritis, Parkinson's disease and in ischemia reperfusion injury. The involvement of gastric acid and pepsin, the commonly known damaging factors are well documented in stress induced ulcers (Das et al., 1993). The actual pathophysiology involved in ulceration still remains obscure. Recently attention has been focused on the role of reactive oxygen species in mediating microvascular disturbances that preceded gastric mucosal injury induced by various types of stress or by ischemia-reperfusion injury.

Despite the relatively high production of free radicals under normal conditions, cellular antioxidants limit the rate of free radical generation. This includes preventive as well as chain breaking antioxidants (Buettner 1993). Antioxidants that prevent and neutralize the oxidant formation include SOD, catalase and glutathione redox cycle enzymes whereas ascorbic acid, tocopherol, and thiols inactivate the oxidizing radicals directly, hence are known as chain breaking antioxidants.
Oxygen free radicals can exert their deleterious effects by lipids peroxidation. Oxidative stress on lipid generates free radicals, which rearrange to form conjugation dienes. The dienes are degraded further and malondialdehyde (MDA) is formed as an intermediate product of lipid peroxidation due to the oxidative deterioration of polyunsaturated lipid.

In the present study pyloric ligation increases the ulcer index, acid content and there was also an increase in the malondialdehyde (MDA) content which has also been reported by others to increased in drug and stress induced ulcers (Ito et al., 1985; Perry et al., 1986; Das et al., 1993; Yoshikawa et al., 1993). Inflammatory and ischemic changes have been observed in the gastric tissue following ulceration (Cutin et al., 1987, Sen. 1995). Anti-ulcer drugs acting through antioxidant echanism may thus decrease MDA content that was observed after AETP pretreatment in pylorus ligated rats.

Role of free radicals and lipid peroxidation has been reported in inflammatory disorders and ischemic myocardial tissue following reperfusion. Neutrophils have been implicated in the development of inflammation and injury in a variety of tissues including the gastric mucosa (Ninemann, 1988). Gastric ulceration due to neutrophil migration to the injured tissue and increase in MPO has been documented (Trevethick et al., 1993; Alican et al., 1995). In the present study it has been demonstrated that neutrophils are not involved in pathogenesis (Avila et al., 1996; Sen, 1995). Superoxide anion thus released from activated neutrophils might cause damage to cell membrane by the generation of hydroxyl radical, which might lead to lipid peroxidation. Gastric haemorrhage and erosions increased TBA-RS, which indicates of lipid peroxidation in the gastric mucosa (Yoshikawa et al., 1993).

Gastric lesions caused by ethanol or pyloric ligation have been attributed to free radical damage, which results in lipid peroxidation. The involvement of oxygen-derived free radicals, in ischemic gastric mucosal damage has been suggested but the exact mechanism of action is not clear. In all cases the rate of oxidation is greatly accelerated resulting in generation of oxygen derived free radicals which
combine with various cellular constituents to form covalent bonds, usually with the thiol groups and hence, produce its damaging effects. Probably free radicals result in lipid peroxidation and damage to intracellular components (Ito and Guth, 1985). In the present study, the extent of lipid peroxidation was assayed by measuring the secondary products such as thio-barbituric acid – reactive substance (TBA-RS) formed in the lipid peroxidation processes. Pretreatment with AETP, significantly reduced the above secondary products, indicating that these drugs possess anti-oxidant capacity in vivo. However, pretreatment with EAETP did not produce a significant reduction of the above secondary products. AELR and AEBR also reduced the TBA-RS but not significantly, indicating AELR and AEBR may lead to lesser activation of scavenging systems for free radicals and active oxygens or free radical generating systems. (Yang et al, 1991).

AETP and EAETP significantly reduced TBA-RS level indicating that these drugs possess antioxidant capacity in vivo. AEBR increase the TBA-RS levels indicating that these drugs may lead to the cellular depletion of anti-oxidants present in the mucosal cells, the inactivation of scavenging systems for free radicals and active oxygens (superoxide dismutase, glutathione peroxidase etc) or the activation of free radicals generating systems (NADPH – cytochrome P450 etc).

Glutathione is an important constituent of intracellular protective mechanism against a number of noxious stimuli including oxidative stress. In pylorus ligated rats, there was a decrease in GSH may be due to its utilization in combating free radical induced damage as GSH is capable of reducing lipid peroxidation (Weiss, 1986) and may also be effective against highly reactive oxygen species like hydroxyl radicals and singlet oxygen. AETP has shown the decrease in GSH level at 20 mg/kg doses. Glutathione is regenerated from oxidized glutathione in presence of enzyme glutathione reductase by utilizing NADPH, which is oxidized to GSSG by enzyme glutathione peroxidase (GPx). However activity of GPx and glutathione reductase was not estimated but increased in glutathione-s-transferase suggests GSH is affected during ulceration (Tanaka and Yuda, 1993) and more GSSG (Sen. 1995) or hydrogen peroxide (H$_2$O$_2$) may get neutralized as glutathione-s-transferase has also shown increased significantly in present study.
Non-enzymic antioxidant tocopherol (vitamin E) acts as free radical quencher and is located in the plasma membranes while ascorbic acid (vitamin C) is present in the cytosol. Vitamin C helps in maintenance of tocopherol levels in membranes (Kosower 1978). Vitamin E, a lipid soluble antioxidant interacts with oxygen and lipid radicals and prevents the propagation of free radical. However, in the present study vitamin E was not estimated but vitamin C was increased after treatment with AETP suggesting that cytosolic antioxidants are affected.

In present study the significant increase in the catalase activity in pylorus ligated rats with AETP suggests that hydrogen peroxide is being neutralized by catalase. It appears that an increase in catalase might be a defensive action of the body against the oxidative injury. AETP 20 mg/kg produced elevated catalase activity significantly. Protection by catalase in ulcer has been reported earlier also.(Vincze et al., 1989, Sen. 1995)

Ulceration induced by pylorus ligation in rat is associated with a marked decline in the antioxidants like SOD, vitamin C and GSH levels, which leads to enhanced production of free radicals and lipid peroxidase, as indicated by increase in MDA content. AETP 20 mg/kg possesses a possible anti-oxidant activity which can be attributed to an interference with Vitamin C, GSH, catalase and glutathione-s-transferase levels as the possible mechanism of action.

In this contention the further study was carried out by analyzing in vitro DPPH radical scavenging activity (%). AETP and EAETP demonstrates dose dependent antioxidant activity, however, the EAETP activity was not as significant as AETP, Our results suggesting the antioxidant and free radical scavenging of Tephrosia purpurea were in perfect corroboration with one other study reported recently (Soni et al., 2003). However the in vitro anti-oxidant activity of AELR and AEBR were less remarkable as compared with AETP which can be attributed to their low solubility in methanol.
The following hypothesis has been laid down as possible mechanism of action for the antioxidant effect of *Tephrosia purpurea* in dotted line.

The above hypothesis is substantiated by our results observed with indomethacin as an ulcerogen, where in AETP, EAETP, AFAETP and AELR exhibited marked anit-ulcer effects. Indomethacin induced gastric ulcers by the inhibiting the synthesis of endogenous cytoprotective prostaglandins (Lanza, 1998). It has also been observed that AETP, EAETP, AFAETP and AELR significantly and dose dependently reduces the extent of gastric ulceration in pylorus ligated rats without affecting the gastric secretion or pepsin activity. These results further point out to cytoprotection as the major mechanism responsible for the anti-ulcer activity of AETP. However EAETP, AFAETP and AELR significantly reduced the gastric secretion and pepsin activity at the dose of 20 mg/kg. AETP and EAETP...
treatment demonstrated significant healing process in indomethacin induced gastric ulcers. Rutin and other flavonoids, flavone and flavanone stimulated prostaglandin E₂ production in isolated gastric mucosal cells (Shen et al., 2002).

Flavonoids possess marked antioxidant property and are reported to act as chemopreventive agent against renal oxidative stress and carcinogenesis induced by N-diethylnitrosamine and KBrO₃ (Khan et al., 2001). Ahmed et al., 1999 have reported that flavonoids like rutin, tephrosin, pongaglabol purpurin, purpuritenin A & B, tephrone and semiglabrin may be responsible for the anti-ulcer activity of *Tephrosia purpurea*.

Eradication of *H. pylori* is also an important approach in the treatment of peptic ulcer. *Terminalia chebula* (Malekzadeh et al., 2001), Licorice extract (Fukai et al., 2002), garlic extract (Wong et al., 1996), turmeric (*Curcuma longa*) and curcumin (Mahady et al., 2002), apple (Kubo et al., 1999), cinnmon extract (Tabak et al. 1999), plantain banana (Goel et al., 2001) and other flavonoids (Bae et al., 1999) possess anti *H. pylori* activity. *Tephrosia purpurea* contains rutin flavonoid which also possess anti *H. pylori* activity (Arima et al., 2002). Inhibitory action of flavonoids on H⁺/K⁺-ATPase activity is related to their ability to complex ATP. Flavone and flavanone stimulats prostaglandin E₂ production in isolated gastric mucosal cells. Further, flavonoids inhibit *Helicobacter pylori* growth in a concentration-dependent manner (Beil et al., 1995). Our preliminary laboratory study (Communicated) suggests the antimicrobial activity of *Tephrosia purpurea*.

The anti-ulcer activity of *Leptadenia reticulata* (Krishna et al., 1975) is ascribed to its flavonoidal constituents like diosmetin; luteolin, isoquercitin, rutin and hyperoside. One of the above flavonoidal constituents rutin is also present in appreciable amounts in our extracts as supplemented by our TLC findings (Ukani et al., 1998). Rutin also possess anti-ulcer and antioxidant activities (La Casa et al, 2000).

Present study with various extracts of Basella *rubra* suggests the cytoprotective effect of AEBR. However it may seen significant ulceroprotective effect on
cysteamine induced duodenal ulcers with 20 mg/kg. Pretreatment with AEBR observed increased in TC/PC ratio as compared to control group. Hexosamine content was also shown significantly increased in gastric juice suggesting the strengthening the mucosal barrier with AEBR pretreatment. Effect with *Basella rubra* on DPPH radical scavenging activity (%), results suggests *Basella rubra* does not possess significant antioxidant activity.