CHAPTER II

LITERATURE REVIEWS

ANATOMY OF STOMACH

Gross Anatomy

The stomach is a "J"-shaped tube connecting the esophagus and the duodenum (Figure 2.1). Its primary functions are the mechanical and chemical disruption of food and its even distribution into the absorption portion of the gastrointestinal tract. It is situated in the upper abdomen, below the diaphragm, and is fixed in two places, at the *esophagogastric junction* above and *gastroduodenal junction* below.

From an endoscopic perspective, the stomach begin at the gastroesophageal junction, where the tubular esophagus ends and the rugal folds of the stomach begin. The lesser and greater curvatures constitute the medial and lateral margins, respectively, of the stomach, and correspond to the regions where the lesser and greater omenta attach. The esophagus open into the cardia. It comprises approximately the proximal 0.5 to 2 cm of the stomach. Stomach consists of the *body* (synonym: *corpus*) and the *fundus*, and extends between the cardia and *antrum*. The fundus consists of the dome of the stomach that lies above an imaginary horizontal line through the diaphragmatic pinch. The antrum occupies the distal fourth of the stomach and extends from an indentation on the lesser curvature, the *incisura angularis* (synonyms: *gastric notch* or *angular notch*), to the *pyloric sphincter* (synonym:

pyloric canal). The pyloric sphincter is a muscular sphincter that appears endoscopically as a small opening leading to the duodenum.

The mucosa of the stomach is thrown up into longitudinal folds (or rugae) that extend the entire length of the stomach.

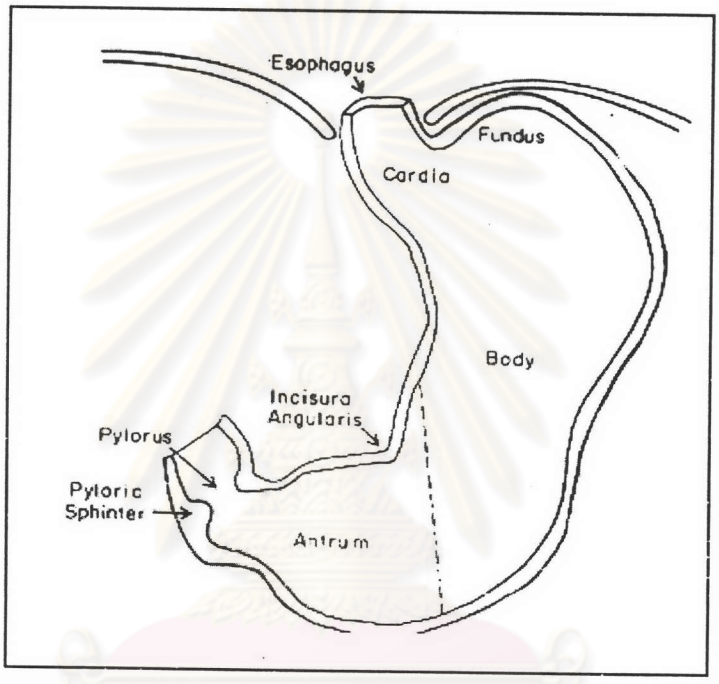

Figure 2.1 The structure of stomach. Demonstrated four major features of Cardia, Fundus, Body, and Antrum. (Marvin&Johns. 1993)

Histology

The wall of the stomach has four layer: the mucosa, submucosa, muscularis propria, and serosa, similar to the rest of the gastrointestinal tract. Morphologically, the mucosa can be separated into three types, namely, cardiac, oxyntic, and antral. These histologically defined types of mucosa roughly correspond to distinct regions of the stomach.

Mucosa

The mucosa of the stomach is made up of epithelium and surrounding connective tissue called the *lamina propria*. The epithelium can be divided into the surface *foveolae* (synonym: gastric pits) and the deeper *gastric glands*. The junction between the foveolae and the glands is called the *neck region*. Gastric glands are divide into three principal gastric regions. The first region, which is only 1.5 to 3 cms. In length, contains the cardiac glands and corresponds with the gastric cardia. The second region (the oxyntic, parietal cell, or acid-secreting portion of the stomach) includes the gastric fundus and body, which about two thirds of the stomach. The third region of the stomach, containing the pyloric or antral glands, corresponds principally with the antrum of the stomach.

Oxyntic glands

The oxyntic glands are segmented into three regions: the isthmus, which contains parietal and surface mucous cells; the neck region, which contains mainly parietal cells and mucous cells; and the base, which contains principally chief cells, some parietal and mucous neck cells, and endocrine cells. The gastric, or oxyntic, glands occupy the largest area of the gastric mucosa and are responsible for acid secretion and most enzyme secretion (Marvin H, 1993). The histopathological of stomach and the oxyntic gland were shown in Figure 2.2 and 2.3, respectively.

The parietal cell play an important role to HCl secretion into stomach. In the normal condition, there are hydrogen ions (H^+) concentration about 10-20 mEq/L, which pH is about 2-3. When stomach was stimulated by various factor, the concentration of H^+ was increased

about 130-150 mEq/L, and pH about 1. Normally, the defense against the massive backflow of acid and consequent mucosal destruction is call the mucosal barrier. This mucosa barrier is composed by epithelial cells with tight junctions and superimposed layer of mucus. The aim of this barrier is to protect the mucosa against damage of deeper structures by hydrogen ions (H^+) and other noxious substances originating from the gastric lumen (Konturek,1997). The endogenous prostaglandin (PGs) play an important role in the maintenance of mucosal integrity, which include continuous secretion of bicarbonate anions (HCO^-_3) and a mucus production in the stomach and duodenum (Brzozowski,2000 ; Konturek,1986). Moreover, PGs can reduce acid secretion, which is the direct action on the oxyntic cells (Harvey, 1982)

The lamina propria comprises the supporting framework for the epithelium. The epithelium rests on a basement membrane. The framework is composed of a fine meshwork of reticulin with occasional collagen and elastin fiber; the supporting capillary network and nerve fibers occupy this space as well. The lamina propria is best seen between the foveolae near the surface. The lymph channels are thought to occupy only the lower region of the lamina propria, near the muscularis mucosae (Lehnert T et al.,1985) .

Muscularis Mucosae, Submucosa, and Muscularis Propria

The mucosa is separated from the submucosa by the *muscularis mucosae.* This is a thin bilayer of smooth muscle composed of an inner circular layer and an outer longitudinal layer.

The submucosa consists of loose connective tissue in which are embedded lymphatics, blood vessels, and scattered mononuclear cells, including mast cells.

The muscularis propria is composed of three layers of smooth muscle: *the outer longitudinal, middle circular, and inner oblique.* The outer longitudinal layer is most concentrated along both curratures. The middle circular layer encircles the body of the stomach and forms the pyloric sphincter distally (Owen DA, 1992). The miner oblique fiber pass down from the fundus over both anterior and posterior walls.

The serosa is a thin covering of loose connective tissue with blood vessels, lymphatics, nerve fiber, and a band of collagen that is covered by a single layer of mesothelium (the visceral peritoneal reflection). The serosa is contiguous with the omentum and "ligaments" attaching the stomach to spleen and liver.

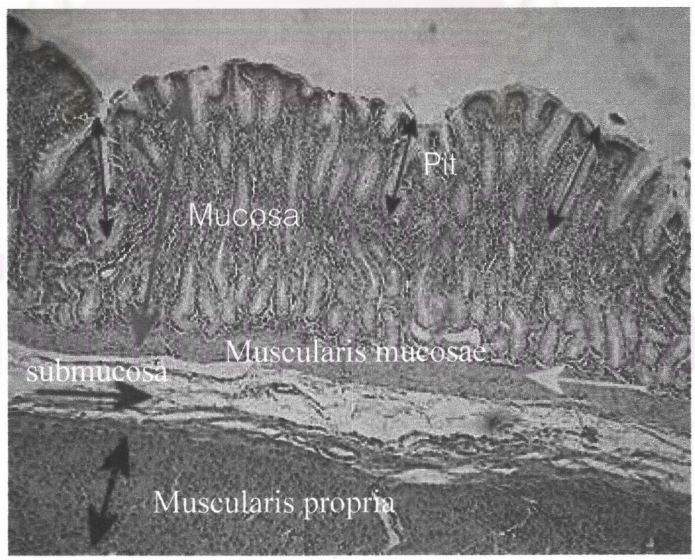

Figure 2.2 The histopathology of normal stomach.
Submucosa is the layer that most blood vessels are located.
(www. 3. umdnj. Edu/histsweb/lab19/lab19pyloric.html.)

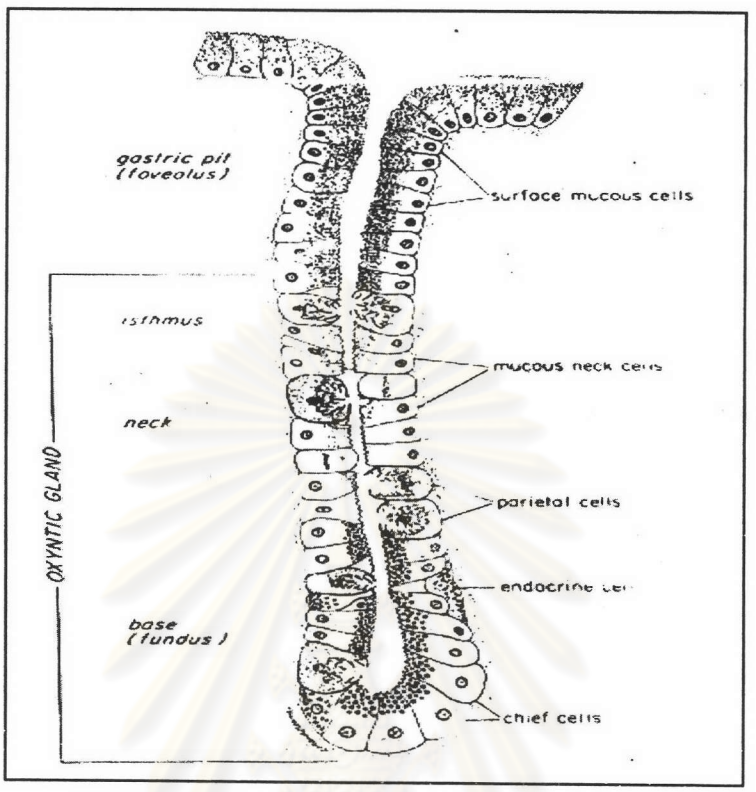

Figure 2.3 The oxyntic gland of stomach from the corpus. (Johnson et al., 1987.)

Vascular Anatomy

The stomach has a rich network of anastomosing vessels derived from the various branches of the *celiac trunk*. The celiac trunk is a short vessel that quickly divides into three arteries: the *left gastric artery*, the *splenic artery*, and the *common hepatic artery*. Each of these divisions supplies a portion of the stomach. The left gastric artery supplies the fundus and left superior portion of the lesser curvature. The splenic artery gives rise to the *short gastric arteries*, which also supply the fundus, and the *left gastroepiploic artery*, which supplies the superior portion of the

greater curvature. The common hepetic artery divides into the gastroduodenal artery and the proper hepatic artery. The gastroduodenal artery gives rise to the *right gastroepiploic artery*, which supplies the inferior portion of the greater curvature of the stomach. The proper hepatic artery divides into three branches, one of which, the right hepatic, gives rise to the *right gastric artery*, which supplies the inferior lesser curvature of the stomach (Piasecki, 1974).

Microcirculation in stomach

Microcirculatrion in gastric mucosa

The stomach has a rich blood supply and the major part of the gastric blood flow is supplies by the microcirculation of gastric mucosa (Guth and Leung, 1987). The mucosal blood flow originates from the submucosal arterioles that break up into capillaries then pass through the mucosa. The feature of capillary network is a honeycomblike pattern just beneath the surface of mucus cells. Blood from capillaries drains into the mucosal venules at lamina propria. These venular branches converge on infrequent mucosal collecting venules, which then pass directly to the submucous venous plexus (Gannon et al., 1984). The microcirculation structure in stomach is show in Figure 2.4.

The bicarbonate anions (HCO^-_3) generated by the parietal cells, rapidly diffuse across the submicron interstitial distance to the proximate capillaries, and readily cross the fenestrated capillary endothelium to enter mucosal capillary blood. Capillary blood flow in the mucosa, beyond parietal cells, will be substantially alkalinized and plays an important role in neutralizing of back-diffusing H+. Therefore, it behaves

as a gastric mucosal defense against damage from acid as shown in Figure 2.4. (Gannon et al., 1984).

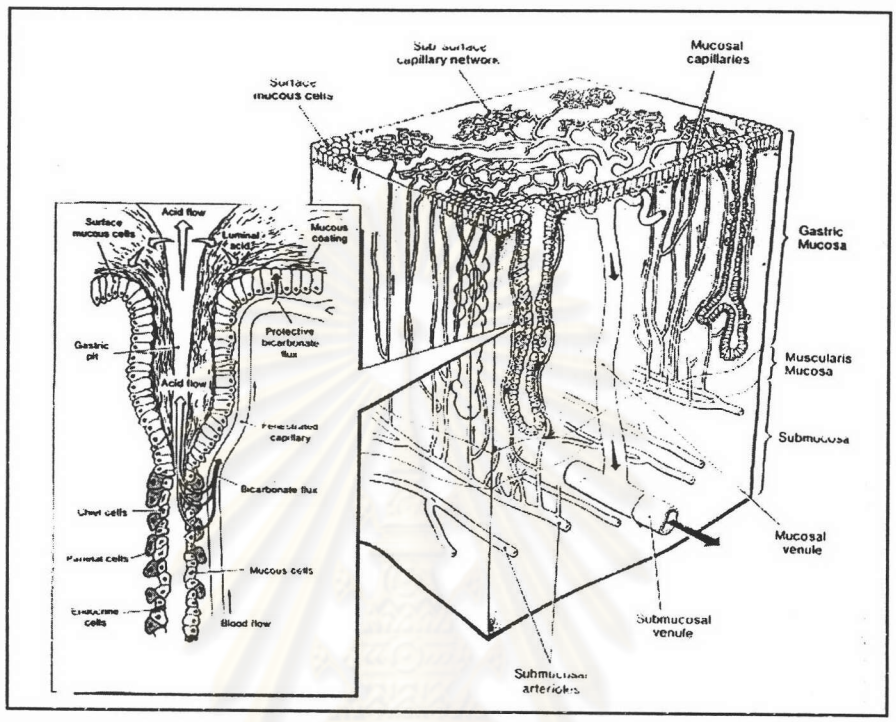

Figure 2.4 Schematic diagram of the vascular organization in oxyntic mucosa (right) and the mechanism of HCO^{-}_{3} (Gannon et al., 1984).

Microcirculation in gastric muscle

The gastric muscle blood flow originates from small artery (SA) in submucosa that break up into submucosal primary arterioles. Submucosal primary arterioles (SMA1) give rise to ascending muscle arterioles, which supply muscle arterioles (MA) in the circular and longitudinal muscle layers. These arterioles run perpendicularly to the muscle fibers and divided into the longitudinal and circular muscle capillaries (MC), which run parallel to the muscle fibers. Capillaries end in muscle venules (MV), which form descending venules through both muscle layers and return

blood to the submucosal primary venules (SMV1) or submucosal small vein (SV) before leaving the submucosa (Peti-Peterdi et al., 1998). The microvasculature in gastric wall was shown as Figure 2.5.

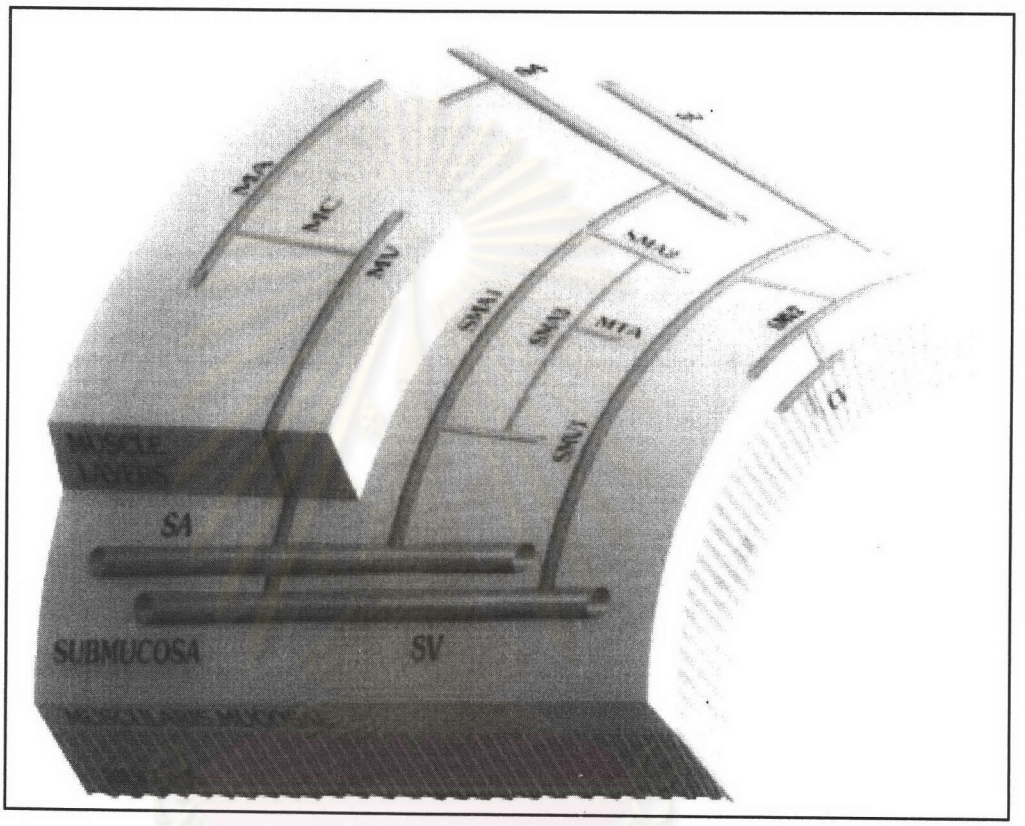

Figure 2.5 Microvasculature in gastric wall. Blood vessels were identified numerically according to their branching order and vascular hierarchy: small artery (SA), submucosal primary (SMA1), secondary (SMA2), and tertiary (SMA3) arterioles, mucosal terminal arteriole MTA), collecting venule (CV), submucosal secondary SMV2) and primary (SMV1) venule, muscle arteriole (MA), capillary (MC), and venule (MV), and submucosal small vein (SV). Common input and output of muscle and mucosal circulation are SMA1 and SMV1, respectively (Peti-Peterdi et al., 1998).

Lymphatic drainage

Lymphatic drainage of the stomach starts in the lower portion of the lamina propria as a periglandular plexus. This pieces the muscularis mucosae linked with the submucosal plexus, and connects with a plexus of lymphatic vessels that lies between the longitudinal and circular muscle fiber. The large lymphatic vessels follow the large arteries to lymph nodes adjacent to the stomach and then drain into the celiac lymph nodes.

The lymphatic drainage is set up so that the right portion of the stomach drains to the lymph nodes adjacent to the greater curvature and the left portion to the lymph nodes adjacent to the lesser curvature.

Nerve supply

The stomach, similar to the rest of the gastrointestinal tract, has both sympathetic and parasympathetic innervation. The sympathetic fiber consist primarily of vasomotor and pain fiber. These nerves have their ganglia in the celiac plexus around the celiac arterial trunk, and the nerve fibers follow the artery and veins. There is also a branch of the left phrenic nerves that innervates the gastric cardiac region. The parasympathetic nerve control the secretory and motor function of the stomach. They are derived from the vagus nerve; the anterior surface originates from the left vagus, which has two branches (gastric and pyloric), and the posterior surface from the right vagus, which also has two main branches (gastric and celiac) (David et al., 1999).

GASTRIC ULCER

Gastric ulcer (GU) is a deep necrotic lesion involving the entire mucosal thickness and the muscularis mucosae (Fred et al., 1995).

Gastric ulcer occurs more frequently in those over 60, and about equally in men and women: a slight female predominance is owing to a greater use of non steroidal anti- inflammatory drugs (NSAIDs) and aspirin in women over 60 to reduce the aches and pains of old age.

Morphology

Acute gastric ulcers are usually less than 1 cm in diameter, are circular and small, and rarely penetrate beyond the mucosa. The ulcer base is frequently stained a dark brown by the acid digestion of extruded blood. They may occur singly or, more than, multiply throughout the stomach and duodenum. The gastric rugal pattern is essentially normal, and the margin and base of the ulcer are not indurated. Microscopically, acute stress ulcers are abrupt lesions, with essentially unremarkable adjacent mucosa. Depending on the duration of the ulceration, there may be a suffusion of blood into the mucosa and submucosa and some inflammation reaction. Conspicuously absent are scarring and thickening of blood vessel, as seen is chronic peptic ulcers. Healing with complete re-epithelialization occurs after the causative factors are removed. The time required for complete healing varies from days to several weeks.

Pathology

A gastric ulcer is a sharp, well-defined break in the mucosa, no different from one in the duodenum except for its site, gastric ulcer is more bigger than duodenal ulcer (DU), usually at the junction of fundic with pyloric mucosa and typically on the lesser curve.

Johnson (1965) subdivided gastric ulcers into three types.

Type I being those ulcers occurring in the body of the stomach without evidence of ulceration or scarring in the prepyloric or duodenal area. This ulcer type, at least, acid and pepsin secretion is either normal or reduced. The most patient is type I.

Type II being those ulcers in the body of the stomach associated with and perhaps secondary to an ulcer or scarring in the duodenum. Ulcer occur after high acid secretion.

Type III prepyloric ulcer occurring to the right of the gastric angulus in the lesser curvature.

Histology

Histologically the wall of a chronic gastric ulcer are usually sharply perpendicular with an abrupt fall the mucosal surface to the crater base. The fourtiered layering originally described for peptic ulcer of the duodenum is also present in the chronic gastric ulcer: a superficial layer of inflammatory cells coating a layer of fibrinoid necrosis that inturn overlies a hypervascular granulation tissue layer and a deep base of dense scar tissue, if the advancing erosion crater base encounters a submucosal artery, the arterial wall can undergo necrosis with resultant risk bleeding.

A variety of factors produce damage of gastric mucosa, including: systemic events such as thermal stress, or local mucosal application of various irritants that are commonly named breakers of gastric mucosal barrier (Brzozowski et al.,2000,1997). The aim of this barrier is to protect the mucosa against damage of deeper structures by hydrogen ions (H^+) and other noxious substances originating from the gastric lumen (Konturek,1997). The imbalance between gastrotoxic agents and protective mechanisms results in an acute inflammation (Kwiecien et al., 2002).

Inflammation

Inflammation and function of Neutrophils and a Macrophages.

When tissue injury occurs, whether caused by bacteria, trauma chemicals, heat, or any other phenomenon, multiple substances that cause dramatic secondary changes in the tissues are released by the injured tissue. The entire complex of tissue changes is called *inflammation.*

Inflammation is characterized by (1) vasodilatation of the local blood vessels with consequent excess local blood flow, (2) increased permeability of the capillaries with leakage of large quantities of fluid in the interstitial spaces, (3) often clotting of the fluid in the interstitial space because of excessive amounts of fibrinogen and other protein leaking from the capillaries, (4) migration of large numbers of granulocytes and monocytes into the tissue, and (5) swelling of the tissue cells. Some of the many tissue products that cause these reactions are histamine, bradykinin, serotonin, prostaglandins, several different reaction products of the complement system, reaction products of the blood-clotting system, and

multiple hormonal substances called lymphokine that are released by sensitized T cells. Several of these substances strongly activate the macrophage system, and within a few hours, the macrophage being to devour the destroyed tissue; at times, the macrophage also further injure the still-living tissue cells. The intensity of the inflammatory process is usually proportional to the degree of tissue injury.

Macrophages and neutrophils response during inflammation

Within minute after inflammation begins, the macrophages already present in the tissues, whether histiocytes in the subcutaneous tissues, alveolar macrophages in the lungs, microglia in the brain, or others, immerdiately begin their phagocytic actions. When activated by the products of infection and inflammation, the first effect is rapid enlargement of each of these cells. Next, many of the previously sessile macrophage break loose from their attachments and become moblie, forming the first line of defense against infection during the first hour or so. The numbers of these early mobilization macrophage often are not great.

Within the first hour or so after inflammation begins, large numbers of neutrophils begin to invade the inflamed area from the blood. This is caused by products from the inflamed tissues that initiate the following reactions; (1) They alter the inside surface of the capillary endothelium, causing neutrophils to stick to the capillary walls in the inflamed area. This effect is called margination. (2) They cause the endothelial cells of the capillaries and small venules to separate easily, allowing openings large enough for neutrophils to pass by diapedesis into the tissue spaces. (3) Other products of the inflammation cause

chemotaxis of the neutrophils toward the injured tissues, as explained in an earlier section. Thus, within several hours after tissue damage begins, the area becomes well supplied with neutrophils. Because the blood neutrophils are already mature cells, they are ready to being immediately their scavenger function for killing bacteria and removing foreign matter (Guyton, 1996).

Neutrophil adherence within the gastric microcirculation and migration into the gastric tissue has long been thought to be a major cause in the pathogenesis of gastric inflammation such as gastric ulcer (Xiao Ru Huang et al., 2001).

Cytokines

The Role of Cytokine on Inflammation

Cytokines are fundamentally involved in the control of the immune and inflammatory responses. They enable the cellular components of these responses to communicate with each other and can also determine the nature of the response. Certain molecules, such as IL-1, IL-6, TNFα and released by activated macrophages. IL-1 and IL-6 have the capacity to stimulate both arms of immune response by activating T cells to produce IL-2 and express the IL-2 receptor, and also by inducing B-cell proliferation, maturation and increased immunoglobulin synthesis. The other major cytokines which include IL-2, IL-4, IL-5, IL-10, IL-13 and γ-interferon (IFN), are predominantly synthesized by activated T lymphocytes. These molecules are now subdivided into the Th1 (IL-2 and IFN) and Th2 (IL-4, IL-5, IL-10 and IL-13) subgroups because of their different actions and effects on the immune response. Th1 cytokine

profile predominates in those patients who successfully eradicate the infection, whereas a Th2 profile is found in those with persistent infection.

Cytokine can work in an autocrine, paracrine or endocrine fashion, and influence not only immune cells but also epithelium, endothelium, mesenchyme and the extracellular matrix. Enhancement of the immune response both locally and systemically is an important feature of most cytokines, but particular IL-1 and TNF. IL-1, IL-6 and TNF all contribute to hepatic acute-phase response, and other systemic effects such as anorexia, fever, anemia and thrombocytosis. (Allan et al., 1997)

Proinflammatory cytokines

Tumor Necrosis Factor Alpha (TNF-α)

Tumor Necrosis Factor Alpha (TNF-α) is a 17 kDa protein produced primarily by macrophages in response to a wide variety of stimuli, including mitogen, cytokine, bacteria, viruses, immune complexes, tumor cells, reactive oxygen species, and platelet-activating factor (PAF) (Aggarawal, 1992; Jaattela, 1991). Besides recognizing antitumor activity. TNF-α has been shown to act as an immunomodulator involved in the activation of neutrophils and the stimulation of endothelial cell adhesion molecules and various multifunctional inflammatory cytokine such as IL-1 and IL-6 (Colletti et al., 1996). TNF-α has been shown to play an important role in the propagation of inflammation by the promotion of leukocyte adhesion to vascular endothelial cells, which enable their migration into the inflamed tissue (Konturek et al., 2000).

Recently, reporting that TNF-α contributed to the gastric damage and leukocyte margination observed after indomethacin administration in the rat (Santucci et al., 1994). Furthermore, the local TNF-α level has been suggested to be critical in tissue and organ damage during the early phase of neutrophil-madiated inflammation (Hashimoto et al., 1994; Von Asmuth et al., 1991).

TNF-α has been shown to upregulated intercellular adhesion molecule (ICAM-1) expression on vascular endothelial cells (Rothlein et al., 1991) and to promote neutrophil adherence to these cells (Morzycki ei al.,1990). Furthermore, TNF-α has been shown to stimulate considerable the adherence of PMN to the vascular endothelium by inducing the expression of β_2 integrins (Lymphocyte function- associated antigen (LFA-1 or CD11a/CD18) (Pohlman et al., 1986; Birdsall et al., 1991; Tracey et al., 1986).

The sequence of events that allows the traveling of leukocytes to site host defense is designated the multistep paradigms of leukocyte recruitment. Involves important events encompassing the transvascular movement of leukocytes: (1) marginaton and capturing of free-follow leukocytes, (2) leukocyte rolling, (3) activation and firm adhesion, (4) spreading transendothelial diapedesis and chemotactic migration of the leukocytes. As shown in Figure 2.6. Different mechanisms appear to mediate leukocyte rolling and adhesion, the former is dependent on selectins expressed on endothelium (P-selectin) and (L-selectins), whereas the latter is dependent on the integrins (CD11/CD18) found on leukocytes and their ligands (ICAM-1, VCAM-1) on endothelial cell (Yang et al., 1996).

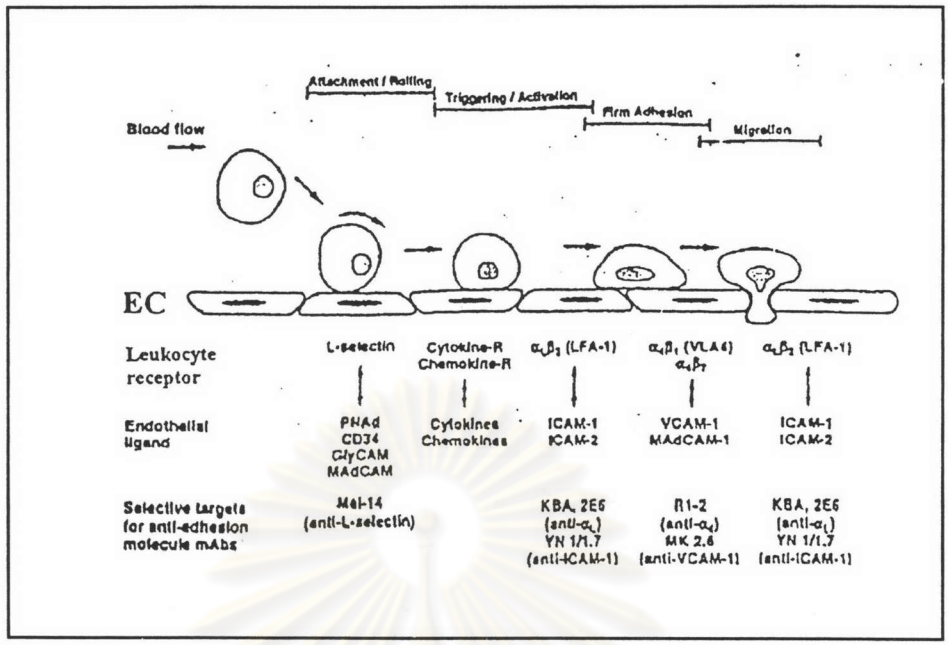

Figure.2.6. The sequence model of leukocyte-endothelial adhesion and transendothelial diapedesis of leukocyte from blood into inflamed tissue (Yang et al., 1996).

Previous studies reported that the infiltrated leukocytes caused further tissue damage leading to the ulcerative lesions after ischemia-reperfusion of the stomach (Wada et al., 1996). Leukocytes could contribute to ethanol induced gastric mucosal injury in several ways; oxygen derived free radical production, adherence to endothelium, and consequent release of vasoactive substances (leukotrienes, platelet activating factor) and by occlusion of the microcirculation (Kalia et al., 1997). Moreover, the leukocyte-endothelial cells interaction play an important role inflammatory process in burn wound rats (Duansak et al., 2003).

Antiinflammatory cytokine

Interleukine-10 (IL-10)

IL-10 is an 18 kDa peptide. IL-10 is produce by a range of cell types including macrophages, monocytes, T and B lymphocytes and tumor cell (Gold man and Velu, 1995). IL-10 shows potent anti-inflammatory properties, including suppression of IL-2 and IFN-γ production by T lymphocytes (Fiorentino et al., 1989; De Prete et al., 1993). IL-10 could inhibited of mitogen induced T cell proliferation and of the effecter function of activated monocytes/macrophages (De Waal Malefyt et al., 1991). Moreover, IL-10 could inhibited of synthesis and gene expression of TNFα, IL-1, IL-6, IL-8 and IL-10 also stimulating factor in monocytes (Fiorentino et al.,1991; De Waal Malefyt et al., 1991). IL-10 also attenuates inflammation by limiting cellular infiltration, B cell aggregation and proliferation are suppressed by IL-10 (Clinchy et al., 1994).

Fiorentino et al. (1991) shown that IL-10 has a significant inhibitory effect on cytokine synthesis by macrophages, as well as causing a marked morphological change in peritoneal macrophage and they suggested that an important role for IL-10, not only in the regulation of T cell response but also as an important modulator of acute inflammatory response elicited by infection or injury.

The other investigators found that IL-10 is antiinflammatory effect in a variety of diseases, such as IL-10 is capable of down regulating secretion and mRNA levels of proinflammatory cytokine by inflammatory bowel disease mononuclear phagocyte in vitro (Schreiber et

al., 1995), suppression of TNFα production in colitis (Karen et al., 1997) and significantly lowers serum levels of macrophage-derived cytokine (IL-1β, Il-6, and TNFα) in acute pancreatitis (Anthony et al., 1997). Moreover, IL-10 also involved in gastric mucosal inflammation in *Helicobacter pylori* infection, which, may be protective, limiting tissue damage cause by inflammation, it may also contribute towards failure of the immune response to eliminate the organism (Bodger et al., 1997).

Aloe vera

Aloe vera, often called the Miracle Plant, the Natural Healer, the Burn Plant, goes by many names which have survived the 4000 or so years during which this amazing medicinal herb has benefited mankind.

Aloe vera (synonym: *Aloe barbadensis* Miller) belongs to the Liliaceae family, of which there are about 360 species, *Aloe vera* is a cactus-like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities (Vogler and Ernst, 1999)

Aloe vera is a short-stemmed succulent herb. The succulent leaves are crowded on the top of their stems, spreading, grayish green and glaucous; spotted when young, 20-50 cms. long, 3-5 cms. wide at the base, tapering gradually to the point tip, 1-2.5 cms. thick; having edeges spiny and bitter latex inside. Flowers borne on the upper part of a slender stalk, 50-100 cms. high. Forms of the species vary in size of leaves and color of flower (Grindlay and Reynolds, 1986).

The epidermis of the leaves has a thick cuticle, and beneath is zone of parenchyma which obtains pericyclic cells. The latex or yellow juice contains within the pericyclic cells. The central bulk of the leaf contains the colorless mucilaginous pulp, made up of large thin-walled mucilaginous cell containing the aloe gel itself (Klein and Penneys, 1988).

Chemical constituents of *Aloe vera*

The fresh leaves of *Aloe vera* are used to obtain two components: (1) a bitter yellow juice (exudates or latex) obtained from the pericyclic cells the beneath the plant's skin (Klein and Penneys, 1988.) Latex contains 1,8 dihydroxyantrquinon derivatives (aloe emodin) and their glycosides (aloins), which are used for their cathartic effects (Fairbarin, 1980) and (2) Aloe gel is obtained from thin-walled mucilaginous cell of the inner central zone of the leaf. The gel contains a variety of organic material thought to contribute to the purported emollient, moisturizing, and healing effects of the gel (Klein and Penneys, 1988). The composition of *Aloe vera* as shown in Table.2.1.

Table 2.1 Constituents of *Aloe vera* (Vogker BK and Ernst E, 1999)

Constituents	Identification
Anthraquinones	Aloin, Barbaloin, Isobarbaloin, Anthranol Aloetic acid, Ester of cinnamic acid, Emodin, Aloe-emodin, Chrysophanic acid, Resistannol
Saccarides	Cellulose, Glucose, Mannose, L-rhamnose, Aldopentose
Vitamins	Vitamin B1, B2, B6, C, Choline, Folic acid, α-tocophepol, β-carotene
Nonessential amino acids	Histidine, Arginine, Hydroxyproline, Aspartic acid, Glutamic acid, Proline, Alanine, Tyrosine
Inorganic compounds	Calcium, Sodium, Chlorine, Manganese, Zinc, Chromium, Potassium sorbate, Copper, Magnesium, Iron
Enzymes	Cyclooxyganase, Oxide, Amylase, Catalase, Lipase, Alkaline phosphatase, Carboxypeptidase
Essential amino acids	Lysine, Threonine, Valine, Leucine, Isoleucine, Phenylalanine, Methionine
Miscellaneous	Cholesterol, Triglycerides, Steroids, β-sitosterol, Lignins, Uric acid, Gibberellin, Lectin-like substance, Salicylic acid, Arachidonic acid

Pharmacological Activities of *Aloe vera*

Wound healing promotion

Collins et al. (1935) demonstrated that the use of *Aloe vera* gel in woman suffering from severe radiation dermatitis who has desquamation, burning and itching on the left side of the scalp and forehead. Treatment with locally applied fresh whole leaf of *Aloe vera* reduced burning and itching after 24 hours. By 5 weeks there was complete healing of the skin of the scalp and forehead with restoration of sensation and absence of scarring. Later, Lushbaugh and Hale (1953) studied the effects of *Aloe vera* on acute radiodermatitis following beta irradiation in albino rabbits. They found that aloe-treated ulcers showed earlier development of necrosis and ulceration and earlier reepithelialization than did nontreated control ulcers. Treatment with aloe was found to hasten both the degenerative and the reparative phases of the lesions, so that healing of aloe-treated ulcer occurred in 2 months, while untreated ulceration were not completely healing 4 months after irradiation.

Goff and Levenstein (1964) assessed the effects of *Aloe vera* extract upon the healing of surgically induced skin wounds in mice. They found that statistically significant increases in wound strength in the *Aloe vera* treated wounds at 9 and 15 days when compare with untreated control group but by 21 days tensile strengths were essentially the same in both group.

El Zawahry et al. (1973) reported three uncontrolled cases of chronic leg ulcers of 5, 7, and 15 years duration, refractory to other treatments, that responded to the fresh gel of aloe leaves. They concluded

that the rapid reduction in ulcer size, with complete healing in two of the three cases supported *Aloe vera*'s healing activity.

Recently, reporting that mannose containing *Aloe vera* promote wound healing (Klein et al., 1988) by increased macrophage activity (Tizard et al., 1989). Stimulation of the macrophage will increase cell and tissue growth, fibroblast proliferation, and fibroblast activity (Diegei et al., 1981; Guyton 1991; Ross and Romrell, 1989).

Macrophages play an important role in decreasing long-term inflammation and stimulation fibroblasts to increase wound healing (Gilman et al., 1990; Balow and Rosenthal, 1973; Diegeimann et al., 1981; Guyton, 1991; Ross and Romrell, 1989). Macrophages promote fibroblast activity and wound repair in a number of ways. They undergo phagocytosis-induce secretion of interleukin-1 and tumor necrosis factor stimulation fibroblast activity (Guyton, 1991; Ross and Romrell, 1989). Ross and Romrell (1989) found that macrophages also release proteases and Glycosaminoglycans (GAGases), which activity function to increase migration of macrophage through connective tissue to the site of the wound, improving macrophage activity and promoting wound repair. Finally, Diegeimann et al. (1981) discussed several other ways that macrophages stimulate collagen depositing, fibroblast proliferation, and tissue repair.

David et al. (1994) concluded that fibroblast activation using *Aloe vera* increase collagen and proteoglycan synthesis, thereby promoting tissue repair without a loss of antiinflammation activity. It is resulting from mannose-6-phosphate containing *Aloe vera*. And they found that

gibberellin, isolate from *Aloe vera*, significant improved wound tensile strength more than 100%.

Recently, Moon E-J et al. (1999) reported that β-sitosterol containing in *Aloe vera* could promoted angiogenesis *in vivo*, which the angiogenic process play an important role in wound healing and tissue regeneration. Moreover, Somboonwong et al. (2000) reported that *Aloe vera* could reduced leukocyte adhesion on endothelial cells and promoted wound healing in second degree burn wound rats.

Antiinflammatory Activity

Previous studied, demonstrated that *Aloe vera* has an effect of anti-inflammation.

In vitro, Fujita and shosuke (1976) reported that aloe significantly inactivated bradykinin. Later in 1979, they found that enzyme to be carboxypeptidase, capable of hydrolyzing bradykinin and angiotensin I. Bradykinin is both a vasodilator and potent pain producing agent at the site of acute inflammation. *In vivo* study of Rebel (1983) demonstrated of the bradykininase activity of *Aloe vera* could hydrolyze bradykinin and angiotensin I to convert into angiotensin II result in suppressing vasodilation and pain.

Heggers et al (1979) found that *Aloe vera* decrease thromboxane B_2 and $PGF_{2\alpha}$ in guinea pig burn wounds while PGE_2 level were increased compare with controls. Thromboxanes and prostaglandins have been shown to elicit platelet aggregation, leukocyte adherence, and vasoconstriction, properties that may be detrimental to burn wound

healing by enhancing ischemia. Thus, *Aloe vera*'s ability to inhibit thromboxane B_2 and PGF_2 for formation in guinea pig burn wounds would preserve dermal circulation and decrease burn wound tissue loss. In addition, Heggers and Robson (1982) demonstrated that *Aloe vera* cream can increase dermal perfusion and apparently decrease thromboxane A_2 quantities in burn wound in guinea pigs. And they demonstrated that *Aloe vera* can enhance tissue survival in frostbite injuries in white rabbit.

Aloe vera containing salicylic acid (Robson et al., 1982), which also antiinflammation. Salicylates are both analgesic and antiinflammation, inhibiting the production of prostaglandins from arachidonic acid by inhibiting cyclooxygenase (Moore and Hoult, 1982). Penneys (1981) demonstrated that both *Aloe vera* gel and commercial *Aloe vera* extract significantly inhibited the oxidation of arachidonic acid in vitro.

In another study, Va'zquez et al. (1996) studied the effect of aqueous, chloroform, and ethanol extract of *Aloe vera* gel on carrageenan-induced edema in the rat paw and neutrophil migration into the peritoneal cavity stimulate by carrageenan. They found that the aqueous and chloroform extracts decrease the edema induced in the hind-paw and the number of neutrophils migration into the peritoneal cavity, whereas the ethanol extract only decreases carrageenan-induced neutrophils. The aqueous extract inhibited prostaglandin E_2 production from [^{14}C]arachidonic acid. The chemical tests performed in the aqueous extract for anthraglycosides, reductor sugars and cardiotonic glycoside. In the ethanol extract, the chemical tests performed for saponins, carbohydrates naftoquinones, sterols, triterpenoids and anthraquinones. In

the chloroform extract, the chemical test performed for sterols and anthraquinones. In the results they suggested that *Aloe vera* gel have antiinflammatory activity by inhibited arachidonic acid production pathway via cyclooxygenase.

Recently, Daunsak et al (2003) found that *Aloe vera* could inhibited the inflammation process following burn injury by reduced leukocyte adhesion and proinflammatory cytokines such as TNF-α, IL-6.

Other activities

Antibacterial and antifungal effects

Lorenzetti et al (1964) studied *in vitro*, *Aloe vera* significantly inhibited growth on plates inoculated with Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium xerose, and Salmonella paratyphi. Moreover, Robson et al (1982) found that 60% concentration of *Aloe vera* extract inhibited Pseudomonas aeruginosa, Klebsiella pneumaniae, Serratia marcescens, Citrobacter species, Enterobacter cloacae, S. pyogenes, and streptococcus agalactiac, 70% for streptococcus aureus, 80% for Enterobacter coli and 90% for streptococcus faecalis and Candida albicans.

Antipruritic effect

Aloe vera contianed magnesium lactate (Hirata, 1977). Magnesium lactate inhibits the *in vivo* conversion of histidine to histamine in mast cell by inhibiting histidine decarboxylase (Lehninger, 1981). Histamine, a known vasodilator, in regarded as one of the major produces of itching in

the skin. Therefore, *Aloe vera* is antipruritic effect by inhibition of histidine decarboxylase by magnesium lactate (Klein and Penneys, 1988).

The effect of *Aloe vera* on gastric ulcer

The pathogenic of the gastric ulcer is generally accepted as resulting from a breakdown of the balance between the aggressive factor such as HCL, pepsin and the defensive factor such as mucus, mucosal barrier, gastric mucosal circulation and prostaglandins (as a cytoprotactive factor) (Mathatanadul, 1995).

Previous study, reporting that *Aloe vera* was the prophylactic and curative effects. It protected the gastric mucosa and its support of mucosal resistance against the sequalae of chemical irritation and its nervous stress without interfering with the gastric pH (Galal et al.,1975; Kandil., 1982). In addition, Robert et al (1979) demonstrated that *Aloe vera* appear to exert this cytoprotective effect via endogenous prostaglandin with prevent the mucosa of the stomach becoming inflamed and necrotic or maintain the cellular intergity of the gastric mucosa when exposed to noxious agents. Prostaglandins leads to increase mucosal blood flow, bicarbonate secretion, and mucus production, thus protecting the gastric mucosa against injury (Hollander, 1994; Wallace et al., 1995; Linder et al., 2000).

Several studies have revealed that *Aloe vera* has antiulcer effect which may be due to its acid reducing properties (Hennessee and Cook, 1994). The observation that *Aloe vera* extract inhibits acid secretion may be due to the presence of lectin in the plant (Blizt et al., 1963). Hirata and Suga (1977) found that aloenin and magnesium lactate in *Aloe vera*

inhibited gastric acid secretion in the rat. Aloctin A are glycoproteins in *Aloe vera*, which inhibited gastric acid, pepsin secretion and reduced gastric ulceration from indomethacin, water-immersion stress (Saito et al., 1989).

Recently, Suvitayavat et al (2004) found that aloe preparation was shown to increase pepsinogen secretion, it has been demonstrated to decrease in gastric acid secretion and increase in mucus secretion. It is possible that the antiulcer effect of aloe gel should be mediated through the decrease in aggressive factors and increase in protective factors.

Furthermore, there is the possibility of a synergistic action between the components of the *Aloe vera* that is responsible for the antigastric ulcer action (Grindlay and Reynolds, 1986; Blitz et al., 1963; Barry, 1983) the mucopolysaccharide and glycoproteins contents which act like the gastric mucin to protect the gastric mucosal from the damage; the antithromboxane B_2 effect of the gel which cause reduction of vasoconstriction and give improved perfusion of gastric mucosal capillaries and the glycoproteins, namely Aloctin-A or Lectin P-2, which have a healing effect on the produced ulcer.

Mahattanadul (1995) investigate the prophylactic and curative effects of *Aloe vera* on gastric ulcer induced by cytodestructing agents; 0.6 NHCl and acetic acid (30 and 100%), in rats. Three preparations of *Aloe vera* gel were used; freshly prepared gel, fresh freeze-dried powder and two month prestored freeze-dried powder. From the experiment, found that three perparations of *Aloe vera* (dose 400 mg/kg/day) had significant efficacy both in protecting gastric mucosa against the injuries caused by necrotizing agent (0.6 NHCl) and healing ulcers already

induced by acetic acid (30 and 100%). In comparision of the curative effect of *Aloe vera* with that of the standard cytoprotecting agent, "sucralfate", using the same treatment and the same dose (400 mg/kg/days), all three preparation of *Aloe vera* gel showed the same curative efficacy as sucralfate on gastric ulcer induced by acetic acid, which these finding suggest that *Aloe vera* gel exerts antigastric ulcer action by directly protecting the gastric mucosa and exerts cytoprotective activity associated with an enhancement of local healing process.

Hypothesis of effect of *Aloe vera* on gastric ulcer

A variety of factors such as stress, various irritants result in gastric mucosal injury and gastric ulcer. The pathogenesis of gastric mucosal injury result in changes of gastric microcirculation, cytokines level leads to gastric inflammation.

In the present, sucralfate is cytoprotective drug which a popular agent used to cure of gastric ulcer.

Aloe vera is a good plant that is composed of various chemicals. The properties of *Aloe vera* are both antiinflammation and promote wound healing. Previous studies demonstrated that *Aloe vera* was cytoprotective effect from various irritants in gastric mucosa and it also inhibited acid secretion, which, promoted gastric ulcer healing.

Thus, it is the hypothesis of this study that *Aloe vera* is able to return to gastric microcirculation and TNF-α, IL-10 levels and promote gastric ulcer healing.

Therefore, the objectives of this study are:

1. To comparative study between effects of *Aloe vera* and sucralfate on leukocyte-endothelial cell interaction.
2. To comparative study between effects of *Aloe vera* and sucralfate on TNF-α and IL-10 levels changes.
3. To comparative study between effects of *Aloe vera* and sucralfate on gastric ulcer healing.

20% acetic acid induced gastric ulcer: the animal model

In this study, the gastric ulcer was induced by 20% acetic acid. Acetic acid produced more severe ulceration resembles to human chronic gastric ulcer (Takagi et al., 1969). From previous study, the gastric ulcer model was induced by oral administration of 1.5 ml 9% acetic acid. into stomach (Liu et al., 1990). In this study, we try to use same method as their study, which after induced gastric ulcer with 9% acetic acid for 1 hour, the stomach was then opened and observed gross appearances of stomach. We found only scattered hemorrhage and mild hyperemia. Thus, we just modified their method by increased concentration of acetic acid to be 20% acetic acid and give by orogastric tube, after induced gastric ulcer for 1 hour, stomach was opened and observe by macroscopic. We found more hemorrhage, edema and red of gastric tissue. After that, we confirmed by histopathology examination, which found that there were moderate erosion and ulcer, congestion and edema of mucosa, and inflammation. Therefore, in this study, we used 20% acetic acid for induced gastric ulcer. The animals were received only one dose of 20% acetic acid.

Sucralfate

Sucralfate is a complex of aluminium hydroxide and sulfated sucrose, which, in the presence of acid, releases aluminium, acquires a strong negative charge and binds to positively charged groups in protein, glycoproteins, etc. Its mechanism of action is thought to involve polymerization and selective binding to necrotic ulcer tissue and protect ulcer from acid, pepsin, and bile. And it also bind to and inactivates pepsin and bile acids. In addition, sucralfate also stimulate the mucosal

protecting mechanism mucus and bicarbonate secretion and prostaglandin production (Bertram 1998; Rang et al., 2003).

Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. ELISAs can provide a useful measurement of antigen or antibody concentration. There are two main variations on this method: The ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen. An ELISA is a five-step procedure: 1) coat the microtiter plate wells with antigen; 2) block all unbound sites to prevent false positive results; 3) add antibody to the wells; 4) add anti-mouse IgG conjugated to an enzyme; 5) reaction of a substrate with the enzyme to produce a colored product, thus indicating a positive reaction. There are many different types of ELISAs. One of the most common types of ELISA is "sandwich ELISA."

The sandwich ELISA measures the amount of antigen between two layers of antibodies. The antigens to be measured must contain at least two antigenic sites, capable of binding to antibody, since at least two antibodies act in the sandwich. So sandwich assays are restricted to the quantitation of multivalent antigens such as proteins or polysaccharides. Sandwich ELISAs for quantitation of antigens are especially valuable when the concentration of antigens is low and/or they are contained in high concentrations of contaminating protein.
(www. Protocol-online. Org/prot/Immunology/ELISA/22k)

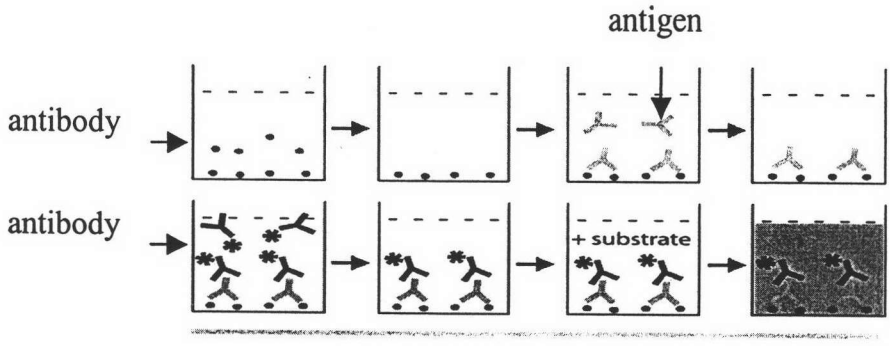

Figure 2.7 The sandwich ELISA technique for detected unknown antigen by binding to two antibodies act in the sandwich. (webmed. Unipv. It/immunology/agabint.html.)