2. REVIEW OF LITERATURE

2.1. Chitosan

Chitosan is a natural mucopolysacharide (Van der Lubben *et al.*, 2001) of marine origin. While it has been in existence for millennia, its current form has just recently been prepared. Chitosan is one of the most available polysaccharides with positive charges found in nature (Xing *et al.*, 2005). Technically speaking, Chitosan is a naturally occurring substance that is chemically similar to cellulose (Barbara, 2005), which is a plant fiber. Chitosan is shown to have superior characteristics and especially flexibility in its use.

2.1.1. Source

Chitosan is obtained from chitin (Shepherd *et al.*, 1997; Krisana *et al.*, 2004), which is believed to be the second most abundant biomaterial after cellulose (Sini *et al.*, 2005). Chitin is widely distributed in the nature. Major sources of chitin are shellfish, clams, krill, oysters, squid, fungi and insects. Chitin content in various sources is given in Table 1 (Allan *et al.*, 1978; Kong, 1975). Amongst several sources, the exoskeleton of crustaceans consist of 15-20 % chitin by dry weight. Chitin found in nature is a renewable bio-resource. It is present not only in animals, but also in plants as cell wall material. Chitin resembles cellulose both in chemical structure and in biological function as a structural polymer. The crystalline structure of chitin has been shown to be similar to cellulose in the arrangements of inter- and intra chain hydrogen bonding. The annual biosynthesis of chitin has been estimated to 10^9 to 10^{11} tons. Chitosan is found in nature, to a lesser extent than chitin, in the cell walls of fungi. Zygomycetes contain both chitin and chitosan (Austin *et al.*, 1981; Rudall, 1969).

Chitosan is prepared by alkaline N-deacetylation of chitin (Kittur *et al.*, 2002). Deacetylation of chitin is done by using concentrated NaOH solution (40-50%).

Source	Quantity harvested 10^3 tonnes	Chitinous waste, 10^3 tonnes	Chitin potential, 10^3 tonnes
Shell fish	1700	468	39
Krill (potential landing)	18200	3640	56
Clam/ Oysters	1390	521	22
Squid	660	99	1
Fungi	790	790	32
Total	22,740	5,118	150

Table 2.1. Chitin content in various sources

2.1.2. Properties

Chitosan occurs as odourless substance. It is an amorphous solid and off-white in colour. The properties of chitosan vary considerably depending on the source and production process. Average molecular weight of chitosan is around $1.2X10^5$ Daltons. Most of the commercial polysaccharides like cellulose, dextran, pectin, alginic acid etc. are neutral or acidic. But chitosan is an abundant basic polysaccharide. Its pH comes around 8 and this basic nature makes it unique for different applications. Chitosan has special properties like polyelectolyte behaviour, polyoxysalt formation, film formation and chelation of metal ions (Austin *et al.*, 1981). The main advantage of Chitosan for different biological applications is that it is nontoxic (Arai *et al.*, 1968) in nature. Chitosan is insoluble in water but soluble in dilute acids. Pure chitosan is not hydrolysed by lysozyme

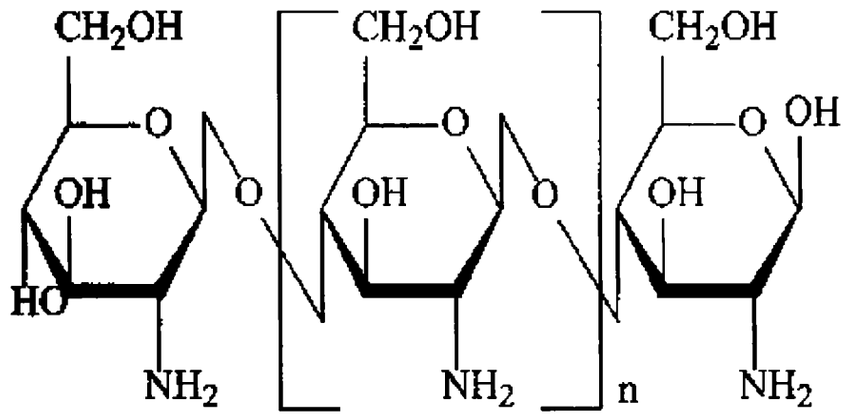
while chitin is hydrolysed. Chitosan undergoes the typical reactions of amines, of which N-acetylation and Schiff reaction are the most important. Although β (1-4) anhydroglucosidic bond of chitosan is also present in cellulose, the characteristic properties of chitosan are not shared by cellulose (Muzarelli, 1978). Chitosan forms aldimines and ketimines respectively with aldehydes and ketones at room temperature. When reacted with ketoacids followed by reduction with sodium borohydride, chitosan produces glucans. N-carboxymethyl chitosan and N-carboxyl benzyl chitosan are examples of glucans derived from chitosan (Muzzarelli, *et al.*, 1982 a,b; Muzarelli and Tanfani, 1982). On hydrogenation with aldehydes, chitosan produces N-alkyl chitosans.In spite of the hydrophobicity of the alkyl chains, N-alkyl chitosans swell in water, but its film forming capacity is retained (Muzarelli *et al.*, 1983). In acidic medium, chitosan develops a violet colour with iodine (Van Wisselingh, 1898; Campbell, 1929; Krishnan and Sundara Rajulu, 1964). This test is used as the detection test for chitosan. An enzymatic method was also put forwarded by Jeuniax (1963 & 1965) for the detection of chitosan. As per the reports of Sundara Rajulu *et al.* (1982), enzymatic test was found more reliable amongst these two tests. Chitosan can be hydrolysed in the presence of chitosanase (Monaghan *et al.*, 1972). According to Fenton and Eveleigh (1981) purified chitosanase from microbes hydrolyses specifically chitosan.

2.1.3. Structure

Chitin, source of chitosan, is a polymer of β (1-4)-N-acetyl-D-glucosamine (Fig. 2.1). Chitin occurs in three polymorphic forms, which differ in the arrangement of molecular chains (Muzarelli, 1977 a). In chitosan, the N-acetyl group of chitin is removed. Chitosan is a polymer of β (1-4)-D-glucosamine (Yasser and Ahmed, 2002)

2.1.4. Applications of chitosan

Since chitosan is nontoxic (Arai *et al.*, 1968), it is suitable for different applications. Chitosan is one of the most abundant natural amino polysaccharides with variety of applications (Majeti and Kumar, 2000) It is having



Chitosan

Fig.2.1. Structure of Chitosan

immunological (Nishimura *et al.*, 1984; Mori *et al.*, 1997) antibacterial (Tokura *et al.*, 1997; Tanigawa *et al.* (1992) and wound healing activities (Okamoto *et al.*, 1993; Kweon *et al.*, 2003; Khnor and Lim, 2003). As chitosan is having antibacterial, haemostatic, fungistatic, antitumoral and anticholesteremic properties (Barbara, 2005), it has wide pharmaceutical applications. Muzarelli *et al.* (1988) studied the application of chitosan in wound dressing since chitosan wound healing property. Chitosan has profound applications in the fields of clarification and purification, chromatography, paper and textiles, photography, food and nutrition, agriculture, cosmetics, biodegradable membranes and biotechnology.

Since chitosan is a long chain polymer, it can wrap solid particles in liquids to bring them together and agglomerate. Thus, chitosan can be used as a coagulant aid. Commercially, chitosan is used for waste water clarification. Free amino group and hydroxyl groups present in chitosan make it suitable for chromatographic applications. Muzarelli (1977) and Allan *et al.* (1972,1975 & 1977) made studies on the application of chitosan on improving the properties of paper. Chitosan can be used for the production of fibers and films (Kunike, 1926). Since chitosan has resistance to abrasion, optical characteristics and behaviour with silver complexes, it has immense applications in photography. Animal feed studies proved that the utilization of whey might be improved if the diet contain small amount of chitinous material. Chitosan feeding studies conducted in chicken, pig, mice, rabbits etc. improved their weights significantly. A thin coating of chitosan in seeds enhances the seed growth. Chitosan coating can be used for the ripening of fruits also. Chitosan can be effectively utilized for the controlled release of drug (Agnihotri and Aminabhavi, 2004). Chitosan is used as a substituent for microcrystalline cellulose (MCC) as a diluent in tablet making by direct compression. Dissolution properties and bioavailability of poorly soluble drugs can be improved by grinding them with chitosan. Enzyme immobilization is another important use of chitosan. Many methods are adopted for enzyme immobilization like entrapment and adaption, fixing by cross-linking etc. There are many advantages when immobilized enzymes are used; enzymic reaction can be stopped at any desired time and small amount of enzyme is

sufficient for large amount of substrate. Since, chitosan is physiologically harmless it can be used in hair cosmetic products instead of synthetic resins (Gross *et al.*, 1982). Chitosan films have good water absorbing property. Chitosan membranes can be used for desalination, ultrafiltration and wastewater treatment (Rutherford and Dunson, 1984). Chitosan is an effective and adequate haemostatic agent even under the most severe conditions of anticoagulation. Chitosan solution is found beneficial for healing "athletes foot" conditions (Allan *et al.*, 1984). Chitosan has good ophthalmologic applications. Both hard and soft contact lenses can be made from chitosan. The effect of chitosan have been investigated in patients with chronic renal failure undergoing long term stable haemodialysis treatment (Jing *et al.*, 1997) Ingestion of chitosan effectively reduced serum cholesterol level and increased serum haemoglobin levels. Significant reduction in urea and creatinine levels in the serum were observed after 4 weeks of chitosan ingestion. The data suggests that chitosan might be effective in the treatment for renal failure patients. Sapelli *et al.* (1986) studied the application of chitosan in dentistry. Knapczyk *et al.* (1989) proved that chitosan is applicable in immunology. Like some plant fibers, chitosan is not digestible; therefore it has no caloric value. No matter how much Chitosan ingest, its caloric count remains at zero. This is a very important property for any weight loss product.

2.2. Glucosamine

Glucosamine is an amino monosaccharide found in most of the tissues in our body. Unlike other forms of sugar in the body, amino sugars are components of carbohydrates that are incorporated into the structure of body tissues, rather than being used as a source of energy. Glucosamine is involved in the formation of the nails, tendons, skin, eyes, bones, ligaments, and heart valves. It also plays a role in the mucous secretions of the digestive, respiratory, and urinary tracts. It is incorporated in the biosynthesis of glycosaminoglycans and proteoglycans, essential for the extracellular matrix of connective tissues. Glucosamine is a nutritional supplement and therefore the authority of the Food and Drug Administration to regulate it is now severely limited (Angell and Kassirer, 1998).

There have been relatively few trials of glucosamine use in humans, and those that have been published are studies that examined the efficacy of glucosamine in the relief of joint pain (Mc Alindon *et al.*, 2000). There have been no reported trials of long-term glucosamine use, and its long-term safety is unknown. Due to favorable, but scientifically inconclusive, reports of glucosamine therapy for arthritis symptoms are less (Loes *et al.*, 1998). Glucosamine is available as an over-the-counter oral supplement, has few adverse side effects, and is relatively inexpensive. Several clinical studies have reported that glucosamine works better in reducing the symptoms of osteoarthritis (Reginster *et al.*, 2001). However, the mechanisms underlying this effect are not yet known.

2.2.1. Source

Exoskeleton of marine organisms are the main source of glucosamine. Chitin is prepared from the shells of crab, shrimp etc. From chitin, glucosamine is prepared by hydrolysis with concentrated acids under drastic conditions (Madhavan, 1992). Glucosamine is found in hyaluronic acid, a compound responsible for the lubricating and shock-absorbing properties of synovial fluid. Glucosamine is synthesized in chondrocytes in the body from glucose and glutamine.

2.2.2. Structure

Glucosamine is an aminomonosacharide. i.e., amino group is attached to a glucose ring (Fig.2.2). Thus comes the name glucose-amine. Chemically glucosamine is 2-amino-2-deoxy-alpha-D-glucose.

2.2.3. Properties

Glucosamine is a colourless and odourless substance with sweet taste. It is crystalline in nature. It is having acidic pH, ranging from 3.5-4.5 and has density of 0.75 g/ml. Specific rotation of glucosamine is 70° and is readily soluble in water. When heated above 80 ^{0}C, it undergoes Maillard reaction and its colour

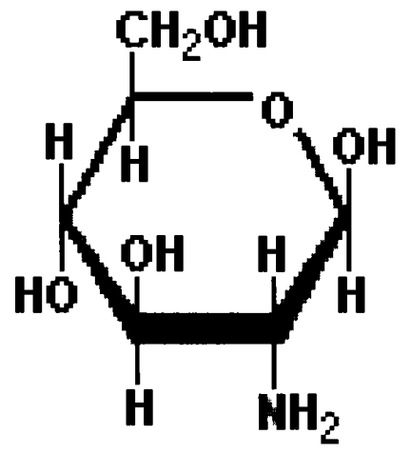


Fig. 2. 2. Structure of β-D-Glucosamine

will be faded. On further heating, glucosamine will be charred. Glucosamine has anti-inflammatory and antioxidant actions. Glucosamine blocks the generation of superoxide free radicals by macrophages. Glucosamine is well tolerated. No severe side effects were reported for glucosamine (Reginster *et al.*, 2001). Glucosamine can increase proteoglycan synthesis (Corinne *et al.*, 1998), which is a fundamental component found in articular cartilage. Proteoglycans synthesized in the presence of glucosamine had smaller glycosaminoglycan chains with a corresponding decrease in lipoprotein retention. This property makes it useful against atherosclerosis (Tannock *et al.*, 2002). Glucosamine inhibits the cartilage-destructive enzyme collagenase. Glucosamine helps in the synthesis of cartilage by increasing key components of cartilage such as glycosaminoglycans. Various reports confirmed that diabetes patients can also consume Glucosamine, which will not increase blood glucose level (Daren *et al.*, 2003). Glucosamine appears to undergo a significant first-pass effect in the liver, which metabolizes a significant proportion of the dose to CO_2, water, and urea (Setnikar *et al.*, 1993)

2.2.4. Applications

Setnikar and colleagues (1984, 1986, 1993) have published several reports on the pharmacokinetics of glucosamine in rats, dogs, and humans. In both humans and rats, oral administration of radio labeled glucosamine results in absorption of more than 90% of the ingested dose (Tannock *et al.*, 2002).

Glucosamine is found largely in cartilage and plays an important role in its health and resiliency. As we age, we lose some of the glucosamine and other substances in cartilage. This can lead to thinning of cartilage and the onset and progression of osteoarthritis. According to Braham (2003), glucosamine supplementation can provide some degree of pain relief and improved function in persons who experience regular knee pain, which may be caused by prior cartilage injury and/or osteoarthritis. Since, glucosamine can increase the synthesis of proteoglycans and glucosaminoglycans, which are necessary ingredients of connective tissue, it is proven to be effective against osteoarthritis

(Timothy *et al.*, 2004; Reginster *et al.*, 2001). Canapp *et al.* (1999) reported that glucosamine was found beneficial against acute synovitis.

Every year about twenty thousand people die from using NSAIDS. The combination of glucosamine and NSAIDs may reduce the doses needed for anti-inflammatory activity as well as the side effects associated with these NSAI drugs (Zupanets *et al.*, 1991). Dettmer (1979) examined the effects of simultaneous administration of glucosamine and chondroitin sulfate on osteoarthritis. This study demonstrated greater effectiveness of this combination therapy. In combination with chondroitin sulfate, it can build blocks for cartilage, up-regulate chondrocyte and reduce the extent of cartilage degradation (Yu Shao *et al.*, 2004).

Fabio *et al.* (2005) synthesized D-glucosaminic acid on a multigram scale by air oxidation of D-glucosamine, catalyzed by the enzyme glucose oxidase. D-Glucosaminic acid is a component of bacterial lipopolysaccharides and a member of the 'chiral pool' and has been used as a starting material for the synthesis of various amino acids.

2.3. Peptic ulcer

Peptic ulcer disease is a very common ailment, affecting one out of eight persons in the United States and commonly seen in adults of India. It is a sore on the lining of the stomach (Fig. 2.3.) or duodenum, which is the beginning of the small intestine. The causes of peptic ulcer have gradually become clear. With this understanding have come new and better ways to treat ulcers and even cure them. These ulcers can occur in the stomach, where they are called gastric ulcers. Or they can occur in the first portion of the intestine. These are called duodenal ulcers. "Peptic Ulcer" is the term used to describe either or both of these two types of ulcers.

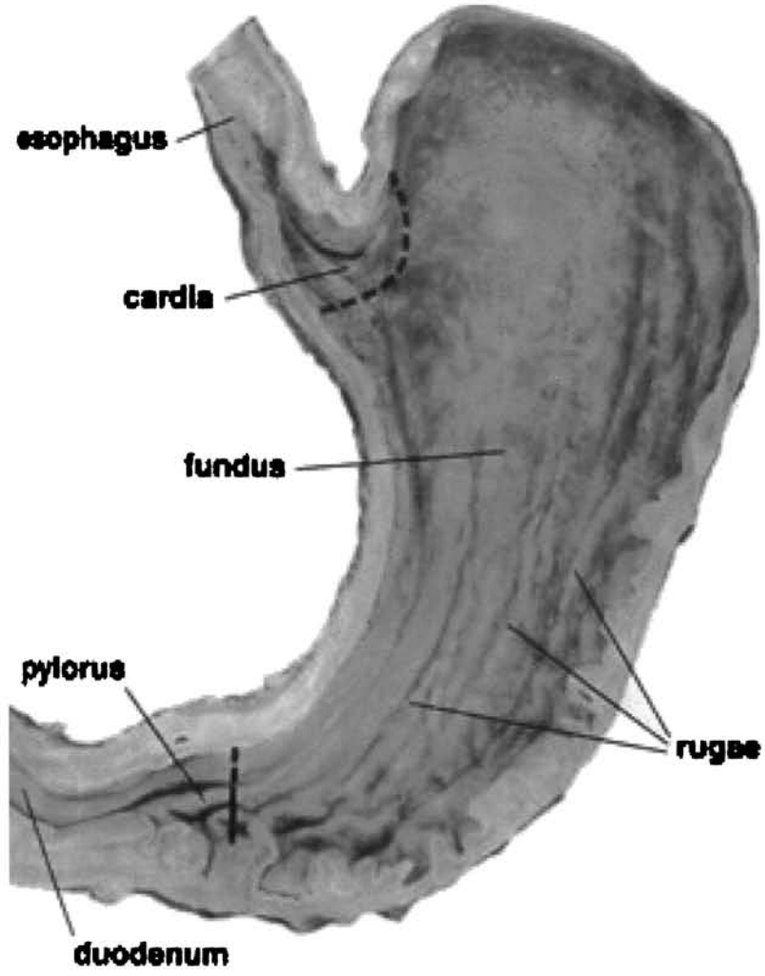


Fig. 2.3. Structure of Stomach

2.3.1. Anatomy and function of the Stomach

The stomach produces a very strong acid. This acid helps to digest and break down food before it enters the small intestine (duodenum). The lining of the stomach is covered by a thick protective mucous layer, which prevents the acid from injuring the wall of the stomach.

2.3.2. Causes of Ulcer

An ulcer is an open wound in the lining of the stomach or intestine, much like mouth or skin ulcers. Peptic ulcers are eventually caused by acid and pepsin, a digestive stomach enzyme.

In the end, it is the production of large amounts of acid in the stomach, passing under the term 'hyperchlorhydria', that causes the injury to the stomach or bowel lining. In a few cases, cancerous tumors in the stomach or pancreas can cause ulcers. Peptic ulcers are not caused by stress or eating spicy food, but these can make ulcers worse. However, a revolutionary and startling recent discovery is that most peptic ulcers result from a stomach infection caused by the bacteria, *Helicobacter pylori* (Fig.2.4). Use of non-steriodal anti-inflammatory drugs (NSAIDs) will also cause ulcers.

2.3.2.1. Helicobacter pylori (*H. pylori*)

Complicating our understanding of stomach ulcers is Barry Marshall and Robin Warren's discovery in 1982 that bacteria are the primary cause of stomach and duodenal ulcers excluding those caused by aspirin or arthritis drugs. This bacterium has a twisted spiral shape and infects the mucous layer lining of the stomach. This infection produces an inflammation in the stomach wall called gastritis. The body even develops a protein antibody in the blood against it. The bacterium is probably acquired from contaminated food or from a drinking glass. It is only after *H. pylori* bacteria injure the protective mucous layer of the stomach, allowing damage by stomach acid, that an ulcer develops. It takes

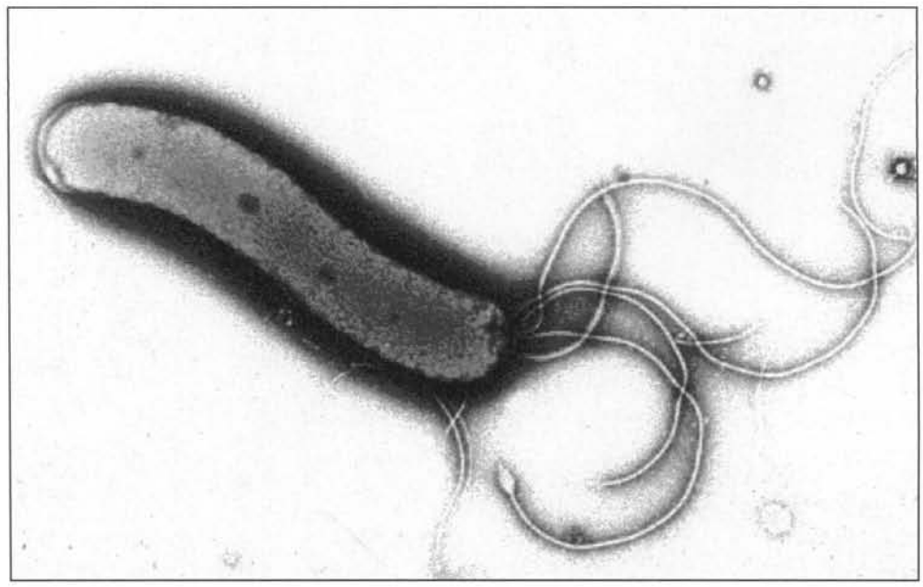


Fig. 2.4. Helicobacter pylori

advantage of the stomach's own mucus for protection. Any acid that does reach the bacteria is converted by *H. pylori*'s urease enzyme in the following reaction

urea + stomach acid + water ⟶ bicarbonate + ammonia

$$C{=}O{\cdot}2NH_2 + H^+ + 2H_2O \longrightarrow HCO_3^- + 2NH_4^+$$

The products of this reaction, bicarbonate and ammonia, are strong bases that further protect the bacteria because of their acid-neutralizing capability. The body's immune system responds to the presence of *H. pylori* and sends infection-fighting cells to the area. However, the neutrophils cannot reach the *Helicobacter pylori* infection because they cannot easily get through the stomach lining. Inflammation in the stomach tissue occurs as the neutrophils die and release superoxide radicals on the stomach wall, damaging tissue. The immune system sends in more nutrients to help the neutrophils, and the *H. pylori* can feed on these nutrients. It may not be the *H. pylori* itself that causes a stomach ulcer, but inflammation in the stomach lining as part of the immune response. Age is also a factor in *H. pylori* infection. In Western countries, children are unlikely to be infected. *H. pylori* infections occur in about 20% of persons below the age of 40 years, and 50% of persons above the age of 60 years.

2.3.2.2. Non Steroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs include ibuprofen, fenoprofen, aspirin, diclofenac, sulindac, diflusinal, naproxen, tolmetin and many others. They can damage the mucous layer of the stomach, after which the stomach acid causes the final injury.

There are two components to NSAID-induced ulceration. First, there is a local acid effect of the dissolved drug. Most NSAIDs are weakly acidic, lipid-soluble compounds. Since the cell membranes on the stomach wall contain lipids for protection against strong acids, they offer little resistance to the lipid-soluble NSAID. The NSAID acts against the cell membrane, increasing its permeability. This results in cell swelling and death. The local acid effect of NSAIDs has been reduced by enteric-coating the drug, delaying dissolution until later in the

digestive process. However, not all NSAIDs are enteric-coated as it increases cost. In addition, enteric-coating does little more than improving the symptoms of upset stomach. Patients must be informed that enteric-coated NSAIDs are still just as likely to cause stomach ulcers as regular NSAIDs. The second and much more significant component to NSAID-induced ulceration is the systemic effect after being absorbed into the bloodstream. NSAIDs inhibit COX-1, reducing prostaglandin production. Normal COX-1 present in stomach tissue produces prostaglandins which:

- increase mucous and bicarbonate production,
- inhibit stomach acid secretion,
- increase blood flow within the stomach wall.

By acting on COX-1, NSAIDs restrict these self-protection mechanisms, allowing stomach ulcers to develop. It is primarily through this mechanism, not a local acid effect, that NSAIDs cause stomach ulcers.

So, *H. pylori* and certain drugs are the two major factors that cause ulcers. In rare cases, a patient will produce very large amounts of acid and develop ulcers. This condition is called Zollinger -Ellison syndrome. Finally, some people get ulcers for unknown reasons.

2.3.3. Other chemicals inducing Ulcer

Other than NSAIDS, certain chemicals can also cause gastrointestinal damage. In a study conducted by Silvana *et al.* (2003) ulcer was induced with indomethacin. Tan *et al.* (2002) induced ulcer by the administration of HCl-ethanol. Reserpine, an alkaloid can also be used for the induction of ulcer (Salvatore *et al.*, 2003). Jianfeng *et al.* (2002) induced ulcer in rat models by using acetic acid.

2.3.4. Symptoms

Ulcers cause gnawing, burning pain in the upper abdomen. These symptoms frequently occur several hours following a meal, after the food leaves the stomach but while acid production is still high. The burning sensation can occur during the night and be so extreme as to wake the patient. Instead of pain, some patients experience intense hunger or bloating. Antacids and milk usually give temporary relief. Other patients have no pain but have black stools, indicating that the ulcer is bleeding. Bleeding is a very serious complication of ulcers.

2.3.5. Diagnosis

A diagnosis of peptic ulcers can be suspected from the patient's medical history. However, the diagnosis should always be confirmed either by an upper intestinal endoscopy, which allows direct examination of the ulcer or by a barium x-ray of the stomach. Rarely an ulcer can be malignant. With endoscopy, a biopsy specimen can be obtained to determine if this is so.

2.3.6. Treatment

Therapy of peptic ulcer disease has undergone profound changes. There are now available very effective medications to supress and almost eliminate the outpouring of stomach acid. These acid-suppresssing drugs have been dramatically effective in relieving symptoms and allowing ulcers to heal. If an ulcer has been caused by aspirin or an arthritis drug, then no subsequent treatment is usually needed. Avoiding these latter drugs, should prevent ulcer recurrence.

The second major change in peptic ulcer disease treatment has been the discovery of the *H. pylori* infection. When this infection is treated with antibiotics, the infection, and the ulcer, do not come back. Increasingly, physicians are not just suppressing the ulcer with acid-reducing drugs, but they are also curing the underlying ulcer problem by getting rid of the bacterial infection. If this infection is not treated, the ulcers invariably recur.

There are a number of antibiotic programs available to treat *H. pylori* and cure ulcers. Working with the patient, the physician will select the best treatment program available. Antacid, H_2 receptor antagonists (ranitidine, cimetidine, nizatidine, famotidine), gastric acid pump inhibitor (omeprazole), barrier agent (sucralfate) etc. were developed to heal ulcers, but they fail to prevent the occurrence of NSAID-induced ulceration (Agrawal, 1995; Blower, 1996). These treatments are often effective in alleviating ulceration symptoms. They are also used in combination with antibiotic treatment for *H. pylori*-induced ulcers. However, the treatments listed earlier do not prevent NSAID-induced ulcers. Only misoprostol, a synthetic prostaglandin, has been shown to prevent NSAID-induced ulcers.

Some plant extracts were also found effective in the treatment of ulcer. Tan *et al.* (2002) proved the gastric cytoprotective anti-ulcer effects of the leaf methanol extract of *Ocimum suave* (Lamiaceae) in rats. Jianfeng *et al.* (2002) found that sea buckthorn (*Hippophae rhamnoides*) seed and pulp oils have both preventive and curative effects against experimental gastric ulcers in rats. Sea buckthorn (*Hippophae rhamnoides*) is a Euro–Asian wild, newly cultivated, edible berry with exceptionally high contents of nutrients and phytochemicals such as lipids, water and fat soluble vitamins, and flavonoids. Silvana *et al.* (2003) reports that *Tanacetum larvatum* has very good gastroprotective effects. Yeel *et al.* (2003) says that crude extract from *Angelica sinensis* (ASCE), which mainly consisted of polysaccharides, has a direct wound healing effect on gastric mucosa.

2.3.7. Ibuprofen

Ibuprofen is in a class of drugs called Non steroidal Antiinflammatory Drugs (NSAIDs) (Tyagi *et al.*, 2005). Ibuprofen is reported to have analgesic properties also (Polat and Karaman, 2005). Ibuprofen works by reducing hormones that cause inflammation and pain in the body. Ibuprofen inhibits cyclooxygenase (COX); thus inhibits prostaglandin synthesis. Ibuprofen appears to have gastrointestinal adverse drug reactions of all NSAIDs.

2.3.7.1. Structure

Ibuprofen is chemically 2-(p-isobutylphenyl) propionic acid (Fig. 2. 5). It has an empirical formula of $C_{13}H_{18}O_2$.

Ibuprofen like other 2-arylpropionate derivatives (including ketoprofen, flurbiprofen, naproxen etc.) contains a chiral carbon in the β-position of the propionate moiety. As such there are two possible enantiomers of ibuprofen with the potential for different biological effects and metabolism for each enantiomer.

Indeed, it was found that S-ibuprofen (sinisteribuprofen) was the active form both invitro and invivo. Logically then, there was the potential for improving the selectivity and potency of ibuprofen formulations by marketing ibuprofen as a single-enantiomer product (as occurs with naproxen, another NSAID).

Further, invivo testing, however, revealed the existence of an isomerase, which converted R-ibuprofen to the active S-enantiomer. Thus, due to the expense and futility that might be involved in marketing the single enantiomer, all ibuprofen formulations currently marketed are a racemic mixture of both enantiomers.

2.3.7.2. Properties

Ibuprofen has a molecular weight of 206.3 g. It has hepatic metabolism and has renal excretion.

Common adverse effects include nausea, dyspepsia, gastrointestinal ulceration/bleeding, raised liver enzymes, diarrhoea, headache, dizziness, salt and fluid retention, hypertension (Rozzi, 2004). Infrequent adverse effects include oesophaveal ulceration, heart failure, hyperkalaemia, renal impairment, confusion, bronchospasm, rash etc. (Rozzi, 2004).

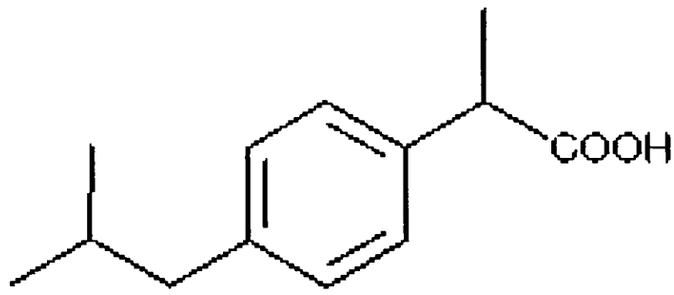


Fig. 2 .5. Structure of Ibuprofen

As with other NSAIDs, ibuprofen has been reported to be a photosensitizing agent (Castell *et al.*, 1987). Ibuprofen however has a very weak absorption spectrum which doesnot reach into the solar spectrum. The molecule contains only a single phenyl moiety and no bond conjugation, resulting in a very weak chromophore system. Ibuprofen, therefore, is only a very weak photosensitizing agent when compared with other members of the 2-arylpropionic acids.

2.3.7.3. Mechanism of action

Nonsteroidal anti-inflammatory drugs work by interfering with the cyclooxygenase pathway (Fig. 2.6). The normal process begins with arachidonic acid a dietary unsaturated fatty acid obtained from animal fats. This acid is converted by the enzyme cyclooxygenase to synthesize different prostaglandin. The prostaglandins go on to stimulate many other regulatory functions and reactionary responses in the body. Earlier research (Tannenbaum, 1996; Vane, 1996; Emery, 1996) have shown that there are two types of cyclooxygenase, denoted COX-1 and COX-2. Each type of cyclooxygenase lends itself to producing different types of prostaglandins.

Different mechanisms stimulate the two types of cyclooxygenase. COX-1 is stimulated continuously by normal body physiology. The COX-1 enzyme is constitutive meaning that its concentration in the body remains stable. It is present in most tissues and converts arachidonic acid into prostaglandins. These prostaglandins in turn stimulate normal body functions, such as stomach mucus production and kidney water excretion, as well as as platelet formation formation. The location of the COX-1 enzyme dictates the function of the prostaglandins it releases (Vane, 1996). For example, COX-1 in the stomach wall produces prostaglandins that stimulate mucous production. In contrast, the COX-2 enzyme is induced. It is not normally present in cells but its expression can be increased dramatically by the action of macrophages the scavenger cells of the immune system (Tannenbaum, 1996) COX-2's most important role is in inflammation. COX-2 is involved in producing prostaglandins for an inflammatory response.

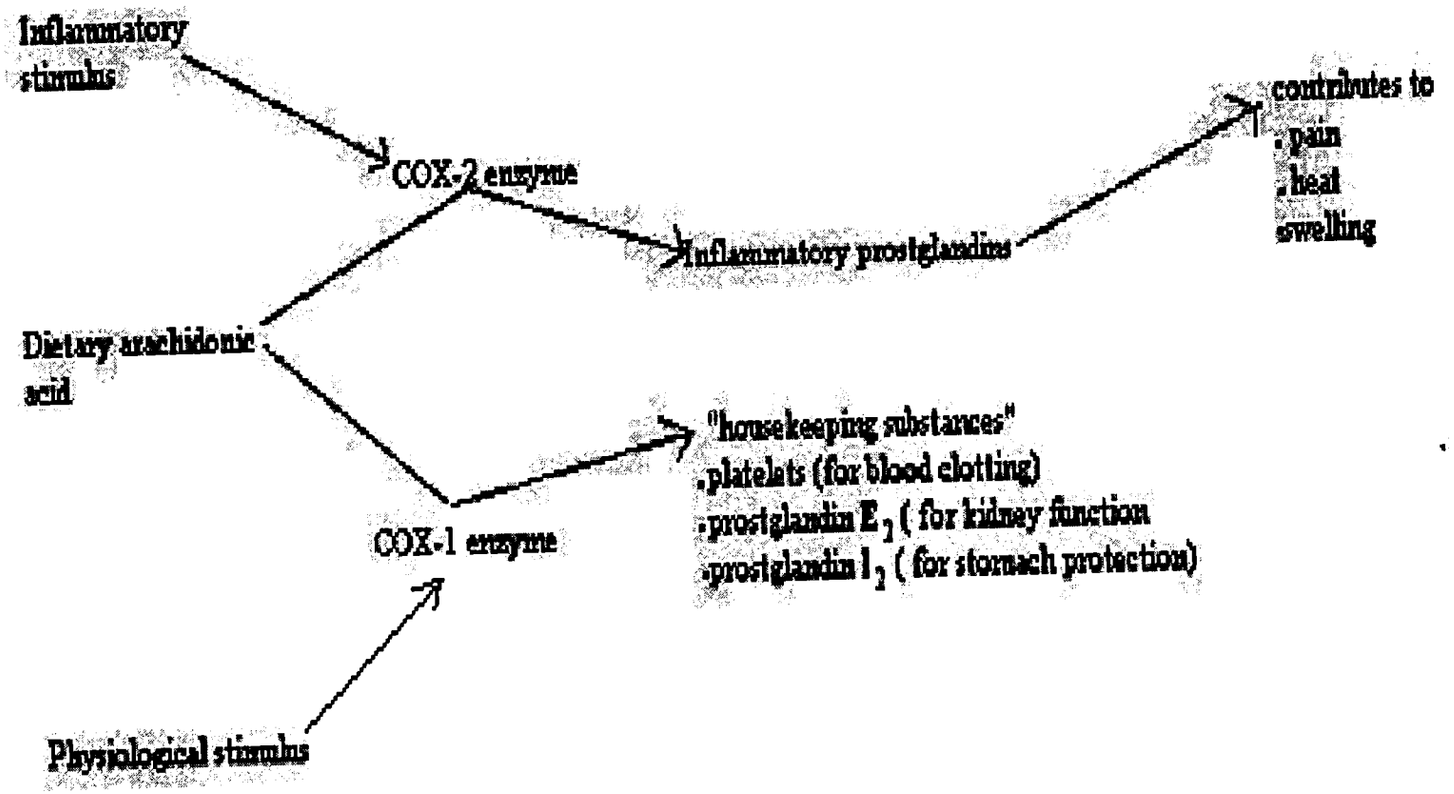


Fig. 2.6. The Cyclooxygenase Pathway

COX-1 is stimulated continually, and COX-2 is stimulated only as a part of an immune response.

2.3.7.4. Biological applications

Ibuprofen is used to reduce the fever, pain, inflammation, and stiffness caused by many conditions, such as osteoarthritis, rheumatoid arthritis, and abdominal cramps associated with menstruation. Ibuprofen is used widely in the community for the relief of headache including migraine. It is also widely marketed as an analgesic agent rather than as an anti-inflammatory and is often used for general pain conditions including those arise from various injuries such as sporting injuries, illness such as influenza, shingles, gout and post operative pain. As with other NSAIDs, ibuprofen inhibits platelet aggregation, but is not used therapeutically for this action since it is a minor and reversible effect. Litkowski *et al.* (2005) studied the analgesic effect of ibuprofen with oxycodone in combination therapy. According to Rostom *et al.* (2005), ibuprofen can cause hepatotoxicity. An earlier study (Hollenz and Labenz, 2004) reported that administration of ibuprofen could lead to gastrointestinal injury like ulcer. Long-term use of ibuprofen will lead to Nephrogenic adenoma, which is an infrequent benign lesion of the urinary system (Scelzi *et al.*, 2004). Dokmeci (2004) proved that Ibuprofen might be a promising new therapeutic avenue for the treatment of neurodegenerative diseases such as Alzheimer's disease (AD).

2.4. Hepatotoxicity

Liver is one of the most important organs in our body. It is the largest gland, which is placed behind the lower right part of ribs. Ribs help in keeping the liver from being injured. Liver is very important to human health and has a lot of functions in the body:

- Stores vitamins, sugars, fats and other nutrients from the food that we eat
- Builds chemicals that the body needs to stay healthy.

- Breaks down harmful substances, like alcohol and other toxic (poisonous) chemicals.
- Removes waste products from the blood.
- Makes sure that the body has just the right amount of other chemicals that it needs.

Many diseases can affect the liver. If anyone has one of these diseases, the liver may not work. Some of the most common diseases that affect the liver are:

1. Viral Hepatitis: Hepatitis is a medical term that means "inflammation (swelling) of the liver." Viruses that attack the liver cause some of the most common forms of hepatitis. Virus attack can be identified by a blood test. Three of the most common viruses that attack the liver are:Hepatitis A virus (HAV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV).

2. Cirrhosis: Cirrhosis is a medical term that means "scarring of the liver." When one has cirrhosis, large parts of the liver are damaged. Because it has been damaged, the liver may not work as well as it should. Cirrhosis of the liver is often the result of drinking too much alcohol. Other common causes of cirrhosis include hepatitis; especially hepatitis C. Cirrhosis can be very dangerous if it is not treated properly.

3. Liver Cancer: Like many other body organs, liver also can get cancer. Liver cancer is a disease in which some of the cells in the liver begin to reproduce faster than they should. These cells form growths called tumors. Having hepatitis B or hepatitis C can increase the chances of getting liver cancer. Liver cancer can be deadly.

4. Hepatotoxicity: Toxicity of liver can be obtained by certain chemicals. Prolonged use of some drugs can also cause hepatoxicity. Millions of people are suffering from hepatic failure. It may lead to death also. Drug induced liver injury was high in patients aged $\geq$35 years (Tasduq *et al.*,

2005). A recent study by Jasmer *et al.* (2002) found that patients >35 years old had a higher risk of grade 3 or 4 hepatotoxicity.

Fulminant hepatitis carries a very high mortality, resulting from acute hepatitis caused by virus infection, alcohol or drugs. Conventional medical therapies can rescue only about 10% of patients with fulminant hepatitis (Hiroaki *et al.*, 2003). Although liver transplantation has improved their mortality, about 40% of these patients die while waiting for liver transplantation (Shakile *et al.*, 2000)

2.4.1. Chemicals inducing hepatotoxicity

Administration of some chemicals can induce hepatotoxicity. Sodium diethyl dithiocarbamate, diclofenac and ketoconazol are three important chemotherapeutic agents that are commonly associated with hepatotoxicity (Amr and Alaa, 2005). One of the most hazardous pollutants called hexachlorobenzene can also cause hepatotoxicity (Billi de Catabbi *et al.*, 2005). Kaufmann (2005) reported that treatment with some drugs causes liver injury. A lot of drugs are devastated from the market due to their induction of hepatotoxicity. Factors affecting susceptibility to drug-induced injury include age, sex, concomitant use of other drugs, and genetic polymorphism in metabolic pathways involved in activation or disposition of therapeutic drugs (Maddrey, 2005). Older persons who are receiving more drugs simultaneously are more susceptible to liver injuries. Guzman *et al.* (2005) studied about the hepatotoxic effect of paroxetine. Galactosamine is capable of inducing wild hepatic failure (Abul *et al.*, 2005). It resembles viral hepatitis. In a study conducted by Senthilkumar and Nalini (2004) hepatotoxicity was induced by the intake of alchohol. Tomiyama *et al.* (2004) studied the effect of DDT in inducing toxicity to liver. Doxorubicin is an anthracycline antibiotic, popularly used in tumour therapy. Administration of doxorubicin was proved to cause hepatotoxicity (Kalender *et al.*, 2005).

Statins are among the most widely prescribed medications in the western world (Eidelman *et al.*, 2002). Their benefit in the primary and secondary prevention of

cardiovascular disease is unequivocal. According to Naga Chalasani (2005), intake of statin will lead to severe liver toxicity. As per the reports of Ishiyama *et al.* (1990) both hepatotoxicity and oxidative stress have been reported in rats following a single dose treatment with diethyl dithiocarbamate.In a study of Crupi *et al.* (2001), liver damage was induced by the use of carbon tetra chloride. Mitra *et al.* (1998) used paracetamol to induce hepatotoxicity in rats.

Herbal drugs have become increasingly popular among patients and physicians (Schuppan *et al.*, 1999) because of the perception that drugs derived from plants are normally safe. However, numerous reports have come forward, which showed hepatic adverse effects induced by plants containing pyrrolizidine alkaloids such as comfrey (Stickel and Seitz, 2000), preparations produced from germander (Larrey *et al.*, 1992), those containing chaparral extracts (Sheikh *et al.*, 1997), and drugs made of Chinese herbs (Yoshida *et al.*, 1996). These plants may contain alkaloids that are biotransformed to toxins. With most of the herbs, hepatotoxic reactions were reversible after withdrawal. Felix *et al.* (2003) studied the hepatotoxic effects of Kava (*Piper methysticum rhizoma*), which is used as a sleeping aid and for the treatment of anxiety disorders and depression.

2.4.2. Mechanism of drug induced liver injury

Mechanisms of drug-induced liver injury are many and varied. With many drugs, intermediary products produced during metabolism are highly reactive and toxic. In these situations, the balance between the rate of production of the metabolite and the effectiveness of the drug may determine whether or not hepatic injury occurs (Maddrey, 2005). Some earlier studies reported that diclofenac induced liver damage through various mechanisms such as mitochondrial permeability transition (Masubuchi *et al.*, 2002), activation of cytochrome P450 (Cantoni *et al.*, 2003) and generation of reactive oxygen species (ROS) (Cantoni *et al.*, 2003: Gomez-Lechon *et al.*, 2003). Ketoconazol has also been reported to mediate hepatotoxicity through chelating GSH with active toxic metabolites (Rodriguez and Buckholz, 2003).

Hepatic DNA fragmentation has been reported in may hepatotoxicity models following treatments with drugs such as acetaminophen (Ray *et al.*, 1992), carbon tetrachloride (Shi *et al.*, 1998) and diquat (Gupta *et al.*, 200). Amr and Alaa (2005) reported that in hepatotoxicity models induced by diethyl dithiocarbamate, diclofenac and ketoconazol, levels of serum ALT and AST has been significantly increased. These three toxic drugs decreased the levels of GSH and SOD. Diethyl dithiocarbamate induced hepatoxicity in rats was associated with reactive oxygen species (ROS) and drug metabolism (Ishiyama *et al.*, 1990).

Other mechanisms of hepatotoxicity are summarized in the following Table 2 (Dominique, 2000)

Liver injuries	Mechanisms
Acute hepatitis	Metabolite-mediated toxicity Metabolite-mediated immunoallergy and/or autoimmunity
Acute cholestasis	Inhibition of biliary secretion
Macrovacuolar steatosis	Decreased secretion of lipoproteins
Microvesicular steatosis	Inhibition of fattyacid mitochondrial beta-oxidation
Phospholipidosis	Inhibition of lysosomal phospholipases
Chronic hepatitis	Metabolite-mediated immune reaction
Vanishing bile duct syndrome	Autoimmune destruction of small bile ducts. Abnormal multidrug resistance protein system
Sclerosing cholangitis	Biliary ischemia caused by arterial lesions
Veno-occlusive disease	Metabolite-mediated endothelial lesions
Persinusoidal fibrosis	Activation of Ito cells

Table 2. Mechanisms of Hepatotoxicity

2.4.3. Treatment

A lot of treatment methods are found popular in the treatment of hepatoxicity. The stem bark of the Betulaceae plant *Alnus japonica*, which is indigenous to Korea, has been used as a popular folk medicine for hepatitis induced by acetaminophen (Kim *et al.*, 2004). Tazduq *et al.* (2005) reported that Silymarin can reduce hepatic toxicity induced by rifampicin, isoniazid and pyrazinamide. Betaine supplementation blocked or significantly attenuated the induction of hepatotoxicity by alpha-naphthylisothiocyanate (ANIT) (Kim *et al.*, 2005). Jigrine was found to be effective against galactosamine induced hepatitis (Abul, 2005). Jigrine, a polypharmaceutical herbal hepatoprotective formulation containing aqueous extracts of 14 medicinal plants, is used in Indian system of medicine (Unani). Kalender *et.al* (2005) reported that Vitamin E and Catechin could reduce the hepatotoxic effects of antitumour drug doxorubicin. Porchezhian and Ansari (2005) studied the heparoprotective effect of *Abutilon indicum* against carbon tetrachloride and paracetamol-induced hepatoxicity.

2.5.Antitubercular drugs

2.5.1. Tuberculosis

Tuberculosis (TB) is an infection caused by two species of Mycobacteria, "Mycobacterium Tuberculosis and Mycobacterium Bovis". Though it can cause disease involving every organ system in the body, it commonly affects the lungs. The disease was scraping the human kind even in the Neolithic period and till the early 20^{th} century. During the 19th century, up to 25 per cent of deaths in Europe were caused by this disease. The death toll began to fall as living standards improved at the start of the 20th century and from the 1940s, effective medicines were developed. However, there are now more people in the world with TB than there were in 1950, and three million individuals may die this year from this disease - mainly in less developed countries. The disease is more common in areas of the world where poverty, malnutrition, poor general health and social disruption are present. Currently, around 1.7 billion people worldwide, a third of the world's population, are infected by Mycobacterium tuberculosis and 3 million

deaths a year is attributable to tuberculosis. Rapid spread of TB in humans is attributed to crowded living conditions that favour airborne transmission. There was a steady decline in the incidence of tuberculosis, especially in the developed countries till early 1980s; but, since then the trend has reversed and an increasing number of cases have been reported.

2.5.1.2. Causes and Pathogenesis

The term tubercle bacillus refers to two species, "*Mycobacterium Tuberculosis* and *Mycobacterium Bovis*". Other species are classified under a typical Mycobacterial Pathogens. Humans are the only reservoir for *Mycobacterium tuberculosis*. Mycobacterium Bovis was transmitted by contaminated milk once, but is no longer so. Though skin infection by inoculation is seen in pathologists and laboratory personnels, almost all infections are due to airborne transmission by inhalation of droplet nuclei. In general, 3 - 4% of infected individuals will develop active disease during the first year after exposure and a total of 5-15% thereafter. The likelihood of developing active disease varies with the intensity and duration of exposure. Malnutrition, alcoholism, renal failure and uncontrolled diabetes favour the progression of infection to active disease. HIV infection is the strongest risk factor today. The tubercle bacilli, once inhaled, reach the terminal air spaces in the lungs, escaping from the host defense mechanism. They multiply locally but are controlled and retained in the lungs by the body's white blood cells. Sometimes infected white cells carry the bacteria to the lymph nodes and from there to the blood stream. Seeding and unchecked proliferation in other organs can cause disease manifestations elsewhere in the body as well. Pulmonary tuberculosis is usually seen in the upper portions of the lung.

2.5.1.3. Symptoms and Signs

The clinical picture depends on the involved organ. Early tuberculosis of the lung is asymptomatic and may be discovered on a chest x-ray by chance. In case of tuberculosis of the lung, the patient will complain of fever, night sweats, fatigue,

cough, sputum production that is sometimes mixed with blood, weight loss and loss of appetite.

Tuberculosis can also appear as swelling of the glands in the neck with or without fever (lymph node TB), back pain, deformity of the spine and weakness in the lower limbs (TB of the spine), fever, headache, vomiting and drowsiness (TB meningitis), joint pain and swelling (TB arthritis), genitourinary symptoms like flank pain and infertility (genitourinary TB).

2.5.1.4. Treatment and Prognosis

With the advent of 'Combination Chemotherapy', successful treatment of tuberculosis is a reality, but problems of drug resistance, selection of an inappropriate regimen and non-compliance hinder effective therapy. WHO recommends a six-month short course therapy consisting of four drugs - rifampicin, isoniazid, ethambutol and pyrazinamide, for the first two months and two drugs - rifampicin and isoniazid, for the next four months.

2.5.2. Isoniazid

Isoniazid, an antibiotic, is used to treat and prevent tuberculosis since, it prevents Tuberculosis bacteria from multiplying in the body. Isoniazid consumption is associated with a lot of side effects like live difficulty breathing, closing of the throat, swelling of the lips, tongue, or face, unusual weakness or fatigue, abdominal pain, yellow skin or eyes, dark urine etc. Isoniazid toxicity is associated with a high mortality rate (Wason, 1981). If isoniazid is taken acutely, as little as 1.5 g can cause toxicity. Doses larger than 30 mg per kg often produce seizures. Ingestion of the drug in amounts greater than 80 to 150 mg per kg can rapidly lead to death (Shannon *et al.*, 1990). A 1-2 % risk of severe and potentially fatal hepatotoxicity (predominantly hepatic necrosis) associated with the use of isoniazid is problematic in the prophylaxis and treatment of tuberculosis. In addition, daily isoniazid administration is associated with mild elevations of liver enzyme activities in plasma up to 20 % of patients (Troy *et al.*, 1998).

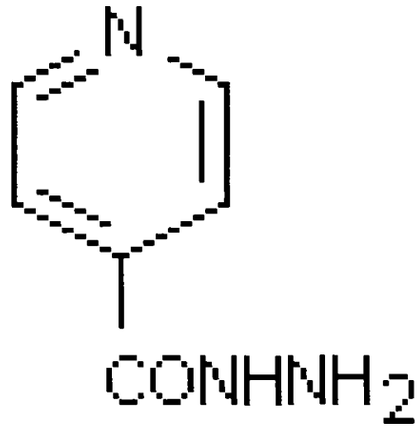


Fig. 2.7. Structure of Isoniazid

2.5.2.1. Structure

Chemically isoniazid is Isonicotinic acid hydrazide. Its molecular formula is $C_6H_7N_3O$. The structure of isoniazid is depicted in Fig. 2. 7.

2.5.2.2. Properties

Isoniazid (INH) has a molecular mass of 137.14g. Isoniazid is a white crystalline powder in occurrence. The substance decomposes on heating and on burning produces toxic fumes including nitrogen oxides. Melting point of Isoniazid comes around 170-173°C. INH is a colourless, odourless, white crystalline powder slowly affected by exposure to air and to light. 1 g isoniazid is soluble in 8 g water. A 10% solution has a pH of 6.0 to 8.0.Isoniazid can induce hepatotoxicity when administered for a long time (Saraswathy and Shyamala Devi, 1999). Hepatitis is due to a toxic metabolite of monoacetyl hydrazine, which binds covalently to liver proteins (Black *et al.*, 1975). In some patients, an allergic mechanism has also been proposed: acetylation of hepatic macromolecules by acetyl hydrazine may lead to the release of antigenic macromolecules, which induce the formation of antibodies directed against the liver (Davies, 1981). Isoniazid interacts with dietary supplements. Isoniazid can interfere with the activity of vitamin B_6 (Goldman and Braman, 1972). Vitamin B_6 supplementation is recommended, especially in people with poor nutritional status, to prevent development of isoniazid-induced peripheral neuritis (inflamed nerves) (Mandell and Petri Jr., 1996). Isoniazid may kill friendly bacteria, in the large intestine, that produce vitamin K. (Holt, 1998). Isoniazid may interfere with the activity of other nutrients, including vitamin B_3 (niacin), vitamin B_{12}, vitamin D, and vitamin E, folic acid, calcium, and magnesium (Werbach, 1997; Holt, 1998).

2.5.2.3. Metabolic pathway of Isoniazid

The major route of isoniazid metabolism is hepatic acetylation by N-acetyl transferase, which produces acetylisoniazed. The rate of acetylation is

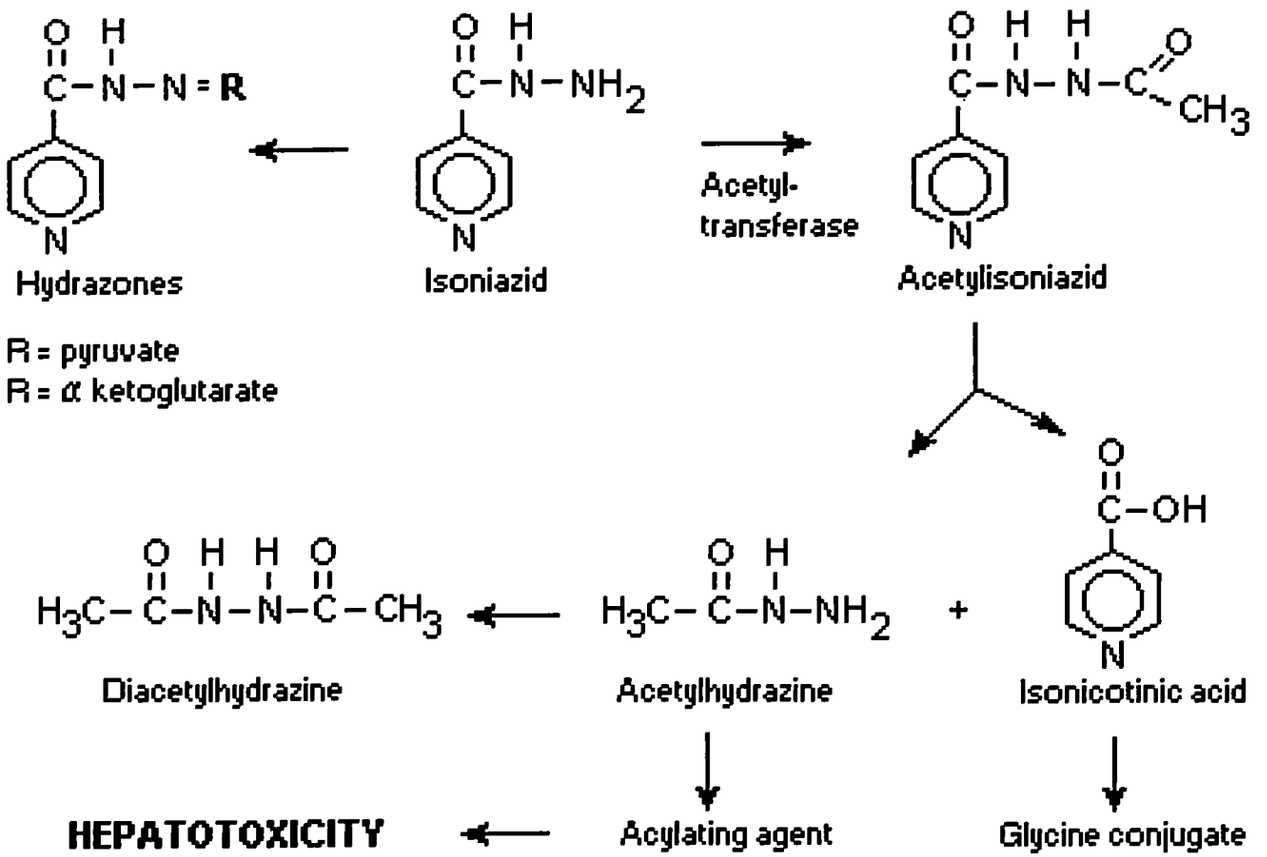


Fig. 2.8. Metabolic pathway of Isoniazid

genetically determined. Acetyisoniazid is further hydrolysed to isonicotinic acid and acetylhydrazine, both of which are excreted in the urine. Isonicotinic acid is conjugated with glycine. Acetylhydrozine is further metabolized to deacetylhydrazine and may be converted by the hepatic microsomal enzymes to the reactive metabolite (presumed to be hydrazine) that are thought responsible for INH-induced hepatotoxicity. Acid labile hydrazones of isoniazid are formed with a-ketoglutarate and pyruvate, but since these do not appear to any extent in the blood, they are thought to be produced in the pointed to cytolytic liver injury in rat studies, as a result of free radical generation, isoniazid bladder (Boxenbaum and Riegelman, 1974). Fig. 2. 8 shows the metabolic pathway of isoniazid.

2.5.2.4. Physiological changes

Some earlier studies have pointed to the cytolytic liver injury in rat studies, as a result free radical generation when isoniazid was used. These cytotoxic materials had been shown to come from lipid peroxidation and the suppression of the antioxidant system (Saraswathy *et al.*, 1998). Studies related to the suppression of the antioxidant system in antituberculosis drugs with rats have also been reported (Skakun and Slivka, 1992). Georgieva *et al.* (2004) reported that lipid peroxidation was found high in isoniazid treated animals.According to Tasduq *et al.* (2005) Alanine aminotrasferase (ALT) and Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) levels were increased when isoniazid was orally administered in rats for induced hepatotoxicity.

2.5.3. Rifampicin

Rifampicin is an antibiotic drug of the rifamycin group. It is typically used to treat mycobacterium infection, including tuberculosis and leprosy and also has a role in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) in combination with fusidic acid.

Rifampicin is a semisynthetic derivative of rifamycin antibiotics which are produced by the fermentation of a strain of *Streptomyces mediterranei*, a species which was first isolated in Italy in 1957 from a soil sample collected in France. The fermentation produces rifamycin B. Rifamycin B is transformed by a series of reactions into 3- formylrifamycin SV, which in turn is condensed with 1-amino-4-methylpiperazine in peroxide-free tetrahydrofuran to give rifampicin.

Rifampicin inhibits DNA-dependent RNA polymerase in bacterial cells by binding its beta subunit, thus preventing transcription of messenger RNA and subsequent translation to proteins.

2.5.3.1. Structure

Chemically rifampicin is 3-[[(4-methyl-1-piperazinyl)imino]methyl)] rifamycin. Structure of rifampicin is given in Fig. 2. 9.

2.5.3.2. Properties

Molecular formula is $C_{43}H_{58}N_4O_{12}$. Molecular weight is 822.96g. Its colour is red to orange. Powder in nature. Odourless. Melting point is 138 to 188 °C. A 1% suspension in water has pH 4.5 to 6.5. Rifampicin is very soluble in water. Solubility in aqueous solution is increased at acidic pH. Freely soluble in chloroform.

Patients receiving antitubercular drugs frequently develop acute or chronic hepatitis (Hussain, 2003). Prolonged use of Rifampicin may affect gastrointestinal system also (Wada, 1998). When rifampicin is administered the levels of ALT was increased. Bilirubin serum levels were also found increasing (Lenaerts, 2005).

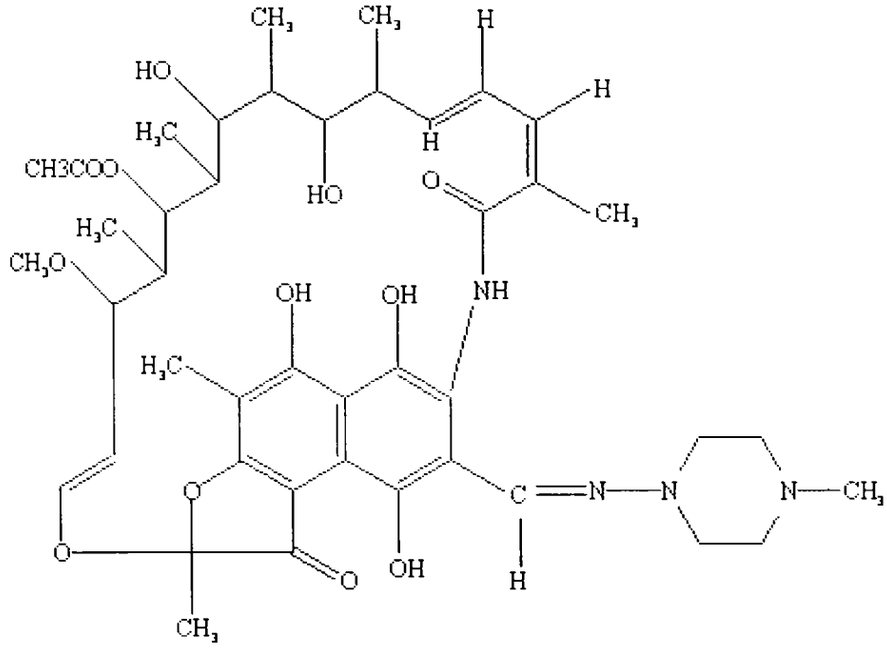


Fig. 2.9. Structure of rifampicin

This is an indication of drug-induced hepatotoxicity. Dawe *et.al* (2004) reported acute renal failure associated with rifampicin administration.

2.5.3.3. Physiological changes

An earlier reported study (Chi *et al.*, 2003) proved that intake of rifampicin increased the level of alanine aminotransferase (ALT). In a study conducted by Prabakan *et al.* (2000) The level of liver mitochondrial protein and the activities of isocitrate dehydrogenase, a-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH dehydrogenase and cytochrome c oxidase were found significantly decreased in rifampicin and isoniazid intoxicated rats.