CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION TO PEPTIC ULCER DISEASE

Our gastrointestinal tract (GIT) is responsible to digest our food, absorb nutrients and excrete unabsorbed waste product. It is the organ that provides a continuous supply of water, electrolyte and nutrients to the whole part of the body. Peptic ulcer disease or PUD is a pathological condition of this organ and refers to the painful spot characterized by the presence of ulcer in any part of GIT which exposed to acid and pepsin. Mostly the organ stomach (gastric ulcer; Figure 1.1) and duodenum (duodenal ulcer; Figure 1.2) are affected by this disease. Benign ulcerative lesion of the stomach and duodenum are collectively called as peptic ulcer disease. The word ulcer derived from the Latin term ‘ulcus’ which means painful spot, whereas the word peptic is derived from the Greek word ‘peptikos’ means digestion [1]. PUD is formed due to the imbalance between the aggressive factors (gastric acid, gastrin, pepsin, Helicobacter pylori, NSAIDs, alcohol, bile, stress, etc.) and defensive factors (mucus, mucosal blood flow, prostaglandin, NO, HCO3⁻ secretion etc.). PUD represents a worldwide health problem due to its high morbidity, mortality and economical factor.

To fully understand the physiology of GIT and its pathological condition, it is necessary to acquire some knowledge on the following important headings given below:-

Figure 1.1: Gastric ulcer

Figure 1.2: Duodenal ulcer
1.1.1 Layers and region of stomach

The stomach is divided into a total of five regions namely cardia, fundus, body, antrum and pylorus (Figure 1.3). Our stomach is made up of about four distinct layers of tissue (Figure 1.4). From the inner side to the outer side of the stomach these are mucosa, submucosa, muscularis and serosa. Mucosa is the inner lining of the stomach, which produces mucus. The injury or erosion to this layer leads to the development of the PUD. The adherent mucus layer provides a defensive barrier against self-digestion of stomach by gastric acid and pepsin [2,3]. The layer submucosa made up of connective tissue and produce digestive hormones where as the layer muscularis and serosa are the muscle and fibrous membrane respectively.

1.1.2 Cells of stomach lumen

The stomach lumen consists of several different specialized cells from which various substances are released (Table 1.1). These secretory substances are taking part either in the pathogenesis of the disease or in the management of disease.

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Substance secreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucus neck cell</td>
<td>Mucus (protects lining)</td>
</tr>
<tr>
<td></td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>Parietal cells</td>
<td>Gastric acid (HCl)</td>
</tr>
<tr>
<td>Enterochromaffin like cells (ECL)</td>
<td>Intrinsinc factor (Ca(^{++}), Vit B12 absorption)</td>
</tr>
<tr>
<td></td>
<td>Histamine (stimulate acid secretion)</td>
</tr>
<tr>
<td>Chief cells</td>
<td>Pepsinogen (Pepsin)</td>
</tr>
<tr>
<td></td>
<td>Gastric lipase</td>
</tr>
<tr>
<td>D cells</td>
<td>Somatostatin (inhibits acids)</td>
</tr>
<tr>
<td>G cells</td>
<td>Gastrin (stimulate acid secretion)</td>
</tr>
</tbody>
</table>
1.1.3 Gastric acid secretion and regulation

There are three phases of gastric acid secretion, the cephalic phase, gastric phase, and intestinal phase (Figure 1.5-18). In the epithelium, gastric glands are present, which secrete acid. During each phase, the secretion of gastric juice can be stimulated or inhibited.

![Layers and cells of stomach and their secretory substances](image)

*Figure 1.4*: Layers and cells of stomach and their secretory substances (Copyright ©2001 Benjamin Cummings, an imprint of Addison Wesley Longman, Inc.) In the epithelium, gastric pits lead to gastric glands that secrete gastric juice. (One gastric glands is enlarged & shown on the right) (a) Different stomach layers; (b) Mucus layer of stomach; (c) Gastric gland

1.1.3.1 Cephalic phase: When we see any food the sensory stimulation trigger the parasympathetic out load via the glossopharyngeal nerve stimulation, i.e. secretion of saliva and vagus nerve stimulation, which initiate gastric and pancreatic secretion.

1.1.3.2 Gastric phase: It starts when food enters to stomach and provide chemical and
mechanical stimulation by the distention in the stomach lumen which initiates enteric neuron to release acetylcholine. Acetylcholine act on parietal cell to release hydrochloric acid (purple color). In gastric phase, there is a stimulation of stress receptor proteins by the smaller peptides and amino acids. Amino acids stimulate G cell to secrete gastrin which act on ECL (enterochromaffin like cell) to release histamine and further activate parietal cell to release acid (blue color) [4]. Presence of food in the stomach also increases pH, which prevents the stimulation of somatostatin (Green color).

1.1.3.3 Intestinal phase:

Chemical stimuli present in the duodenum initiate this phase. The presence of proton, high osmolarity, nutrient in intestinal lumen, fats in duodenum stimulate three regulatory pathways namely neural, hormonal and paracrine which in turn increases pancreatic secretion and decreases gastric secretion.

![Figure 1.6: Gastric phase [5]](image)

![Figure 1.7: Intestinal phase [5]](image)

1.1.3.4 Cellular mechanism:

Acetylcholine, gastrin and histamine are the three agonists for the physiology of gastric acid secretion and our parietal cells are having three different receptors for these three agonists for e.g. muscarinic receptor (M3) for acetylcholine, cholecystokine receptor for gastrin and histamine receptor for histamine. Acetylcholine is released by cholinergic terminals, whereas gastrin from G cell and histamine from ECL. Both acetylcholine and gastrin activate second messenger to release calcium ions. Elevated cAMP level and intracellular
calcium, enhance hydrochloric acid secretion by activating \( \text{Na}^+/\text{K}^+ \) pump, \( \text{H}^+/\text{K}^+ \) ATPase and chloride channels in apical membrane. Acetylcholine is having both the excitatory effect on G cell and inhibitory effect on D cell. Gastrin increases the secretion of somatostatin, which release from the D cell act as a regulatory molecule that inhibits acid secretion. It reduced the cAMP level, therefore, inhibit acid release. On the other hand somatostatin also blocks gastrin receptor to release gastrin as a result no further acid secretion.

1.1.4 Pathogenesis of peptic ulcer disease

There are several factors that are responsible for the pathogenesis of PUD (Figure 1.9). The factors may be environmental, microbial, pharmacological, psychological, genetical etc. [6-9]. The risk factors for PUD are *Helicobacter pylori* infection, use of non-steroidal anti-inflammatory drugs, stress, smoking, alcohol etc. These factors are either increase gastric acid secretion or impaired mucosal barrier protection. Brief mechanisms of individual risk factors involved in the pathogenesis of PUD are described in figure no.1.10 – 1.14.

1.1.5 Symptoms of PUD

- Burning pain in the middle or upper stomach
- Bloating
- Dyspepsia
- Heartburn
- Coffee ground emesis
- Nausea or vomiting
- Vomiting blood
- Melena
- Weight loss

1.1.6 Diagnosis of PUD

- Test for *H. pylori*
- Endoscopy
- X-ray
- Biopsy
Figure 1.8: Phases of gastric secretion. During each phase, the secretion of gastric juice can be stimulated or inhibited [10].
1.1.7 Treatment available

In the later stage of the 20th century, PUD was treated with antacid, anti secretory agents, proton pump inhibitors and antibiotics. But in the 21st century, when the role of stress and diet in the pathogenesis of PUD is understood, there is a revolution in the treatment of PUD with hospitalization, bed rest and proper diets. By the 1950s, antacid therapy had become the treatment of choice for PUD with 80% of respondents in duodenal ulcer healing after 4 weeks of therapy. The introduction of the histamine (H2) receptor antagonist (cimetidine) in 1977 became choice of treatment with a good ulcer healing rate (80% to 95%), after 6 to 8 weeks of therapy. The other H2 blockers available in the market are Ranitidine, Famotidine, Nizatidine. Probably the chord of success in ulcer healing was achieved with the availability of proton pump inhibitors (PPIs) (Omeprazole, Lansoprazole, Pantoprazole, Rabeprazole, Lansoprazole, Dex Lansoprazole) in the 1980s. The PPIs decrease gastric acid secretion through inhibition of H⁺/K⁺-ATPase, the proton pump of the parietal cell. In the 20th century PUD was considered as a chronic, incurable disorder due to its recurrences on discontinuation of treatment. There was another fruitful success in the management of PUD after knowing the relationship between H. pylori and PUD by Marshall and Warren in the year 1982. Antimicrobials available for the treatment of H. pylori are Clarithromycin, Metronidazole, Amoxicillin, Tetracycline etc. Apart from the above described treatment, mucous protective agents (sucralfate, Bismuth salts), prostaglandin analogs are also available.
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Figure 1.9: Pathogenesis of Peptic Ulcer Disease

Figure 1.10: Pathogenesis of PUD due to the attack of *H. pylori*
Figure 1.11: Mechanism involved in pathogenesis of PUD by NSAIDs

Figure 1.12: Mechanism involved in stress ulceration
**Figure 1.13:** Mechanism involved in smoking ulceration

**Figure 1.14:** Mechanism involved in alcohol ulceration
1.2 INTRODUCTION TO PLANT

*Paederia foetida* L. known as Prasarani in Sanskrit belongs to the family Rubiaceae. It is an extensive smelling perennial climber. Profile [11-13] of *P. foetida* L. is described below:

1.2.1 Synonyms

*Paederia chinensis* Hance

*Paederia scandans* (Lour.) Merr

*Paederia tomentosa* Blume

![Figure 1.15(a): *Paederia foetida* L. leaf](image)

![Figure 1.15(b): *Paederia foetida* L. dried leaf](image)

1.2.3 Plant Part used

Leaf, root, bark, fruit

1.2.4 Ayurvedic Properties and Action:

- **Rasa** - Tikta (bitter)
- **Guna** - Guru (heavy)
- **Veerya** - Ushna (hot)
- **Vipaka** - Katu (pungent)
- **Karma** - Vedanasthapana, Shothahara, Stabdhatanashaka, Nadibalya, Vatanulomana, Raktaprasadana, Mootrala, Ashmaribhedana, Vrishya, Balya, Sandhaneeya, Saruka.
1.2.5 Vernacular Names

English : Skunk vine, Stinkvine, Chinese fever vine, King's Tonic
Sans : Prasarni, Sarani, Prasarani, Gandhapatra
Hindi : Gandhali
Beng : Gandha bhaduli, Gandhal
Mar. : Hiranvel
Guj : Gandhana
Tel. : Savirela
Tam. : Penarisangai
Kan. : Hesarane
Mal. : Talanili
Oriya : Gandali
Assam : Bedoli sutta, Bhedilata, paduri-lata

1.2.6 Macroscopical and Microscopical Character

Leaf - Simple, petiolate, stipulate, 10-15 cm long, 5-6 cm broad, somewhat glabrous, ovate, entire, apex acute or cuspidate.

Midrib - composed of single layered epidermis covered with cuticle; ground tissue, consisting of 2-5 layered of collenchyma towards the upper and lower side and the rest are parenchyma; crescent-shaped vascular bundle present with xylem towards upper side and phloem towards lower side.

Lamina - shows a dorsiventral structure; epidermis single layered covered with striated cuticle; uniseriate covering trichomes and paracytic stomata present; mesophyll composed of single layered palisade cells and 3-4 layered spongy tissue; vein islet number 5-10 per sq. mm, palisade ratio 6.75-14.2.

Leaf- Petiole - shows a similar structure as midrib but differs in trichomes, comparatively smaller, starch grains, oil globules and raphides of the calcium oxalate present

Root - Tap root 2-4 cm long, 0.5-2 cm thick, cylindrical or sub cylindrical, tortuous, dark brown; odour disagreeable and foetid. Mature root shows 6-13 layers of cork, secondary cortex 5-16 layers of thin-walled, phloem appears as wedge-shaped, cambium 1-3 layered,
starch grains, oil globules and raphides of calcium oxalate present in a few cells of secondary cortex, phloem, xylem and medullary rays are present.

**Stem** - Slender, sub-erect with diffuse branching, up to 4 cm thick; longitudinal anastomosing wrinkles, ridges and a few transverse cracks and circular lenticels, fracture, fibrous; odour- foetid, 7-11 layers of cork composed of rectangular cells, secondary cortex 6-9 layers, pericyclic fibres present in singles or in groups, cambium 1-2 layers, pith, secondary cortex, phloem, xylem and medullary rays contain starch grains, oil globules and raphides of calcium oxalate.

**Flower** - Violet to pink; bracteate, pedicellate, bisexual, calyx campanulate, corolla funnel-shaped, usually pubescent, ovary turbinate, two celled containing one ovule.

**Fruit** - Berry, orbicular, ellipsoid, five lines on each side, two seeded,

**Seed** - Compressed, smooth,

1.2.7 Chemical Constituents

Asperuloside (Figure 1.17a), deacetylasperulside, paederosidic acid, paederoside (Figure 1.17b), scandoside (Figure 1.17c), (iridoid glycosides), ceryl alcohol, hentriacontane, hentriacontanol, palmitic acid, methyl mercaptan, campesterol (Figure 1.17d), ß-sitosterol (Figure 1.17e), stigmasterol (Figure 1.17f), ursolic acid, carotene, vitamin C, protein, amino acids: arginine, cystine, histidine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine, epifriedelinol, friedelan-3-one etc. are present in *P. foetida*.

1.2.8 Pharmacological activities

Antiinflammatory, antispasmodic, anticancer, anthelmintic, hepatoprotective.

1.2.9 Formulations and preparations

*Prasarnini taila, Dashamoolarishta, Prasarini leha, Kubja prasarini taila, Narayana taila, Pushparaja prasarini taila, Trishatiprasarni taila, Saptashatika prasarini taila, Ekadashashatika prasarini taila, Maharaja prasarini taila, Mahamasha taila, Rheu Capsule (Ban Lab, Bombay)*

1.2.10 Safety aspects

Considered as safe due to its traditional use.
Figure 1.17: (a) Chemical Structure of Asperuloside, (b) Structure of Paederoside, (c) Structure of Scandoside, (d) Structure of Campesterol, (e) Structure of β-sitosterol, (f) Structure of Stigmasterol
1.3 INTRODUCTION TO STANDARDIZATION AND VALIDATION

Medicinal plants play a major source of therapeutic agents for the alleviation of human diseases since our ancestor times. India is considered as “Botanical Garden of the world.” Indian flora and fauna consist of more than 2200 species of medicinal and aromatic plants. Due to the increase in the interest of use of medicinal plants throughout the world, there is a manifold increase of medicinal plants based industries, which are growing at a rate of 7-15% annually. Though, India has the largest number medicinal and aromatic plant producer, but the contribution in herbal industry less than 12%. In India most of the traditional knowledge about medicinal plants was in the form of oral knowledge that has been eroded or distorted due to the persistent invasions and cultural adaptations.

1.3.1 Features that restricting development of herbal medicine in India

- Lack of standardization
- Lack of validation of standardized method
- Deficient of market contacts
- Marketing is inefficient, imperfect, selective and opportunistic

Due to these reasons, there is a drastic reduction in the monograph on crude drugs and plant products in Indian Pharmacopoeia. There is a prevalence of using plants and plant based products in various contemporary and traditional systems of medicine, without any written documentation or regulation. To meet the demand of international market it is mandatory to standardize the product or method and validate it in writing document form. Now days R&D thrust in the pharmaceutical sector focuses on the standardization and validation of the developed standardized techniques.

1.3.2 Standardization

Standardization is a system that ensures a predefined amount of quality, quantity and therapeutic effect of the ingredients present in each dose [14]. It is an important aspect of maintaining and assessing the quality, purity and safety of herbal product/extract/raw
material/ formulation to attain the desired therapeutic effect [15]. A standardized herbal extract means a measurable marker substance or substances present, which is extracted from the herb. The markers present in an extract may be active or inactive. The first Indian National Health Policy 1983 claims that India is the richest source of herbs and the drugs should be standardized [16].

1.3.3 Need of Standardization

The revival of interest in natural drugs and the herbal drugs started in the last decade, mainly because of the wide spread and belief that green medicine is healthier than synthetic one. This leads to the rapid spurt of demand for health products of traditional medicine, which leads to the market of medicinal plants for commercialization. To fulfill this need there is a practice of indiscriminate and unscientific collection practices, without any consideration for the quality control of the material. Thus decreases in the therapeutic efficacy and quality of the final product. Hence standardization plays an important role in the quality assurance of herbal drugs and their products. Raw plant material contains various chemical constituents, thus sometimes standardizing the herbal drug require more than one analytical technique [17].

1.3.4 What is Validation

Validation is a concept that has been evolving continuously its first formal appearance in the United States in 1978 [18]. The validation of an analytical procedure is the process of confirming that the analytical procedure employed for a test of pharmaceuticals is suitable for its intended use. In other word, the validation of an analytical procedure requires to demonstrate scientifically that risks of errors in different analytical steps are acceptably small [19]. Validation is a basic requirement to ensure quality and reliability of the results for all analytical applications. The object of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose, determine by means of well-documented experimental studies [20].

1.3.5 Reasons for validation
There are two important reasons for validating assays in the pharmaceutical industry. The first, and by to the most important, is that assay validation is an integral part of the quality-control system. The second is that current good manufacturing practice regulation requires assay validation [21]. In industry, it would be difficult to confirm that the product being manufactured is uniform and that meet the standards set to assure fitness for use. The varying nature of the differences between the analytical development laboratory and quality control laboratory is a good reason for the validation program in pharmaceutical analysis.

1.3.6 **Benefits of method validation** [17]

- A fully validated process may require less in-process control and end-product testing.
- It deepens the understanding of processes, decrease the risks of processing problems, and thus assure the smooth running of the process.

1.3.7 **Steps in method validation**

- Develop a validation protocol, or operation procedure for the validation.
- Define the application, purpose and scope of the method.
- Define the performance parameter and acceptance criteria.
- Define validation experiments.
- Verify relevant performance characteristic of equipment.
- Quality materials (for e.g. standard and reagents).
- Perform pre validation experiments.
- Adjust method parameter or/and acceptance criteria if necessary.
- Performed full internal and external validation equipments.
- Developed SOP for executing the method in the routines.
- Define criteria for revalidation.
• Define the type and frequency of system for suitability test and/or analytical quality control (AQC) for the routine.

• Document validation experiment and result in the validation report.

1.3.8 Today's validation requirements

![Diagram showing relationships between ICH/USP, GMPs, and FDA]

The objectives of ICH as laid down in their terms of reference in their early years were:

• To provide a forum for constructive dialog between and among regulatory authorities and the pharmaceutical industry on the real and perceived differences in the technical requirements for product registration in the EU, United States, and Japan

• To identify areas in which modification in technical requirements or greater mutual acceptance of R&D procedure could lead to a more economical use of human, animal and material resources without compromising safety

• To make recommendations on practical ways to achieve greater harmonization in the interpretation and application of technical guidelines and requirements for registration [20].

There are two guidelines of validation issued by the US (FDA), one for the applicant, the other for inspecting and reviewing. The first one is also intended to ensure that the analytical
procedure can be applied in an FDA laboratory and therefore requires a detailed description of the procedure, reference material as well as a discussion of the potential impurities etc. The second guidelines focus on RP- chromatography and provides a lot of details with regard to critical methodological issues as well as some indication of acceptability of results [20].

There are specific guidelines given by ICH, FDA, and USP. There are different validation characteristics normally evaluated for the different types of test procedure:

- Specificity
- Linearity
- Range
- Accuracy
- Precision (Repeatability, Intermediate precision, Reproducibility)
- Limit of detection
- Limit of quantification