Review of Literature
3. REVIEW OF LITERATURE


Botanical name: *Helicteres isora* Linn.
Family: Sterculiaceae

*Helicteres isora* is a large shrub or small tree up to 5 meters in height with grey bark and young shoots clothed with stellate hairs.

Distribution: Widely distributed from southern China to India (Common in Central and Western India as far west as Jammu), Burma, Malaya, South East Asia, Australia, West Indies, Central Peninsula

Parts used: Root, bark, and fruit

VERNACULAR NAMES

Sanskrit: Avarani, Mrigashinga
Hindi: Bhendu, Jonkphal, Kapasi, Maraphali, Marorphali, Marosi
English: East Indian screw-tree
Bengal: Antamora, Atemora
Bombay: Kawun, Kevana, Kewan, Khiran
Burma: Khungiche, Thuguaykhyae, Thungeche
Gujarati: Murdasing
Konkani: Kivani
Madras: Valambiri
Malayalam: Ishvaramuri, Kaivalanara, Kaiyuna, Valampiri
Marathi: Kewan, Muradsing
Persian: Kishtburkisht, Pechaka
Punjab: Kupasi, Marorphali
Tamil: Vadampiri, Valamburi, Valampuri, Valumberi
Telugu: Adasamanti, Adasyamali, Gubadarra, Gubalada, Kavanchi, Nulitada, Nuliti
Urdu: Marorphali
Leaves: Bifarious, 7.5-12.5 by 5-10 cm, oblong, obovate or roundish, cordate, suddenly and shortly acuminate, closely dotted on both surfaces with stellate hairs, more or less irregularly crenate-serrate.

Petiole: 6-9 mm long; stipules subulate, 6 mm long

Flowers: 2.5-3.8 cm long, distinctly bilabiate, in axillary clusters of 2-6 together; pedicles very short, stellately tomentose; bracts small, subulate, hairy. Calyx tubular, 2 cm long, somewhat 2-lipped, stellately pubescent without, curved, laterally compressed, mouth wide; teeth triangular, unequal. Petals red at first, fading to lead colour, very unequal, closely reflexed on the calyx, separate but with the claws closely hooked together. Staminal column fused with the gynophore, much exerted, suddenly deflexed; anthers 10, in a ring round the ovary. Ovary conical, on a curved gynophore 3.8 cm long; style as long as the ovary, deflexed.

Fruit: Greenish brown, 5 follicles, beaked, 5-6.3 cm long, linear, twisted together into the form of a screw, stellately tomentose.

Seeds: Numerous, angular; testa loose, wrinkled
Ethnopharmacology of *Helicteres isora*

- In India, dried bark and leaf of *H. isora* mixed with decoction of Gymnema sylvestre leaves are used in diabetes (Khan & Singh, 1996), while hot water extract of bark and seed or whole plant is also used for diabetes (Jain & Sharma, 1967).
- Fruits of *H. isora* are soaked in boiling mustard oil, cooled and rubbed on body of an infant with rickets (Girach et al, 1994).
- In India, fruits are also used to treat infantile diarrhea and earache (Singh & Ali, 1992; Jain, 1989).
- In Malaysia, dried fruit is eaten as an aphrodisiac in male while hot water extract of dried fruit is given to female after child birth (Burkill, 1966).
- Ramchandran and Nair (1981) reported the use of leaves of *H. isora* by natives of Cannanore district, Kerala for skin ailments including eczema.
- In Saudi Arabia, *H. isora* is also used as traditional medicine. Hot water extract of *H. isora* is used as antispasmodic, nervine tonic, antipyretic, antidysenteric, antiparalytic, antidiarrhoeal (Al-Yahya, 1986).
- In Rayalseema area of Andhra Pradesh, root powder is mixed with turmeric powder and applied externally for cuts and wounds (Nagaraju & Rao, 1990).
- Singh et al, 1984 reported the use of *H. isora* in empyema, snakebite, diabetes.
- In Thailand, fresh root is crushed and used as a poultice in inflammation (Panthong et al, 1986).
- In Karnataka, decoction of stem bark made with pepper is used for cough, throat infections (Bhandari et al, 1995).
- Fruits and roots are also used in polio and cholera by the tribal of Khunti area of Bihar (Topho & Ghosh, 2003).
Phytochemical studies of *Heliceteres isora*

- Barik et al (1981) estimated diosgenin (0.33 %) in free state as well as glycosidic form, and found not to be admixed with other steroid sapogenins like other sources of diosgenin.
- Singh et al (1984) isolated tetracontanol, β-sitosterol, and tetracontanoic acid from the leaves of *H. isora*.
- Satake et al (1999) reported the presence of 4'-O-β-D-glucopyranosyl rosmarinic acid, 4-4'-O-di-β-D-glucopyranosyl rosmarinic acid, 2R-O-(4'-O-β-D-glucopyranosyl caffeoyl)-3-(4-hydroxyphenyl) lactic acid (4'-O-β-D-glucopyranosylisorinic acid), and rosmarinic acid.
- Cytotoxic compounds like cucurbitacin B, and isocucurbitacin B were isolated from roots of *H. isora* (Bean et al, 1985).
- Tezuka et al (2000) isolated six neolignans, the helicetins, and elucidated their structures by spectral analyses. Helicetins are dimeric (7.5', 8.2')-neolignans with a bicycle (2.2.2) octene C-framework.
- Kamiya et al (2001) reported the presence of five flavonoid glucuronides form fruits of *H. isora* mainly isoscutellarein 4'-methyl ether 8-O-β-D-glucuronide, isoscutellarein 4'-methyl ether 8-O-β-D-glucuronide 6''-n-butyl ester, isoscutellarein 4'-methyl ether 8-O-β-D-glucuronide 2''-sulfate, isocutellarein 4'-methyl ether 8-O-D-glucuronide 2'', 4''-disulfate, isoscutellarein 8-O-β-D-glucuronide 2'', 4''-disulfate.
- Various triterpenes like α- amyrin, β- amyrin, bauerenol acetate, betulic acid, taraxerone, friedelin, friedelinol, isorin, lupeol, oleonolic acid are also reported to be present in *H. isora* (Dan & Dan, 1988; Qu et al, 1991).
Pharmacological studies of *Helicteres isora*

- Venkatesh et al (2003) studied the antihyperglycemic activity of *H. isora* roots in normal and alloxan-induced diabetic rats. Among various fractions (aqueous ethanol, chloroform, ethyl acetate, butanol, and left over aqueous) the butanol fraction was found to have maximum antihyperglycemic activity, while none of extracts was found to have antihyperglycemic activity in normal rats.

- Chakrabati et al (2002) reported that ethanolic extract of *H. isora* root caused significant reduction in plasma glucose, triglyceride, and insulin levels in insulin resistant and diabetic C57/BL/KsJdb/db mice. While in normoglycemic and mildly hypertriglyceridemic Swiss albino mice, the extract also showed significant reduction in plasma triglyceride and insulin levels, without affecting plasma glucose level. An ethanolic extract showed activity distinctly different from glibenclamide and acarbose but similar to troglitazone in these models. In high fat fed hamster model, the extract showed significant reduction in plasma lipid levels.

- Pohocha and Grampurohit (2001) reported the antispasmodic activity of *H. isora* in vitro on guinea pig ileum against acetylcholine, histamine, and BaCl₂. The antispasmodic activity was also studied in vivo by observing the gastrointestinal motility in mice.

- Dhawan & Saxena (1958) reported the uterine stimulant effect *H. isora* in rat.
3.2 PEPTIC ULCER DISEASE

Peptic ulcer disease refers to a group of disorders characterized by circumscribed lesions of the mucosa of the gastrointestinal tract (especially the stomach and duodenum). Peptic ulcers are chronic but may have acute exacerbation. They are most often solitary and occur at any level of the GIT exposed to the proteolytic action of acid pepsin. If acid and pepsin are diverted from an established ulcer, the ulcer will heal and not recur (Grossman, 1980). Therefore, a peptic ulcer may be broadly ascribed to one or both of the following factors; namely, an abnormality of acid and pepsin secretion and a predisposition of the mucosa to ulcerate when exposed to digestive juice. Treatment may be designed to reduce the acid secretion rate, neutralize the acid secreted, inhibit peptic activity, or to maintain the integrity of the mucosa.

Peptic ulcers have a distinct crater that is visible or can be scanned by radiological or endogenous examination of the upper GIT. Microscopic study reveals that these ulcers penetrate the muscularis mucosa and that acute and chronic inflammatory cell infiltrate surrounds the crater. Causes of ulcers are numerous but the end point in all peptic ulcers is disruption of the mucosa in the peptic ulcer disease, there is shift in the balance between mucosal damaging mechanisms and mucosal protecting mechanisms. Duodenal ulceration was found to be strongly associated with raised or high normal output of acid, however this relationship was not found to apply to gastric ulceration which was attributed to impaired mucosal resistance.

Factors that contribute the peptic ulcer disease can be classified as: (Dole, 1994)

Defensive factors:

(A) Gastric mucosal barrier

- Gastric mucosa
- Gastric and duodenal secretion of bicarbonate
- Chemical groups such as sulphhydryl groups
- Hydrophobic cell membrane
- Mucosal cell turnover or mucosal proliferation
- Mucosal restitution
- Gastric mucosal blood flow and angiogenesis
(B) Endogenous prostaglandins

(C) Others

- Gamma amino butyric acid (GABA)
- Secretin
- Somatostatin
- Cholecystokinin
- Epidermal growth factor
- Nitric oxide

Aggressive factors

(A) Endogenous factors

- Gastric acid secretion
- Pepsin
- Gastrin hypersecretion
- Gastrointestinal motility
- Free radicals

(B) Exogenous factors

- Aspirin and NSAIDS
- Smoking
- Helicobacter pylori infection
- Alcohol
- Caffeine
- Psychological stress

3.2.1 Defensive factors

Gastric mucosal barrier

The gastric mucosal barrier was one of the first defenses discovered and was originally described as simply the prevention of back diffusion of acid from the gastric lumen into the stomach wall.

(a) Mucus

- The gel produced by mucus cells protects against mechanical shearing forces, assists in transport of food particles, retain water near the mucosa and provides the unstirred layer that impedes diffusion of hydrogen ions. Mucus of gastric juice occurs
in two forms, visible mucus and dissolved mucus. The visible (insoluble) mucus can be separated by filtration or centrifugation while dissolved mucus can be separated by the addition of two volumes of acetone or alcohol to the gastric juice which can be estimated in terms of total carbohydrates and protein content of gastric juice (Ivy et al, 1950). The visible or insoluble mucus and soluble mucus are secreted by the surface epithelial cells of mucus membrane and chief cells lining the neck of fundic gland tubules respectively (Babkin, 1944; Illingworth, 1953). Mucus gives a uniform cover of water insoluble gel over the gastro duodenal mucosal surface. The thickness being 200μm in man (Allen et al, 1984). While readily permeable to both H+ and HCO3-, the mucus gel provides a stable unstirred layer on the mucosal surface in which HCO3- secreted by the epithelial cells is prevented from mixing with the bulk of the acid. Luminal acid is therefore neutralized before it reaches the mucosal cells. This in turn provides favorable microenvironment over the areas of gastric damage to allow re-epithelisation of mucosa. Mucus protects the epithelial cell against sudden osmotic changes and in stomach, it prevents the corrosive effect of acid and pepsin. This substance also provides some degree of chemical protection as it is alkaline and is capable of neutralizing considerable amount of acid (Davenport, 1966). The real barrier to acid-pepsin digestion is the mucosal barrier. A layer of mucus that consists of glycoproteins and mucopolysaccharide makes some contribution to the mucosal barrier.

**Individual components of gastric mucosal barrier**

A complete understanding of the biology of gastrointestinal mucus requires knowledge of its molecular components, their structure and behavior (Florey, 1962).

The various groups of protein and glycoproteins in gastric mucus have been characterized in dog fundic secretion (DeGraef, 1971; Fouad & Waldron-Edward, 1979).

(a) Proteins (Horowitz & Hollander, 1961; Degraef, 1971)
(b) Pepsin (Horowitz & Hollander, 1961)
(c) A sulphated amino polysaccharide (SPAS): Chondroitin-4-sulphate (DeGraef & Glass, 1968; Woussencolle & DeGraef, 1969)
(d) Acidic glycoproteins resistant to pepsin or papain digestion, precipitable with detergents like cetylpyridinium chloride and stained with basic dye like alcian blue (DeGraef & Glass, 1969). These glycoproteins are found mostly in the
visible mucus but small amounts are present as a coater soluble fraction (Hakkinen et al, 1965; Parmar et al, 1986). They contain 20% proteins and 80% carbohydrates, among which glucosamine, galactosamine, galactose, fucose, and sialic acid have been found. They also contain 1-45 sulphate residues per 100 hexosamines, and are thus classified as sulphated glycoproteins (SGP).

(e) Glycoproteins are resistant to pepsin digestion but not precipitated by detergents and are unstained faintly by basic dyes (DeGraef & Glass, 1969). These materials are similar in their general composition with the SGP but contain less or no sulphate residue or contain sulphate residue inaccessible to detergents or basic dyes. Like the SGP they are found mostly in the visible mucus.

(f) Unidentified dissolved glycoproteins and glycopeptides.

The viscous and gel forming properties of mucus secretion are derived from mucin glycoprotein constituents, which in adherent gastroduodenal mucus gel constitute about 5% (50 mg mucin/ml) by weight of the secretion (Bell et al, 1985; Allen et al, 1984). The mucus secretion also contains protein, lipid and nucleic acid, much of it is derived from exfoliated epithelial cells and bacteria. Mucins alone can account for the viscous and gel forming properties of the secretion. However, protein (Clamp & Creeth, 1984), lipid (Murthy et al, 1984), and nucleic acid (Mall et al, 1988) have been shown to enhance the viscous properties of mucin in vitro.

The chief cells secrete the SPASs, while the sulphated and at least parts of unsulphated glycoproteins are derived from the mucus cells of the surface and crypts (Gerard, 1968). Mucus cells of the neck of fundic glands and some parietal cells secrete neutral mucosubstances.

Water-soluble mucus has got three-dimensional structure. The water-soluble mucus can be separated by gel filtration into high and low molecular weight mucoprotein components A and B respectively. These mucoproteins have a central protein core with carbohydrates side chains attached giving a ‘bottle brush’ structure (Clamp et al, 1978). This polymeric structure consists of glycoprotein subunits joined by disulfide bridges from their protein cores. These protein cores are composed of glycosylated protease resistant regions and non-glycosylated regions, which are essentially carbohydrates free, contain the inter-chain disulfide bridges and are
susceptible to attack by proteases. The linkages between carbohydrate and protein in the mucus glycoprotein is O-glycosidic and the monosaccharide involved in N-acetyl-galactosamine that is attached to either serine or threonine. Native human mucus glycoprotein has a high molecular weight of about two million dollars and is formed by polymerization of four glycoprotein subunits joined by disulfide bridges. The carbohydrates side chain comprise over 80% by weight of the glycoprotein molecules and their presence is compatible with the high degree of hydration essential for the special rheological properties of the molecule (Goel and Bhattacharya, 1991).

The mucus gel layer is a complex secretion containing number of substances for eg. Inorganic material, proteins including specific proteins such as lysosome, secretory IgA, Lactoferrin and so on and high molecular weight glycoprotein. The latter component, mucin or mucus glycoprotein confers on mucus its characteristic properties and is generally considered to be the most important single factor in mucosal protection.

Mucus consists of glycoproteins synthesized by goblet cells, protein mucopolysaccharide and blood group substances (Horowitz, 1967). It consists of 1% by weight of salts and other dialyzable components, 0.5-1% of free proteins and similar quantum of carbohydrates rich glycoprotein and 95% or more of water (Goel & Bhattacharya, 1991). The glycoprotein molecules have got a protein chain to which several disaccharide side chains are attached. Depending on the presence of sialic acid residue glycoprotein may be acidic or neutral. The principle glycoproteins may be acidic or neutral. The principle glycoproteins of human gastric juice contain carbohydrate components composed of mainly galactose, galactosamine, glucosamine, fucose and proteins which contains chiefly the amino acids serine, threonine, praline, and alanine (Schrager, 1969; Allen and Snary, 1972). There are approximately 600 carbohydrates side chains per molecule of glycoprotein. Altered synthesis of hexosamine containing compound have been shown to influence gastroduodenal ulcer (kent, 1962). Factors that impair the integrity of this mucus barrier form a vital link in the pathogenesis of gastric ulceration.

The importance of altered mucosal defense mechanism in duodenal ulcer patients is controversial. The concentration of the duodenal mucosal prostaglandin 6-keto-PGF is actually increased in patients with duodenal ulcers, as is the luminal secretion of PGE. Both of these prostanoids have cytoprotective effects, and then secretion may be altered as a response to ulceration. Data implying the importance of
other cytoprotective factors in the cause of duodenal ulcer are less convincing although this may be due to the difficulty in assessing these factors (Perry & Molinoff, 1990).

The mucus gel protects against mechanical shearing forces, assists in transport of food particles, retains water near the mucosa and provides an unstirred layer that impedes diffusion of hydrogen ions (Goel & Bhattacharya, 1991). Mucus protects the epithelial cell against sudden osmotic changes and in stomach, it prevents corrosive effect of acid and pepsin. This also provides some degree of chemical protection as it is alkaline and is capable of neutralizing considerable amount of acid before it reaches the mucosal cells (Davenport, 1966).

(b) Gastric and duodenal secretion of bicarbonate

Bicarbonate secreted into the mucus gel helps to maintain a neutral pH immediately adjacent to the mucosa in spite of the low luminal pH in the stomach. Results with different techniques indicate that bicarbonate secretion by intact gastric mucosa is a metabolism dependent process. Uptake of bicarbonate at the serosal membrane of the gastric surface epithelial cells depends on the maintenance of a transmembrane Na⁺ gradient and it may occur by NaHCO₃ co-transport. The latter co-transport has been suggested to be part of the acid-base transport process of duodenal mucosa (Simson et al, 1981; Isenberg et al, 1993) and in a small intestinal cell line. Transport of HCO₃⁻ across the apical epithelial membrane very probably occurs by Cl⁻ /HCO₃⁻ exchange. The inhibition of the gastric alkaline secretion in vitro (Flemstrom, 1987) by acetazolamide may reflect inhibition of intracellular hydratin of CO₂ or possibly an effect on membrane translocation of HCO₃⁻.

Duodenal secretion of bicarbonate is an important aspect of duodenal mucosal defense. Proximal duodenal bicarbonate secretion per unit of surface area is nearly five times greater than in gastric mucosa and nearly twice that seen in the distal duodenum. Experimental models of duodenal ulceration induced in the rat with cysteamine or mepirizole are associated with reduced secretion of alkali (Flemstrom, 1987). The existence of an active gastric and duodenal bicarbonate secretion creates a pH-gradient from the luminal acid to near neutrality at the surface of the epithelial cells thereby acting as an important defense mechanism. Bicarbohydrate is secreted throughout the stomach by surface epithelial cells, which contains large amounts of
carbonic anhydrase. As these cells also secrete mucus, a high pH mucus-bicarbonate blanket covers the gastric epithelium (Heatley, 1959).

As luminal hydrogen ion diffuses toward this blanket, hydrogen ions are converted to CO$_2$ and H$_2$O by carbonate ion. Furthermore, as pepsin diffuses toward the mucosa, its activity declines as the pH in the microenvironment near the surface of the mucosa approaches neutrality.

(c) Chemical group such as sulphydryl groups

Non-protein sulphydryl compounds (SC) are abundantly present in gastric epithelium. Reduced glutathione, the major component of sulphydryl compounds is capable of binding reactive free radicals which accumulate during tissue ischaemia and injury induced by noxious agents like alcohol. Reduced glutathione induces gastric mucosal protection by increasing SC levels. SC blocking agents can reduce the cytoprotective effect of prostaglandins in stomach. The precise role of SC in gastroduodenal mucosal protection remains unelucidated, however, they appear to be involved in prostaglandin synthesis and prostaglandin receptor activation, and it may also directly influence membrane permeability, cell release and effects of mediators likely to be involved in inducing mucosal damage (Szabo, 1984).

(d) Hydrophobic cell membrane

Liposomal suspensions of surface-active phospholipids significantly protected the gastric mucosa from acid induced gastric damage in rats (Lichtenberger et al, 1985). One of the important properties of the stomach wall is its hydrophobicity inspite of its hydrophobic mucoid layer. This is attributed to the surface active phospholipids (SAPs) present largely as the intergranular matrix material of unsecreted mucus, which in any case provides the major resistance to hydrogen ions diffusing from the lumen of the stomach to the vital organelles of surface mucus cells. Stress ulcers are associated with a change in the lipid profile of gastric mucosa (Slomiany et al, 1975). While each of the barrier breakers display greater affinity for SAP. Bile salts chemically complex with SAPs while NSAIDs inhibit the production of prostaglandin controlling SAP synthesis (Allen et al, 1984).
(e) **Mucosal cell turnover or mucosal proliferation**

The rapid proliferation of the gastric mucosa plays an important role in mucosal protection during normal state and following mucosal damage. In the latter situation, the undifferentiated neck cells proliferate, migrate towards the lumen and differentiate into surface epithelial cells. Other cells migrate downwards to the depth of the gland and chief cells (Goel and Bhattacharya, 1991).

(f) **Mucosal restitution**

The process of restitution involves rapid re-epithelisation characterized by the migration of remaining viable surface mucus cells and mucus neck cells from the crypts to cover the damaged surface. The morphological healing is accompanied by physiological return of function characterized by concomitant flux of bicarbonate ions across the epithelium. This restitution process is inhibited by luminal pH below 4. Prostaglandins do not appear to be involved in restitution (Svanes et al, 1984).

(g) **Gastric mucosal blood flow and angiogenesis**

Gastric mucosal blood flow protects the mucosa by ensuring the delivery of optimum quantum of oxygen, nutrient and bicarbonate to surface epithelial cells and removing H+ ions which have permeated the mucus-bicarbonate and epithelial barrier (Rosam et al, 1986). Angiogenesis, the formation of new blood vessels is a tightly regulated component of normal growth and wound healing. It prevents the mucosal damage by ulcers and maintains blood flow. Uncontrolled angiogenesis is a driving force in the growth of solid tumors (Mitchell & Wilks, 1992).

(h) **Endogenous prostaglandins**

Endogenous prostaglandins (PGs) are important in the defense of the gastric mucosa (Goel & Bhattacharya, 1991). In the past they were classified as ‘cytoprotective’ to describe the ability of PG to augment gastroduodenal mucosal resistance to injury, independent of acid secretion inhibition. PGs are present in large quantity in the mucus membrane of fundus, body and pylorus of stomach and duodenum (Konturek, 1981). PGE1 and PGE2 inhibit basal histamine and pentagastric stimulated acid secretion in human subjects and other species (Becker et al, 1973).
PGs enhance the mucosal resistance to injury under certain conditions perhaps by increasing blood flow (Gaskill et al, 1982), stimulating secretion of mucus and bicarbonate (Hogan et al, 1994), strengthening the gastric mucosal barrier, decreasing the gastric motility, increasing the release of endogenous mediators of gastric cytoprotection such as sulphhydryls and epidermal growth factor (Szabo et al, 1981), scavenging of free radicals (Szabo, 1984), decreasing release of endogenous mediators of gastric injury (vasoactive amines and leukotrienes) and stimulation of cellular growth and repair (Hawkley & Ramtin, 1985).

OTHERS

(a) Gamma amino butyric acid (GABA)

CNS mechanism are important in the regulation of acid secretion, GABA and GABA mimetics have an ulcer attenuating effect not associated with central sedation or decreased acid secretion but could be through augmenting gastric mucosal defense (Goel & Bhattacharya, 1996).

(b) Secretin

Secretin inhibits gastrin but not histamine-stimulated gastric secretion in Heidenhain pouch dogs (Gillespie & Grossman, 1964). A dose-response study to gastrin and to gastrin plus a fixed dose of secretin, on gastric acid response in Heidenhain pouch dogs showed a non-competitive inhibition of secretion by gastrin. This particular result is interpreted as evidence that secretin acts at a receptor site different from on affected by gastrin (Johnson & Grossman, 1969). In humans, secretin inhibits the gastric secretory response to a low dose of pentagastrin and histamine but not to a high dose of pentagastrin or histamine (Chey et al, 1970). Direct cholinergic stimulation of the parietal cell is not inhibited by secretin (Way, 1970). Secretin is a GI hormone released from the mucosal cells of the duodenum. It stimulates the pancreatic secretion, flow of bile and pepsin secretion. But it inhibits gastric acid secretion and motility.

(c) Somatostatin

Its site of occurrence is in the hypothalamus, gastric mucosa and pancreatic islets. It is also present in nerves of GIT. Its function is to inhibit gastric and pancreatic secretion and motility. Somatostatin inhibits the release of growth hormone
and thyrotrophin from the anterior pituitary and insulin and glucagon from the pancreas, and decreases the release of most GI hormones. Octreotide a long-acting analogue of somatostatin can be of use in pancreatitis and in bleeding from esophageal varices and gastric bleeding due to stress gastritis (Rang et al, 1995b). Somatostatin acts locally to regulate secretion of acid by decreasing the secretory capacity of the parietal cell and by decreasing the amount of gastrin released from antral G cells (Berne & Levy, 1988).

(d) Cholecystokinin (CCK)

CCK is the hormone responsible for contraction of the gall bladder. CCK is elaborated in the duodenum in response to food. It supplements the action of secretin to produce alkaline pancreatic juice and retards gastric emptying (Guyton, 1986).

(e) Epidermal growth factor

It appears that epidermal growth factor (EGF) is specially adapted for ulcer healing. EGF has been shown to be identical to urogastrone (URO) and is now designated as URO/EGF. URO/EGF promotes wound healing accelerates peptic ulcer healing and augments crypt cells production rate in the gastrointestinal tract. It stimulates DNA synthesis and the uptake of amino acids by skin fibroblasts. It is also a potent inhibitor of gastric secretion (Sakamoto et al, 1985).

(f) Nitric oxide

Nitric oxide is concerned with vagus nerve and mediate relaxation of stomach, ileocolic junction, internal anal sphincter and the peristaltic contraction wave. It may also be associated with gastric ulceration (Rastogi et al, 1995).

3.2.2 Aggressive factors

Endogenous factors

(a) Gastric acid secretion

Increased gastric acid secretion has been believed to be the major cause of peptic ulceration and presently the antisecretory drug like H₂ blockers and proton pump inhibitors are the major groups of drugs used in the treatment of peptic ulceration.
Patients who develop gastric ulcers in the body or fundic mucosa may have low peak acid secretory capacity. In fact, basal acid secretion, nocturnal secretion, titrable acid secretin in response to meal, and maximal acid output as determined by histamine stimulation are all lower in patient with ulcers above the angularis when compared with the rates of secretion in healthy controls. Similarly, a decreased density of gastrin secreting cells in the antrum and reduced circulating levels was seen in patients undergoing antrectomy for gastric ulcer when compared with controls (Perry and Molinoff, 1990). The common causative factor in duodenal ulceration is an increased delivery of acid to the duodenum. An increase in acid secretion both in basal state and in response to stimulants has been described in patients with duodenal ulcers. Basal acid secretion is variable, but is most consistently elevated at night. This nocturnal hypersecretion of acid in duodenal ulcers has important therapeutic implications. Maximal acid secretion is variable, even among duodenal ulcer subjects. When duodenal ulcer patients are compared with controls, there is a significant increase in acid secretion in the ulcer patient group. The presence of increased acid secretion in both the cephalic phase (Sham feeding and insulin hyperglycemia) and gastric phase (gastric distension) of acid secretion suggests that an increased parietal cell mass is present in many patients (Richardson, 1983).

(b) Pepsin

In the presence of sufficient acid to activate the conversion of pepsinogen to pepsin, the proteolytic activity of the pepsin is enhanced. Several lines of evidence suggest that there is also hypersecretion of pepsinogen in some patients with duodenal ulcer. These patients have increased circulating levels of pepsinogen, increased levels of pepsinogen in gastric secretions and an increased rate of pepsin secretion that parallels hypersecretion of acid (Hersey, 1987).

(c) Gastrin hypersecretion

Hypersecretion of acid and pepsin may be due to the increased activity of gastric in patients with duodenal ulcer disease. The peak level and time integrated serum concentration of gastrin is elevated in these patients even in absence of gastrinoma or gastric surgery. In light of these, frequency of acid hypersecretion is significant, suggesting that feedback inhibitory systems are impaired. The inhibition
of gastrin secretion in response to somatostatin is less in ulcer patients than in control (Arnold, 1974).

(d) Gastroduodenal motility

The importance of abnormal gastroduodenal motility in the development of gastric ulcers also distinguishes gastric concentration of bile salts in the basal state and after a meal is increased in patients with gastric but not duodenal ulcers. Pyloric sphincter pressures are low, and the pyloric contractile response to duodenal acidification is impaired in patients with gastric but not duodenal ulcers. Thus, gastric ulcers may be the result of the reflex of duodenal contents on to an atrophic gastric mucosa with impaired acid secretory capacity.

Motility factors, particularly an enhanced rate of emptying of liquids, have been suggested to play an important role in the development of duodenal ulcers. In several studies the rate of acid delivery to the duodenum has been shown to be enhanced, although the results from other studies suggest that the importance of motility in the development of ulcers is not well resolved. Pyloric sphincter pressures are similar in duodenal ulcer subjects and controls (Wormsley, 1974).

The specific agent responsible for mucosal damage is not known, although most studies implicate bile salts. Bile salt conjugates have been shown to have the capacity to disrupt the mucosal barrier. These motility abnormalities are an important contributory factor in many patients with gastric ulcers in that they permit the retrograde movement of duodenal contents, primarily bile salts (Perry & Molinoff, 1990).

(e) Free radicals

Acute inflammation is a complex process characterized by vascular permeability changes with interstitial edema and neutrophil infiltration of a tissue with resultant production of oxygen radicals. Recent studies in rat showed that oxygen derived free radicals are directly implicated in the mechanism of acute and chronic gastroduodenal ulceration and that scavenging them stimulates the healing of ulceration (Salim, 1990).
Exogenous factors

A large number of therapeutic agents when ingested irritate the stomach. Aspirin is being at the top of the list. Even agents in this group include multivalent cations (such as iron and calcium), antibiotics (such as erythromycin and tetracyclines) and CVS medications (such as reserpine and quinidine).

(a) Aspirin and NSAIDs

Gastric erosion develops when therapeutic doses of aspirin are ingested on a regular basis. Both aspirin and NSAIDs inhibit the synthesis of prostaglandins, which have an important role in normal mucosal defense and mucus secretion. Aspirin decreases epithelial permeability across the mucosa (Perry & Molinoff, 1990).

(b) Corticosterone

A convincing association between corticosteroids and peptic ulcers disease is difficult to substantiate. Corticosteroids decrease the mucosal barrier and increase acidity. Steroids reduce the rate of shedding of gastric mucosal cells by decreasing the rate of cell renewal. Steroids potentiate the development of ulcers from other causes (eg. Aspirin ingestion) and impair normal defense mechanism (Maudlin, 1980).

(c) Smoking

Smoking has an unequivocally adverse effect on the epithelium. Smoking increases the risk of developing a duodenal ulcer by five-fold than the non-smokers. Smoking also significantly reduces the probability of spontaneous ulcer healing and perhaps the rate healing in the response to treatment (Perry & Molinoff, 1990).

(d) Helicobacter pylori infection

Infection with Helicobacter pylori has now been accepted as the cause of the overwhelming majority of cases of non-immune gastritis. H. pylori are gram –ve, spiral, microaerophile organism. It spreads by oro- oral, gastro- oral, faecal- oral routes. H. pylori can colonize in gastric epithelium. The organism is adapted to its ecological niche, resting on the surface of gastric epithelial cells beneath the layer adherent mucus. The spiral shape and motility conferred by its flagella may help to distribute it over the gastric mucosa. Scanning electron micrograph shows striking evidence of the damage to the gastric epithelium produced by H.pylori.
H. pylori display potent urease activity, a property that may have important pathogenic implications (Eaton et al, 1990). Urease hydrolyses urea to ammonia, which is cytotoxic and provides alkaline environment around the microorganism, due to which H. pylori survives in the harsh environment of the stomach. Also a consequence of generating ammonia, it may disturb normal negative feedback of acid to antral G cells (Levi et al, 1989). H. pylori also produces catalase, proteases, lipases, phospholipase, adhesins, and toxins that may reduce important mucosal defense by degrading mucus layer and damaging the lipid containing epithelial cell membrane.

Infection with H. pylori produces important abnormalities in the secretion of GI hormones from the gastric antrum and these abnormalities have far reaching consequences on gastric pathophysiology and the cause of ulceration. H. pylori infection is associated with increased serum gastrin concentration. H. pylori have been shown to induce histamine release from human mast cells by an IgE mediated reaction. In addition to this, the inflammatory response to H. pylori itself may release histamine from mast cells. Moreover, it has also been reported that H. pylori express an enzyme that produces a potent agonist action at H3- receptors (N-methyl histamine). H3- receptors are involved in regulation of acid and somatostatin and exert negative feedback on histamine synthesis and release. Thus, production of this agonist by H. pylori may lead to decreased histamine synthesis (which might account for the low antral histamine content found in H. pylori positive patients with duodenal ulcer) and an inhibitory effect on the somatostatin content of antral D cells.

There are a number of alternative methods for proving the presence of active Helicobacter pylori whose results correlates reasonably well where gastroscopy is being performed anyway. Barium meal method is also used very frequently that coats the craters or holes of an ulcer and shows them as white spots on the X-rays. This procedure is painless and no anesthetic is needed. However, barium meal method is less accurate than endoscopy in about 30% cases they provide an incorrect diagnosis. It can also not detect the presence of H. pylori. Rapid urease test (CLO-test), polymerase chain test (PCR), 14C- urea breath test, culture of biopsy material, staining of antral biopsies and recently IgG ELISA serology tests is also used for rapid detection (Schrader et al, 1993; Missewice & Harris, 1997).
(f) Alcohol

High concentrations of alcohol causes denuding of the superficial mucosal cells extending to the midfundic glands, resulting in intra-mucosal haemorrhage, increased permeability and to a lesser extent an inflammatory infiltrate. Alcohol is a contributing factor in over one third of patients presenting with acute upper GI hemorrhage. Further gastric erosions caused by ethanol have been attributed to free radical damage, which results due to lipid peroxidation products (Salim, 1990).

(g) Caffeine

Caffeine acts synergistically with histamine (but not pentagastrin) to stimulate acid secretion. It also enhances the secretion of pepsin (Perry & Molinoff, 1990).

(h) Psychological stress

Of the common acid peptic diseases, none has a greater risk of leading to severe morbidity or mortality than does stress-related mucosal disease (SRMD). These lesions are characterized by the presence of superficial haemorrhage and ulceration involving the gastric mucosa of patients with multisystem disease, shock or sepsis. In contrast to other peptic ulcer disease, pain occurs uncommonly in most patients present with signs of bleeding. Gastric mucosal injury in patients is found who are under stress because of serious injuries, CNS, trauma or burns etc.

Several features distinguish the mucosal lesions in patients under extreme of physiologic stress from those who have the more typical chronic peptic ulcer disease. These lesions occur in a setting of trauma, shock, burn, sepsis, and multiple organ failure in patients with no previous history of ulcer disease. Stress lesions tend to be multiple in contrast to the one or two lesions typically found in patients with duodenal or gastric ulcers. SRMD is a diffuse mucosal abnormality that occurs throughout the stomach, although it is predominantly in the acid-secreting mucosa. In contrast, typical peptic ulcers occur in the non-acid secreting mucosa, the gastric antrum, and the duodenum. SRMD is a superficial mucosal lesion involving the upper half of the gastric glands in contrast to typical peptic ulcers that can penetrate through the intestinal wall. Perforations are distinctly uncommon in SRMD but occur in 1% to 2% of patients with gastric and duodenal ulcers when bleeding occurs. In SRMD, therefore only the venous capillaries are involved in contrast to the layer vessels involved with other peptic ulcers (Robert & Kauffman, 1983). The overall outcome in
patients with SRMD is dependent primarily on the outcome of their underlying disease. Once the acute catastrophe has been cleared, the mucosal injury repairs itself without sequellae. In contrast to typical gastric and duodenal ulcers, there is no tendency toward recurrence in the absence of recurrence of the underlying disease (Robert & Kauffman, 1983).

The specific mechanisms involved in the development of SRMD are incompletely understood, but most likely involve multifactorial impairment of mucosal defense system. Key to initiation of this lesion is the reduction in mesenteric blood flow that accompanies systemic hypotension and shock. The decrease in gastric mucosal blood flow results in localized anoxia and ischaemic changes. This in turn reduces the ability of the gastric epithelium to undergo the active metabolic processes necessary for mucus and bicarbonate synthesis, cell turnover, and the maintenance of mucosal ionic pumps needed to maintain mucosal integrity (Perry & Molinoff, 1990).

During stress changes in plasma corticosterone and gastric mucosal integrity are widely reported and it seems that peripheral and central mechanisms regulate these changes (Maudlin, 1980). Much interest has recently been generated on the immunological changes during stress with reports indicating that the immune status of the organism is actually modified by experimental stresses. The CNS, besides crucial for stress, also regulates immune function and studies show that common neural substrates like the hypothalamus are clearly involved in such CNS-immune system interactions.

Neuropharmacological data have shown that complex neurochemical mechanisms regulate stress responses and transmitters like GABA and endogenous opiates are crucially involved. For e.g. benzodiazepines which modulate GABA and opioid antagonists modify several stress responses like gastric ulcer formation and plasma corticosterone (Perry & Molinoff, 1990).

3.2.3 Role of gastric mucosal blood flow (GMBF) in affording gastroprotection

In 1856 Virchow suggested that aseptic vascular occlusion of small nutrient vessels in the mucosa or submucosa produced by spasm, thrombosis, embolism or endocarditis might be the cause of localized necrosis and subsequent ulcer formation in stomach. Because of the funnel shape of the ulcer lesion, Hauser (1883) thought it resembled anatomically an infarcted area, caused possibly by an occlusion of one of the small arteries as it passed from the submucosa to the mucosa. A number of
substances which produced embolisation of the smallest vessels (fat (Baronsnofsy et al., 1945), or drugs capable of constricting the first blood vessels, such as large doses of adrenaline (Friedman, 1915) or vasopressin (Nedzel, 1938)) can produce acute lesions of the stomach and duodenum. Such lesions, however, usually heal rapidly. Serotonin may also be playing a part in the peptic ulcer by influencing local mucosal blood flow. Both serotonin and its precursor, 5-hydroxytryptamine (Haverback & Bogdanski, 1957) have been shown to produce ulceration in the glandular portion of the rat in spite of the fact that they also reduce gastric secretion (Shay et al., 1959).

The stomach, like all organs of the body, requires an adequate blood flow to maintain its structural and cellular integrity and to conduct its physiological function. The severity of mucosal damage was inversely dependent on mucosal blood flow if all other conditions were kept constant. Because of the extensive metabolic requirements for the elaboration and secretion of hydrogen ions from the parietal cell, gastric mucosal blood flow can be a prime factor in the regulation of gastric secretion and protection of mucosa. Mucosal blood flow, tissue integrity and mucosal defense are interrelated in the maintenance of intramucosal acid-base neutrality (Kivilaasko et al., 1978). The importance of mucosal blood flow in the defense of the gastric mucosa against injury has been demonstrated in haemorrhagic shock models (Starlinger et al., 1981; Leung et al., 1985). When the mucosal epithelial cell layer is intact, minimal level of the hydrogen ions secreted into lumen diffuse back into the gastric tissue. At a luminal pH 2 or above, bicarbonate mucus secretion from surface epithelial cells from an unstirred layer is buffering such acid back diffusion (Flemstrom, 1987). Any remaining hydrogen ions, that gain entry tissue are buffered by the bicarbonate derived from blood or from the alkaline tide generated by parietal cell activity are also removed by microcirculation (Whittle, 1993).

There is increasing evidence to suggest that these be among the first steps in the pathogenesis of gastric ulceration although it remains debatable whether capillary damage and local ischaemia at the site of ulceration are the cause or the consequence of mucosal injury. This not only applies in the case of lesions induced by chemical ulcerogens (Szabo, 1987), but possibly also with more physiologically relevant stress induced lesions. Local molecular weight phospholipids, platelet activating factor (PAF), which reduces mucosal blood flow and cause haemorrhagic erosions in the gastric mucosa (Rosam et al., 1986), greatly potentiates mucosal damage brought about by low intraluminal concentrations of ethanol or bile salts. Thus, any limitations
of microvascular blood flow can greatly enhance the susceptibility of the mucosa to disruption by topical irritants as well as itself provoking mucosal injury.

Gastric damage which results inadequate vascular perfusion may reflect the period of relative anoxia and hence disturbances in cell metabolism and integrity. Direct evidence supporting the critical importance of an intact mucosal microcirculation in the maintenance of mucosal integrity is provided by findings that microvascular congestion and thrombosis provokes mucosal ulceration in rat stomach (Rosam et al, 1986). However, such damage may also involve the subsequent local release of tissue damaging mediators, among which oxygen derived free radicals are likely candidates. Free radicals are generated in the gastric mucosa and other tissues during reperfusion following ischaemia. Direct evidence for their role in the pathogenesis of acute gastric ulceration is provided by the finding that local intra-arterial infusion of the naturally occurring oxygen-radical generating system, hypoxanthine/xanthine oxidase, caused gross mucosal damage in the stomach even in the absence of luminal acid (Stein et al, 1989). Preventing their formation by the xanthine oxidase inhibitor allopurinol or enhancing disposal of free radicals with scavengers such as superoxide dismutase and dimethyl sulfoxide alleviates the mucosal damage provoked by haemorrhagic shock (Smith et al, 1987).

3.2.4 Neuronal mediators in gastric mucosa

Neuronal derived vasoactive mediators play a strong influence in the process underlying damage and protection of gastric mucosa. Local neuronal activity could provoke the inappropriate release of vasoconstrictor mediators such as adrenaline or neuropeptide Y (NPY), while stimulation of sensory neurons release the neuropeptide calcitonin gene-related peptide (CGRP) involved in protective vasodilatation. Pathological events that enhance the neuronal release of CGRP and possibly other protective sensory neuropeptide would thus be expected to lead to mucosal injury (Whittle, 1993).

An increase in gastric mucosal blood flow following vagal stimulation has been observed to precede the secretion of acid, indicating a direct vasodilator action (Guth & Smith, 1975). The gastric vasodilatation induced by vagal stimulation can be blocked by hexamethonium and reduced but not abolished by atropine in doses sufficient to abolish the response to acetylcholine (Kitagawa et al, 1987). This could
suggest the release of a vasodilator mediator other than acting on non-muscarinic sites following vagal stimulation.

Local nor-adrenergic, not-cholinergic (NANC) neuronal process within the gastric mucosa modulates its ability to withstand noxious challenge. Thus, local infusion of tetradoxin (through the left gastric artery), which did not itself induce gastric mucosal injury, substantially potentiated the haemorrhagic damage following local administration of (platelet aggregating factor) PAF (Espluges et al, 1989) suggesting the involvement of NANC neuronal pathway. Further, local infusion of tetradoxin also potentiated the mucosal injury induced by intraarterial administration of a vasoconstrictor thromboxane mimetic as well as that brought about by intragastric application of acidified ethanol, again indicating the involvement of a local neuronal mechanism in the regulation of mucosal integrity.

CGRP acts as endogenous vasoactive mediator involved in the regulation of gastric mucosal blood flow and integrity. Activation of sensory neurons following acid back diffusion induced by intragastric application of acid, ethanol is associated with protective gastric mucosal hyperaemia. The release of vasodilator neuropeptide CGRP from afferent sensory neurons has been suggested to be a protective mechanism in gastric mucosa. A pungent extract of red pepper capsaicin, was found to deplete functional ablation (Green & Dockray, 1988). In addition it is reported that capsaicin pretreatment, which itself did not injure the mucosa, enhanced mucosal damage following a number of proulcerogenic procedures including acid distension and pylorus ligation as well as challenge with indomethacin, ethanol and PAF (Espluges et al, 1989). The predominant neuropeptide localized by immunohistochemical techniques in capsaicin sensitive neurons in the rat stomach is CGRP and such neurons are found in close proximity to the submucosal microvasculature. This neuropeptide occurs principally in the form of α-CGRP in the sensory neurons innervating gastrointestinal tissue; local infusion of CGRP prevented the vascular and haemorrhagic injury by intra arterial infusion of endothelin-1. Intravenous administration of higher dose of CGRP increased blood flow in the rat and rabbit stomach. Moreover, intra-arterial infusion of α-CGRP in rat increased resting mucosal blood flow. Thus, CGRP has the profile of actions compatible with its proposed role as an endogenous vasoactive mediator involved in the regulation of gastric blood flow and integrity (Whittle, 1993).
In the gastrointestinal tract, NPY like immunoreactivity has been found in sympathetic nerves with blood vessels and in enteric neurons originating from myenteric and submucosal plexus. Investigations on the possible physiological role of NPY in the gastrointestinal tract have included studies on its effects on non-vascular smooth muscle tone. NPY induced vasoconstriction in the vascular bed of the spleen, small and large intestine. In addition, local intra-arterial infusion of NPY reduces rat gastric mucosal blood flow. NPY, like noradrenaline affects the gastric microcirculation by actions directly on gastric vascular smooth muscle, but through activation of a distinct receptor type (Whittle, 1993).

3.2.5 Endothelium-derived factors and gastric ulcers

The endothelium is a rich of local vasoactive mediators capable of modulating the tone and integrity in microcirculation. Thus the local release of the vasoconstrictor endothelin-1 (ET-1) may be initial event in some forms of mucosal injury (Peskar et al, 1992) or be released as a consequence of endothelial injury thus augmenting and perpetuating the original insult (Morales et al, 1992) which could be by stimulating formation of PAF along with the activation of the fibrinolytic system (Kurose et al, 1992). Capsaicin pretreatment substantially elevated mucosal damage provoked by ET-1, while close arterial infusion of CGRP inhibited the damage. Interactions between CGRP and ET-1 in the mucosal microcirculation are not only due to opposing vasoactive properties but may also reflect more complex event involving release of local mediators and actions as the continuity of the vascular endothelium (Whittle & Lopez-Belmonte, 1991).

Nitric oxide

Endothelin cells also release another highly labile humoral vasodilator substance nitric oxide (NO), originally known as endothelium derived relaxing factor (EDRF) that mediates the vascular relaxation induced by agents such as acetylcholine and bradykinin. Endogenous NO plays an important role in the modulation gastric mucosal blood flow (GMBF) associated with acid secretion under resting and stimulated conditions and hence in the regulation of gastric mucosal integrity, regulation of vascular tone and neurotransmission.

Critical interaction exists between endogenous NO, sensory neuropeptides and prostanoids, all of which appear to subserve modulation function in the regulation of
gastric mucosal integrity (Whittle et al, 1990). There could be a partial dependence upon endothelial NO for the vascular relaxation induced by CGRP since CGRP is an endothelin dependent vasodilator in some vascular beds (Brain et al, 1985). Alterations in the balance between these vasodilator mediators may thus be implicated in the pathogenesis of peptic ulcer disease.

Cromoglycate pretreatment affords gastric mucosal protection against ethanol induced damage by the maintenance of a critical level of endogenous NO levels, prevention of depletion of non-protein sulphhydrals, maintenance of GMBF and stabilization of mast cells (Mobark Ali, 1995). Thus interplay between these diverse endogenous mediators at the level of endothelial barrier in the microvasculature is involved in preserving the endothelial tissue integrity which in turn helps to maintain adequate microvascular blood flow. The regulation of microvascular tone and integrity is thus of critical importance for the conduct of the physiological responses of the stomach and in the prevention of mucosal injury by both endogenous and exogenous aggressors.

**Prostacyclines**

The synthesis of the labile vasodilator cyclo-oxygenase product prostacycline (PGI$_2$) from the fatty acid precursor, arachidonic acid was originally identified in endothelial cells. Prostacyclines exerts protective actions on gastric mucosal damage in a number of experimental models (Whittle et al, 1981). Increase in local blood flow by prostacycline would be beneficial in maintaining the functional integrity of gastric tissue and defense mechanisms, especially under conditions of relative ischaemia in the mucosa (Whittle & Vane, 1987). Prostacycline increased the ratio of blood-flow to acid output during intravenous infusion of antisecretory doses in the conscious dog. Protection of the gastric microvasculature or cytotoxic mediators may be important mechanisms underlying the so called cytoprotective actions of prostacyclins and other prostanoids (Brughton-Smith & Whittle et al, 1978).
3.3 MECHANISM OF HEPATOTOXICITY

Drugs continue to be pulled from the market with disturbing regularity because of late discovery of hepatotoxicity. Such unexpected toxicities appear to be the consequences of the unique vascular, secretory, synthetic, and metabolic features of the liver. About 75% of hepatic blood comes directly from the gastrointestinal viscera and spleen via the portal vein. Portal blood brings drugs and xenobiotics absorbed by the gut directly to the liver in concentrated form. Drug-metabolizing enzymes detoxify many xenobiotics but activate the toxicity of others. Hepatocytes are highly reliant on ATP for ureagenesis, gluconeogenesis, and fatty acid metabolism among many other metabolic processes. In fasted individuals with low hepatic glycogen content especially, hypoxia, mitochondrial inhibition and damage to mitochondrial DNA lead to hepatocellular necrosis.

The liver synthesizes, concentrates, and secretes bile acids and excretes other toxicants, such as bilirubin. Drug-induced injury to hepatocytes and bile duct cells can lead to cholestasis. Cholestasis, in turn, causes intrahepatic accumulation of toxic bile acids and excretion products, which promotes further hepatic injury. Fortunately, the liver has enormous regenerative capacity, but regeneration of hepatocytes lost by necrotic and apoptic cell death may mask detection of drug-induced injury. Furthermore, the active proliferative response of hepatocytes makes the liver an important target of carcinogens.

Hepatic nonparenchymal cells, the Kupffer, sinusoidal endothelial, and stellate (fat-storing or Ito) cells, and newly recruited leukocytes, i.e., monocytes and neutrophils, also contribute to the pathogenesis of hepatic toxicity. Kupffer cells and neutrophils are a source of proinflammatory cytokines and chemokines and of reactive oxygen and nitrogen species, which promote oxidative stress in injury, induced toxicants and ischemia/reperfusion. Kupffer cells also play a key role in hepatic injury due to ethanol consumption. The uniquely fenestrated sinusoidal endothelial cell is selectively vulnerable to cold ischemia/reperfusion injury to cause graft failure after transplantation and to cancer chemotherapy agents to cause veno-reactive disease. Activated stellate cells synthesize collagen whose overproduction leads to hepatic fibrosis and cirrhosis.
3.3.1 Bile acid-induced hepatocyte apoptosis

Bile formation is an essential function of the liver, and failure of bile formation is a pathophysiological process termed cholestasis. Retention of bile constituents within the hepatocyte during cholestasis is associated with hepatocyte apoptosis (Patel et al., 1998). Although the mechanisms of cholestasis associated with hepatocyte apoptosis are likely complex and multifactorial, hydrophobic bile acids are especially hepatotoxic, and they accumulate in the liver in cholestatic disorders (Rodrigues et al., 1998). The intrinsic hepatotoxicity of these hydrophobic, sterol-derived molecules is apparent in children who have a mutation in the bile salt excretory pump in the canalicular membrane (Strautnieks et al., 1998). The failure to secrete bile acids into bile results in liver injury, cirrhosis, and death from liver failure (Strautnieks et al., 1998). This unfortunate human disease highlights the toxicity of bile acids in humans.

In cultured rat hepatocytes, the hydrophobic bile acid glycochenodeoxycholate, GCDC, at pathophysiologically relevant concentrations (20-100 μM) induces apoptosis, as documented by cell shrinkage, nuclear condensation and lobulation, caspase activation, DNA fragmentation, and phosphatidylserine externalization (Patel et al., 1994). Thus, bile acids provide a valuable model to dissect the mechanisms of liver cell apoptosis and the role of apoptosis in liver injury from endogenous toxicants.

Apoptosis occurs by one of two pathways: (1) a death-receptor pathway, and (2) the mitochondrial pathway (Green, 1998). To determine if death-receptor pathways contribute to bile acid-mediated apoptosis, hepatocytes from tumor necrosis factor-receptor 1 (TNF-R1) and Fas-deficient mice were exposed to GCDC. TNF-R1 and Fas are the predominant death receptors expressed by hepatocytes (Faubion and Gores, 1999). Hepatocytes from Fas-deficient lpr mice were resistant to GCDC-mediated apoptosis, whereas TNF-R1-deficient hepatocytes readily underwent apoptosis. Unexpectedly, hepatocytes from Fas ligand-deficient mice were also sensitive to GCDC-stimulated apoptosis (Faubion et al., 1999). These data implicate mechanism for bile acid–related liver injury. To further test this concept, the bile ducts of wild type and Fas-deficient mice were ligated to produce severe extrahepatic cholestasis. Caspase 8, an initiator cysteine-aspartate protease in apoptosis, was activated in wild type animals but not Fas-deficient mice. Bile duct ligated Fas-
deficient animals also had less apoptosis, decreased liver injury, and improved survival as compared to wild type mice (Miyoshi et al, 1999). Thus, Fas activation appears to play a dominant role in bile acid cytotoxicity.

**Fig H1-Bile acid-induced hepatocytes apoptosis.** Bile acids are normally secreted rapidly by hepatocytes by transporters located in the canalicular membrane. In cholestasis, secretion is impaired, resulting in elevated concentration of toxic bile acids (TBA) within hepatocytes. At pathophysiologic concentrations, toxic bile acids trigger translocation of intracellular Fas bearing vesicles to the plasma membrane where they self-aggregate in the absence of ligand. Activated Fas receptor complexes on the plasma membrane then cause caspase 8 activation and an apoptotic cascade.

How do bile acids cause Fas activation? Potential mechanisms include alterations in Fas synthesis, Fas compartmentation, and Fas trimerization in the plasma membrane. However, toxic bile acids did not increase Fas synthesis. Rather, bile acids promoted rapid transport of cytoplasmic vesicular Fas to the plasma membrane in a microtubular-dependent manner (Sodeman et al, 2000). Bile-acid induced apoptosis was dependent upon this translocation of Fas to the plasma membrane. Whether Fas translocation is sufficient to trigger spontaneous association of Fas receptor death domains is unclear. Nonetheless, toxicant-induced transport of intracellular death receptors to the plasma membrane is a new paradigm for cell death. In summary, bile acids accumulate in the liver when canalicular transport is impaired, which results in translocation of cytoplasmic Fas to the plasma membrane where these receptors self-aggregate and trigger cell death by apoptosis (fig-1).
3.3.2 Adhesion molecules and oxidant stress in inflammatory liver injury

Sepsis/endotoxemia, alcoholic hepatitis, ischemia-reperfusion injury, and certain drug-induced liver toxicities are characterized by systemic and local inflammation with recruitment of macrophages and neutrophils into the liver vasculature (Jaeschke and Smith, 1997; Jaeschke et al, 1996; Laskin and Laskin, 2001). The main function of these phagocytes is to destroy invading microorganisms and to remove dead cells and cell debris in preparation for tissue regeneration. Because of the nature of the toxic mediators generated by these phagocytes, healthy cells may also be affected, which can aggravate the original liver injury. Therefore, it is important to understand the mechanism involved in the activation, recruitment, and cytotoxicity of these phagocytes in the liver.

Previous work during the last 10 years characterized a role for neutrophils in the pathophysiology of inflammatory liver injury, and many aspects that are relevant for neutrophil-mediated cytotoxicity also apply to mononuclear cells. Neutrophils cytotoxicity also applies to mononuclear cells. Neutrophils can be recruited into the hepatic vasculature by local tissue injury and CXC chemokine generation (Lawson et al, 2000b; Maher et al, 1997) or the systemic exposure to inflammatory mediators, including tumor necrosis factor-α (TNF-α), IL-1, complement factors, platelet activating factor and CXC chemokines (Jaeschke, 1997). Each of these mediators upregulates β2 integrins on neutrophils (Jaeschke, 1997). In liver, neutrophils accumulate in sinusoids adhere to venular endothelial cells (Chosay et al, 1997). In general, recruitment of neutrophils into sinusoids dose not depend on cellular adhesion molecules (CAMs; Jaeschke, 1997) but appears to result from mechanical trapping due to rheological changes in neutrophils, active vasoconstriction in sinusoids and swelling of the sinusoidal lining cells (Jaeschke, 1997). However, subsequent steps of firm adhesion to endothelial cells, transmigration and adherence to hepatocytes are dependent on CAMs, including ICAM-1 and VCAM-1 (Essani et al, 1995, 1997). Expression of E-selectin on endothelial cells activates neutrophils during transmigration (Lawson et al, 2000a). Neutrophils adhesion and extravasation in sinusoids do not involve PECAM-1 or P- or L-selectin. In contrast, neutrophil rolling and adhesion in postsinusoidal venules are dependent on P- and L-selectin and ICAM-1, respectively (Jaeschke, 1997; Lawson et al, 2000a). CAMs are differentially expressed and are cytokine-inducible on all liver cell types (Jaeschke, 1997).
leukocytes, members of the $\beta_2$ (CD 18)-integrin family are critical for neutrophil-mediated injury (Jaeschke et al, 1993). LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) are involved in transmigration and adhesion to hepatocytes (Jaeschke and Smith, 1997). Upregulation of Mac-1 is a prerequisite for neutrophils cytotoxicity (Jaeschke et al, 1993). Adherence of neutrophils to target cells through Mac-1 triggers release of proteases and prolonged oxygen formation. Neutrophils are rarely cytotoxic when present in sinusoids and must transmigrate into the subsinusoidal space to cause tissue injury (Chosay et al, 1997). In order to transmigrate and attack, neutrophils must receive a chemotactic signal, CXC chemokines generated by hepatocytes can trigger a neutrophil-induced injury (Maher et al, 1997). Furthermore, lipid peroxidation products are highly chemotactic (Curzio et al, 1986) and may be responsible for the continuation and amplification of the injury (Liu et al, 1994). Recently, apoptotic cell death of hepatocytes was identified as a potent stimulus for neutrophil extravasation and enhancement of endotoxin-induced injury (Jaeschke et al, 1998; Lawson et al, 1998). In human alcoholic hepatitis, apoptotic hepatocytes colocalize with neutrophils, which correlates strongly with the severity of tissue damage (Ziol et al, 2001). Thus, hepatocytes apoptosis and neutrophil extravasation may be important events in alcoholic liver injury.

Despite the improved understanding of neutrophil-mediated hepatotoxicity, the molecular mechanism of cell death remains controversial (Jaeschke, 2000). In vitro studies using neutrophil-hepatocyte cocultures have identified proteases as the critical mediators of cell injury (Jaeschke et al, 1996; Jaeschke and Smith, 1997). In support of this concept, protease inhibitors attenuate neutrophil hepatotoxicity in vivo (Jaeschke and Smith, 1997). Recent data also suggest that neutrophil-derived reactive oxygen species can induce an intracellular oxidant stress in hepatocytes that triggers necrotic cell injury in less than 1 h (Jaeschke et al, 1999). Similar results can be obtained with a macrophage-derived oxidant stress in the liver (Bilzer et al, 1999). The mechanism of injury does not involve gross lipid peroxidation (Jaeschke et al, 1999) but may be caused by the opening of the membrane permeability transition pore and the collapse of the mitochondrial membrane potential (Nieminen et al, 1995). In addition to causing cell injury, reactive oxygen species promote inflammation by enhancing the activation of the transcription factor NF-κB, which controls the formation of cytokines, chemokines, and adhesion molecules (Jaeschke, 2000).
Liver PMN
CD11b/CD18 upregulation

EC, PC, KC
Adhesion Molecules
± ROS
Transmigation
Adherence to PC

Kupffer Cells
± ROS

Liver Cell Necrosis

Drug Toxicity
Trauma
Endotoxin
Bacteria

C5a

TNF
IL-1

ROS
LPO
CXC

ROS
Proteases
Antiproteases

Liver PMN
CD11b/CD18 upregulation

Fig-H2: Mechanism of neutrophil-induced liver injury. Tissue trauma, endotoxin, and bacteria, trigger formation of inflammatory mediators such as complement factors (C5a), cytokines (TNF-α, IL-1) and CXC chemokines. Each of these factors can upregulate expression of β2 integrin (CD11/CD18) and prime neutrophils (PMN) for ROS formation. C5a also stimulates Kupffer cells (KC) to release ROS. In addition, cytokines activate expression of adhesion molecules on endothelial cells (EC) and hepatocytes (PC). If primed neutrophils receive a chemotactic signal from the parenchyma, they will transmigrate and adhere to hepatocytes. This leads to the final activation of neutrophil with degranulation (protease release) and adherence-dependent oxidant stress, which causes cell necrosis. Mediators generated during cell injury, such as lipid peroxidation products (LPO) and chemokines, become chemotactic signals for further neutrophil activation and transmigration.

In summary, drug toxicity, tissue trauma, ischemia-reperfusion, sepsis, and other pathophysiological events activate both neutrophils and Kupffer cells directly or through activation of complement (Fig-2). Kupffer cells release cytotoxic mediators, such as reactive oxygen species and proinflammatory mediators, such as cytokines and chemokines. Complement factors (eg. C5a) and cytokines prime and activate neutrophils to promote their recruitment into the hepatic vasculature. If chemotactically stimulated, neutrophils extravasate and adhere to preenchymal cells, which induce necrotic cell death through release of reactive oxygen and proteases. Adhesion molecules on neutrophils (β2 integrins, especially CD11b/CD18) and ICAM-1 on endothelial cells and hepatocytes are essential for neutrophil migration, extravasation, and oxidant production. Cytokines can induce hepatic adhesion molecule and chemokine formation, which in turn is modulated by oxidant stress. The growing insight into mechanisms of neutrophil cytotoxicity in vivo should lead to new
therapeutic strategies to prevent neutrophil-induced tissue injury without paralyzing host-defense functions.

3.3.3 CYP2E1-Dependent toxicity in HepG2 cells

Cytochrome P4502E1 (CYP2E1), the ethanol-inducible form, metabolizes and activates many toxicologically important substrates, including ethanol, carbon tetrachloride, acetaminophen, and N-nitrosodimethylamine, to more toxic products (Guengerich et al, 1990; Koop, 1992). CYP2E1-dependent ethanol metabolism produces oxidative stress through generation of reactive oxygen species (ROS), a possible mechanism by which ethanol is hepatotoxic (Bondy, 1992; Dianzani, 1985). Introduction of cytochrome P4502E1 by ethanol is a central pathway by which ethanol generates oxidative stress, and in the intragastric model of ethanol feeding a prominent induction of CYP2E1 occurs along with significant alcohol liver injury (Morimoto et al, 1994; Nanji et al, 1994). Lipid peroxidation also occurs, and ethanol-induced liver pathology correlates with CYP2E1 levels and elevated lipid peroxidation, which is blocked by inhibitors of CYP2E1.

An approval to understand the effects and actions of CYP2E1 is to express human CYP2E1 in cell lines. CYP2E1 overexpressing HepG2 cell lines were established by retroviral infection methods (E9 cells) and by plasmid transfection methods (E47 cells; Chen and Cederbaum, 1998; Dai et al, 1993). E9 and E47 cells express CYP2E1 at levels of about 10 and 45 pmol/mg microsomal proteins, respectively. Compounds actively metabolized by CYP2E1 to reactive intermediates, such as acetaminophen or carbon tetrachloride, were toxic to CYP2E1-overexpressing HepG2 cells but not to control cells, which validates the model for study of CYP2E1-dependent toxicity (Dai and Cederbaum, 1995a, b).

Ethanol, iron, and polyunsaturated fatty acids, such as arachidonic acid (but not monoenoic acids such as oleic acid), were considerably more toxic to CYP2E1-overexpressing E9 cells than MV5 control cells (Chen et al, 1997; Sakurai and Cederbaum, 1998; Wu and Cederbaum, 1996). Toxicity was concentration- and time-dependent and associated with lipid peroxidation. Antioxidants, especially inhibitors of lipid peroxidation, prevented toxicity. The toxicity correlated with CYP2E1 levels and was enhanced after transfection with a sense CYP2E1 plasmid and diminished after transfection with an antisense CYP2E1 plasmid. CYP2E1-dependent cell killing was apoptotic, associated with activation of caspase 3 (a major effector caspase in
apoptosis), and blocked by pancaspase inhibition (Chen et al, 1997; Sakurai and Cederbaum, 1998; Wu and Cederbaum, 1999). Bcl-2 is a proto-oncogene that blocks cytochrome c release during apoptotic signaling through mitochondria, and transfectin with a plasmid containing Bcl-2 prevented the apoptosis, implicating a mitochondrial pathway for apoptosis (Chen et al, 1997). Iron and arachidonic acid decreased mitochondrial membrane potential in the CYP2E1-overexpressing cells and lowered cellular ATP levels. HepG2 cells were also infected with adenoviruses containing catalase cDNA and catalase cDNA directed to mitochondria by the 27-amino acid peptide leader sequence of manganese superoxide dismutase. Both catalase constructs protected CYP2E1-overexpressing E47 cells against iron and arachidonic acid toxicity (Bai and Cederbaum, 2001).

Glutathione (GSH) is a critical cellular antioxidant. After GSH depletion with buthionine sulfoximine (BSO), the toxicity of ethanol, iron, arachidonic acid, and acetaminophen was strikingly enhanced (Chen and Cederbaum, 1998; Chen et al, 1997; Sakurai and Cederbaum, 1998; Wu and Cederbaum, 1996, 1999). BSO treatment of CYP2E1-overexpressing E47 cells caused toxicity even in the absence of an added toxicant (Chen and Cederbaum, 1998). CYP2E1 inhibitors and antioxidants prevented cell killing, which was partly apoptotic and partly necrotic. Surprisingly, GSH in E47 cells was increased compared to C34, E9, and MV control cells. Increased GSH represented increased GSH synthesis due to transcriptional activation of the gamma-glutamyl cysteiny1 synthase gene and was blocked by antioxidants (Mari and Cederbaum, 2000). Activity, protein, and mRNA levels for other antioxidant enzymes, such as catalase, α- and microsomal glutathione transferases, were increased in E47 cells (Mari and Cederbaum, 2001). Upregulation of these antioxidant genes may reflect an adaptive mechanism to detoxify CYP2E1-derived oxidants.
Fig-H3: Role of cytochrome P4502E1 in oxidative stress after ethanol. Ethanol increases levels of CYP2E1, largely by a posttranscriptional mechanism involving stabilization against degradation. CYP2E1, a loosely coupled enzyme, generates reactive oxygen species such as super oxide radical and hydrogen peroxide during its catalytic cycle. In the presence of iron, which is increased after ethanol treatment, more powerful oxidants including hydroxyl radical, ferryl species, and 1-hydroxyethyl radical are produced. These various oxidants can promote toxicity by protein oxidation and enzyme inactivation and by damage to cell membranes via lipid peroxidation and enzyme inactivation and by damage to cell membranes via lipid peroxidation and production of reactive lipid aldehydes, such as malondialdehyde and 4-hydroxynonenal. Mitochondria appear to be among the critical cellular organelles damaged by CYP2E1-derived oxidants. A decrease of mitochondrial membrane potential and perhaps the mitochondrial membrane permeability transition causes release of proapoptotic factors resulting in apoptosis. Some CYP2E1-derived reactive oxygen species eg. H2O2, LOOH, MDA, HNE, are diffusible and may exit hepatocytes and enter other liver cell types, such as stellate cells, and stimulate these cells to produce collagen and elicit a fibrotic response.

Hepatic stellate cells are central to the fibrotic response of the liver to injury, and ROS activate stellate cells (Friedman, 2000). Since CYP2E1 produces ROS, ethanol-induced CYP2E1 expression may promote collagen type I biosynthesis by stellate cells. However, CYP2E1 is mostly present in hepatocytes, whereas stellate cells contain low levels of CYP2E1. Accordingly, a coculture model involving HepG2 cells and stellate cells was developed (Mari et al, 2001). A time-dependent increase in collagen type I was observed when stellate cells were coincubated with C34 control cells, which was further elevated when stellate cell were coincubated with CYP2E1-overexpressing E47 cells. However, little type I collagen was released into the incubation medium from the C34 plus stellate cell coculture. By contrast, E47 plus stellate cell cocultures secreted much more type I collagen protein. These experiments suggest that CYP2E1-overexpressing E47 cells generate diffusible...
mediators that promote type I collagen synthesis and release by stellate cells. Catalase and vitamin E markedly decreased type I collagen synthesis by both cocultures and completely blocked the increased collagen production by the E47 coculture. These results suggest that E47 cells release ROS, such as H₂O₂ and lipid peroxidation products, which stimulate type I collagen synthesis by stellate cells.

HepG2 cells expressing CYP2E1 have proven to be a valuable model to characterize the biochemical and toxicological properties of CYP2E1. Induction of CYP2E1 by ethanol appears to be one of the central pathways by which ethanol generate a state of oxidative stress. Figure 3 depicts a working hypothesis of the role of CYP2E1 in ethanol-induced oxidative stress and hepatotoxicity. While several mechanisms likely contribute to alcohol-induced liver injury, the linkage between CYP2E1-dependent oxidative stress, mitochondrial injury, and increased collagen formation by stellate cells may make an important mechanistic contribution to the toxic action of ethanol on the liver.

3.3.4 Peroxinitrite in drug-induced hepatotoxicity

In overdose, the analgesic/antipyretic acetaminophen produces centrilobular hepatic necrosis (Mitchell et al, 1973a). Cytochrome P450 metabolism to N-acetyl-p-benzoquinone imine (NAPQI) is a critical step. NAPQI reacts with hepatic glutathione (GSH) leading to its depletion by as much as 90% (Mitchell et al, 1973b). Additionally NAPQI covalently binds to proteins as acetaminophen-cysteine adducts (Cohen et al, 1997). Immunochemical studies indicate that the cellular site of covalent binding correlates with the toxicity (Hart et al, 1995; Roberts et al, 1991).

Recent work shows that nitrated tyrosine occurs in hepatic centrilobular cells. These adducts colocalize in cells containing the acetaminophen protein adducts (Hinson et al, 2000, 1998). Peroxynitrite, a highly reactive nitrating and oxidizing species formed by the rapid reaction of nitric oxide (NO) and superoxide, produces nitrated tyrosine (Beckman, 1996; Pryor and Squadrito, 1995). Since acetaminophen-protein adducts correlate with development of necrosis (Hart et al, 1995; Roberts et al, 1991), it follows that nitration of tyrosine correlates with necrosis.

Recent evidence suggests that activated Kupffer cells are mechanistically important in NO and superoxide formation. Pretreatment of rats and mice with macrophage activators (gadolinium chloride, dextran sulfate, LPS, or dichloromethylene, diphosphonate) dramatically decreased acetaminophen toxicity
(Blazska et al, 1995; Goldin et al, 1996; Laskin et al, 1995; Laskin and Pendino, 1995; Michael et al, 1999; Winwood and Arthur, 1993). Neither gadolinium chloride nor dextran sulfate decreased acetaminophen protein binding, but both decreased nitration of tyrosine (Mitchell et al, 1999). However, other cellular sources of NO and superoxide may be important. Hepatocytes and stellate cells express inducible nitric oxide synthase (iNOS; Muriel, 2000), and acetaminophen induces iNOS in rat hepatocytes (Gardner et al, 1998). Endothelial cells constitutively express eNOS (Muriel, 2000). Various sources produce superoxide, including damaged mitochondria (Knight et al, 2001).

The importance of iNOS in acetaminophen toxicity was investigated by utilizing iNOS knockout mice (Michael et al, 2001). Although serum ALT levels (a biomarker of liver toxicity) was less in iNOS knockout mice than in wild type mice after acetaminophen treatment, histology showed no significant differences in hepatotoxicity. Acetaminophen induced an approximate 5-fold induction of NO synthesis (serum nitrate plus nitrite) in wild type mice, and the increase in serum nitrate plus nitrite paralleled increases in serum ALT. Increased NO synthesis was not observed in iNOS knockout mouse, although a small increase in nitrotyrosine residues was observed. Nitrotyrosine in the knockout mouse was in centrilobular areas, which suggested involvement of constitutively expressed NOS. Consistent with previously reported data, acetaminophen did not increase lipid peroxidation in wild type mice (Kamiyama et al, 1993). By contrast, hepatic lipid peroxidation (malondialdehyde) increased in iNOS knockout mice (Michael et al, 2001).

It is hypothesized that the initial step in acetaminophen toxicity is metabolism to NAPQI, leading to depletion of GSH and covalent adduct formation, as previously proposed. In wild type mice, induction of NO synthesis and superoxide generation occurs subsequently, leading to peroxynitrite formation. Ordinarily, GSH detoxifies peroxynitrite (Sies et al, 1997). However, after GSH depletion of by NAPQI, peroxynitrite nitrates protein tyrosine and may oxidize other macromolecules. In vitro acetaminophen competes with tyrosine for reaction with peroxynitrite, but in vivo peroxynitrite reacts rapidly with protein tyrosine in wild type mice. In iNOS knockout mice, superoxide increases after acetaminophen but not NO synthesis. Superoxide then causes lipid peroxidation. Thus acetaminophen toxicity may be mediated by nitration in wild type mice and by lipid peroxidation in iNOS knockout mice (Fig-4).
Indeed, by reacting with superoxide NO may prevent lipid peroxidation in wild type mice (Rubbo et al, 1994).

These data indicate the importance of peroxynitrite as a mediator of hepatotoxicity and suggest that nitric oxide is important in controlling superoxide levels. Depending on GSH status, nitric oxide may induce a toxification or detoxification mechanism. With hepatotoxins like acetaminophen, bromobenzene, chloroform, and allyl alcohol that deplete hepatic GSH, peroxynitrite formation promotes toxicity. However with hepatotoxins that cause lipid peroxidation but do not deplete GSH, such as carbon tetrachloride, NO may scavenge superoxide by forming peroxynitrite, which is then detoxified by GSH.

3.3.5 Hepatotoxicity due to mitochondrial dysfunction

Microvesicular steatosis

Primary and secondary mitochondrial dysfunction is an important mechanism of drug-induced microvesicular steatosis, nonalcoholic steatohepatitis, nonalcoholic steatohepatitis (NASH), and cytolytic hepatitis (Fromenty and Pessayre, 1995). Severe impairment of mitochondrial fatty acid β-oxidation causes microvesicular steatosis, characterized by accumulation of tiny lipid vesicles in the cytoplasm of hepatocytes (Fromenty and Pessayre, 1995). Because of poor mitochondrial oxidation, nonesterfied fatty acids (NEFAs) accumulate in the liver and become esterified into triglycerides. Hepatic triglycerides, perhaps emulsified by a rim of amphilic NEFAs, amass as small lipid vesicles (Fromenty and Pessayre, 1995). The sudden onset or aggravation of mitochondrial dysfunction leaves no time for the progressive coalescence of tiny lipid droplets into the large fat inclusions of macrovasculuar steatosis.

Microvascular steatosis is the histological hallmark of severe metabolic perturbations causing energy shortage. Inhibition of β-oxidation itself deprives cells of their most important source of energy during fasting. Furthermore, NEFAs and their dicarboxylic acid metabolites directly impair mitochondrial energy production (Froment and Pessayre, 1995). Finally, disruption of hepatic mitochondrial β-oxidation decreases delivery of hepatic ketone bodies and glucose to peripheral tissues (Froment and Pessayre, 1995). The resulting deficiency of energy substrates may cause renal failure, pancreatitis, coma, and death (Froment and Pessayre, 1995).
Fig-H4: Postulated mechanism of acetaminophen (APAP)-induced hepatotoxicity. Hepatocytes, Kupffer cells, and endothelial cells all participate in the production of reactive nitrogen and oxygen species. The reactive levels of nitric oxide (NO) and super oxide (O₂⁻) determine whether the mechanism of hepatic necrosis is dependent on protein nitrosylation or lipid peroxidation. GSH, glutathione; HOONO, peroxynitrite; NOS, nitric oxide synthase; CYP450, cytochrome P450.

Damage to mitochondrial DNA (mtDNA) and direct inhibition of mitochondrial respiration also inhibit β-oxidation (Fromenty and Pessarye, 1995). β-oxidation consumes NAD⁺ and transforms it into NADH. Mitochondrial respiration reoxidizes NADH into the NAD⁺ that is required for β-oxidation. Therefore, impairment of respiration inhibits β-oxidation. Thus, various endogenous and exogenous substances impair mitochondrial β-oxidation to cause microvesicular steatosis through different mechanisms. Oxidative stress after ethanol causes damage to mitochondrial proteins, lipids, and DNA. mtDNA depletion occurs in ethanol-treated mice (Mansouri et al, 1999). In humans, these oxidative lesions cause mtDNA deletions (Mansouri et al, 1997). Interferon-α and nucleoside analogs (dideoxynucleosides, fiafluridine) impair mtDNA transcription and replication, respectively (Lewis and Dalakas, 1995; Shan et al, 1990). DNA polymerase γ incorporates nucleoside reverse transcriptase inhibitors into mtDNA, an event that blocks mtDNA replication, eventually causing mtDNA depletion.

Salicylic acid and valproic acid sequester CoA, which is needed to form thio esters with fatty acid (Deschamps et al, 1991; Kesterson et al, 1948). 2,4-Diene-
valproyl-CoA, a reactive metabolite of valproic acid, may also inactivate β-oxidation enzymes (Kassahun and Abbott, 1993). Several drugs inhibit β-oxidation, including tetracycline derivatives (Labbe et al, 1991), glucocorticoids (Letteron et al, 1997), the nonsteroidal antiinflammatory drugs ibuprofen and pirprofen (Freneaux et al, 1990; Geneve et al, 1987), the antidepressant drugs amineptine and tianeptine (Fromenty et al, 1989; LeDinh et al, 1988), the antianginal cationic amphiphilic drugs amiodarone, perhexiline, and diethylaminoethoxyhexestrol (Berson et al, 1998; Deschamps et al, 1994; Fromenty et al, 1990), as well as female sex hormones or pregnancy (Grimbert et al, 1993).

These metabolic effects may combine to block mitochondrial β-oxidation and trigger microvascular steatosis. For example, Reye’s syndrome occurs after a viral infection in children taking aspirin and/or having a latent inborn defect in β-oxidation enzymes (Fromenty and Pressayre, 1995). Likewise, acute fatty liver of pregnancy is more frequent in women whose fetus has a genetic deficiency in long-chain 3-hydroxy-acyl-CoA dehydrogenase (Ibdah et al, 1999).

Nonalcoholic steatohepatitis (NASH). NASH develops progressively in patients with chronic, macrovascular, or microvesicular steatosis, leading to liver cell death, Mallory bodies, polynuclear cell infiltrates, fibrosis, and cirrhosis (Pessayre et al, 2001). NASH occurs in patients with the obesity/hypertriglyceridemia/insulin resistance syndrome, or can be induced by chronic amiodarone, perhexiline, or diethylaminoethoxyhexestrol administration (Pessayre et al, 2001). These cationic amphiphilic drugs concentrate electrophoretically into mitochondria to inhibit β-oxidation and respiration (Berson et al, 1998). Respiratory inhibition leads to ROS formation by mitochondria to cause lipid peroxidation of fat deposits (Berson et al, 1998). Similarly in alcohol abuse, increased ROS formation causes lipid peroxidation and steatohepatitis (Pessayre et al, 2001). Both lipid peroxidation and ROS-induced cytokine release (TGF-β, TNF-α, IL-8) may contribute to the development of NASH (Pessayre et al, 2001).

Cytolytic hepatitis

Cytolytic hepatitis is a severe liver lesion that can cause liver failure and may involve mitochondrial uncoupling or respiratory inhibition (Berson et al, 1996; 2001). Another mechanism of is onset of the mitochondrial permeability transition (MPT)
caused by opening of permeability transition (PT) pores in the mitochondrial inner membrane. PT pore opening causes mitochondrial depolarization, uncoupling, and large amplitude swelling and can lead to both necrotic and apoptotic cell death (Lemasters et al, 1998; Pessayre et al, 1999). PT pore opening in all mitochondrial of a cell causes ATP depletion, which prevents apoptosis (an energy-requiring process) and causes necrosis (Lemasters et al, 1999; Pessayre et al, 1999). In contrast, PT pore opening in only some mitochondria permits ATP synthesis by the unaffected mitochondria, thus preventing necrosis. In mitochondria undergoing the MPT, however, matrix swelling and outer membrane rupture causes release of mitochondrial cytochrome c, which activates caspases in the cytosol to cause apoptosis (Bradha, et al, 1998; Feldmann et al, 2000; Hatano et al, 2000).

Drugs cause the MPT through diverse mechanisms. Some compounds, such as ROS (Nieminen et al, 1995), thio crosslinkers, bile acids (Botla et al, 1995), atracyloside (Halestrap and Davidson, 1990), betulinic acid (Fulda et al, 1998), and lonidamide (Ravagnan et al, 1999), may directly include PT pore opening, whereas other drugs, such as salicylic acid and valproic acid, may facilitates PT pore opening by calcium (Lemasters et al, 1998). Other drugs, such as anticancer drugs, cause Fas ligand expression in hepatocytes to initiate Fas- and MPT-dependent fratricidal killing (Muller et al, 1997).

The most frequent mechanism of cytolytic hepatitis is cytochrome P450-dependent formation of reactive metabolites that cause direct toxicity or immune reactions. Reactive metabolites may cause DNA damage and overexpression of p53 and Bax, as well as glutathione depletion, protein thiol oxidation, and increased cytosolic Ca^{2+} (Haouzi et al, 2000). Bax overexpression, disulfide formation, and increased mitochondrial Ca^{2+} all promote MPT and cell death (Haouzi et al, 2000). Covalent binding of reactive metabolites to hepatic proteins can also trigger an immune response. Cytotoxic T lymphocytes kill their targets by 3 mechanisms: cell surface Fas ligand expression, formation of TNF-α, and release of granzyme B (Pessayre et al, 1999). All three events trigger the MPT to cause death of target cells (Bradham et al, 1998; Feldmann et al, 2000; Hatano et al, 2000; Pessayre et al, 1999).
CONCLUSION

In summary, several mechanisms initiate liver cell damage and aggravate ongoing injury processes. Mitochondria are prominent targets for the hepatotoxicity of many drugs. Dysfunction of these vital cell organelles results in impairment of energy metabolism and an intracellular oxidant stress with excessive formation of reactive oxygen species and peroxynitrite. In addition to mitochondria, induction of cytochrome P450 isoenzymes such as CYP2E1 also promote oxidant stress and cell injury. Once hepatocellular function is impaired, accumulation of bile acids causes additional stress and cytotoxicity. Cell injury, gut-derived endotoxin or a combination of both also activate Kupffer cells and recruit neutrophils into the liver. Although responsible for removal of cell debris and part of the host-defense system, under certain circumstances these inflammatory cells initiate additional liver injury. However, cell injury and death is not only determined by the nature and dose of a particular drug but also by factors such as an individual’s gene expression profile, antioxidant status, and capacity for regeneration. Because of the many direct and indirect mechanisms of drug-induced cell injury in the liver, hepatotoxicity remains a major reason for drug withdrawal from pharmaceutical development and clinical use.
3.4. DIABETES AND MEDICINAL PLANTS: PRESENT STATUS AND FUTURE PROSPECTUS (Tiwari et al, 2002)

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, negative nitrogen balance, and sometimes ketonaemia. It causes a number of complications like retinopathy, neuropathy, and peripheral vascular insufficiencies. The dramatic increase in the prevalence of diabetes can be attributed to several factors. Globally, diabetes has shadowed the spread of 'modern lifestyle' and can be linked to an increasingly overnight and sedentary population. The chronic metabolic disorder that afflicts 150 million people is set to rise to 300 million by 2025. There are two major forms of diabetes. Type 1 or insulin-dependent diabetes mellitus is an autoimmune genetic disease resulting from an absolute deficiency of insulin due to destruction of insulin-producing pancreatic β cells. Type 2 or non-insulin-dependent diabetes mellitus is a multifactorial disease which is characterized by insulin resistance associated not only with hyperinsulinaemia and hyperglycemia but also with atherosclerosis, hypertension and abnormal lipid profile, collectively accounts for 90-95% of the diagnosed cases of the disease. There is no single approach to treat this disease and usually a combination therapy is adopted from different approaches (Vats et al, 2005).

Despite the great strides that have been made in understanding and management in this disease, serious problems like diabetic retinopathy, diabetic nephropathy and lower extremity amputation continue to confront patients and physicians. The graph of diabetes-related mortality is rising unabated. Certain population subgroups have prevalence rates of disease approaching 50% and this is strongly related to the epidemic of obesity and socio-economic inequalities that plague our society.

Defects in carbohydrates metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia. This presents moving therapeutic target that requires a range of different agents to address the different features of the disease at different stages of its natural history. Although biomedical science has unraveled substantially the pathobiological processes involved in causing/fostering
Review of literature

diabetes, and has designed therapeutic agents with a range of action to fight hyperglycemia, the efficacy of these therapeutic agents is compromised in several ways. For individual agents act only on part of the pathogenic process and only to a partial extent. This may be the reason that even after so much advancement in understanding the disease process and availability of a wide range of therapeutic agents, the disease is still progressing.

Multiple defects in the pathophysiology of diabetes are mostly imprecisely understood, and therefore warrant not isolating a single drug target to the reversal of all or majority of aspects of the disease, as biological systems are too complex to be fully understood through conventional experimentation and also because they are nonlinear. They also may have properties that are not obvious from biological consideration alone. For example, though hyperglycemia is a classical risk factor for the development of diabetic complications, there is no consensus regarding the pathogenic links between hyperglycemia and diabetic complications. There are a number of equally tenable hypotheses on the origin of complications beyond hyperglycemic consideration. Therefore, the unidirectional therapeutic approach in the management of diabetes does not appear to be the way to address this problem.

On the other hand, the therapeutic approach of several traditional medicinal systems is more holistic. The fundamental mechanisms of these medicinal systems are still unexplainable using modern tools. The medicinal preparations in traditional medicines contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms.

3.4.1 Impaired carbohydrate metabolism and hyperglycemia

Carbohydrates from various dietary sources are the primary exogenous source of glucose. Glucose is the main fuel energy requirement of the body. Therefore, a continuous supply of glucose is necessary to ensure proper function and survival of all organs. Hence, mammals have evolved sophisticated systems to maintain glucose levels in the blood within tight limits, despite large fluctuation in food intake. Homeostatic mechanisms are in place to maintain blood glucose levels within a very narrow range (around 5 mM), protecting the body against hypoglycemia during periods of fasting and against excessively high levels following the ingestion of a high carbohydrate diet. These goals are met chiefly through the hormonal modulation of the production of glucose by the liver and the peripheral uptake of glucose by skeletal
muscle, heart muscle and fat. When mammals fast, glucose homeostasis is achieved by triggering expression of gluconeogenic genes in response to glucagons, and when they take a carbohydrate-rich diet, the function is taken over by insulin for its uptake and utilization peripherally. The impairment in glucose metabolism, therefore, may lead to physiological imbalance and warrants proper management.

Starting from the carbohydrate ingestion, breakdown into the monosaccharides, their building blocks glucose, sucrose, and fructose to the downhill utilization of glucose for generation of energy, Figure-1 presents in brief a systematic sketch of the pathways involved in carbohydrate metabolism. Any vitiation, therefore, in normal glucose metabolic pathway may lead to the impaired glucose metabolism, the onset of hyperglycemia and subsequently, diabetes mellitus. This flow chart also presents points, where modern therapeutics targets and traditional medicines have shown their therapeutic potential.

After the breakdown of carbohydrates by digestive enzymes, the released glucose becomes the primary stimulus for the β-cells of the pancreatic islets. Prolonged exposure of pancreatic islets to elevated glucose concentration has been shown both in vitro and in vivo to impair glucose-stimulated insulin release. Glucose stimulation of pancreatic islet of β-cells initiates a cascade of events resulting in insulin secretion and is dependent on an increase in intracellular Ca\(^{2+}\). This increases the phosphoinositide hydrolysis, inositol 1, 4, 5-triphosphate (IP-3) production and mobilizes Ca\(^{2+}\) from intracellular IP-3 sensitive Ca\(^{2+}\) stores in the pancreatic β-cells. Regulation of glucose metabolism is a key aspect of metabolic homeostasis and insulin is the dominant hormone influencing this regulatory system. One of the major effects of insulin is to enhance overall glucose disposal, and this is achieved by stimulation of glucose uptake into the target tissues. This task is facilitated by insulin-sensitive glucose transporter (GLUT-4), which is uniquely expressed in skeletal muscles, cardiac muscles and adipose tissues action. This action of insulin in the regulation of glucose homeostasis in post-absorptive state is a very important function in maintaining euglycemia and preventing hyperglycemia. The molecular and cellular biology of GLUT-4 is a complicated science. Further, understanding of GLUT-4 biology may provide novel therapeutics in insulin resistance in Type 2 diabetes, for GLUT-4 is the key facilitator responsible for the maintenance of euglycemia in the body. Glucose-stimulated release of insulin and insulin-guided metabolism of glucose are therefore, the primary balancing factor in maintaining euglycemic state in
the blood. However, this homeostatic relationship is disturbed when glucose remains at supraphysiological level for a protracted period of time, a consequence referred to as glucose toxicity.

The establishment of association between toxic effects of elevated concentration of glucose on β-cells’ function, changes in key constituents of insulin gene expression and insulin synthesis reveals that among several operating mechanisms, the potential mechanism is chronic oxidative stress accelerating overt generation of reactive oxygen species (ROS) that adversely affects the islets’ functions. ROS can also be formed by chronic exposure to hyperglycemia that involves non-enzymatic glycation proteins and the formation of products that in turn lead to the generation of ROS.

Chronic oxidative stress due to hyperglycemia may therefore play an important role in progressive β-cell dysfunction. Studies have demonstrated that this can be ameliorated by antioxidants of varied nature. Furthermore, it is important to note that pancreatic islets themselves have low expression of antioxidant enzymes, and might be susceptible to ROS. Radical scavenger/antioxidants, therefore, may find varied application at this juncture.

3.4.2. Hyperglycemia-induced oxidative stress and diabetic complications

Hyperglycemia alone does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycemia, which is mediated and complicated through oxidative stress. Diabetic hyperglycemia causes of a variety of pathological changes in small vessels, arteries and peripheral nerves. Vascular endothelial cells become primary vulnerable targets of hyperglycemic damage as glucose continuously flows through them. Hyperglycemia increases the production of ROS inside the aortic endothelial cells. Hyperglycemia-induced activation of protein kinase-C (PK-C) isoforms, increased formation of glucose-derived advanced glycation end products, and increased glucose flux through aldose reductase pathways are some of the known biochemical mechanisms of hyperglycemia-induced tissue/organ damage.

However, the belief that these metabolic pathways have their independent origin has undergone some changes recently. It has been proposed a single unifying hypothesis linking these mechanisms by which elevated concentrations of glucose perturb cellular properties in a fundamental way. Hyperglycemia aggravates
Figure 5: Sketch of pathways of carbohydrate metabolism and targets where imbalance/insufficiencies in function lead to hyperglycemia and resultant diabetic syndrome. S-Glut, Sodium glucose co-transporter-1; GIP, gastrointestinal peptide; VIP, vasoactive intestinal peptide; EIA, Entero-insular axis; glu-R, Glucose receptor; IR, Insulin receptor, IR-s, Insulin receptor substrate; TK, Tyrosine kinase enzyme; PTP, Protein phosphotyrosine phosphatase; TNF, Tumor necrosis factor; Ald-Red, Aldose reductase; Hk, Hexokinase; LPL, Lipoprotein lipase, Brush border-basolateral membrane of the intestinal epithelium; (1), α-Glucosidase inhibitors; (2), Sulphonyl ureas; (3), Biguanides, and (4), Aldose reductase inhibitors are the available therapies at the particular points and synthetic medicines available. (A) denotes points where phytochemicals of various or similar nature have been shown to demonstrate multiple activities.
endothelial ROS generation by a variety of mechanisms. Suppression of intracellular mitochondrial, ROS over-production by use of low-molecular weight inhibitors and antioxidants prevents glucose-induced activation of PK-C, formation of advanced glycation end-products, sorbitol accumulation and activation of cytokines. This study has opened a new avenue to make a radical approach for the treatment of diabetic complications.

ROS increases the generation of TNF-α expression and aggravates oxidative stress. Increased liberation of cytokines like TNF-α and interleukins has been implicated in the pathogenesis of insulin resistance. TNF-α is a putative inhibitor of tyrosine phosphorylation on insulin receptor and post-receptor signaling intermediates. TNF-α is a pleiotropic cytokine involved in many metabolic responses in both normal and pathophysiological states. It has a central role in obesity, modulating energy expenditure, fat deposition and insulin resistance. TNF-α may produce insulin resistance by a decrease in autophosphorylation of insulin receptor, conversion of insulin receptor substrate-1 into an inhibitor of insulin receptor tyrosine kinase activity, decrease in GLUT-4 transporter in muscle cells, increase in circulating fatty acid, altering β-cell function and also increase in triglycerides and decrease in high density lipoprotein. TNF injection to healthy individuals reduces insulin sensitivity by inducing hyperglycemia without lowering plasma insulin levels. Adipocytes exposed to TNF become insulin-resistant, since insulin is not able to stimulate hexose transport. This appears to be the consequence of down-regulation in expression of GLUT-4, the insulin stimulable glucose transporter. Antioxidants and polyphenolic compounds have been shown to scavenge free radicals, reduce oxidative stress and decrease the expression of TNF-α. Therefore, phytochemicals appear to manipulate by various indirect mechanisms, the complications of diabetes mediated through oxidative stress, ROS or TNF-α.

The nuclear targets/transcription factors that regulate glucose homeostasis represent a potentially large class of therapeutic targets. However, they represent rather more complex targets for therapeutic intervention than metabolic targets as they are usually expressed in multiple tissues and potentially regulate large number of genes. Furthermore, the precise mechanisms of these targets and behaviours of transcription factors in regulation biological functions are poorly understood. The long-term safety aspects of therapies developed on these targets await time-testing.
Diabetic Syndrome

Hyperglycemia

Diabetic complications

Previously believed independent pathways

Oxidative Stress

Pathological changes in small vessels, arteries and peripheral nerves

Increased generation of reactive oxygen species from mitochondria

leads to now realised single unifying mechanism

- Activation of PK-c isoforms
- Increased glycation end-product
- Increased glucose flux via aldose reductase Pathway
- Increased hexosamine synthesis
- O-glycation of Sp-1
- PAI-1 expression

Fig-2: Single unifying mechanism of oxidative stress due to persistent hyperglycemia, which leads to overt generation of ROS in mitochondria. This results in a variety of harmful oxidative products previously believed to be originated in dependently. These oxidative products are known to complicate the diabetic pathology. The relevance of each of the pathways is supported by animal studies in which pathway-specific inhibitors prevent various hyperglycemia-induced abnormalities. Normalizing levels of mitochondrial ROS have been shown to prevent formation of the above products.
3.4.3. Multiple approaches of phytomedicines in combating diabetic disorders.

Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in regimen for treatment of this devastating disease. The most challenging goal in the management of patients of diabetes mellitus is to achieve blood glucose level as close to normal as possible. In addition, post-prandial hyperglycemia (PPHG) or hyperinsulinemia are independent risk factors for the developing of macrovascular complications of diabetes mellitus. This section presents a composite view of the multiple target beneficial effects of the plant medicines/phytochemicals.

Glucose absorption

Starting from the very beginning of carbohydrate metabolism, release of glucose and transport across the intestinal brush membrane down to the blood stream, has attracted much attention recently as potential targets to control PPHG. In this category, majority of recent studies report the potential antidiabetic medicinal plants on inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase and manipulation of glucose transporters. A wealth of literature has emerged now, showing the potential effect of phytochemicals in inhibiting α-amylase and α-glucosidase. Kobayashi et al reported screening of various plants for α-amylase inhibitory activity and the resultant in vivo PPHG activity.

Tea polyphenolics, apart from their much-cited antioxidant activities, also have been reported to inhibit α-amylase and sucrase, and have been shown to be the principle substances for suppressing PPHG. Furthermore, these polyphenolics also inhibit glucose transport across the intestine by inhibiting sodium glucose co-transporter-1 (S-GLUT-1). (+) catechin, (-) epicatechin, (-) epigallocatechin and epicatechin gallate, isoflavones from soybeans, polyphenolics compounds, tannic acid, chlorogenic acid, crude saponin from Gymnema sylvestre and other saponins from several plant extracts have been shown to possesses potent S-GLUT-1 mediated inhibition of glucose and antihyperglycemic activity. The manipulation of S-GLUT-1 mediated transport along with α-amylase and α-glucosidase inhibitory activity by plant phenolics make them hence, very exciting candidates in the control and management of hyperglycemia.

Recently, Mastuda et al studied in detail the structure activity relationship among saponins isolated from various sources and their hypoglycemic activity. The 3-
O-glucuronic acid moiety of oleonolic acid possesses strong hypoglycemic activity. The 28-ester glucoside moiety however, reduces the activity. The 3-O-glucuronic acid glycosides are more potent than 3-O-glucopyranosyl analogues. The 6'-methyl esters of glucuronic acid moiety strongly reduce hypoglycemic activity. Regarding mechanism of action, the amounts propose that these compounds act as hypoglycemic by delaying the transfer of glucose from the stomach to the small intestine, the main site of glucose absorption and by inhibiting the glucose transport at the site of intestinal brush border membranes. Drugs that reduce PPGH by suppressing the absorption of carbohydrate are effective in NIDDM prevention and treatment.

It is envisaged therefore, that there are several approaches to retard glucose uptake in the small intestine: (a) by inhibiting digestive enzymes, (b) by inhibiting active transport of glucose across intestinal brush border membrane and (c) by delaying the gastric emptying rate of gastrointestinal content. The water-soluble dietary fibres, guar gum, pectin, polysaccharides contained in plants have been reported to increase the viscosity of gastrointestinal content, thereby decreasing the gastric emptying rate and suppressing/delaying the digestion and absorption of carbohydrates.

The α-glucosidase inhibitors are currently the most commonly used oral agents for ameliorating PPHG because of the lack of hypoglycemic threat, and more importantly because of the prospect of blood glucose control without hyperinsulinemia and body weight gain. At present three glucosidase inhibitors—acarbose, miglitol and voglibose—are available for the treatment of patients with Type 2 diabetes mellitus. Inhibition of glucosidase and amylase should result in delayed carbohydrate digestion and glucose absorption, with attenuation of PPGH excursion. It has been reported that α-glucosidase inhibitors usually do not alter the total amount of carbohydrate absorbed and therefore, do not cause any net nutritional calorific loss, and they act mostly by slowing down the carbohydrate digestion.

KK-A Y mice have genetically determined obesity, and diabetic complications like hyperglycemia, hyperinsulinemia, glucosuria and severe insulin resistance. These symptoms increase with age at least up to 16 weeks. This model therefore has become a very close model to mimic NIDDM. Takki et al have reported using this model that apart from many activities reported for glycerrhizin, the main substance from licorice root, it also inhibits S-GLUT-1-mediated glucose transport, suppresses rise in fasting blood glucose and insulin level and improves glucose tolerance.
β-cell regeneration and insulin releasing activity

Alloxan and streptozotocin (STZ) are the chemicals used conventionally to produce diabetes and hyperglycemia in experimental animals by selectively destroying β-cell. These chemicals induce necrosis to islet β-cells through free radical-mediated damage. It has been a difficult task to regenerate β-cells once they are destroyed. However, a crude extract *Pterocarpus marsupium*, (an Ayurvedic medicinal plant advocated for diabetes), in the form of water decoction has been reported have protective and restorative effect in alloxan-induced diabetic rats by Chakravarty et al. The same authors later isolated the active principle as (-) epicatechin form *P. marsupium* which was shown to possess preventive as well as restorative properties of β-cells against alloxan-induced damage. These results were substantiated by histological observations. The regeneration of β-cells' normal function was evidenced by blood sugar values in these animals. Jahromi et al identified some more flavonoids from *P. marsupium* as liquiritigenin and pterosupin, and reported hypolipidemic properties of these phytochemicals in experimental animals.

*Gymnema sylvestre*, an Indian medicinal plant, has long been known to possess antidiabetic activities. It is popularly known in Hindi as ‘gurmar’ meaning sugar destroyer. Extracts of *G. sylvestre* have been reported to possess a variety of actions related to the antidiabetic properties like reduction in insulin requirement possibly by enhancing endogenous insulin availability, improving vitiated blood glucose homeostasis, better control of hyperlipidemia associated with diabetes, reduction in amylase activity in serum, increase in β-cell function as shown by higher levels of serum C-peptide. The water-soluble alcoholic extracts of *G. sylvestre* leaves were found to regenerate β-cells in pancreatic islets of STZ-induced diabetic rats. Water-soluble alcoholic extracts of *G. sylvestre* leaves have also been reported to potentiate insulin release from pancreatic β-cells in different animal models representing hyperglycemia and diabetes. The dried powder of *G. sylvestre* was found not only to regulate the blood sugar homeostasis in alloxan-induced diabetic rats but it also increased the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway.

Ignacimuthu and Amalraj reported the antidiabetic and antihyperlipidemic effect of *Zizyphus jujuba* in alloxan-induced diabetic rats, which was fairly comparable to that of glibenclamide. The authors propose that alkaloid barberine
present in the leaves of the plant may be responsible for its hypoglycemic activity and suggest that chemical constituents in Z. jujuba may have the ability to release insulin from pancreatic β-cells and also have the potential to protect it from alloxan-induced damage in experimental animals.

*Trigonella foenum-graceum* L. fenugreek seeds have been reported to possess hypoglycemic and hypolipidemic properties in animal experiments as well as in human and clinical cases. Recently, Ravikumar and Anuradha reported the antioxidant property of fenugreek seeds in diabetic rats.

*Ocimum sanctum* and *O. album* have been observed to decrease the fasting and postprandial blood and urinary glucose levels in Type 2 diabetic patients. The dried powder of leaves also mildly reduced cholesterol level.

There are several reports where medicinal plants have been found to possess hypoglycemic, antidiabetic, hypolipidemic and antioxidant activities. Similarly, there may be several unexplored plants that may contain more yields of active principles known to possess multiple activities in this regard compared to the plant material reported in the classical literature of traditional medicines.

**Aldose reductase pathway inhibitors**

Aldose reductase, the key enzyme of the polyol pathway, has been demonstrated to play an important role in etiopathology of diabetic complications such as neuropathy, cataract, nephropathy and retinopathy. Aldose reductase catalyses the reduction of glucose into sorbitol. Sorbitol does not readily diffuse across the cell membrane and intracellular accumulation of sorbitol is responsible for cataract in diabetic complications. The inhibitors of aldose reductase (sorbinil, tolrestate) have been proved to improve the diabetic complications in experimental animals and clinical trials. Several plant-derived flavonoids, apart from possessing their common antioxidant activity, have been reported to inhibit aldose reductase activity and impart beneficial action in diabetic complication. Similarly, these phytochemicals may also contribute beneficially in mitigating glucose auto oxidation, glycation, and act against the major contributors for increased free radicals generation in diabetic lens. Recently, Lim et al have identified butein as the most promising antioxidant and aldose reductase inhibitor for prevention and treatment of diabetic complications. Similarly, flavanone and flavonol glucosides isolated from a plant popularly known as ‘plant insulin’ (*Myrcia multiflora* - a Brazilian medicinal plant have been reported to possess
aldose reductase inhibition, α-glucosidase inhibition and potential for hypoglycemic activity in alloxan-induced diabetic animals.

**Antioxidant defense**

The antioxidant defense system represents a complex network with interaction, synergy and specific tasks for a given antioxidant. Recent studies show that majority of the plasma antioxidants are depleted in Type 2 diabetes patients. The depletion of antioxidants in the diabetic patients was independent of body mass index and dietary intake and this depletion is a major cause of diabetes-related complications and onset of other disease conditions like atherosclerosis and coronary heart disease. Apart from acting on carbohydrate metabolic targets compounds present in medicinal plants alone or in combination, possess a variety of beneficial activities and have the potential to impart therapeutic effect holistically in complicated disorders like diabetes and its complications.

The oxidative stress and resultant tissue damage are hallmark of chronic diseases and cell death, and diabetes is not the exception. Baynes and Thorpe have recently discussed some of the paradigms of oxidative stress in diabetes. Whether oxidative stress occurs at an early stage in diabetes, preceding the appearance of complications, or whether it is merely a common consequence of the tissue damage reflecting the presence of complications, is a matter of scientific debate. But it true that oxidative stress plays an important role in diabetic complications. Baynes and Thorpe argue that treatment of diabetes with antioxidant therapy is like applying water to a burning house and is certainly helpful in limiting the conflagration. Obviously, if antioxidant therapy is helpful in relieving symptoms and complications in a diabetic patient based on the present evidences, the physician will consider this aspect first to relieve and improve his patient. Finding out the real cause the understanding becomes the secondary consideration to be explained and addressed later. Present therapeutic strategies mostly try to relieve the clinical manifestations of diabetes and its complications. The major challenge in diabetes research is to define not only the cause-effect relationship between various risk factors and complications, but also to comprehend the effects of pharmaceutical agents that are beneficial in the management of diabetic complications. Nonetheless, the rationale for the therapeutic use of antioxidants in cases of diabetes and other critical disease conditions is emerging fast.
3.4.4. Present status and future prospects

Diabetes is becoming something of a pandemic and despite the recent surge in new drugs to treat and prevent the condition, its prevalence continues to soar. Perhaps the most worrying aspect of all is that the rise is even reflected in children. Although several drugs targeted for carbohydrate hydrolyzing enzymes (pseudosaccharides), release of insulin from pancreatic β-cells (sulphonyl urea), glucose utilization (biguanides), insulin sensitizers, PPARγ agonists (glitazones) are in clinical practice, the growing diabetes market observes a number of changes. The glitazones are meant to target the problem of insulin resistance and enhance insulin action at the cellular level; however, some of these drugs are linked to liver toxicity (triglitazone) including a number of deaths from hepatic failure and raising the symptoms and risk factors of heart disease leading to heart failure (rosiglitazone). Therefore, as the long term of risk and effect on the complications of diabetes related with these drugs are not yet clear, UK Drug and Therapeutic Bulletin warrants that patients taking glitazones to monitored for signs of heart failure.

On the other hand, traditional medicinal plants with various active principles and properties as discussed in this article have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer. The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of β-cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them into novel therapeutics.

The multifactorial pathogenicity of diabetes demands multi-modal therapeutic approach. Thus, future therapeutic strategies require the combination of various types multiple agents. Polyherbal formulations have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves due to incorporation of plant medicines with diverse pharmacological actions. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. Traditional medicinal preparations therefore, should not be considered just as a collection of therapeutic recipes. They are formulated and prepared keeping in mind the conditions of sickness and the healing properties of individual ingredients. It is important therefore, that herbal medicines and preparations should be taken with the consideration of their holistic therapeutic
Review of literature

approach. The multiple activities of plant-based medicinal preparations meant for diabetes offer enormous scope for combating the threat of the diabetic epidemic.

To achieve a blockbuster status, clear evidence of the advantage over the existing therapy is the most important requirement of the day. The ability of modern medicine and healthcare systems to adequately manage symptoms of chronic and terminal disease is a central theme. The systematic reviews and meta analysis of clinical trials are the foundation of their success. Unfortunately, despite the apparent supremacy in terms of multiple therapeutic approaches of herbal medicines, well-organized, rigorous clinical trials evidences are not adequately available in order to advocate their scientific merit and supremacy over the existing drugs. Though the markets for herbal medicines are booming and evidence for effectiveness is growing, it is also being simultaneously counterbalanced by adequate regulation. Therefore, the product standardization, efficacy, safety, and therapeutic risk/benefit associated with the use of herbal medicines need proper evaluation. A sound basic and rigorous clinical investigation to confirm and advocate the excellence over the existing therapies of traditional medicinal plants, preparations, mechanism(s) of action, and therapeutic effects is absolutely required.