Gastric ulceration:

A gastric ulcer is a break in the normal tissue that lines the stomach. Gastric ulcer develops when the delicate balance between some gastroprotective factors and aggressive factor is lost. The aggressive factors are either endogenous like hydrochloric acid, pepsin, refluxed bile, leukotrienes and reactive oxygen species or exogenous like alcohol, steroidal and nonsteroidal anti-inflammatory drugs and drugs which stimulate gastric acid and pepsin secretion, stress and tension and Helicobacter pylori. The mucosal defense against these aggressive factors is contributed by mucusbicarbonate barrier, surface active phospholipids, prostaglandin, mucosal blood flow, cell renewal and migration, antioxidants and antioxidant enzymes, and some growth factors [17].

**Fig. 1: Pathogenesis of gastric (or peptic) ulcer**
Hyperacidity and gastric ulcer

Hyperacidity or acid dyspepsia simply means increase of acidity in the stomach. The human stomach secretes hydrochloric acid which is necessary for the digestion of food. When the stomach contains an excessive amount of hydrochloric acid, then the condition is called as hyperacidity or acid dyspepsia. Hyper-acidity is one of the causes of peptic ulcer and gastritis. Uncontrolled hydrochloric acid secretion and ulceration in the stomach due to various factors are serious global problem today [17] but the acid factor is not the sole cause for the development of gastric ulcer.

Helicobacter pylori and gastric ulcer

A major causative factor in 60% of gastric and up to 90% of duodenal ulcers, is chronic inflammation due to *Helicobacter pylori* that colonizes the antral mucosa. *H. pylori* is a type of bacteria-a germ that may cause infection. *H. pylori* infection is common, particularly in developing countries, and often begins in childhood. In Western countries the prevalence of *Helicobacter pylori* infections roughly matches age (i.e., 20% at age 20, 30% at age 30, 80% at age 80 etc.). Prevalence is higher in third world countries. Transmission occur by food, contaminated groundwater, and through human saliva (such as from kissing or sharing food utensils) [38]. Symptoms usually don't occur until adulthood, although most people never have any symptoms. *H. pylori* causes more than half of peptic ulcers worldwide [38]. The bacterium causes peptic ulcers by damaging the mucous coating that protects the stomach and duodenum. Damage to the mucous coating allows powerful stomach acid to get through to the sensitive lining beneath. Together, the stomach acid and *H. pylori* irritate the lining of the stomach or duodenum and cause an ulcer. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis (type B gastritis), resulting in a defect in the regulation of gastrin production by that part of the stomach, and gastrin secretion can either be increased, or as in most cases, decreased, resulting
in hypo- or achlorhydria. Gastrin stimulates the production of gastric acid by parietal cells and, in *H. pylori* colonization, responses that increase gastrin will also cause increased gastric acid secretion that can contribute to the erosion of the mucosa and therefore ulcer formation.

**Stress and gastric ulcer**

Researchers also continue to look at stress as a possible cause, or at least complication, in the development of ulcers. There is debate as to whether psychological stress can influence the development of peptic ulcers [39]. Burns and head trauma, however, can lead to physiologic stress ulcers, which are reported in many patients who are on mechanical ventilation [40].

An expert panel convened by the Academy of Behavioral Medicine Research concluded that ulcers are not purely an infectious disease and that psychological factors do play a significant role. Researchers are examining how stress might promote *H. pylori* infection. For example, *Helicobacter pylori* thrive in an acidic environment, and stress has been demonstrated to cause the production of excess stomach acid [41]. This was supported by a study on mice showing that both long-term water-immersion-restraint stress and *H. pylori* infection were independently associated with the development of peptic ulcers [42]. A study of peptic ulcer patients in a Thai hospital showed that chronic stress was strongly associated with an increased risk of peptic ulcer, and a combination of chronic stress and irregular mealtimes was a significant risk factor [43].
**Drugs and gastric ulcer**

Gastric ulcer has been associated with the use of alcohol, steroidal and non-steroidal anti-inflammatory drugs and drugs which stimulate gastric acid and pepsin secretion [17].

**Ethanol-induced gastric ulceration:**

Ingestion of ethanol is the predisposing cause of acute hemorrhagic gastric erosions in human [17]. Ethanol lowers the concentration of non-protein sulphydryls specially GSH [17], thereby exerting ulcerogenic effect by increasing ROS formation [17].

**Gastric ulceration due to anti-thyroid drugs:**

Antithyroid drugs specially mercaptomethylimidazole (MMI), used to treat hyperthyroidism, is a potent inducer of gastric acid secretion [44]. MMI can induce acid secretion *in vivo* or isolated gastric mucosa or in gastric gland preparation where the effect can also be mimicked by addition of micromolar concentration of $\text{H}_2\text{O}_2$ [45]. Since MMI augments both acid and luminal pepsin content [45], this drug is potentially dangerous to aggravate gastric ulcer.

**Steroidal anti-inflammatory and gastric ulceration:**

Steroidal anti-inflammatory drugs, like dexamethasone is a widely used drug in the medication of different diseases having various genomic and non-genomic actions [46]. However, these have been reported to have different adverse effects specially on gastric mucosa. It has been reported by Bandyopadhyay U et al. (1999) that dexamethasone stimulates both basal and mercaptomethylimidazole-induced gastric acid secretion. It aggravates the severity of ulcer under different experimental conditions such as pylorus ligation or pylorus-esophagus ligation through its inhibitory role on the prostaglandin synthetase and peroxidase activity, the two important protective enzymes of the gastric mucosa [47].
**Non-Streoidal Anti-inflammatory Drug (NSAID)-induced gastric ulceration**

Non Steroidal Anti-inflammatory Drugs (NSAIDs) have been reported to affect the entire gastrointestinal tract [6] and are responsible for a high level of dyspepsia [7]. A recent report claimed that approximately 2000 patients per annum may die as a result of NSAID-induced ulcer bleeding and perforation in the UK [5]. Worldwide, more than 30 million or even more people consume NSAIDs daily and of these 40% of the patients are more than 60 years of age. Known additional risk factors include advanced age, previous history of ulceration, concomitant use of steroids, higher doses of NSAIDs and the use of more than one NSAIDs at a time, concomitant administration of anticoagulants, and co-existing serious systemic disorders [1]. The anti-inflammatory actions of NSAIDs are exerted through the inhibition of cyclooxygenase responsible for prostaglandin synthesis. However, inhibition of gastro-protective prostaglandin synthesis in the stomach brings about ulceration due to weakening of the mucosa and free radical generation [33].

Nonsteroidal anti-inflammatory drugs do possess analgesic, antipyretic (fever-reducing) and anti-inflammatory effects. These drugs have the ability to inhibit fever, pain, and inflammation by blocking the formation of prostaglandins by inhibiting the rate-limiting enzyme cyclooxygenase [48-52]. There are at least fifteen NSAIDs available in the market at present and, because of their increased potency to inhibit cyclooxygenase activity, they are frequently prescribed for individuals suffering from chronic inflammation and pain [53]. There are several different types of NSAIDs. Some of the examples are listed below:

- **Salicylates:** aspirin, diflunisal, salsalate
- **Arylalkanoic acids:** diclofenac, indomethacin
- **2-Arylpropionic acids (profens):** ibuprofen, ketoprofen, naproxen, carprofen
- **Pyrroles:** ketorolac
- **Enolic acids (oxicams):** piroxicam, meloxicam
- Sulphonanilides: nimesulide
- Napthylalkanones: nabumetone

Non-steroidal anti-inflammatory drugs which are commonly used as pain killer in the treatment of rheumatoid arthritis and many other acute and chronic inflammatory conditions cause gastric mucosal damage [17]. These drugs apart from causing inhibition to COX-2 also inhibit COX-1 which produces gastro-friendly prostaglandins. The best studied drug, aspirin, by inhibiting prostaglandin synthesis, interferes with the protective mechanism such as mucus and bicarbonate secretion, surface epithelial hydrophobicity and mucosal blood flow [54]. These changes permit back diffusion of acid through the breached surface to destroy cells, arteries, capillaries and veins causing hemorrhagic ulcer. Enhancement of leukotriene synthesis by NSAIDs also exhibit sufficient damaging effect [55]. Aspirin also decreases mucosal ATP synthesis and cell turnover process [17]. The changes brought about by NSAIDs, as described above, in totality can induce gastric damage through generation of ROS [56] and inhibiting cell proliferation [56, 17, 57]. Non-steroidal anti-inflammatory drugs also inhibit gastric peroxidase [60], an important gastric antioxidant enzyme found in rodents [58] as well as in humans, and may increase H2O2 and 'OH to cause oxidative mucosal damage [60, 59].

Fig.3: NSAID: Mechanism of action
**Piroxicam:**

| Systematic (IUPAC) name:                                                                                     |
| (8E)-8-[hydroxy-(pyridin-2-ylamino)methylidene]-9-methyl-10,10-dioxo-10\(\lambda^6\)-thia-9 azabicyclo          |
| [4.4.0] deca-1,3,5-trien-7-one                                                                               |
| Formula: C_{15}H_{13}N_{3}O_{4}S                                                                               |
| Mol. mass: 331.348 g/mol                                                                                      |
| Half-life: 45 hours in man                                                                                     |
| 5.8 hours in rat                                                                                              |
| Excretion: Urine and feces                                                                                     |
| \(LD_{50}\) of piroxicam given orally in the normal rat: \(\sim\) 270 mg/kg bw (for single dose)               |

![Fig. 4: Structure of piroxicam](image)

**Piroxicam** is a member of the oxicam group of NSAIDs. According to the Biopharmaceutic Drug Classification System (BCS) proposed by Amidon et al. [62], piroxicam is a class 2 drug with low solubility and high permeability. Its pharmacokinetic pattern is characterized by slow and gradual absorption via the oral route and a long half-life of elimination, rendering a prolonged therapeutic action. Piroxicam like other NSAIDs, is a non-selective COX inhibitor possessing both analgesic and antipyretic properties. It undergoes enterohepatic circulation [61].

Gastrointestinal bleeding is also related to the type of NSAID and the dosage [63]. Piroxicam, a classic nonselective, COX-1 preferent NSAID, is widely used by patients requiring anti-inflammatory intervention [1]. High ulcerogenic potential of this oxicam anti-inflammatory is because of its ability to decrease the synthesis of prostaglandins through the inhibition of COX-1 and forces clinicians to limit its use on many occasions [60]. It has been reported that approximately, 30% of all patients receiving daily doses of 20 mg of piroxicam experience side effects including inflammation of the stomach mucosa and ulceration [64]. Possible involvement of oxidative stress in piroxicam-induced gastric lesions has also been indicated recently [60, 10].
Reactive Oxygen Species and Gastric Ulceration

The seemingly paradoxical consequences of the beneficial and harmful effects of oxygen (O2) have been shown for several decades. While more than 95% of the O2 taken in by the aerobic organisms is fully reduced to water (H2O) during the process of mitochondrial respiration, a small percentage (<5%) of the O2 consumed is converted to semireduced species i.e. the superoxide anion free radicals (O2•−), Hydrogen peroxide (H2O2) and Hydroxyl free radicals (‘OH). These species are collectively referred to as Reactive Oxygen Species (ROS) which can be highly toxic, and their interactions often with cellular macromolecules bring about oxidative damage [17]. The most toxic of the ROS is the ‘OH which is often formed when O2•− and H2O2 are exposed to the trace transition metals iron or copper via metal catalyzed Haber-Weiss reaction:

\[
\begin{align*}
\text{Fe}^{3+} + \text{O}_2^{•−} & \rightarrow \text{Fe}^{2+} + \text{O}_2 \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^{−}
\end{align*}
\]

The net result is therefore,

\[
\text{O}_2^{•−} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \cdot\text{OH} + \text{OH}^{−}
\]

The mechanism of ROS formation and how the cellular antioxidant systems defend against accumulating ROS (Fig. 5) have already been reviewed [65, 66].
Involvement of ROS in the pathogenesis of gastric ulceration was first evident from the studies of ischemia-reoxygenation-induced gastric mucosal injury [67]. A growing body of experimental and clinical evidence suggests that gastric mucosal damage by ethanol, NSAIDs, and by Helicobacter pylori is mediated through ROS [17]. Moreover, ROS may play an important role in gastric ulceration induced by several kinds of stress. Reactive Oxygen Species also decreases the level of endogenous antioxidants such as GSH, α-tocopherol and ascorbate and, make the mucosa more prone to oxidative damage. The pathogenesis of gastric mucosal lesions by water-immersion restraint stress and burn shock in rat is associated with increased lipid peroxidation [17]. Furthermore, cold-restraint stress has been shown to alter the level of various damaging and cytoprotective factors of rat gastric mucosa to cause gastric ulceration [32].

Although the involvement of ROS in gastric lesions caused by various types of stress has been reported [17], detailed investigation on the role of
ROS in cold restraint stress induced gastric ulceration has been studied recently wherein researchers have shown the causal role of specific oxygen-derived free radical in mediating gastric damage during stress. In this work, stress induced gastric ulceration has been shown to be associated with increased levels of lipid peroxidation and a decreased endogenous GSH content of the rat gastric tissue. This work has further shown that there occurred an activation of SOD and inactivation of gastric peroxidase (GPO) following cold-restraint stress [17] - a condition suitable for generation of $\text{H}_2\text{O}_2$ and formation of more reactive $\cdot\text{OH}$ which causes antioxidant depletion and lipid peroxidation [68]. This observation was supported by other researchers as well [32].

Yoshikawa et al. [67] reported suppression of both lipid peroxidation and gastric mucosal injury induced by ischemia reperfusion, after administration of SOD and catalase, indicating the role of ROS in the damage. Similarly, astaxanthin has been shown to provide protection against naproxen-induced and stress-induced gastric ulceration by reducing the level of lipid peroxide and free radicals indicating again the role of ROS in gastric damage [69, 70]. Lipid peroxidation caused by $\cdot\text{OH}$ is increased in gastric lesions induced by ethanol, Indomethacin [17], ischemia-reperfusion [67], water immersion and burn shock [17]. Dimethyl sulfoxide (DMSO), a specific $\cdot\text{OH}$ scavenger, reduces the gastric mucosal injury produced by cold-restraint stress [32] indicating a critical role of $\cdot\text{OH}$ in the mucosal damage. It has been shown by direct measurement using DMSO that $\cdot\text{OH}$ is actually generated in the gastric mucosa under stress [68]. The plausible role of $\cdot\text{OH}$ in the generation of stress-ulcer is shown in Fig.6. Stress causes both sympathetic and parasympathetic stimulation of the stomach, which induces and increased motility and muscular contraction leading to vascular compression and mucosal ischemia [17]. Sympathetic stimulation causes direct arteriolar vasoconstriction and, thus greatly reduces the blood flow to the stomach leading to local hypoxia and near or actual ischemia. The ischemic condition increases the leakage of electrons from the mitochondrial electron transport chain [17] and facilitates the availability of “redox-active” copper or iron. Increased $\text{O}_2^-$ production leads to elevated
levels of $\text{H}_2\text{O}_2$ (caused by dismutation reaction catalyzed by SOD), which, in conjunction with $\text{O}_2^\cdot\text{'}$ generates $\cdot\text{OH}$ via the metal catalyzed Haber-Weiss reaction. Hydroxyl radicals thus generated oxidizes important cellular constituents such as structural and functional proteins, membrane lipids, and depletes cellular glutathione. Lipid peroxidation causes loss of membrane fluidity, impaired ion transport and finally loss of cellular functions [17].

Stress also causes inactivation of prostaglandin synthetase (currently known as Cyclooxygenase-1 [COX-1]) – a key enzyme in gastro-protection against all forms of insults to the gastric mucosa. It has been shown that COX-1, but not COX-2, is constitutively expressed throughout the gastrointestinal tract (GIT) in several species including humans [71]. Prostaglandins (especially PGI$_2$ and PGE$_2$) formed by COX-1 have important cytoprotective effects on the gastrointestinal mucosa. The cytoprotective action is complex and multi factorial. Both PGI$_2$ and PGE$_2$ reduce gastric acid secretion from stomach parietal cells, increase mucosal blood flow, and stimulate the release of viscous mucus [72]. It is generally believed that gastrointestinal toxicity of classical NSAIDs is due to inhibition of COX-1 activity.

Fig.6: Proposed mechanism of stress-induced gastric ulceration [Bandyopadhyay D, Chattopadhyay A. Reactive Oxygen Species-Induced ulceration: Protection by Melatonin. Curr Med Chem 2006; 13: 1187-1202.]
Recent clinical trials have shown that the use of two COX-2 inhibitors, celecoxib and rofecoxib, is associated with effective anti-inflammatory relief in osteoarthritis and rheumatoid arthritis as well as with a significantly lower incidence of ulcer complications [73-75]. Prolonged use of COX-2 inhibitors has been shown to severely affect heart and kidneys [76, 77]. Additional studies have also revealed that COX-2 inhibitors also inhibit COX-1 [77]. The question before the researcher is whether COX-1 inhibitors can be used as treatment for inflammation in combination with some drugs, antioxidant(s) or natural extracts which will minimize the gastro-toxic effects of these anti-inflammatory drugs?

A number of anti-ulcer drugs such as antacids, anticholinergics, proton pump inhibitors, H2 receptor antagonists [78, 79], cytoprotectives and prostaglandin analogues [80] are available for treatment of ulcer but all these drugs have side effects and limitations. Thus, research into new medicine that enables the development of alternative therapies for the treatment of gastric ulcer is of paramount importance, and in this context, plant extracts are among the most promising source of new treatments for this complaint [81-83] or some well known antioxidant with anti-inflammatory properties, like melatonin [84] can be used alone or in combination with well known medicinal herbs, like tulsi at the minimum possible doses.

<table>
<thead>
<tr>
<th>Antacids</th>
<th>Action</th>
<th>Side-effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bicarbonate</td>
<td>Most potent, rapidly acting</td>
<td>Rarely prescribed now a days, systemic alkalosis, rebound hyperacidity, belching, precipitate congestive heart failure, edema.</td>
</tr>
<tr>
<td>Aluminium hydroxide</td>
<td>Weak, slowly acting, increases mucus secretion, absorbs and inactivate pepsin</td>
<td>Cause delay in gastric emptying, constipation, hypophosphatemia, encephalopathy, osteodystrophy, proximal myopathy in patients with renal impairment.</td>
</tr>
<tr>
<td>Magnesium carbonate</td>
<td>Less rapidly reactive with acid</td>
<td>Laxation</td>
</tr>
<tr>
<td>Magnesium trisilicate</td>
<td>Poorly reacts with acid</td>
<td>Laxation</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>Neutralizes HCl rapidly and effectively</td>
<td>Belching, abdominal distention and flatulence, hypercalcemia, marked rebound hyperacidity.</td>
</tr>
<tr>
<td>Antacid combinations</td>
<td>Slow acting</td>
<td>Constipation, laxation</td>
</tr>
</tbody>
</table>

Table 1: Some of the antacids in use [Bandyopadhyay D, Chattopadhyay A. Reactive Oxygen Species-Induced ulceration: Protection by Melatonin. Curr Med Chem 2006; 13: 1187-1202.]
Tulsi: The Queen of Herbs

*Ocimum sanctum*

Familiar name: Holy Basil
Bengali and Hindi name: Tulsi
Sanskrit name: Tulsi

Tulsi, Queen of Herbs, the legendary “Incomparable One” of India is one of the holiest and most cherished of the many healing and health-giving herbs of the Orient. The holy or sacred basil ‘Tulsi’ is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani systems of holistic health and herbal medicine of the East [85].

An impressive array of health promoting, disease preventing and life prolonging properties of ‘Tulsi’ have been described and documented over five millennia. Medical, religious and culinary use of ‘Tulsi’ has also been documented for centuries in China and the rest of Asia, the Middle East, North Africa and Australia [85].

Tulsi initially met with mixed reactions in Europe. After a period of cultural assimilation, the plant became known to Christians as sacred or holy basil, and was hailed as “The King of Herbs” by European herbalists and physicians, as well as cooks. The name *basil* is likely derived from Greek words referring to “royalty” or “king” [85].

At least three types of Tulsi are encountered which are cultivated in India – Rama Tulsi (green leafed, most common), Krishna Tulsi (dark green to purple leafed) and Bana Tulsi (forest variety). Rama Tulsi is mostly being used as medicinal plant in Ayurveda. Its scientific name is *Ocimum sanctum*.
**Taxonomy**

Domain: Eukaryota  
Kingdom: Plantae  
Phylum: Magnoliophyta  
Class: Magnoliopsida  
Subclass: Lamiales  
Order: Lamiales  
Family: Lamiaceae  
Genus: Ocimum  
Species: sanctum  

*Fig. 8: Tulsi (Ocimum sanctum) plant*

“The Elixir of Life”, Tulsi has been traditionally employed in hundreds of different formulations for the treatment of a wide range of disorders, including those of the mouth and throat, lungs, heart, blood, liver, kidney, and the digestive, metabolic, reproductive and nervous systems [85]. Tulsi is commonly used to treat coughs, colds and flu, head and ear aches, rheumatism and arthritis, malaria, fever, allergies, and various skin diseases, to reduce the toxicity of various poisons, including insect and reptile bites, to expel intestinal parasites, repel insects and purify the air [85].

Current scientific research offers substantial evidence that Tulsi protects against and reduces stress; enhances stamina and endurance; increases the body’s efficient use of oxygen; boosts the immune system; reduces inflammation; protects against radiation damage; lessens aging factors; supports the heart, lungs and liver; has antibiotic, antiviral and antifungal properties; enhances the efficacy of many other therapeutic treatments; and provides a rich supply of antioxidants and other nutrients [85].

Research indicates that Tulsi has a very high safety margin with exceptionally low toxicity, providing general beneficial effects at doses without adverse reactions or other undesirable side effects [86].

Overall, Tulsi is a premier adaptogen, helping the body and mind to adapt and cope with a wide range of physical, emotional, chemical and...
infectious stresses, and restore disturbed physiological and psychological functions to a normal healthy state [85].

**Melatonin**

<table>
<thead>
<tr>
<th>Systematic (IUPAC) name:</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-[2-(5-methoxy-1H-indol-3-yl) ethyl] ethanamide</td>
</tr>
<tr>
<td>Biochemical Name: N-acetyl-5-methoxy tryptamine</td>
</tr>
<tr>
<td>Formula: C13H16N2O2</td>
</tr>
<tr>
<td>Mol. mass: 232.278 g/mol</td>
</tr>
<tr>
<td>Metabolism: Hepatic via CYP1A2 mediated 6-hydroxylation</td>
</tr>
<tr>
<td>Excretion: Urine</td>
</tr>
<tr>
<td>Average physiological plasma levels: 10-60pg/mL</td>
</tr>
</tbody>
</table>

**Fig. 9: Structure of melatonin**

Melatonin (N-acetyl-5-methoxy tryptamine), a tryptophan derivative, was first isolated from bovine pineal glands and was structurally identified in 1958 by Aaron Lerner [48]. It is a naturally occurring compound found in microbes, plants and animals including humans [87, 22].

In mammals, melatonin is synthesized and secreted into the blood by the pineal gland in the brain. The pineal production of melatonin in vertebrates exhibits an unambiguous circadian rhythm with its peak near the middle of scotophase and basal levels during the photophase. Known as the "hormone of darkness", it is secreted in darkness in both day-active (diurnal) and night-active (nocturnal) animals [88].

Melatonin was found to be a sleep promoter [89, 90], a chemical signal of light and darkness (Zeitgeber) as well as a regulator of photoperiod-dependent seasonal reproduction in some vertebrates [89]. The pineal melatonin production in mammals including humans changes with age and its amount waning with the advancement of age (Fig. 10) [91].
**Biosynthesis of melatonin:**

The amino acid L-tryptophan is considered to be the primary precursor of melatonin biosynthesis [18]. Melatonin is biosynthesized through several enzymatic steps including tryptophan 5-hydroxylation, decarboxylation, N-acetylation and O-methylation. Alternatively, but at lower flux rates, melatonin can also be formed via O-methylation of serotonin and subsequent N-acetylation of 5-methoxytryptamine or by O-methylation of tryptophan followed by decarboxylation and N-acetylation [18].

![Chemical structures of tryptophan, 5-hydroxytryptophan, serotonin, N-acetylserotonin, and melatonin.](image)

**Fig. 10:** The synthesis and catabolism of melatonin [Carpentieri A, DeBarboza GD, Areco V et al. New perspectives in melatonin uses. Pharmacol Res 2012; 65(4): 437-444].

The hydroxy indole-O-methyl transferase (HIOMT) is one of the major enzymes regulating melatonin biosynthesis. There are two main pathways in the catabolism of melatonin. About 60% of melatonin is hydroxylated to 6-hydroxymelatonin, which undergoes further conjugation to form either 6-sulfomelatonin or 6-hydroxymelatonin glucuronide. Furthermore, about 15% is metabolized to the N1-acetyl-5-methoxy-kynuramine (AMK), while about 25% of melatonin remains unchanged. All the metabolites are
excreted into urine and, as in melatonin synthesis there is a circadian rhythm in the excretion, with higher rates during darkness [92].

Originally, melatonin was believed to be synthesized exclusively in the pineal gland of vertebrates including humans. However, melatonin of extra-pineal origin has also been identified [35, 17]. It is produced by a variety of peripheral cells such as bone marrow cells, ganglionic cells of retina, lymphocytes, gastric mucosal cells and epithelial cells [18]. The biosynthetic pathways in these cells have been established [18]. Usually, the melatonin concentration in these cells is much higher than that found in the blood but it does not seem to be regulated by the photoperiod [18]. It is speculated that the local melatonin synthesized in the tissues is consumed up as a protective measure against oxidative stress [18]. Melatonin has been shown to reach and bind to melatonin receptors in the brains of chicks that ingested a plant feed such as rice reported to be rich in melatonin [93]. Further, consumption of walnuts has been shown to elevate plasma melatonin level in humans [18].

The well-documented effects of melatonin and its metabolites as antioxidants have shown that they protect cells, tissues and organs from oxidative damage induced by ROS as well as from nitrogen-based reactants [94, 95]. Melatonin is particularly effective in neutralizing the hydroxyl radical (•OH) which attacks DNA, proteins and lipids leading to a variety of disorders [25, 96]. Melatonin also detoxifies superoxide anion free radical (O2•−) [97], nitric oxide (NO•), peroxynitrite anion (ONOO•) [98], hypochlorous acid (HOCl) [99], the haemoglobin oxoferryl radical [101], ABTS+ cation radical and possibly the peroxyl radicals (LOO•) [102], all of which cause cell damage [100]. In addition, melatonin inhibits inducible nitric oxide synthetase (iNOS) [103] and stimulates several antioxidant enzymes [104]. Additionally, it increases the efficiency of the electron transport chain and, as a consequence, likely reduces electron leakage and the generation of free radicals [105]. Due to its antioxidative actions, melatonin protects against heavy metals [106] and other toxic agents and it works synergistically with exercise to improve stroke volume [107].
Melatonin receptors:

Many biological effects of melatonin are produced through activation of melatonin receptors. The first melatonin receptor gene, expressed in *Xenopus laevis* melanophores, was cloned in 1994 [108]. After that many melatonin receptors and receptor fragments have been cloned from different animal classes. The length of melatonin receptor proteins is 346-420
amino acids and their molecular weights are 39-47 kDa [109]. Since melatonin receptors are located in the cell membrane, melatonin regulates the function of the cell through G-protein-regulated effectors. Based on their DNA and amino acid sequences, the melatonin receptors can be divided into three subtypes. Two of these, MT1 and MT2, are expressed in mammals, and the third, Mel1c, is expressed in birds, amphibians and fish [111]. Two of these melatonin receptors (MT1 and Mel1c) has similar pharmacological specificity: 2-iodomelatonin > melatonin > 6-chloromelatonin > 6-hydroxymelatonin > N-acetyl-5-hydroxytryptamine > serotonin. MT2 receptors differ only in that the affinity of 2-iodomelatonin, melatonin and 6-chloromelatonin is equal for this receptor [110].

**Melatonin as an antioxidant:**

Tan et al. in 1993 have shown for the first time that melatonin does possess free radical scavenging ability in *in vitro* system [25]. The ability of melatonin to serve as an antioxidant at both physiological as well as pharmacological concentrations *in vivo* in humans and different experimental models of oxidative stress have been demonstrated [97]. Some of the recent reports have shown that pharmacological doses of melatonin protects against stress- and drug-induced gastric ulceration in experimental rats through its antioxidant mechanism(s) [35]. Melatonin can function as a pervasive and powerful antioxidant with a particular role in the protection of nuclear and mitochondrial DNA [106]. The antioxidant properties of melatonin and its possible regulatory effects on ROS production and redox signaling have been proposed to play a key role in antagonizing the mitochondrial pathway of apoptosis [107]. In the recent years, several findings support the antioxidant effect as well as a direct role of melatonin in mitochondrial homeostasis [112]. This latter action of melatonin may contribute to melatonin’s protective effects in degenerative disorders such as Parkinson’s disease, Alzheimer’s disease, epilepsy, aging, ischemia-reperfusion and sepsis, all of which involve mitochondrial dysfunction as a primary or secondary cause of the disease [111]. Melatonin’s ability to provide protection against formation of ulcer in the stomach has been
shown in different models of oxidative stress [32-35] and is currently an emerging area of research. Recently, evidence has been provided to show that melatonin protects against restraint-cold stress induced gastric ulceration in rats not only by scavenging hydroxyl radical (·OH) but also possibly by altering the activities of three key gastric antioxidant enzymes, i.e., gastric peroxidase (GPO), catalase and superoxide dismutase (SOD) [32, 60]. Understanding the protective role of melatonin against ulceration of the gastric mucosa is an interesting task since it is a highly conserved natural molecule present both in plants and animals and its pharmacological doses have been found to be non-toxic.