EFFECTS OF THYROIDECTOMY AND THYROXINE TREATMENT ON GASTRIC ULCER HEALING IN RATS

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ABSTRACT

Embryologic and phylogenic relationships have been established between the thyroid gland and the gastrointestinal tract. Although previous studies suggested that thyroxine treatment influences the secretion of gastric acid and ulcerogenesis in man and experimental animals, there is no available information on its role on gastric ulcer healing processes. The effects of thyroidectomy and thyroxine treatment on healing of gastric ulceration were investigated.

Two hundred and sixty male albino rats (160 – 200g) were divided into: control, thyroxine treated (100µg/Kg/day, p.o) and thyroidectomised animals with or without thyroxine replacement. Thirty-five days post-treatment, the plasma levels of thyroxine, triiodothyronine and thyroid stimulating hormone were estimated by radioimmunoassay to confirm thyroid states. Experimental ulcers were then induced in the stomach of animals by serosal application of acetic acid (0.5ml, 80%). The following indices of ulcer healing were determined in five rats per group on each of days 3, 7 and 10 post-induction: ulcer area by planimetry, ulcer depth and width by histomorphometry, tissue regeneration by histology. Acid secretion was studied by continuous perfusion method. Lipid peroxidation and white blood cell counts were determined by spectrophotometry and haemocytometry respectively. The DNA damage was studied by electrophoresis and visualised by UV exposure after ethidium bromide staining. Data were analysed using Students t-test at $p = 0.05$.

The rate of ulcer healing was significantly higher in thyroxine-treated ($0.8 \pm 0.1 mm^2$/day) and lower in thyroidectomised ($0.3 \pm 0.1 mm^2$/day) rats when compared with control ($0.5 \pm 0.1 mm^2$/day) by day 10. There were significant reductions in ulcer width and depth in thyroxine-treated animals ($69.3 \pm 1.5\%$ and $65.7 \pm 1.4\%$ respectively) when compared with control ($40.5 \pm 2.2\%$ and $53.9 \pm 1.6\%$) and thyroidectomised rats ($34.1 \pm 0.5\%$ and $35.6 \pm 7.5\%$). Histology

revealed gastric ulceration in all groups on day 3, with presence of inflammatory cells at ulcer bed. By day 7, marked reduction in the inflammatory cell and greater fibroblast proliferation were observed in control and thyroxine-treated but not in thyroidectomised animals. On day 10, there was marked epithelial regeneration in thyroxine-treated animals, while thyroidectomised animals had abundant inflammatory cells. Basal and histamine-stimulated gastric acid secretion declined over the healing period at a higher rate in thyroxine-treated rats (77.4 ± 2.6% and 48.3 ± 3.4% respectively) when compared with control (65.0 ± 0.0% and 30.4 ± 1.9%). The reduction in neutrophil-lymphocyte ratio accompanying healing was highest in the thyroxine-treated (65.0 ± 2.5%) and lowest in thyroidectomised rats (20.1± 1.7%) when compared with control (28.3 ± 2.8%). Thyroxine significantly reduced lipid peroxidation from 271.3 ± 9.9nmol/mg protein on day 3 to 120.0 ± 13.9nmol/mg protein on day 10 and suppressed acetic acid induced DNA fragmentation, suggesting its antiapoptotic role during ulcer healing. The deficiencies observed in thyroidectomised animals were restored by therapy with exogenous thyroxine.

Thyroidectomy delayed ulcer healing while thyroxine treatment accelerated it. Thyroxine had anti-inflammatory action in the rat stomach, decreased gastric hyperacidity, promoted fibroblast and epithelial cell proliferation.

Keywords: thyroxine treatment, thyroidectomy, gastric ulcer, healing.

Word count: 497

DEDICATION

This research work is dedicated to:

THE ALMIGHTY GOD

For the priviledge, provision and grace to accomplish this task

My Wonderful Parents

ENGR. (ELDER) THEOPHILUS OLADIPO ADENIYI AND MRS JANET DELE ADENIYI

For their love, encouragement and support

And

My Loving Wife

MRS. KYENMU ADENIYI

For her love, encouragement and support

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CERTIFICATION

I certify that **MR. OLASUPO STEPHEN ADENIYI** carried out this work titled **"EFFECTS OF THYROIDECTOMY AND THYROXINE TREATMENT ON GASTRIC ULCER IN RATS"** under my supervision in the Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

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CHAPTER ONE

INTRODUCTION

Gastric ulcer, a type of peptic ulcer, is one of the most prevalent gastrointestinal disorders. It is a deep defect in the gastric (stomach) wall penetrating the entire mucosal thickness and the muscularis mucosa (Tarnawski, 2000). Pathophysiology of ulcer is due to an imbalance between aggressive factors and local mucosal defensive factors. The main causes of gastric ulceration include: Infection with *Helicobacter pylori* (Marshall and Warren 1984; Kusters *et al.*, 2006), use non streroidal anti-inflammatory drugs (NSAIDs) e.g aspirin (Gabriel and Bombardier, 1990), smoking, psychological stress and free oxygen radicals (Olaleye *et al.*, 2007).

It is estimated that the prevalence of peptic ulcer in the United States is 1.5% of population (Munnangi and Sonnenberg, 1997), in Denmark, the prevalence is 2.6% (Hein *et al.*, 1997), in Israel, 4.7% (Rennert and Peterburg, 2001), and in Sweden 4.1% (Aro *et al.*, 2006). Data is lacking about the incidence and prevalence of ulcer in Asia and Africa. In Nigeria, hospital cases of peptic ulcer are reported (Ameh and Nmadi, 1998; Onyekwere *et al*, 2008). Endoscopy report from Jos, Plateau State, Nigeria revealed that duodenal and gastric ulcers were found in 17.3% and 4.9% patients respectively (Malu *et al.*, 1994). The annual financial burden of peptic ulcer disease in the US, including direct and indirect costs, is estimated as US$3.4 billion (Sandler *et al.*, 2002).

Thyroid hormones (thyroxine [T_4] and triiodotyronine [T_3]) have long been recognized as a major regulator of the oxidative metabolism of energy producing substrates (food or stored substrates like fat and glycogen) by the mitochondria. Recently 3,5 and 3,3' diiodothyronines, collectively called T_2, which are active metabolite of T_3 (Moreno *et al.*, 2002) are now

considered to participate in action of thyroid hormones. Thyroid hormones increase the flux of nutrients into the mitochondria as well as the rate at which they are oxidized, by increasing the activities of the enzymes involved in the oxidative metabolic pathway (Moreno *et al.*, 2002). Thyroid hormones affect nearly all systems of the body. Thyroid hormones influence growth in intrauterine life and they are important for body growth after birth (Cabello and Wrutniak, 1989). Childhood hypothyroidism results in growth arrest, delayed bone age, epiphyseal dysgenesis and short stature (Chiesa *et al.*, 1994). Hyperthyroidism results in increased systolic contractile activity (Dieckman and Solaro, 1990) and shortens diastolic relaxation (lusitropic activity), whereas diastole is prolonged in hypothyroid states in all mammalian species (Mintz *et al.*, 1991).

A relationship exists between the thyroid gland and the gastrointestinal tract; Phylogenetically, thyroid cells are derived from primitive iodide-concentrating gastroenteric cells (Kupper *et al.*, 2008). Embryologically, the thyroid gland appears as an epithelial proliferation in the floor of the pharynx at the base of the tongue, at a point later indicated by the foramen cecum. The effects of thyroid hormones on gastrointestinal functions are well reported.

Hypothyroidism is associated with dysphagia, decrease in the amplitude and velocity of peristalsis of oesoghagus, decrease in lower esophageal sphincter pressure, prolonged gastric emptying (Kahraman *et al.*, 1997). On the other hand, hyperthyroidism accelerates small intestine and large intestine transit time, but there was no significant difference in gastric emptying between hyperthyroid and euthyroid subjects (Wegener *et al.*, 1992). Thyroxine has been reported to increase basal and histamine stimulated gastric acid secretion, while thyroidectomy caused a decrease (Adeniyi and Olowookorun, 1989; Rafsanjani *et al.*, 2003). Thyroidectomy decreased the number of parietal cells while thyroxine administration increased it

(Adeniyi and Olowookorun, 1989). Thyroid hormones affect nutrient absorption in such a way that hyperthyroidism increased glucose absorption, while hypothyroidism decreased glucose absorption (Olaleye and Elegbe, 2005; Loeb, 1996).

Thyroid disorders were thought to be rare in Africans in the early 1960s. However the 1970s witnessed an upsurge in reported cases of thyroid disorders in Africans (Ogbera *et al.*, 2007). In endocrinology clinics in Nigeria, thyroid disorders are the second most common endocrine disorders seen (Ogbera *et al.*, 2007, Ojule *et al.*, 1998, Adesunkanmi and Makinde 2003). Ogbera *et al.*, (2007) reported that the prevalence of thyrotoxicosis in Lagos, Nigeria was 1.6%. They reported that within 15 months, a total of 78 patients with thyroid disorders were seen. Hypothyroidism was present in five (7%), Graves disease/hyperthyroidism in 63 (84%), and euthyroid in 10 (9%).

The effects of thyroid hormone on ulcer formation has been reported; Thyroid hormones reduce formation of stress ulcers in rats when given before or at beginning of the stress (Koyunca *et al.*, 2002). Oluwole and Saka (2007) reported gastroprotective effects thyroid hormones against ulcer induction. These reports suggest that thyroid hormones play a role in gastric ulceration, however there is no available information about the effects of these hormones on gastric ulcer healing.

Gastric ulcer healing consists of reconstruction of mucosal architecture and is a dynamic, active process of filling the mucosal defects with epithelial and connective tissue cells. Ulcer healing process include: inflammation, tissue formation (granulation tissue formation, angiogenesis, and re-epithelialization), and tissue remodeling (Tarnawski 2000). All these processes are controlled by growth factors, transcription factors and cytokines (Tarnawski 1993, Vanwijck, 2001).

Effects of thyroid hormones on healing in other tissues of the body are contradictory. Thyroxine treatment was reported to accelerate healing of laryngeal fistula in post operative hypothyroid patients (Talmi *et al.*, 1989), accelerated healing of experimental myocardial infarction in rats (Kranz *et al.*, 1976), accelerated skin wound healing (Lennox and Johnston, 1973), stimulated epidermal proliferation, dermal thickening, and hair growth (Safer *et al.*, 2001; Safer, 2005). Mehregan and Zamick (1974) reported that additional thyroid hormone stimulated the rate and quality of wound healing in euthyroid rats while hypothyroidism retarded wound healing on the skin 2-fold relative to euthyroidism (Safer *et al.*, 2004; Lennox and Johnston, 1973).

However, Pirk *et al.*, (1974) noted no change in wound healing with euthyroid hamsters receiving thyroxine (ip). Cannon (1994) reported that hypothyroidism did not diminish wound strength in pigs, and Ladenson *et al.*, (1984) failed to detect wound healing deficits in hypothyroid humans after surgery.

Gastric acid has long been associated as a causative factor of gastric and duodenal ulcer, for instance, about 50% of gastric ulcer patients are pepsin and acid hypersecretors (Szabo, 1988) and duodenal ulcer patients usually secrete more acid (Wolfe and Soll, 1988). Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost. Furthermore, the corrosive property of gastric acid and increased peptic activity is sufficient to aggravate ulcer, interfere with the process of restitution, resulting in the conversion of superficial injury to deeper mucosal lesion and inactivating acid-labile growth factors important for maintenance of mucosal integrity and repair of superficial injury (Wallace and Muscara, 2001)

Therefore, inhibition of gastric acid secretion is considered to be an important step to healing of gastric ulcer (Sachs *et al.*, 1993). Histamine H_2 receptor antagonists (Fock *et al.*, 2008) and proton pump inhibitors such as omeprazole, lansoprazole, pantoprazole, and rabeprazole are extensively used for therapeutic control of peptic-ulcer disease: enhance ulcer healing, and prevent ulcer recurrence caused by stress (stress-related erosive syndrome), nonsteroidal antiinflammatory drugs, and *Helicobacter pylori* infection (Wolfe and Sachs, 2000).

White blood cell counts have been used as an indicator by clinicians to monitor progress of healing in patients (Haffor, 2010). White blood cells are important for healing. They do this by ingesting materials that need to be removed from the body. These include debris from body tissues and dead cells. Elevated WBC count was reported to be found in a group of rats in which gastric ulcer healing was accelerated (Haffor, 2010). A physiologic immune response of circulating white blood cells to various stressful events, such as tissue injury, severe trauma, major surgery, burns, is characterized by elevation of neutrophils and decline in lymphocyte counts. The neutrophil-lymphocyte ratio (NLR) provides an indicator of inflammatory status (Halazun *et al.*, 2008). Zahorec (2001) reported that the lowest values of lymphocyte counts were observed in the most severe group (severe sepsis syndrome or septic shock, major surgery, trauma). Improvement of the clinical status coincides with a gradual increase in lymphocyte counts and concomitant decrease in neutrophil counts. Although up to date search of information revealed that neutrophil-lymphocyte ratio has not been applied to healing of ulcer.

Reactive oxygen species have been implicated in the pathogenesis of many diseases in the body including gastric ulcer through oxidative damage (Halliwell and Gutteridge, 1986). Lipid peroxidation, an important parameter for $^{\cdot}OH$-induced oxidative damage of membrane, is increased in gastric lesions caused by ethanol (Pihan *et al.*, 1987), indomethacin (Yoshikawa *et*

al., 1993), and water immersion stress (Yoshikawa *et al.*, 1986). Inhibition of lipid peroxidation is associated with accelerated rate of ulcer healing in rats (Rodriguez *et al*., 2006).

Recent studies also indicate that programmed cell death or apoptosis plays a significant role in gastric ulceration. Gastric mucosal lesions caused by stress, indomethacin, ethanol, and *H. pylori* are also due to increased cell death by apoptosis (Fuji *et al*., 2000, Konturek *et al*., 1999). However for healing of ulcer to occur, apoptosis decrease at the initial stage of the healing process to allow cell proliferation to occur, but later during healing, apoptosis increase to clear the wound site of inflammatory cells, blood vessels and fibroblasts in granulation tissue (Brown *et al*., 1997).

As mentioned earlier, thyroid hormones prevent ulcer formation when given before ulcer was induced in animals (Koyunca *et al.*, 2002; Oluwole and Saka 2007). This reveals that thyroid hormones play a role in ulcerogenesis. The other side of ulcer pathophysiology is the process involved in the healing of an ulready formed ulcer. Tarnaswki (2000), in a comprehensive review of ulcer healing in the gut, stated that certain processes must take place at the molecular level before healing could take place. These include cell proliferation, cell migration and angiogenesis. Other workers have buttressed this fact (Luo et al, 2003; Tarnawski 2000; Vanwijck, 2001). There appears to be no available information about the roles of thyroid hormones on gastric ulcer healing. Furthermore, although acid secretion has long been known to play significant role in ulcerogenesis, its exact role in ulcer healing is not fully understood.

In the light of the grey areas highlighted in the pathophysiology of ulcer healing, the present study was designed to achieve the followng objectives:

1. To investigate the exact roles of thyroid hormones in gastric ulcer healing
2. To investigate the role of acid secretion in the ulcer healing processes in the stomach

3. Depending on the outcome of the first to objectives, to attempt to elucidate the possible mechanism of the actions

The above stated objectives will be investigated by:

1. Measuring the dimensions of ulcer by planimetry and histomorphometry
2. Assessing the characteristics and quality of healing by histological method
3. Assessing the role of gastric acid secretion during ulcer healing
4. Assessing the role of blood cells during ulcer healing
5. Assessing cellular activities during ulcer healing by biochemical study (lipid peroxidation) and apoptotic study.

CHAPTER TWO

LITERATURE REVIEW

2.1. The Thyroid Gland

2.1.1. The Thyroid Gland and Thyroid Hormones

Goiter is very common in many parts of the world and was recognized long before the thyroid gland itself. Enlarged thyroids are said to have been known in China in about 2700 BC and the Chinese seem to have used burnt sponge and seaweed for its treatment since 1600 BC (Leoutsakos, 2004). In vertebrates, the thyroid gland is one of the largest endocrine glands. Normally not visible on inspection, the thyroid gland can be palpated during swallowing when it is upwardly displaced. Thyroid tissue is highly vascular and is composed of follicular cells that synthesize and secrete 2 major hormones: thyroxine (T_4) and triiodothyronine (T_3). These hormones are sometimes collectively referred to as thyroid hormone (Dabon-Almirante and Surks, 1998).

2.1.2. Anatomy of Thyroid Glands

The thyroid glands, weigh 2-3 grams in neonates and 18-60 grams in adults (Bargman, 1932), and is increased in pregnancy. It is a butterfly-shaped organ and is composed of two cone-like lobes or wings, *lobus dexter* (right lobe) and *lobus sinister* (left lobe), connected via the isthmus. The organ is situated on the anterior side of the neck, lying against and around the larynx and trachea (Kamath, 2010), reaching posteriorly the oesophagus and carotid sheath. It starts cranially at the oblique line on the thyroid cartilage (just below the laryngeal prominence, or 'Adam's Apple'), and extends inferiorly to approximately the fifth or sixth tracheal ring. It is

difficult to demarcate the gland's upper and lower border with vertebral levels because it moves position in relation to these during swallowing.

The thyroid gland is covered by a fibrous sheath, the *capsula glandulae thyroidea*, composed of an internal and external layer. The external layer is anteriorly continuous with the *lamina pretrachealis fasciae cervicalis* and posteriorolaterally continuous with the carotid sheath. The gland is covered anteriorly with infrahyoid muscles and laterally with the sternocleidomastoid muscle. On the posterior side, the gland is fixed to the cricoid and tracheal cartilage and cricopharyngeus muscle by a thickening of the fascia to form the posterior suspensory ligament of Berry (Yalçin and Ozan, 2006). Between the two layers of the capsule and on the posterior side of the lobes, there are on each side two parathyroid glands.

The thyroid is supplied with arterial blood from the superior thyroid artery, a branch of the external carotid artery, and the inferior thyroid artery, a branch of the thyrocervical trunk, and sometimes by the thyroid ima artery, branching directly from the brachiocephalic trunk (Wissig, 1964). The venous blood is drained via superior thyroid veins, draining in the internal jugular vein, and via inferior thyroid veins, draining via the *plexus thyroideus impar* in the left brachiocephalic vein. Lymphatic drainage passes frequently the lateral deep cervical lymph nodes and the pre- and parathracheal lymph nodes. The gland is supplied by parasympathetic nerve input from the superior laryngeal nerve and the recurrent laryngeal nerve.

2.1.3. Evolution of Thyroid Gland

Thyroid cells are phylogenetically derived from primitive iodide-concentrating gastroenteric cells (endostyle) which, during evolution, migrated and specialized in uptake and storage of iodine in follicular cellular structures, in order to adapt organisms from iodine-rich sea

to iodine-deficient land. Venturi *et al.*, (2000) suggested that iodide has an ancestral antioxidant function in all iodide-concentrating cells from primitive algae to more recent vertebrates. This ancestral antioxidant action of iodides has been experimentally confirmed by Küpper *et al.*, (2008). When some primitive marine chordates started to emerge from the iodine-rich sea and transferred to iodine-deficient fresh water and finally land, their diet became iodine deficient.Therefore, during progressive slow adaptation to terrestrial life, the primitive vertebrates learned to use the primitive thyroxine to transport antioxidant iodide (Kupper *et al.*, 2008) into the cells. Therefore, the remaining triiodothyronine (T_3), the real active hormone, became active in the metamorphosis and thermogenesis for a better adaptation of the organisms to terrestrial environment (fresh water, atmosphere, gravity, temperature and diet).

2.1.4. Embryological Development of Thyroid Gland

In the fetus, at 3–4 weeks of gestation, the thyroid gland appears as an epithelial proliferation in the floor of the pharynx, at the base of the tongue between the tuberculum impar and the copula linguae, at a point later indicated by the foramen cecum. The thyroid then descends in front of the pharyngeal gut as a bilobed diverticulum through the thyroglossal duct. Over the next few weeks, it migrates to the base of the neck. During migration, the thyroid remains connected to the tongue by a narrow canal, the thyroglossal duct (Neas, 2003).

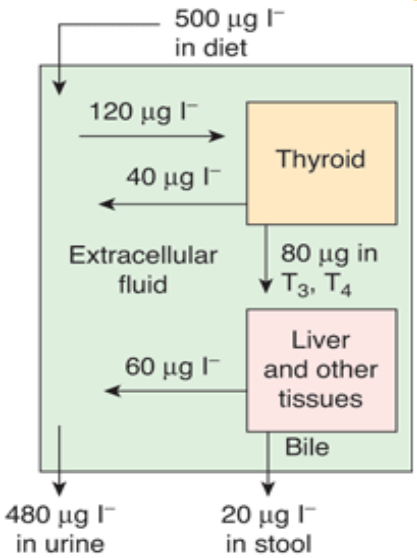
Thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) start being secreted from the fetal hypothalamus and pituitary at 18-20 weeks of gestation, and fetal production of thyroxine (T_4) reach a clinically significant level at 18–20 weeks (Eugster and Pescovitz, 2004). Fetal triiodothyronine (T_3) remains low (less than 15 ng/dL) until 30 weeks of gestation, and increases to 50 ng/dL at term (Eugster and Pescovitz, 2004). Fetal self-sufficiency

of thyroid hormones protects the fetus against brain development abnormalities caused by maternal hypothyroidism (Zoeller, 2003). However, preterm births can suffer neurodevelopmental disorders due to lack of maternal thyroid hormones and their own thyroid gland being insufficiently developed to meet their postnatal needs (Berbel *et al.*, 2010).

2.1.5. Synthesis of Thyroid Hormones

Iodine Requirement

Iodine is an essential raw material for thyroid hormone synthesis. The minimum daily iodine intake that will maintain normal thyroid function is 150 µg in adults (US National Research Council, 1989) However, the average dietary intake of iodine is approximately 500 µg/d, and about 120 µg/d enter the thyroid. The thyroid secretes 80 µg/d in the form of T_3 and T_4 and 40 µg/d diffuses back into the extracellular fluid (ECF). Normally, most of the iodides are rapidly excreted by the kidneys.



Source: Ganong, 2001.

Figure 2.1. Body requirement and excretion of iodine

Iodide Trapping

This is the first step in thyroid hormone synthesis. The basolateral membrane of thyrocytes possesses a pump called Na^+/I^- symport. The symport transports two Na^+ ions and one I^- ion into the cell with each cycle, against the electrochemical gradient for I^-. Concentration of TSH is the strongest regulator of iodide trapping symport mechanism (Dohan *et al.*, 2003).

Thyroglobulin

Thyroglobulin, a glycoprotein molecule with a molecular weight of about 335,000 is synthesized by the thyroctes (Venturi *et al.*, 2000). Each molecule contains about 70 tyrosine residues.

Oxidation of Iodide

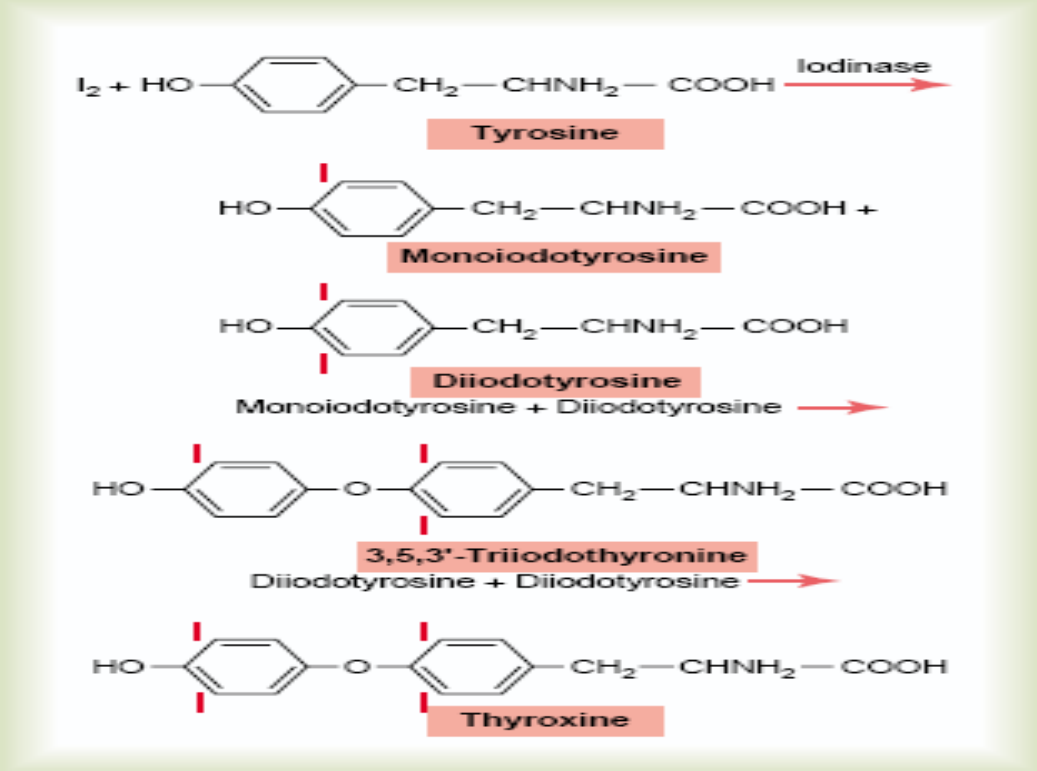
Once iodide is inside the thyroid cells, it is converted into an oxidized form of iodine, either nascent iodine (I^0) or I_{3-} by enzyme peroxidase. Oxidized iodine is capable of combining directly with the amino acid tyrosine (De La Vieja *et al.*, 2000)

Organification of Thyroglobulin

The binding of iodine with the thyroglobulin molecule is called organification of the thyroglobulin. Thyroid peroxidase catalyzes the iodination of tyrosine residues. Tyrosine is first iodized to monoiodotyrosine (MIT) and then to diiodotyrosine (DIT) (Marino and McCluskey, 2000).

Coupling Reaction

Iodotyrosine residues become coupled with one another (thyroid peroxidase is involved in coupling reaction). Thyroxine (T_4) is formed by condensation of two molecules of DIT and Triiodothyronine (T_3) is formed by condensation of MIT with DIT. A small amount of reverse T_3, RT_3 is also formed, probably by condensation of DIT with MIT. The major hormonal product of the coupling reaction is the molecule thyroxine that remains part of the thyroglobulin molecule. Triiodothyronine represents about one fifteenth of the final hormones (Marino and McCluskey, 2000).



Source: Elsevier. Guyton and Hall: Textbook of Medical Physiology 11e – www.studentconsult.com

Figure 2.2. Chemistry of thyroxine and triiodothyronine formation

Storage of Thyroid Hormones

Each thyroglobulin molecule contains up to 30 thyroxine molecules and a few triiodothyronine molecules. Stored thyroid hormones maintain the body's requirement of T_3 and T_4 for up to 2-3 months (Marino and McCluskey, 2000).

Release of Thyroid Hormones

Thyroglobulin itself is not secreted into the circulation. Thyroglobulin is digested by pinocytosis mechanism at the apical membrane. Triiodothyronine and thyroxine are released; they then diffuse into the capillaries through the basal surface. During digestion of thyroglobulin, iodothyronins are also freed but not released in to the blood. Enzyme deiodinase cleaves iodine from them making it available for more thyroid hormone synthesis inside the gland. About 93 per cent of the thyroid hormone released from the thyroid gland is normally thyroxine, only 7 per cent is triiodothyronine. At the target tissue, most of T_4 is converted to T_3 (Laubery, 1980).

Thyroid Hormone Transport and Protein Binding

Once in circulation, T_3 and T_4 bind to plasma proteins. These proteins include: thyroxin-binding globulin, thyroxin-binding prealbumin, albumin (Distefano and Fisher, 1979). Free T_3 and T_4 are the physiologically active forms. About 99.98% of the T_4 in plasma is bound, the free T_4 level is only about 2 ng/dL. T_3 is not bound to quite as great an extent; 0.2% (0.3 ng/dL) is free. The remaining 99.8% is protein-bound (Distefano and Fisher, 1979).

2.1.6. Secretion of Thyroid Hormones

Thyroxine and triiodothyronine are regulated through a feedback loop that involves the hypothalamus, the anterior part of the pituitary gland, and the thyroid gland. The initiating hormone, thyrotropin-releasing hormone, is synthesized and stored in the hypothalamus (Ladenson *et al.*, 2000). Thyrotropin-releasing hormone circulates to the anterior part of the pituitary gland, where it stimulates the release of thyroid-stimulating hormone (TSH). Thyroid-stimulating hormone binds to receptor sites located on the thyroid gland, which produces, secretes, and stores T_3 and T_4. Stimulation by TSH results in an immediate release of stored thyroid hormones into the circulation. Concurrent effects of TSH on the thyroid gland include; increase in the uptake and oxidation of iodine, increase in the synthesis of T_3 and T_4 (Ladenson *et al.*, 2000). Under normal circumstances, the feedback loop ensures that circulating levels of thyroid hormones are appropriate for metabolic needs. Disruption of the feedback loop can result in a life-threatening excess or deficit of T_3 and T_4. It has recently been recognized that there exist two additional active metabolites of T_3: 3,5 and 3,3' diiodothyronines, collectively called T_2 (Moreno *et al.*, 2002).

2.1.7. Mechanism of Action of Thyroid Hormones

2.1.7.1. Genomic Actions of Thyroid Hormones

Thyroid hormones enter cells through membrane transporter proteins. Once inside the nucleus, the hormone binds its receptor, and the hormone-receptor complex interacts with specific sequences of DNA in the promoters of responsive genes (Brent, 1994). The effect of the hormone-receptor complex binding to DNA is to modulate gene expression. In contrast to steroid hormone receptors, thyroid hormone receptors bind DNA in the absence of hormone, usually

leading to transcriptional repression. Hormone binding is associated with a conformational change in the receptor that causes it to function as a transcriptional activator (Tsai and O'Malley, 1994).

Receptor Structure

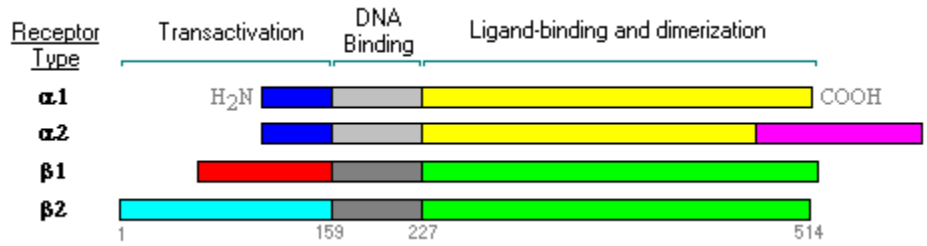
Mammalian thyroid hormone receptors are encoded by two genes, designated alpha and beta. Further, the primary transcript for each gene can be alternatively spliced, generating different alpha and beta receptor isoforms. Currently, four different thyroid hormone receptors are recognized: alpha-1, alpha-2, beta-1 and beta-2 (Zhang and Lazer, 2000).

Thyroid hormone receptors encapsulate three functional domains:

i. A transactivation domain at the amino terminus that interacts with other transcription factors to form complexes that repress or activate transcription. There is considerable divergence in sequence of the transactivation domains of alpha and beta isoforms and between the two beta isoforms of the receptor.

ii. A DNA-binding domain that binds to sequences of promoter DNA known as hormone response elements.

iii. A ligand-binding and dimerization domain at the carboxy-terminus (Zhang and Lazer, 2000).



Source: Bowen. Thyroid hormone receptors: thyroid and parathyroid gland. online

Figure 2.3: Structure of thyroid hormone receptors

The DNA-binding domains of the different receptor isoforms are very similar. The alpha-2 isoform has a unique carboxy-terminus and does not bind triiodothyronine (T_3). Almost all tissues express the alpha-1, alpha-2 and beta-1 isoforms, but beta-2 is synthesized almost exclusively in hypothalamus, anterior pituitary and developing ear. Receptor alpha-1 is the first isoform expressed in the conceptus, and there is a profound increase in expression of beta receptors in brain shortly after birth (Tsai and O'Malley, 1994; Zhang and Lazer, 2000).

Thyroid hormone receptors bind to short, repeated sequences of DNA called thyroid or T_3 response elements (TREs), a type of hormone response element. Thyroid hormone receptors bind to TRE DNA regardless of whether they are occupied by T_3. However, the biological effects of TRE binding by the unoccupied versus the occupied receptor are dramatically different. In general, binding of thyroid hormone receptor alone to DNA leads to repression of transcription, whereas binding of the thyroid hormone-receptor complex activates transcription. (Zhang and Lazer, 2000).

2.1.7.2. Nongenomic Actions of Thyroid Hormones

Thyroid hormone actions have been mechanistically defined in terms of expression of specific genes that is modulated by the intranuclear complex of 3,5,3.-triiodo-L-thyronine (T_3) with its nuclear receptors. These are designated genomic mechanisms. Another set of mechanisms exist that are initiated at or near the plasma membrane and do not primarily involve gene transcription (Smith *et al.*, 1989; Sun *et al.*, 2000) or may have consequences in the cell nucleus. There are other nongenomic actions of iodothyronines that involve the mitochondrion (Moreno *et al*., 2002), the actin cytoskeleton (Farwell *et al.*, 1995), certain cytoplasmic proteins (Ashizawa *et al.*, 1991) and ribosomes (Puymirat *et al.*, 1995). Nongenomic and genomic

mechanisms of thyroid hormone action interface in the cell nucleus, where nuclear hormone receptors are acted upon by signal transduction pathway kinases whose activities are regulated from outside the nucleus by iodothyronines. Nongenomic actions are usually rapid in onset (seconds to minutes), do not require protein synthesis and are independent of nuclear TR. A number of these actions are at least equally responsive to T_4 and T_3, and in some cases are more responsive to T_4 (Sun *et al.,* 2000).

Actions of thyroid hormone at the plasma membrane that are nongenomic in mechanism include increased activities of the Na^+/H^+ exchanger (antiporter) (Incerpi *et al.,* 1999), Na^+ current (Sun *et al.,* 2000), inward rectifying K^+ current and Ca^{2+}-ATPase (calcium pump) (Smith *et al.,* 1989). Such actions may be relevant to regulation of intracellular pH and $[Ca^{2+}]i$ or to modulation of excitability in muscle cells. The nongenomic effect of T_3 on Na^+/H^+ exchanger has been well-studied in rat myoblasts and has been shown to enhance the ability of the cell to recover from an acute acid load by increasing the rate of export of protons. Thyroxine is more active than T_3 in stimulating membrane Ca^{2+}-ATPase activity in a variety of tissues. Acute cardiovascular responses to thyroid hormone occur in organs (Yoneda, 1998), intact animals (Davis and Davis, 2002) and man (Schmidt *et al.,* 2002).

The cell surface receptor for thyroid hormone has qualities of a family of structural membrane proteins (integrins) that connect the cell to extracellular matrix and are also linked intracellularly to the mitogen-activated protein kinase (MAPK) cascade. Genomic actions are exerted by L-3,5,3-tri-iodothyronine (T_3) while L-thyroxine (T_4), reverse T_3 (rT_3) or L-3,5-di-iodothyronine (T_2) have predominantly nongenomic activity (Davis and Davis 1996).

2.1.8. Peripheral Effects of Thyroid Hormones

2.1.8.1. Effects of Thyroid hormones on Metabolic Rate

Triiodothyronine is considered the physiologically active hormone, and T_4 is converted peripherally into T_3 by the action of the enzyme deiodinase. The bulk of the body's T_3 (about 80%) comes from this conversion. In addition to T_3, it has recently been recognized that there exist two additional active metabolites of T_3: 3,5 and 3,3' diiodothyronines, collectively call T_2. Studies have shown that T_2 may be more effective in raising resting metabolic rate when hypothyroid subjects are treated with T_3, than when normal (euthyroid) subjects are given T_3. Therefore in normal subjects, T_2 may be the principal active metabolite of T_3 (Moreno *et al.*, 2002).

Thyroid hormone has long been recognized as a major regulator of the oxidative metabolism of energy producing substrates (food or stored substrates like fat, muscle, and glycogen) by the mitochondria (Magnus-Levy, 1895). Triiodothyronine and diiodothyronine increase the flux of nutrients into the mitochondria as well as the rate at which they are oxidized, by increasing the activities of the enzymes involved in the oxidative metabolic pathway. The increased rate of oxidation is reflected by an increase in oxygen consumption by the body. Triiodothyronine and diiodothyronine appear to act by different mechanisms to produce different results. Diiodothyronine is believed to act on the mitochondria directly, increasing the rate of mitochondrial respiration, with a consequent increase in ATP production (Moreno *et al.*, 2002). Triiodothyronine on the other hand acts at the nuclear level, inducing the transcription of genes controlling energy metabolism, primarily the genes for so-called uncoupling proteins, or UCP (Lebon *et al.*, 2001). The time course of these two actions is quite different. Diiodothyronine begins to increase mitochondrial respiration and metabolic rate immediately. Triiodothyronine

on the other hand requires a day or longer to increase BMR since the synthesis of new proteins, the UCP, is required (Moreno *et al.*, 2002).

Ways by which the increased ATP promotes an increase in metabolic activity, include:

i. Increased Na^+/K^+ATPase. This is the enzyme responsible for controlling the Na^+/K^+ pump, which regulates the relative intracellular and extracellular concentrations of these ions, maintaining the normal transmembrane ion gradient. It was estimated that this effect may account for up to to 10% of the increased ATP usage (Lanni *et al.*, 2001).

ii. Increased Ca^{2+}-dependent ATPase. The intracellular concentration of calcium must be kept lower than the extracellular concentration to maintain normal cellular function. ATP is required to pump out excess calcium. It has been estimated that 10% of a cell's energy expenditure is used just to maintain Ca^{2+} homeostasis. (Moreno *et al.*, 2002)

iii. Substrate cycling. Hyperthyroidism induces a futile cycle of lipogenesis/lipolysis in fat cells. The stored triglycerides are broken down into free fatty acids and glycerol, then reformed back into triglycerides again. This is an energy dependent process that utilizes some of the excess ATP produced in the hyperthyroid state (Sestoft, 1980). Futile cycling has been estimated to use approximately 15% of the excess ATP created during hyperthyroidism (Sestoft, 1980)

iv. Increased Heart Work. This puts perhaps the greatest single demand on ATP usage, with increased heart rate and force of contraction accounting for up to 30% to 40% of ATP usage in hyperthyroidism (Freake and Oppenheimer, 1995)

Triiodothyronine has the ability to uncouple oxidation of substrates from ATP production. Triiodothyronine is believed to increase the production of so called uncoupling proteins. Uncoupling protein is a transporter family that is present in the mitochondrial inner membrane, it uncouples respiration from ATP synthesis by dissipating the transmembrane proton gradient as heat. Instead of useful ATP being produced from energy substrates, heat is generated instead. This would contribute to T_3 induced thermogenesis, with a resulting increase in basal metabolic rate (Lebon *et al*., 2001). To make up for the deficit in ATP production more substrates must be burned for fuel, resulting in fat loss. Unfortunately, along with the fat that is burned, some protein from muscle is also catabolised for energy. Muscle glycogen is also more rapidly depleted, and less efficiently stored during hyperthyroidism. This may account for some of the muscle weakness generally associated with T_3 use (Lebon *et al*., 2001).

Administration of T_3 has been shown to upregulate beta 2 adrenergic receptor in fat tissue. An enzyme called Hormone Sensitive Lipase (HSL) is the rate-controlling enzyme in lipolysis. The body produces two catecholamines, epinephrine and norepinephrine, which bind to the beta 2 receptor and activate HSL (Kraemer and Shen, 2002). The upregulation of the beta 2 receptor due to T_3 results in an increased ability of catecholamines to activate HSL, leading to increased lipolysis.

In vitro, animal, and human studies have all demonstrated that T_3 administration increases growth hormone production (Lovejoy *et al.*, 1999). Growth hormone is calorigenic, therefore, elevated growth hormone may contribute to some of the fat burning associated with T_3 administration.

2.1.8.2. Effects of Thyroid Hormones on Growth

Growth hormone does not always control growth rate. For instance, it does not clearly influence intra-uterine growth. Moreover, growth hormone does not always clearly stimulate somatomedin production, particularly during food restriction, fetal life, in hypothyroid animals or sex-linked dwarf chickens (Cabello and Wrutniak, 1989). In such situations, this phenomenon is associated with a reduced T_3 production, suggesting a significant influence of thyroid function on growth hormone action, and more generally, on body growth. Reports demonstrated that thyroid hormone is strongly involved in the regulation of body growth. In species with low maturity at birth, such as the rat, T_4 and T_3 affect postnatal growth eleven days earlier than the appearance of growth hormone influence. In contrast to growth hormone, thyroid hormone significantly influences fetal growth in sheep (Cabello and Wrutniak, 1989). Moreover, the body growth rate is clearly stimulated by T_3 in dwarf animals. In addition to its complex metabolic effects involved in the general mechanisms of body growth, thyroid hormone stimulates the production of growth factors, particularly epithelial growth factor and nerve growth factor. Moreover, it affects growth hormone and somatomedin production and also their tissue activity. All these results strongly suggest that it would be difficult to study growth hormone regulation and physiological effects without taking thyroid function into account (Cabello and Wrutniak, 1989).

Thyroid hormone (T_3) is essential for normal skeletal development. Childhood hypothyroidism results in growth arrest, delayed bone age, epiphyseal dysgenesis and short stature (Bucher *et al*., 1985; Chiesa *et al.*, 1994). Thyroid hormone replacement induces catch-up growth, though maximum predicted height may not be reached and any height deficit correlates with the duration of untreated hypothyroidism (Rivkees *et al.*, 1988). Untreated childhood

thyrotoxicosis causes accelerated growth and advanced bone age with premature closure of the growth plate and short stature (Segni and Gorman, 2001).

2.1.8.3. Effects of Thyroid Hormones on the Central Nervous System

Brain development is thyroid hormone sensitive, not only in the neonatal period but also prior to birth (Heyerdahl 1991). Development of different areas of central nervous system has been associated with the timing and duration of thyroid hormone deficiency, suggesting that there are critical periods during which various parts of the brain are sensitive to thyroid hormone supply (Rovet *et al.*, 1992). It is well established that the thyroid status of neonates and children has a significant long-term impact on their behaviour, locomotor ability, speech, hearing and cognition (Legrand 1986). Delay in restoring normal thyroid status in the neonate can lead to irreversible damage.

In intact rats, thyroxine and triiodothyronine increase brain excitability (Paola *et al*, 1955). Concomitantly with brain excitability variations, thyroxine and triiodothyronine produce alterations in brain electrolyte metabolism, the most important of which are those concerned with Na distribution (decreased extracellular Na, decreased ratio of extracellular to intracellular Na, increased Na space and increased Na intracellular concentration) (Paola *et al*, 1955).

2.1.8.4. Thyroxine and the Cardiovascular System

Influences of increased thyroid hormone on cardiovascular function were noticed more than 200 years ago. In 1785, a British physician, C. Parry, noted for the first time an association between the swelling of the thyroid area and heart failure (Pary, 1825). Parry described eight

cases, all women, with a thyroid enlargement, a rapid heartbeat, and palpitations, and four were judged to have cardiac enlargement.

Overall, changes in thyroid hormone status influence cardiac action by four different routes: (1) T_3, exerts a direct effect on cardiac myocytes by binding to nuclear T_3 receptors influencing cardiac gene expression; (2) T_3 exerts nongenomic actions; (3) T_3 may influence the sensitivity of the sympathetic system; and (4) T_3 leads to hemodynamic alterations in the periphery that result in increased cardiac filling and modification of cardiac contraction (Klein and Levey, 2000; Klein and Ojamaa, 2000).

The mRNA coding for the ryanodine channel, the calcium channel of the sarcoplasmic reticulum, is markedly up-regulated by thyroid hormones. The increased number of ryanodine channels results in T_3-induced increases of calcium release from the sarcoplasmic reticulum during systole and probably accounts, in large part, for the increased systolic contractile activity of the hyperthyroid heart. Thyroid hormones also lead to a marked increase in cardiac actin, and troponin I (Dieckman and Solaro, 1990)

Triiodothyronine markedly shortens diastolic relaxation (lusitropic activity), whereas diastole is prolonged in hypothyroid states in all mammalian species (Mintz *et al.*, 1991). Triiodotyronine markedly increases expression of the sarcoplasmic reticulum Ca^{2+}ATPase (SERCa2) gene under in vivo conditions. Release of calcium and its reuptake into the sarcoplasmic reticulum are critical determinants of systolic contractile function and diastolic relaxation (Fazio *et al.*, 2004). Several plasma-membrane ion transporters, such as Na^+/K^+-ATPase, Na^+/Ca^{2+} exchanger, and voltage-gated potassium channels are also regulated by thyroid hormones, thus coordinating the electrochemical and mechanical responses of the myocardium (Ojamaa *et al.*, 1999).

Extranuclear or nongenomic actions of thyroid hormones on the heart: In contrast to T_3 effects mediated by nuclear receptors, which take at least 0.5–2.0 h to demonstrate, T_3-induced changes in ion flux can be demonstrated within several minutes (Davis and Davis, 2002). For example, T_3 addition leads to a rapid recruitment (within 4 min) of slowly inactivating sodium channels in cardiac myocytes. Triiodothyronine also stimulates the Ca^{2+}ATPase activity as well as the calcium movement across the membrane, which are due to changes in calcium channels (Segal, 1990).

There is however a hypothesis that some T_3 effects are mediated by an increased activity of the sympathoadrenal system or an increased responsiveness and sensitivity of cardiac tissue to normal sympathomimetic stimuli (Bilezikian and Loeb, 1983).

Thyrotoxicosis may be associated with as much as a 50% decline in systemic vascular resistance and T_3 is capable of causing rapid relaxation of vascular smooth muscle cells in culture (Park *et al.,* 1997). Triiodothyronine may directly regulate vascular resistance, which, in turn, causes alterations in blood pressure and cardiac output (Danzi and Klein, 2002). In hyperthyroid animals, arterial resistance decreases and venous tone increases, leading to an augmented return of blood to the heart (Goldman *et al.*, 1984).This leads to an increase in mean circulatory filling pressure, no change in blood volume, and a decrease in venous compliance, whereas hypothyroid animals showed a decrease in mean circulatory filling pressure and blood volume but no change in venous compliance. Thyroid dysfunction alters blood pressure: hyperthyroidism has only minor effects on mean arterial blood pressure, because increases in systolic pressure, caused by increased stroke volume, are offset by decreases in diastolic pressure, due to peripheral vasodilatation (Klein and Ojamaa, 1994).

2.1.8.5. Effects of Thyroid Hormones on Muscle Functions

Slight increase in thyroid hormone usually makes the muscles react with vigor (Udaya and Tan, 2003), but when the quantity of hormone becomes excessive, the muscles become weakened because of excess protein catabolism. Conversely, lack of thyroid hormone causes the muscles to become sluggish, and they relax slowly after a contraction. One of the most characteristic signs of hyperthyroidism is a fine muscle tremor. The tremor can be observed easily by placing a sheet of paper on the extended fingers and noting the degree of vibration of the paper. This tremor is believed to be caused by increased reactivity of the neuronal synapses in the areas of the spinal cord that control muscle tone. The tremor is an important means for assessing the degree of thyroid hormone effect on the central nervous system (Udaya and Tan, 2003).

2.1.8.6. Effects of Thyroid Hormones on Reproductive System

The reproductive system has been regarded as relatively resistant to the effects of thyroid dysfunction. This view has been challenged by recent evidence, though most of the consequences are minor and reversible. In males, thyrotoxicosis may increase total concentration of serum testosterone, although free testosterone is usually normal. In some men with thyrotoxicosis, oestrogen production is increased (Ridgway et al., 1982). Basal serum gonadotrophin concentrations are usually normal in adult males with thyroid dysfunction, but increased sensitivity of gonadotrophin secretion to GnRH has been described in thyrotoxic patients, and the reverse in hypothyroidism. These changes are reversible when euthyroidism is achieved (Donnelly and White, 2000).

Abalovich et al. (1999) found that of 21 patients with hyperthyroidism, 43% had a low total sperm count, and the majority had sperm motility problems. Following treatment of thyrotoxicosis, sperm density and motility improved but sperm morphology remained unchanged. Hypothyroidism is associated with decreased libido or impotence (Wortsman, 1987).

In females, hyperthyroidism results in increased levels of steroid hormone binding globulin (SHBG) (Tulchinsky and Chopra, 1973). Plasma oestrogen levels may be twofold or threefold higher in hyperthyroid women during all phases of the menstrual cycle. Mean plasma levels of testosterone and androstenedione are elevated. Mean LH levels in both the follicular and luteal phases are significantly higher in hyperthyroid women than in normal females. Baseline FSH levels may be increased, although this is refuted by some studies (Tulchinsky and Chopra, 1973). In women with hypothyroidism, the binding activity of SHBG in plasma is decreased, so that plasma concentrations of testosterone and oestradiol are decreased, although their unbound fractions are elevated. The alterations in steroid metabolism disappear when the euthyroid state is restored (Tulchinsky and Chopra, 1973).

Amenorrhoea, oligomenorrhoea, hypomenorrhoea, and anovulation can occur in hyperhtyroidism. In women of fertile age, hypothyrodism results in changes in cycle length and amount of bleeding, that is, oligo- and amenorrhea, polymenorrhea, and menorrhagia. Severe hypothyroidism is commonly associated with diminished libido and failure of ovulation (Shomon, 2003).

2.1.9. Effects of Thyroid Hormones on Gastrointestinal Tract and Nutrient Metabolism

2.1.9.1. Effects of Thyroxine on Gastric Gut Growth and Development

Thyroid hormones stimulate the biochemical processes involved in cell growth. Thyroxine increases the mitotic activity of cells especially in the crypts of digestive system in experimental animals such as rat (Fatemeh *et al.,* 2003). Moreover, thyroxine plays an important role in the gastric development (Neil *et al.,* 2008). In developing animals, hypothyroidism results in decreased mucosal thickness and villous height, weight, and protein content of the small intestine (Blanes *et al.,* 1977). Experimental hyperthyroidism in developing animals leads to mucosal hypertrophy and epithelial hyperplasia.

2.1.9.2. Effects of Thyroxine on Intestinal Transit Time

Thyroid hormone affects gastrointestinal transit time, such that hyperthyroidism accelerated small intestine and large intestine transit time, but there was no significant difference in gastric emptying between hyperthyroid and euthyroid subject (Wiley *et al*, 1978; Wegener *et al.,* 1992). Hypothyroidism is associated with dysphagia, decrease in amplitude and velocity of peristalsis of oesoghagus, decrease in lower esophageal sphincter pressure, prolonged gastric emptying (Kahraman *et al.,* 1997), intestinal transit time (Shafer *et al.,* 1994), decrease amplitude of muscular contractions of the sigmoid colon and rectum.

2.1.9.3. Effects of thyroid hormones on gastric acid secretion

Thyroxine has been reported to increase gastric acid secretion, while thyroidectomy decreased it (Adeniyi and Olowookorun, 1989; Rafsanjani *et al.,* 2003). Thyroidectomy decreased the number of parietal cells while thyroxine administration increased it (Adeniyi and

Olowookorun, 1989). Gastric acid secretion decreased in rats whose thyroxin level had been lowered by the administration of thiouracil and this effect was removed after the administration of thyroxine (Tseng and Johnson, 1986).

2.1.9.4. Thyroid hormones and Nutrient Absorption

Thyroid hormones affect intestinal absorption of glucose, in a way that hyperthyroidism increases glucose absorption, while hypothyroidism decreases glucose absorption (Olaleye and Elegbe, 2005; Loeb, 1996). Thyroid hormone stimulates both Na^{+}-K^{+}-ATPase activity and electrogenic Na absorption in the intestine (Giannella, *et al.*, 1993).

2.1.9.5. Thyroxine and glucose metabolism

Thyroid hormones enhance the utilization of carbohydrates. They stimulate both the synthesis and use of glucose. Glucose intolerance was observed in up to 57% of patients with thyrotoxicosis (Loeb, 1996), overt diabetes mellitus ensues in 2% to 3% (Loeb, 1996). Pre-existing diabetes almost always aggravates during thyrotoxicosis (Loeb, 1996). These patients are prone to ketoacidosis and need more insulin. Thyroid hormones also promote dissipation of body fat and glycogen and/or protein (Gander, 1990). Thermogenic effects of thyroid hormones are accompanied by an increased peripheral and splanchnic utilization of glucose. The increased glucose requirement is balanced by the increase in hepatic glucose output through augmented gluconeogenesis and glycogenolysis (Loeb, 1996). Hypothyroidism may lead to hypoglycemia due to the fall in insulin requirement, however, in a mild form it seems that it does not affect the sensitivity to catecholamines, which in turn could alter insulin action (Greenspan, 1997).

2.1.9.6. Lipid metabolism

Increased thyroid hormone levels stimulate fat mobilization, leading to increased concentrations of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triglycerides are inversely correlated with thyroid hormone levels - one diagnostic indication of hypothyroidism is increased blood cholesterol concentration (Engelken and Eaton, 1981).

2.2.1. Peptic Ulcer

An ulcer is a deep defect in the esophageal, gastric, duodenal or intestinal wall penetrating the entire mucosal thickness and the muscularis mucosae (Tarnawski, 2001). Histologically, an ulcer consists of two major structures; a distinct ulcer margin formed by the adjacent non-necrotic mucosa - the epithelial component and granulation tissue at the ulcer base, which consists of fibroblasts, macrophages and proliferating endothelial cells forming microvessels (Tarnawski, 2001).

Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, *Helicobacter pylori* and non steroidal anti-inflammatory drugs (NSAID's) and local mucosal defensive factors (mucus, bicarbonate, blood flow and prostaglandins). Integrity of gastro duodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors (Hoogerwerf and Pasricha, 2001).

2.2.1.1. Classification

Based on location include;

i. Oesophageal ulcer
ii. Gastric ulcer
iii. Duodenal ulcer (www.cybermedicine2000.com)

2.2.1.2. Signs and Symptoms

i. Abdominal pain, classically epigastric with severity relating to mealtimes, after about 3 hours of taking a meal (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it (Foster, 2003).

ii. bloating and abdominal fullness

iii. waterbrash (rush of saliva after an episode of regurgitation to dilute the acid in esophagus)

iv. nausea, and copious vomiting (Foster, 2003).

v. loss of appetite and weight loss (Foster, 2003)

vi. haemetemesis (vomiting of blood); this can occur due to bleeding directly from a gastric ulcer, or from damage to the esophagus from severe/continuing vomiting.

vii. melena (tarry, foul-smelling feces due to oxidised iron from hemoglobin).

viii. rarely, an ulcer can lead to a gastric or duodenal perforation, which leads to acute peritonitis. This is extremely painful and requires immediate surgery (Foster, 2003).

2.2.1.3. Complications of Ulcer

i. Gastrointestinal bleeding is the most common complication (Cullen *et al.*, 1997). It occurs when the ulcer erodes one of the blood vessels, such as the gastroduodenal artery.

ii. Perforation (a hole in the wall) often leads to catastrophic consequences. Erosion of the gastro-intestinal wall by the ulcer leads to spillage of stomach or intestinal content into the abdominal cavity. Perforation at the anterior surface of the stomach leads to acute peritonitis. The first sign is often sudden intense abdominal pain. Posterior wall perforation leads to pancreatitis; pain in this situation often radiates to the back (Wang *et al.*, 2010).

iii. Penetration is when the ulcer continues into adjacent organs such as the liver and pancreas (Peptic Ulcer, 2007).

iv. Scarring and swelling due to ulcers causes narrowing in the duodenum and gastric outlet obstruction. Patient often presents with severe vomiting (Irabor, 2005).

v. Cancer is included in the differential diagnosis (elucidated by biopsy). Helicobacter pylori as the etiological factor makes it 3 to 6 times more likely to develop stomach cancer from the ulcer (Peptic Ulcer, 2007).

2.2.1.4. Risk Factors of Peptic Ulcer:

i. Heredity

ii. Older age (Lanza *et al.*, 2009)

iii. Chronic pain, from any cause such as arthritis, fibromyalgia, repetitive stress injuries (like carpal tunnel syndrome), or persistent back pain, causing long-term use of aspirin or NSAIDs (Lanza et al, 2009).

iv. Alcohol abuse

v. Diabetes may increase risk of having *H. pylori* (Kuster *et al.*, 2006).

vi. Lifestyle factors, including chronic stress, coffee drinking (even decaf), and smoking, increases the susceptibility to damage from NSAIDs or *H. pylori* if one is a carrier of the organism.

2.2.2. Epidemiology of Peptic Ulcer

Helicobacter pylori infection was originally identified as the main cause of peptic ulcer; however, as the prevalence of *H. pylori* infection has declined in Western countries, gastric ulcer has become more commonly associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid (ASA) (Yuan *et al.*, 2006; Ramakrishnan and Salinas, 2007).

In this literature, studies reporting data earlier than 1980 were excluded to focus on recent trends in peptic ulcer disease epidemiology.

Table 2.1. Incidence of peptic ulcer disease extrapolated to the general population, or as a proportion of patients registered in primary care

Incidence of Peptic Ulcer

Country	Annual incidence of PUD, % (years)
Belgium	0.19 (2002/2003)† (Bartholomeeusen *et al.*, 2007).
UK	0.10 (1995–1999)† (Garcia Rodruigez and Hernandez-Diaz , 2004)
Denmark	0.15 (2002)‡ (Lassen *et al*, 2006)
Spain	0.14 (2000)† (Perez-Aisa *et al.*, 2005)
US	0.17 (1999)‡ (Lewis *et al*, 2002)
Finland	0.07 (1999)‡ (Paimela *et al*., 2002)
The Netherlands	0.03 (2003)‡ (Post *et al.*, 2006).

PUD, peptic ulcer disease.

† Reported as a proportion of patients registered in primary care.

‡ Reported as a proportion of the general population.

Table 2.2. Population-based prevalence of peptic ulcer disease

Country	Prevalence of PUD (%) (Year study conducted)
Sweden	4.1 (1998–2001) (Aro *et al.*, 2006*)*
UK	0.12 (1998) (Kang *et al.*, 2002*)*
US	1.1 (1994/1995) (Levin *et al.*, 1997*)*
US	1.5 (1995) (Sonnenberg and Munnangi, 1997)
Israel	4.7 (1998) (Peterburg and Rennert, 2001)
Denmark	2.6 (1977–1991) (Hein *et al.*, 1997*)*
UK	0.10 (2000–2002) (Kang *et al.*, 2006*)*

Peptic ulcer disease remains a relatively common condition worldwide. Each year in the United States, about half a million people develop a peptic ulcer (Ramakrishnan and Salinas, 2007). In terms of future work, an estimate of the global population prevalence of symptomatic as well as asymptomatic PUD, including the associated risk symptoms and potential risk factors, would yield important information on burden of the disease and aid its management. Such data, although scarce, are available from Europe, whereas similar data from Asia and Africa are still lacking.

2.2.2.1. Prevalence of peptic ulcer in Nigeria

In Nigeria, hospital cases of peptic ulcer are reported (Ameh and Nmadi, 1998; Onyekwere et al, 2008). However, data is lacking about incidence and prevailence of ulcer in the country. Report from two hundred and forty-three Nigerian patients referred for endoscopy at the

Jos University Teaching Hospital, Nigeria revealed that duodenal and gastric ulcers were found in 42(17.3%) and 12(4.9%) patients respectively (Malu *et al.*, 1994).

2.2.3. Causes of Peptic Ulcer

2.2.3.1. Helicobacter pylori and Ulcer

Helicobacter pylori, (previously named *Campylobacter pyloridis*) a bacteria found in the stomach was identified in 1982 by Barry Marshall and Robin Warren. They found that it was present in patients with chronic gastritis and gastric ulcer, conditions that were not previously believed to have a microbial cause (Marshall and Warren 1984). It was also linked to the development of duodenal ulcers and stomach cancer. However, over 80 percent of individuals infected with the bacterium are asymptomatic and it has been postulated that it may play an important role in the natural stomach ecology (Blaser, 2006).

More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and incidence is decreasing in Western countries. Individuals infected with *H. pylori* have a 10 to 20% lifetime risk of developing peptic ulcers and a 1 to 2% risk of acquiring stomach cancer (Kuster *et al.*, 2006).

Helicobacter pylori consist of a large diversity of strains. Study of the *H. pylori* genome is centered on attempts to understand pathogenesis, the ability of this organism to cause disease. Approximately 29% of the loci are in the "pathogenesis" category of the genome database. Two of sequenced strains have an approximately 40 kb-long Cag pathogenicity island (a common gene sequence believed responsible for pathogenesis) that contains over 40 genes. This pathogenicity island is usually absent from *H. pylori* strains isolated from humans who are carriers of *H. pylori*, but remain asymptomatic (Baldwin *et al.*, 2007). The *cagA* gene codes for

one of the major *H. pylori* virulence proteins. Bacterial strains that have the *cagA* gene are associated with an ability to cause ulcers (Broutet et al, 2001). The *cagA* gene codes for a relatively long (1186 amino acid) protein. The *cag* pathogenicity island (PAI) has about 30 genes, part of which code for a complex type IV secretion system.

To colonize the stomach, *H. pylori* must survive the acidic pH of the lumen and use its flagella to burrow into the mucus to reach its niche, close to the stomach's epithelial cell layer (Amieva and El-Omar, 2008). To avoid being carried into the lumen, *H. pylori* senses the pH gradient within the mucus layer by chemotaxis and swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface (Schreiber *et al.*, 2004). *Helicobacter pylori* are also found on the inner surface of the stomach epithelial cells and occasionally inside epithelial cells (Petersen and Krogfelt, 2003).

Helicobacter pylori produce large amounts of the enzyme urease. Urease breaks down urea (which is normally secreted into the stomach) to carbondioxide and ammonia. The ammonia is converted to ammonium by taking a proton (H^+) from water, which leaves only a hydroxyl ion. Hydroxyl ions then react with carbon dioxide, producing bicarbonate, which neutralizes gastric acid. The ammonia produced is toxic to the epithelial cells, and, along with the other products of *H. pylori*—including proteases, vacuolating cytotoxin A (VacA), and certain phospholipases—damages those cells (Smoot, 1997).

Duodenal and stomach ulcers result when the consequences of inflammation allow the acid and pepsin in the stomach lumen to overwhelm the mechanisms that protect the stomach and duodenal mucosa from these caustic substances. The type of ulcer that develops depends on the location of chronic gastritis, which occurs at the site of *H. pylori* colonization (Dixon, 2000). The acidity within the stomach lumen affects the colonization pattern of *H. pylori*, and therefore

ultimately determines whether a duodenal or gastric ulcer will form. In people producing large amounts of acid, *H. pylori* colonizes the antrum of the stomach to avoid the acid-secreting parietal cells located in the corpus (main body) of the stomach (Kuster *et al.*, 2006). The inflammatory response to the bacteria induces G cells in the antrum to secrete the hormone gastrin, which travels through the bloodstream to the corpus (Blaser and Atherton 2004). Gastrin stimulates the parietal cells in the corpus to secrete even more acid into the stomach lumen. Chronically increased gastrin levels eventually cause the number of parietal cells to also increase, further escalating the amount of acid secreted (Schubert and Peura, 2008). The increased acid load damages the duodenum, and ulceration may eventually result. In contrast, gastric ulcers are often associated with normal or reduced gastric acid production, suggesting the mechanisms that protect the gastric mucosa are defective (Schubert and Peura, 2008). In these patients, *H. pylori* can also colonize the corpus of the stomach, where the acid-secreting parietal cells are located. However chronic inflammation induced by the bacteria cause further reduction of acid production and, eventually, atrophy of the stomach lining, which may lead to gastric ulcer and increases the risk for stomach cancer (Suerbaum and Michetti, 2002).

About 50-70% of *H. pylori* strains in Western countries carry the *cag* pathogenicity island (*cag* PAI) (Peek and Crabtree, 2006). Western patients infected with strains carrying the *cag* PAI have a stronger inflammatory response in the stomach and are at a greater risk of developing peptic ulcers or stomach cancer than those infected with strains lacking the island (Kuster, 2006). Following attachment of *H. pylori* to stomach epithelial cells, the type IV secretion system expressed by the *cag* PAI "injects" the inflammation-inducing agent, peptidoglycan, from their own cell wall into the epithelial cells. The injected peptidoglycan is

recognized by the cytoplasmic pattern recognition receptor (immune sensor) Nod1, which then stimulates expression of cytokines that promote inflammation (Viala *et al*., 2004).

The type IV secretion apparatus also injects the *cag* PAI-encoded protein CagA into the stomach's epithelial cells, where it disrupts the cytoskeleton, adherence to adjacent cells (possibly contributing to apical junction barrier disruption), intracellular signaling, cell polarity, and other cellular activities (Backert and Selbach, 2008). Pathogenic strains of *H. pylori* have been shown to activate the epidermal growth factor receptor (EGFR), a membrane protein with a tyrosine kinase domain. Activation of the EGFR by *H. pylori* is associated with altered signal transduction and gene expression in host epithelial cells that may contribute to pathogenesis. *Helicobacter pylori* also damage the mucosal defense system by reducing the thickness of the mucus gel layer and diminishing mucosal blood flow.

2.2.3.2. Non Steroidal Antiinflammatory Drugs (NSAIDs) Induced Injury

The upper gastrointestinal tract damage caused by NSAIDs has been referred to as an `epidemic' (Gabriel and Bombardier, 1990). This is in part attributable to the widespread use of these drugs, particularly by patients with osteo-arthritis and rheumatoid arthritis. In general, the properties of NSAIDs that contribute to ulcerogenesis can be divided into two categories: (1) topical irritancy, and (2) the suppression of prostaglandin synthase activity. In addition, the presence in the stomach and duodenum of acid and, in some cases, *Helicobacter pylori*, may contribute to the ability of NSAIDs to damage the mucosa.

2.2.3.2a. Topical irritation of mucosa

i. Studies in the 1960s by Davenport suggested that aspirin could directly damage the gastric epithelium (Davenport, 1969). The breaking of the `barrier' permitt the back-

diffusion of acid into the mucosa, which eventually leads to the rupture of mucosal blood vessels. These topical irritant properties were subsequently found to be predominantly associated with those NSAIDs with a carboxylic acid residue (Davenport, 1969).

ii. The unionized forms of these NSAIDs can enter epithelial cells in the stomach and duodenum. Once in the neutral intra-cellular environment, the drugs are converted to an ionized state and cannot diffuse out. This has been referred to as `ion trapping' (Fromm, 1987). As the drug accumulates within the epithelial cell, the osmotic movement of water into the cell results in swelling of the epithelial cell, eventually to the point of lysis (Fromm, 1987).

iii. Various NSAIDs have been shown to uncouple mitochondrial respiration (Mahmud *et al.,* 1996), leading to a depletion of ATP and therefore a reduced ability to regulate normal cellular functions, such as the maintenance of intracellular pH. Thereby, damaging the gastroduodenal epithelium

iv. NSAIDs are able to decrease the hydrophobicity of the mucus gel layer in the stomach. Lichtenberger, proposed that this layer is a primary barrier to acid-induced damage in the stomach (Lichtenberger,1995). NSAIDs were shown to associate with the surface-active phospholipids within the mucus gel layer, thereby reducing its hydrophobic properties (Lichtenberger *et al.,* 1995). These investigators further demonstrated that the mucus gel layer in the stomach of rats and mice given NSAIDs was converted from a non-wettable to a wettable state.

Attempts have been made to produce NSAIDs with reduced topical irritant effects. These include formulations in slow-release or enteric coated tablets, as well as the preparation of the drug as a pro-drug that requires hepatic metabolism in order to be active (e.g. sulindac).

However, the incidence of gastroduodenal ulceration with pro-drugs is comparable to that seen with standard NSAIDs (Hawthorne *et al*., 1991). Topical irritant properties of NSAIDs are not paramount in terms of their ability to induce frank ulceration in the stomach. However, one cannot completely exclude this possibility.

2.2.3.2b. Suppression of Prostaglandin Synthesis

Endogenous prostaglandins are involved in the regulation of mucus, bicarbonate secretion, mucosal blood flow and epithelial cell proliferation by the gastric and duodenal epithelium. Non steroidal anti-inflammatory drugs work by interfering with the cyclooxygenase pathway. Different mechanisms stimulate the two different types of cyclooxygenase (cyclooxygenase 1 [COX-1] and cyclooxygenase 2 [COX-2]). Cyclooxygenase 1 is stimulated continuously by normal body physiology (Masferrer *et al.*, 1996). The concentration of COX-1 enzyme in the body remains stable. It is present in most tissues and converts arachidonic acid to prostaglandins. These prostaglandins in turn stimulate body functions, such as stomach mucous production (protective) and increase gastric blood flow. In contrast, the COX-2 enzyme is induced. It is not normally present in cells but its expression can be increased dramatically by the action of macrophages, ischemia and topical irritation (Davies *et al.*, 1997; Maricic *et al.*, 1999). Cyclooxygenase 2 plays a very important role in inflammation. Cyclooxygenase 2 is involved in producing prostaglandins for an inflammatory response. Non steroidal anti-inflammatory drugs temporarily blocks the attachment site for arachidonic acid on the cyclooxygenase enzyme, thereby preventing it from converting arachidonic acid to prostaglandin (Wallace *et al*., 2000)

The component of mucosal defence that appears to be most profoundly altered by NSAIDs is the gastric microcirculatory response to injury. When the mucosa is exposed to an irritant, or when superficial epithelial injury occurs, mucosal blood flow substantially increases. This is probably a response aimed at removing any toxins or bacterial products that enter the lamina propria, neutralizing back-diffusing acid and contributing to the formation of a microenvironment at the surface of the mucosa that is conducive to repair (Wallace and Granger, 1996).

Prostaglandins of the E and I series are potent vasodilators that are continuously produced by the vascular endothelium, so the inhibition of their synthesis by a NSAID leads to a reduction in vascular tone (Gana *et al.*, 1987). Non steroidal anti-inflammatory drugs administration to rats also resulted in a rapid and significant increase in the number of neutrophils adhering to the vascular endothelium in both gastric and mesenteric venules (Asako *et al.*, 1992). The adherence of neutrophils to the vascular endothelium is accompanied by an activation of these cells, leading to the release of proteases (e.g. elastase and collagenase) and oxygen-derived free radicals (e.g. superoxide anions). These substances may mediate much of the endothelial and epithelial injury caused by NSAIDs.

2.2.3.3. Stress and Ulcer

Psychological stress is not only empirically associated with ulcers, but is a very plausible risk factor for ulcer disease. Stress can be caused by a number of factors, including; emotionally disturbing circumstances or events, surgery, physical injury, such as a severe burn, severe illness or trauma. Stress ulcers occur in the stomach and the duodenum. Gastric acid output is correlated with psychological distress in patients with and without ulcers (Feldman *et al.*, 1992), and

increased enormously during intense military training (Oektedalen *et al.*, 1984). Compared with healthy people, patients with duodenal ulcers are particularly likely to respond to laboratory stressors by secreting more acid (Bresnick *et al.*, 1993). Under stress, the amount of acid reaching the duodenum may increase further because gastric motility has changed or meals have been missed. People affected by stress may also smoke more, sleep less, and take more non-steroidal anti-inflammatory drugs, thereby increasing their susceptibility to ulcer by mechanisms that are not related to acidity. Stress could facilitate the evolution of *H. pylori* infection into ulcer by producing gastric hyperchlorhydria (Lee *et al.*, 1995). Stress could reduce mucosal defences to *H. pylori* invasion through behavioural mediators such as cigarette smoking (Martins et al, 1989). Stress could increase the chances of ulceration in duodenal mucosa that have already been weakened by the effects of *H. pylori* infection simply by increasing the acid load which flows past. Stress induced acid secretion could promote *H. pylori* colonisation of the duodenal bulb by neutralising the inhibitory effect of bile (Hans *et al.*, 1996)

2.2.3.4. Smoking and Gastric Ulcer Formation

Cigarette smoking is associated with peptic ulceration in humans. Experimental findings suggest that cigarette smoking increases xanthine oxidase activity, leukotrienes, and nitric oxide production and also neutrophil infiltration in the gastric mucosa. It also reduces blood flow, prostaglandin production, epithelial cell proliferation, and formation of blood vessels in the tissue. These actions are important for ulcer formation and healing. The evidence thus far available strengthens the hypothesis that cigarette smoke is indeed harmful to gastric mucosa (Ma *et al.*, 1998). Smoking potentiates aggressive factors (gastric acid and pepsin secretion, reflux of bile salts, *Helicobacter pylori infection*) and attenuates defensive mechanism of gastric

and duodenal mucosa (bicarbonates, epidermal growth factor and gastric mucus secretion, mucosal blood flow). In this way smoking could disturb the balance between aggressive and defensive factors and predispose to peptic ulcer (Budzynski and Swiatkowski, 1996).

2.2.4. Methods of Assessment of Experimental Erosions and Ulcers

2.2.4.1. Pylorus ligated (Shay) rats:

This is perhaps the oldest animal model of gastric ulcers developed by Shay *et al.*, (1945). Albino rats weighing 150-200 g were fasted for 24-36 hours prior to pyloric ligation, care being taken to avoid coprophagy. Under light ether anaesthesia the abdomen is opened by a small midline incision below the xiphoid process, pyloric portion of the stomach is slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach is replaced carefully and the abdominal wall closed by interrupted sutures. Drugs are administered subcutaneously immediately after pyloric ligation. The animals are deprived of both food and water during the postoperative period and are sacrificed at the end of 19 hours after operation. Stomachs are dissected out, contents are drained into tubes and subjected to analysis for pH and free and total acidity. The stomachs are then cut open along the greater curvature and the inner surface is examined for ulceration. The ulcer index is calculated as below:

Ulcer Index = 10 / X

where X = Total mucosal area / Total ulcerated area.

Usually circular lesions, linear lesions and petechiae are seen. The method has great predictive value for anti-ulcer agents in the human disease though, the ulcers in this model are localised in the rumenal area of the stomach whereas in the human disease the glandular stomach and duodenal region are most commonly involved.

2.2.4.2.Stress Ulcers

Gastrointestinal erosion is one of the consistent findings in man and in experimental animals subjected to different types of stress. The major advantages of these preparations over pylorus ligation are: they do not require anaesthesia or surgery, they bring central nervous system into play and the lesions produced by these methods are located in the glandular region of the stomach whereas in the pylorus ligated rats, the lesions occur mostly in the rumen of the rat stomach.

i. **Restraint ulcers:** The method described by Brodie and Hanson (1960). Albino rats of either sex weighing 150-200 g are housed in separate cages and divided into groups. The animals are deprived of food for 36 hours before experimentation. Each rat is then placed in a piece of galvanised steel window screen of appropriate size. The screen is moulded around the animal and held in a place with wire staples. The limbs are put together in pair and tightened with adhesive tape so that the animal cannot move. The drugs under investigation are administered 30 min before subjecting the animal to restraint. At the end of 24 hours period the animals are removed from the screen and killed using overdose of ether. The stomach is removed from the body, opened along the greater curvature, cleaned and spread on card board with the mucous surface upwards avoiding corrugation. Tracing paper is placed over the stomach and the outline of stomach and the areas of erosions of ulceration are traced on it. This method has been successfully used for studying the healing of ulcers as well (Parmar and Hennings, 1964). Brodie (1971) concluded that the technique was a useful one but that several disadvantages were still present, including the fact that the

lesions did not penetrate the muscularis mucosa and as such were not ulcers in true sense and the fact that the technique appeared to be somewhat species- specific.

ii. **Water immersion-induced restraint ulcer:** In this method male Wistar rats, fasted for 24 hours are immobilised in a stress cage and then immersed to the level of xiphoid process in a water bath (23°C) for 16 hours. Test drugs are administered 30 min prior to stress. Each animal is then sacrificed by a blow on the head, stomach is removed, filled with 1% formalin for 10 min. The ulcer index can be estimated by measuring the total length of the lesions (Li *et al.*, 1998).

iii. **Cold and restraint ulcers:** In this method Wistar rats are deprived of food for 12 hours, they are then immobilised in a stress cage and forced to remain in a cold room (4-6°C) for 3 hours (Vincent *et al.*, 1977). The animals are sacrificed by a blow on the head and the ulcer index is calculated as described for restraint ulcers (Tariq *et al.*, 1987). The test drugs are administered 30 min before immobilising the animals.

iv. **Gastric erosion following short-term stress and concurrent administration of nonsteroidal anti-inflammatory drugs (NSAIDs):** Wistar rats are deprived of food for 24-36 hours before the experiments. The substances (in 1% carboxymethylcellulose) to be investigated are administered via gastric intubation at the same time as the intraperitoneal injection of a NSAlD e.g indomethacin (0.2-4 mg/kg), diclofenac (1.25-12.0 mg/kg) or aspirin (3.12-25 mg/kg) (Parmar *et al.*, 1984). The rats are placed in a stress cage and immersed to the level of xiphoid

process in a water bath (23°C) for 7 hours. The animals are then sacrificed and evaluated for the ulcer index (Parmar *et al.*, 1984).

v. **Swimming stress ulcers:** Male Wistar rats fasted for 24-36 hours are forced to swim inside the vertical cylinders (height 30 cm, diameter 15 cm) containing water up to 15 cm height, maintained at 23°C. Three hours after the stress, they are removed from the cylinders and sacrificed by a blow on the head. The ulcer index is determined as described by Weischer and Thiemer, (1988). Test drugs are administered 30 min prior to stress.

vi. **Activity stress ulcers in rats:** If young adult rats are individually housed in running wheel activity cage, allowing continuous access to the wheel, and fed only one hour each day, some of these animals will die within 4-16 days. An interesting feature of this phenomenon is that rats, demonstrating high activity levels which die, reveal extensive lesions in the glandular stomach. Since these glandular lesions resembled the "stress ulcer" reported by other workers and since the activity was shown to be instrumental in their development, these lesions have been designated as "activity stress ulcers". The method has been described in details by Pare (1975) and is of limited value in the evaluation of anti-ulcer activity of new drugs as it is time consuming and needs continuous supervision of the animals in the activity cages.

2.2.4.3. Histamine induced gastric ulcers in guinea pigs

In guinea pigs, histamine produces gastric ulceration in 100% animals along with increased volume of gastric secretion and marked enhancement of free and total acidity. The

percentage of area of ulceration and its intensity are reproducible and the model provides another species for studying the anti-ulcer and anti-secretory activities of novel compounds. Male guinea pigs weighing 300-400 g are fasted for 36 hours (water allowed). Gastric ulceration is induced by injecting 1 ml of histamine acid phosphate (50mg base) i-p. Promethazine hydrochloride 5mg is injected i.p. 15 min before and 15 min after histamine to protect the animals against histamine toxicity. The drugs under investigation are given p.o,. or s.c. 30-45 min before histamine injection. The animals are sacrificed four hours after histamine administration and the stomach is dissected out. The gastric contents are subjected to analysis and the stomach is cut open and the degree of ulceration is graded (Barret *et al.,* 1953).

2.2.4.4. Gastric mucosal damage by NSAIDs in rats

Gastric ulceration in rats is induced by drugs and the ability of several agents to either protect against or aggravate this ulceration is observed. The compounds under investigation are administered 30 min to 1 hour before the noxious challenge. The animals are sacrificed after a prescribed period which may vary with different agents and the stomachs are examined for the presence of mucosal lesions. The incidence and grading of the severity of lesions are done according to different methods (Parmar *et al.,* 1984, Parmar *et al.,* 1985, Parmar *et al.,* 1987)

i. **Aspirin:** Aspirin is suspended in 1% carboxymethylcellulose in water (20 mg/ml) and administered orally (gavage) in a dose of 500 mg/kg in 36 hours fasted rats. Four hours later the animals are sacrificed. The stomachs are removed and opened along the greater curvature to determine the ulcer index. Ulcer index is measured by the method of Ganguly and Bhatnagar, (1973). The administration of aspirin results in the

production of gastric mucosal damage mainly in the glandular segment of the stomach in 100% of the animals. The majority of the gastric lesions are gastric erosions i.e. superficial mucosal lesions not penetrating the muscularis mucosa.

ii. **Phenylbutazone:** This is given in a similar fashion as aspirin in a dose of 100 mg/kg, p.o (suspension) or i.p.(solution). Two doses are given at an interval of 15 hours. Six hours after the second dose, the animals are sacrificed and assessed for the gastric mucosal damage. The drugs for studying gastroprotective effects are administered 30 min before each dose of phenylbutazone (Sanyal *et al.*, 1964).

iii. **lndomethacin and Ibuprofen:** These are also administered in a similar fashion. lndomethacin is given in a dose of 10 mg/kg, p.o. while ibuprofen is given in the doses of 200 mg/kg, p.o. at 15 hours intervals. The animals are sacrificed 6 hours after indomethacin administration and 6 hours after the second dose of ibuprofen. The drugs for studying gastro-protective effects are given 30 min before each dose of the ulcerogens (Parmar *et al.*, 1984).

2.2.4.5. Reserpine Induced solitary chronic gastric ulcers

Animals are deprived of solid food for 24 hours with water *ad libitum* and housed in cages with wide mesh wire bottoms to prevent coprophagy. Reserpine 5mg/kg/day is administered for 5 days and they are sacrificed after two weeks (Salim, 1992). All the rats develop solitary chronic gastric ulceration. These ulcers are oval or round situated at or immediately adjacent to the lesser curvature in the pre-antral region. They are sharply

demarcated, punched out, of vertical edges, a black base depressed below the mucosal surface, and of diameters ranging from 2-3 mm (Salim, 1992).

2.2.4.6. Serotonin-induced gastric mucosal lesions

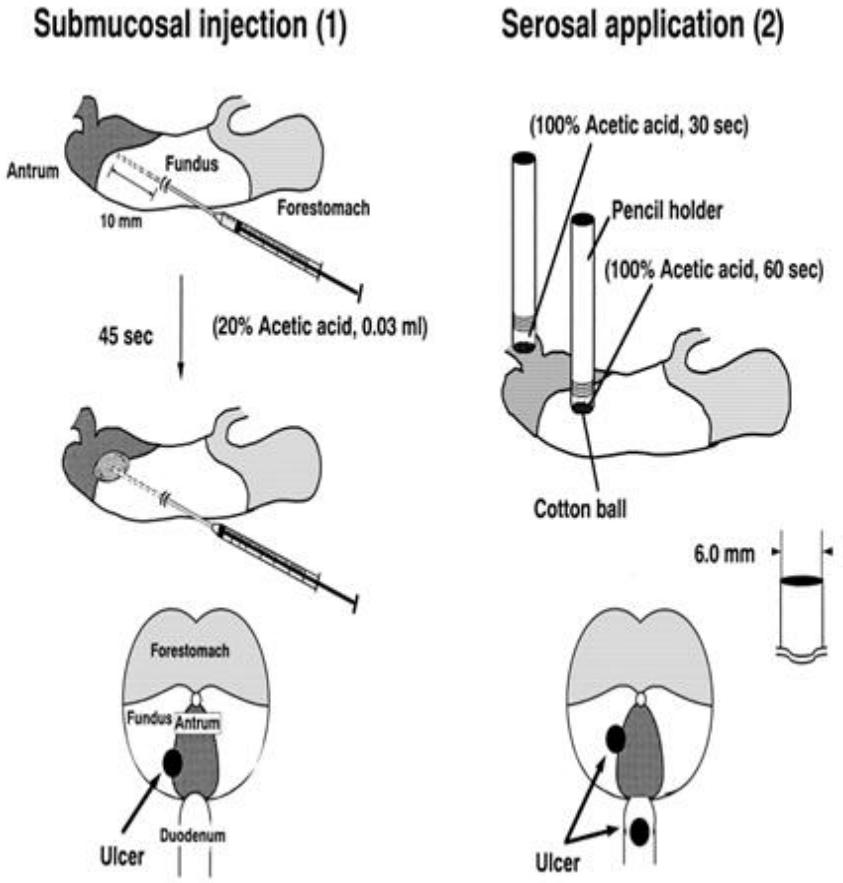
Described by Wilhelmi (1957). Male rats of Wistar strain are used for the experiments. The animals are fasted for 24 hours prior to the experiments, water being provided *ad libitum.* Serotonin creatinine sulphate is dissolved in saline and injected to rats. s.c. A dose of 20 mg/kg dose of serotonin is found to induce a moderate but evident gastric lesion. The lesions are located mainly at the side of the greater curvature of the corpus.

2.2.4.7. Acetic Acid Induced Ulcer

Since the development of acetic acid ulcer in 1969, the acetic acid ulcer model has become well established in the scientific community (Tagaki *et al*., 1969). The reasons underlying the model's frequent use as the chronic ulcer model of choice appear to be as follows. 1) The ulcer induction procedure is quite simple, readily resulting in ulcers of consistent size and severity at an incidence of 100%. 2) The ulcer models highly resemble human ulcers in terms of both pathological features and healing mechanisms. Indeed, spontaneous relapse of healed ulcers is frequently observed, just as in peptic ulcer patients. 3) The ulcers respond well to various anti-ulcer drugs, such as acid pump inhibitors, sucralfate, and several growth factors (Okabe and Amagase, 2005). Several models of acetic acid induced ulcer has been developed as follows:

i. **Type 1 Ulcer Model.** The procedure begins with laparotomy of ether anesthetized rats via midline epigastric incision. After the stomach is exposed, 20% acetic acid (0.02ml) is injected into the submucosal layer of the glandular portion of the anterior

wall (Fig. 2.4) with a microsyringe (0.05ml). At the time of injection, a thumb is placed tightly on the inserted needle so as to avoid solution leakage. After injection, the needle is withdrawn, but the thumb is maintained at the injection site for 45 s to prevent acetic acid leakage upon removal of the needle. Accuracy of the injection is easily confirmed by observation of wheal-like swelling at the injection site. Five days after acid injection, round/oval, deep ulcers (approximately 50 - 60mm^2) are observed in the antrum or in the area near the junction of the antrum and corpus corresponding to the injected area (Tagaki *et al*., 1969). The 5th day after acid injection is designated as the first day of ulceration. Notably, acetic acid ulcers produced with this model persist for more than 250 days without intervention. Occasionally, animals subjected to the injection of the acetic acid solution died due to gastric perforation on the second or third day after operation. All rats that lived for greater than 5 d after operation survived for the remainder of the experiment (Tagaki *et al.*, 1969). Following this procedure, the external surface of the ulcerated region strongly adhere to the liver. Such adhesion appears to represent the only shortcoming of this acetic acid ulcer model, as, in humans, the ulcer base seldom adheres to surrounding organs.



Source: Okabe and Amagase, 2005

Figure 2.4: Schematic Drawing of the Experimental Procedure for Producing Acetic Acid Ulcers in Small Animals. (1) Submucosal injection of acetic acid solution into the rat stomach (Type 1 ulcer). (2) Local application of acetic acid solution to the serosa of either the stomach or duodenum (Type 2 ulcer).

ii. **Type 2 Ulcer Model:** Under ether anaesthesia, laparatomy is performed and the stomach of the animals is exposed. Acetic acid (0.2 ml of 100%) is focally applied to the serosal surface for 30 to 60 s (Fig. 2.4). This method can be used to induce ulcer in both the duodenum and the stomach. Type 2 ulcer model has the similar shortcoming as the Type 1 ulcer model, that is, the ulcer base adheres to the surrounding organs, notably the liver (Okabe *et al.*, 1971).

iii. **Type 3 Ulcer Model (Kissing Ulcer):** The adhesion problem in Type 1 and Type 2 ulcer models necessitated exploration of alternative methods. In type 3 ulcer model, round forceps (ID 9 mm) were developed to clamp the fundic area. Acetic acid solution was injected into the clamped portion through the distal antrum. Two deep, round ulcers, one on the anterior wall and the other on the posterior wall, developed in the area that had been exposed to the acetic acid solution. The bases of such ulcers do not adhere to the liver or any other surrounding organ. This ulcer model is therefore more similar to human ulcers than Type 1 or Type 2 ulcer models. Nonetheless, in contrast to Type 1 and Type 2 ulcer models, the Type 3 ulcer model completely healed within 6 to 8 weeks after acid application and failed to exhibit relapse. The reason that Type 3 ulcers completely heal remains unclear, it was postulate that the lack of adhesion at the ulcer base results in permanent healing (Tsukumi and Okabe, 1996).

iv. **Type 4 Ulcer Model:** In order to produce a single ulcer via intraluminal application of acetic acid, the procedure for Type 3 ulcer model was slightly modified as follows. First, the anterior and posterior walls of the gastric corpus were clamped together with forceps that were used for the Type 3 ulcer model. A mixture of 60% acetic acid (0.1 ml) and 0.2 ml of air was then injected into the clamped lumen with an injection needle through the distal antrum, approximately 3 mm proximal to the pylorus (Amagase and Okabe, 2002). The clamped stomach was horizontally positioned so that the injected air rose to the upper half and the acetic acid solution gravitated to the bottom half. Given such positioning, the acetic acid solution only contacted the lower half of the clamped mucosa, which resulted in development of a single ulcer in the

posterior mucosa. After 45 seconds, the mixture of acetic acid solution and air was removed from the stomach and the abdomen was closed. A clearly defined deep, round ulcer consistently developed in the corpus of only the posterior wall 3 days after acid application; the anterior mucosa remained essentially intact in each animal.

2.2.5. Gastric Ulcer Healing

Ulcer healing is a complex process, which involves cell migration, proliferation, reepithelialization, angiogenesis, and matrix deposition, all ultimately leading to scar formation (Tarnawski, 2000; Vanwijck, 2001). The classic model of ulcer (wound) healing is divided into three or four sequential, yet overlapping phases (Stadelmann *et al.*, 1998):

(1) Haemostasis (not considered a phase by some authors),

(2) lag or inflammatory,

(3) proliferative and

(4) Maturation and remodeling (Quinn, 1998).

2.2.5.1. Hemostasis

When the stomach is first ulcerated, blood comes in contact with collagen, triggering blood platelets to begin secreting inflammatory factors (Rosenberg and de la Torre, 2006). Platelets also express glycoproteins on their cell membranes that allow them to stick to one another and to aggregate, forming a mass (Midwood *et al.*, 2004).

Fibrin and fibronectin cross-link together and form a plug that traps proteins and particles and prevents further blood loss (Sandeman *et al.*, 2000). This fibrin-fibronectin plug is also the main structural support for ulcer until collagen is deposited (Midwood *et al.*, 2004). Migratory

cells use this plug as a matrix to crawl across, and platelets adhere to it and secrete factors (Midwood *et al*., 2004). The clot is eventually lysed and replaced with granulation tissue and then later with collagen.

The platelets, which are present in the highest numbers shortly after wound, release a number of substances into the blood, including extracellular matrix (ECM) proteins, cytokines and growth factors (Rosenberg and de la Torre, 2006). Growth factors stimulate cells to speed their rate of division. Platelets also release other proinflammatory factors like serotonin, bradykinin, prostaglandins, prostacyclins, thromboxane, and histamine, (Stadelmann *et al.*, 1998) which serve a number of purposes, including to increase cell proliferation and migration to the area and to cause blood vessels to become dilated and porous.

2.2.5.2. The lag or Inflammatory Phase:

Within an hour after ulcer (wound), polymorphonuclear neutrophils (PMNs) arrive at the ulcer site and become the predominant cells for the first two days after the injury occurs. They are attracted to the site by fibronectin, growth factors, and substances such as kinins. Neutrophils phagocytise debris and bacteria and also kill bacteria by releasing free radicals in what is called a 'respiratory burst' (Greenhalgh, 1998). They also cleanse the ulcer by secreting proteases that break down damaged tissue. Neutrophils usually undergo apoptosis once they have completed their tasks and are engulfed and degraded by macrophages (Martin and Leibovich, 2005). Other leukocytes to enter the area include helper T cells.

Macrophages replace PMNs as the predominant cells in ulcer bed. They are attracted to the wound site by growth factors released by platelets and other cells. Monocytes from the bloodstream enter the area through blood vessel walls (Lorenz and Longaker, 2003). Numbers of

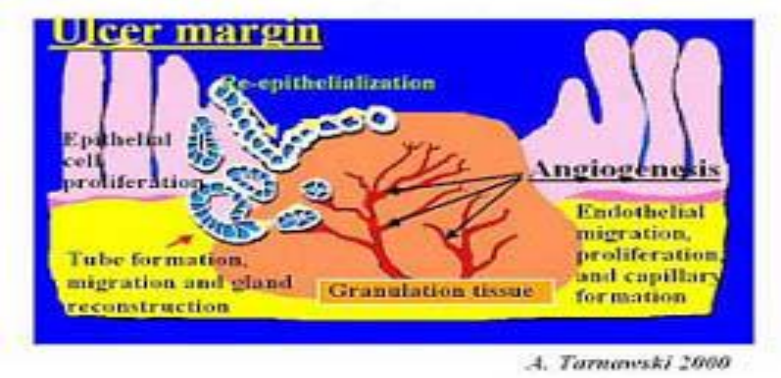
monocytes in the wound peak one and a half days after the injury occurs (Santoro and Gaudino, 2005). Once they are in the injury site, monocytes mature into macrophages.

The main role of macrophage is to phagocytize bacteria and damaged tissue (de la Torre and Sholar, 2006), debride damaged tissue by releasing proteases (Deodhar and Rana, 1997) and secrete a number of factors such as growth factors and other cytokines, especially during the third and fourth post-wounding days. These factors attract cells involved in the proliferation stage of healing to the area (Rosenberg and de la Torre 2006), although they may restrain the contraction phase (Newton *et al.*, 2004). They also stimulate cells that reepithelialize the ulcer, create granulation tissue, and lay down a new ECM (Mercandetti and Cohen, 2005).

As inflammation dies down, fewer inflammatory factors are secreted, existing ones are broken down, and numbers of neutrophils and macrophages are reduced at the wound site (de la Torre and Sholar, 2006). These changes indicate that the inflammatory phase is ending and the proliferative phase is underway (de la Torre and Sholar, 2006). Inflammation lasts as long as there is debris in the wound. Thus the presence of dirt or other objects can extend the inflammatory phase for too long, leading to a chronic wound.

2.2.5.3. Proliferative Phase:

About two or three days after the injury occurs, fibroblasts begin to enter the ulcer site, marking the onset of the proliferative phase even before the inflammatory phase has ended (Falanga, 2005).



Source: Tarnawski, 2000

Figure 2.5: Healing of gastric ulcer

Angiogenesis

Angiogenesis also called neovascularization - formation of new microvessels from preexisting vessels – is essential for healing of chronic gastroduodenal ulcers (Folkman and D'Amore, 1996). It occurs concurrently with fibroblast proliferation when endothelial cells migrate to ulcer site (Kuwahara and Rasberry, 2007). Because the activity of fibroblasts and epithelial cells requires oxygen and nutrients, angiogenesis is imperative for other stages in wound healing. The tissue in which angiogenesis has occurred typically looks red (is erythematous) due to the presence of capillaries (Kuwahara and Rasberry, 2007). Stem cells of endothelial cells, originating from parts of uninjured blood vessels, develop pseudopodia and push through the ECM into the ulcer site to establish new blood vessels (Greenhalgh, 1998).

The growth of granulation tissue and generation of new microvessels through angiogenesis is stimulated by vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), angiopoietins and possibly by other growth factors and cytokines, including interleukin 1 (IL-1) and tumor necrosis factor-alpha (TNF-α) (Tarnawski, 2000). Endothelial

growth and proliferation is also directly stimulated by hypoxia, and presence of lactic acid in the wound (Falanga, 2005). Vascular endothelial growth factor is a fundamental regulator of angiogenesis. Its binds to at least two specific receptors; VEGF-R1 or flt-1 and VEGF-R2 or flk-1/KDR expressed mainly on endothelial cells (Ferrara, 1999), initiates phosphorylation of numerous cytosolic proteins involved in signal transduction that triggers endothelial cell proliferation, migration and microvascular tube formation (Ferrara, 1999).

To migrate, endothelial cells need collagenases and plasminogen activator to degrade the clot and part of the ECM (de la Torre and Sholar, 2006). Zinc-dependent metalloproteinases digest basement membrane and ECM to allow cell migration, proliferation and angiogenesis (Lansdown *et al.*, 2001).

When macrophages and other growth factor-producing cells are no longer in a hypoxic, lactic acid-filled environment, they stop producing angiogenic factors (Greenhalgh, 1998). Eventually blood vessels that are no longer needed die by apoptosis (Romo and Pearson, 2005).

Collagen deposition

Simultaneously with angiogenesis, fibroblasts begin to accumulate in the ulcer site. Fibroblasts begin entering the wound site two to five days after ulceration as the inflammatory phase is ending (de la Torre and Sholar, 2006). By the end of the first week, fibroblasts are the main cells in ulcer bed (Stadelmann *et al.*, 1998).

Origins of these fibroblasts are thought to be from the adjacent uninjured mucosa tissue (although new evidence suggests that some are derived from blood-borne, circulating adult stem cells/precursors) (Song *et al.*, 2010). Initially fibroblasts utilize the fibrin cross-linking fibers (well-formed by the end of the inflammatory phase) to migrate across the wound, subsequently

adhering to fibronectin (Romo and Pearson, 2005). Fibroblasts then deposit ground substance into the wound bed, and later collagen, which they can adhere to for migration (Rosenberg and de la Torre, 2006).

Granulation tissue functions as rudimentary tissue, and begins to appear in the wound already during the inflammatory phase, two to five days post wounding, and continues growing until the wound bed is covered. Granulation tissue consists of new blood vessels, fibroblasts, inflammatory cells, endothelial cells, myofibroblasts, and the components of a new, provisional extracellular matrix (ECM). (Collagens are the most abundant structural proteins of extracellular matrix (Rosenberg and de la Torre, 2006).

Fibroblasts also secrete growth factors that attract epithelial cells to the wound site. Hypoxia also contributes to fibroblast proliferation and excretion of growth factors, though too little oxygen will inhibit their growth and deposition of ECM components, and can lead to excessive, fibrotic scarring.

Collagen deposition is important because it increases the strength of the wound; before it is laid down, the only thing holding the wound closed is the fibrin-fibronectin clot, which does not provide much resistance to traumatic injury (Greenhalgh, 1998). Also, cells involved in inflammation, angiogenesis, and connective tissue construction attach to, grow and differentiate on the collagen matrix laid down by fibroblasts (Ruszczak, 2003).

Even as fibroblasts are producing new collagen, collagenases and other factors degrade it. Shortly after ulceration, synthesis exceeds degradation, so collagen levels in the ulcer increase, but later production and degradation become equal so there is no net collagen gain (Greenhalgh, 1998). This homeostasis signals the onset of the later maturation phase. Granulation gradually ceases and fibroblasts decrease in number in the wound once their work is done (DiPietro and

Burns, 2003). At the end of the granulation phase, fibroblasts begin to commit apoptosis, converting granulation tissue from an environment rich in cells to one that consists mainly of collagen (Stadelmann *et al.*, 1998).

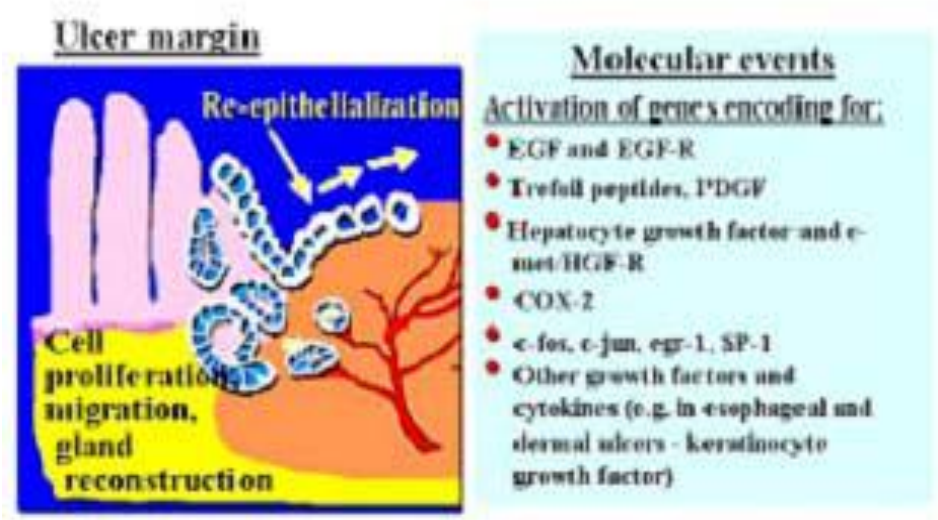
Epithelialization

The formation of granulation tissue in an ulcer allows the reepithelialization phase to take place, as epithelial cells migrate across the new tissue to form a barrier between the ulcer and the environment (Romo and Pearson, 2005). Migration can begin as early as a few hours after wounding. However, epithelial cells require viable tissue to migrate across, so if the ulcer is deep it must first be filled with granulation tissue (Mulvaney and Harrington, 1994).

Migration of epithelial cells over the wound site is stimulated by lack of contact inhibition and by chemicals such as nitric oxide (Witte and Barbul, 2002). Before they begin to migrate, cells must dissolve their desmosomes and hemidesmosomes, which normally anchor the cells by intermediate filaments in their cytoskeleton to other cells and to the ECM (Santoro and Gaudino, 2005). Integrins, which normally anchor the cell to the basement membrane by its cytoskeleton are released from the cell's intermediate filaments and relocate to actin filaments to serve as attachments to the ECM for pseudopodia during migration (Santoro and Gaudino, 2005). Epithelial cells detach from the basement membrane and are able to enter the ulcer bed (Falanga, 2005).

The epithelial cells lining glands of the ulcer margin undergo de-differentiation, express epidermal growth factor receptor (EGF-R) and actively proliferate (Tarnawski, 1993; Tarnawski, 2000). These cells migrate from the ulcer margin onto the granulation tissue to re-epithelialize the ulcer base. In addition, the epithelial cells from the base of the ulcer margin form tubes

composed of ulcer-associated cell lineage, which invade granulation tissue migrate toward the surface, branch and undergo transformation into gastric glands within the ulcer scar (Tarnawski, 2000, Tarnawski *et al.*, 1990). Growth factors are the major stimuli for cell proliferation, division, migration and re-epithelialization (Cotran *et al.*, 1999, Vanwijck *et al.*, 2001). In addition to the initial pool of growth factors derived from the platelets, macrophages and injured tissue, ulceration triggers in cells lining mucosa of the ulcer margin, genes encoding for the growth factors e.g.epidermal growth factor, hepatocyte growth factor, vascular endothelial growth factor, platelet derived growth factor and Cox2, in a well synchronized spatial and temporal manner. These growth factors produced locally, activate epithelial cell migration and proliferation via autocrine and/or paracrine actions. Okamoto *et al.*, (2002) demonstrated that bone marrow cells can re-populate epithelium of human gastrointestinal tract and this process is increased by ~ 50-fold in gastric ulcer. The cell migration is dependent on the transcription factors and cytoskeletal rearrangements (Chai *et al.*, 2004). The cytoskeleton (actin filaments, microtubules, intermediate filaments, focal adhesions and their associated proteins) plays an important role in cell structure, shape and mobility (Chai *et al.*, 2004). Cell migration requires polymerization of G-actin into F-actin and formation of stress fibers.



Source: Tarnawski, 2000

Figure 2.6: Gastric ulcer healing

2.2.5.4. Maturation and remodeling:

When the levels of collagen production and degradation equalize, the maturation phase of tissue repair is said to have begun (Greenhalgh, 1998). During maturation, type III collagen, which is prevalent during proliferation, is gradually degraded and the stronger type I collagen is laid down in its place (Dealey, 1999). Originally disorganized collagen fibers are rearranged, cross-linked, and aligned along tension lines (Lorenz and Longaker, 2003). The onset of the maturation phase may vary extensively, depending on the size of the wound (Mercandetti and Cohen, 2005), ranging from approximately 3 days to 3 weeks. The maturation phase can last for a year or longer, similarly depending on wound type (Mercandetti and Cohen, 2005).

2.3. Gastric Acid Secretion

The stomach has two types of glands: the pyloric and oxyntic glands. The pyloric glands secrete mainly mucus for protection of the pyloric mucosa from the stomach acid. They also secrete the hormone gastrin. The oxyntic gland is made up of three types of cells: (1) mucous neck cells which secrete mainly mucus; (2) peptic or chief cells, which secrete pepsinogen and (3) parietal or oxyntic cells which secrete hydrochloric acid and intrinsic factor. The parietal cells secrete an acid solution that contains about 160 millimoles of hydrochloric acid per liter (Yao and Forte, 2003), which is almost exactly isotonic with the body fluids. The pH of this acid is about 0.8, demonstrating its extreme acidity. At this pH, the hydrogen ion concentration is about 3 million times that of the arterial blood.

2.3.1. Mechanism of Hydrochloric Acid Secretion

i. Chloride ion is actively transported from the cytoplasm of the parietal cell into the lumen of the canaliculus, and sodium ions are actively transported out of the canaliculus into the cytoplasm of the parietal cell. These two effects together create a negative potential of -40 to -70 millivolts in the canaliculus, which in turn causes diffusion of positively charged potassium ions and a small number of sodium ions from the cell cytoplasm into the canaliculus (Yao and Forte, 2003).

ii. Water becomes dissociated into hydrogen ions and hydroxyl ions in the cell cytoplasm. The hydrogen ions are then actively secreted into the canaliculus in exchange for potassium ions: this active exchange process is catalyzed by H^+,K^+- ATPase (Forte and Zhu, 2010). This ATPase is magnesium-dependent, and not inhibitable by

ouabain. In addition, the sodium ions are actively reabsorbed by a separate sodium pump.

iii. Water passes into the canaliculus by osmosis because of extra ions secreted into the canaliculus. Thus, the final secretion from the canaliculus contains water, hydrochloric acid at a concentration of about 150 to 160 mEq/L, potassium chloride at a concentration of 15 mEq/L, and a small amount of sodium chloride (Yao and Forte, 2003).

iv. Finally, carbon dioxide, either formed during metabolism in the cell or entering the cell from the blood, combines under the influence of carbonic anhydrase with the hydroxyl ion to form bicarbonate ions. These then diffuse out of the cell cytoplasm into the extracellular fluid in exchange for chloride ions that enter the cell from the extracellular fluid (Yao and Forte, 2003).

2.3.2. Control of Acid Secretion

Parietal cells have receptors for three stimulants of acid secretion, reflecting a triumverate of neural, paracrine and endocrine control:

i. Acetylcholine (muscarinic type receptor)
ii. Gastrin (Feldman, 1997)
iii. Histamine (H2 type receptor) (Soll and Berglindh, 1994)

Histamine from enterochromaffin-like cells may well be the primary modulator, but the magnitude of the stimulus appears to result from a complex additive or multiplicative interaction of signals of each type (Soll and Berglindh, 1994). For example, the low amounts of histamine released constantly from mast cells in the gastric mucosa only weakly stimulate acid secretion,

and similarly for low levels of gastrin or acetylcholine. However, when low levels of each are present, acid secretion is strongly forced. Additionally, pharmacologic antagonists of each of these molecules can block acid secretion (Samuelson and Hinkle, 2003).

Histamine's effect on the parietal cell is to activate adenylate cyclase, leading to elevation of intracellular cyclic AMP concentrations and activation of protein kinase A (PKA). One effect of PKA activation is phosphorylation of cytoskeletal proteins involved in transport of the H^+/K^+ ATPase from cytoplasm to plasma membrane. Binding of acetylcholine and gastrin both result in elevation of intracellular calcium concentrations (Samuelson and Hinkle, 2003).

Several additional mediators have been shown to result in gastric acid secretion when infused into animals and people, including calcium, enkephalin and bombesin. Calcium and bombesin both simulate gastrin release, while opiate receptors have been identified on parietal cells. It is unclear whether these molecules have a significant physiologic role in parietal cell function.

A variety of substances are capable of reducing gastric acid secretion when infused intravenously, including prostaglandin E_2 and several peptides hormones, including secretin, gastric inhibitory peptide, glucagon and somatostatin. Prostaglandin E_2, secretin and somatostatin may be physiologic regulators. Somatostatin inhibits secretion of gastrin and histamine, and appears to have a direct inhibitory effect on the parietal cell (Samuelson and Hinkle, 2003).

The stimulation of gastric acid secretion is controlled in 3 phases:

i. **Cephalic Phase.** The sight, smell, taste or even thought of food causes impulses from the cerebral cortex, amygdala and hypothalamus to send impulses through the vagus nerve. This triggers gastric acid production and secretion (Lloyd and Debas, 1994).

ii. **Gastric Phase.** Food in the stomach triggers reflexes and stimulates gastrin secretion. This then stimulates gastric acid production and secretion (Lloyd and Debas, 1994).

iii. **Intestinal Phase.** Food in the duodenum (small intestine) causes the duodenal mucosa to secrete gastrin and this continues to stimulate secretion of small amounts of gastric acid. However, food in the duodenum primarily inhibits gastric acid secretion through other mechanisms (Lloyd and Debas, 1994).

Gastric Acid Inhibition

Nerve reflexes and hormones slow gastric emptying and inhibit gastric acid secretion due to :

i. Acidic gastric contents in the duodenum.

ii. Distension of the small intestine (duodenum).

iii. Fat and protein breakdown products in the small intestine (duodenum) (Chris, 2010).

2.3.3. Role of Gastric acid in Peptic Ulcer

The role of acid in gastroduodenal pathogenesis has been extensively studied. Although gastric ulcer patients show normal or reduced level of acid secretion, duodenal ulcer patients usually secrete more acid (Wolfe and Soll, 1988). In fact, "no acid, no ulcer" is the dictum for duodenal ulcer. Although the secreted acid itself is not sufficient for ulcer formation, its corrosive property and increased peptic activity is sufficient to aggravate the ulcer. Even the

normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost. Hence, acid suppression by omeprazole is a common practice to control gastroduodenal lesions (Wolfe and Sachs, 2000). In animals, the role of acid in gastric lesions has been studied using some animal models such as stress or nonsteroidal antiinflammatory drug-induced gastric ulcer. Stress itself inhibits gastric acid secretion through a central nervous reflex mechanism (Esplugues *et al*., 1996). Restraint or water immersion stress significantly decreases acid secretion induced by pylorus ligation (Hayase and Takeuchi, 1986), therefore, acid plays a minor role in stress ulcer. Administration of antacids to neutralize secreted acid does not protect stress ulcer, suggesting that factors other than acid are involved in stress ulcer formation. On the other hand, indomethacin-induced gastric damage, acidity may be increased because of decreased biosynthesis of prostaglandin (Evbuomwan and Bolarinwa, 1993). Also, Ca^{2+} blockers, nifedipine and verapamil, which significantly reduced gastric acid secretion, significantly attenuated stress gastric ulcer formation (Glavin, 1988).

2.3.4. Gastric Acid and Ulcer Healing

Histamine H_2 receptor antagonists and proton pump inhibitors (Horn, 2000) such as omeprazole, lansoprazole, pantoprazole, and rabeprazole are extensively used for therapeutic control of acid-related disorders including gastroesophageal reflux disease and Zollinger-Ellison syndrome and for peptic-ulcer disease caused by stress (stress-related erosive syndrome), nonsteroidal antiinflammatory drugs, and *Helicobacter pylori* infection (Wolfe and Sachs, 2000). Inhibition of gastric acid secretion by these compounds is considered to be an important step to control the disorders (Sachs et al, 1993). Proton pump inhibitors inhibit acid secretion by irreversibly interacting with the H^+-K^+-ATPase, the terminal proton pump of the parietal cell

(Sachs, 1997). Proton pump inhibitors were found to accumulate in parietal cell secretory canaliculi resulting in an antisecretory effect that lasts much longer than that of H_2 receptor antagonists. Brzozowski et al. (2000) noticed that the suppression of gastric acid secretion by omeprazole or ranitidine prevents the progression of gastric erosions into ulcers, and the addition of exogenous acid restores the progression of the acute lesions into gastric ulcers, indicating that gastric acid plays a key role in ulcerogenesis. Proton pump inhibitors (PPIs) are the most potent acid suppressants available and are significantly more effective than histamine H_2 receptor antagonists (Fock *et al.*, 2008).

2.4. Blood and Healing

White blood cell counts have been used as an indicator by clinicians to monitor progress of healing in patients (Haffor, 2010). White blood cells are important for healing. They do this by ingesting materials that need to be removed from the body. These include; debris from body tissues and dead cells. Cell mutation is generally kept in check by properly functioning white blood cells. However, if that cell count is too low, the white blood cells already spread thin on their main assignment (immune system activities) - will be unable to fight abnormal cell multiplication and, thus, increase the risk for cancerous and noncancerous cell proliferation. Elevated WBC count was reported in a group of rats in which gastric ulcer healing was accelerated (Haffor, 2010).

2.4.1. Neutrophil Lymphocyte Ratio (NLR)

The events that lead to the inflammatory response are characterized by the recognition of the site of injury by inflammatory cells, specific recruitment of subpopulation of leukocytes into the tissue, and removal of the offending agent.

The physiologic immune response of circulating white blood cells to various stressful events as tissue injury, severe trauma, major surgery, burns, sepsis syndrome, is characterized by elevation of neutrophils and decline in lymphocyte counts. The neutrophil-lymphocyte ratio (NLR) provides an indicator of inflammatory status (Halazun *et al.*, 2008).

Zahorec (2001) reported that the lowest values of lymphocyte counts were observed in the most severe group (severe sepsis syndrome or septic shock) as compared with other groups and improvement of clinical status coincides with an increase in lymphocyte counts above 10 % of the WBC population.

Zahorec (2001) found out that the improvement of the clinical status following major surgery, severe infection, systemic inflammation, sepsis, injury or trauma coincides with a gradual increase in lymphocyte counts (more than 1.000/mm3 or higher than 10 % of WBC population) and concomitant decrease in neutrophil counts (less than 8.000/mm3 or less than 80 % of WBC). The persistency of neutrophilia (more than 90 %) and sustained lymphocytopenia (less than 5 % of WBC, or less than 500/mm3) lasting more than 5 to 7 days may lead to further complications e.g. to the development of multiple organ dysfunction syndrome. The development of neutrophilia and lymphocytopenia is caused and maintained by many factors (Sterzl, 1988).

Lymphocytopenia - a significant decrease in circulating lymphocyte count after severe trauma, major surgery, severe sepsis and systemic inflammation has been described by many authors (O.Mahony, 1984; Dionigi *et al.*, 1994; Jilma *et al.*, 1999). The causes involve margination and redistribution of lymphocytes within the lymphatic system and marked accelerated apoptosis (Ayala *et al.*, 1996; Hotchkiss *et al.*, 1999).

Lymphocytopenia is induced by: hormones, cytokines and chemokines. Lymphocytes have many receptors on their cell membranes: alpha- and beta-adrenoceptors, dopamine, histamine, cholinergic and kinin receptors, which play a very important role in the regulation of inflammatory response. Lymphocyte populations are very sensitive to neuroendocrine hormones as adrenaline, noradrenaline, histamine, cortisol and prolactin (Sterzl, 1988; Dionigi *et al.*, 1994). High serum concentration of these hormones during surgery leads to lymphocytopenia (Dionigi *et al.*, 1994).

Neutrophilia the opposite phenomenon during systemic inflammation, is caused by demargination of neutrophils, delayed apoptosis of neutrophils, and stimulation of stem cells by growing factors (G-CSF). (Mahidhara and Billiar, 2000).

Neutophil-Lymphocyte Ratio and Ulcer

There is no literature on the use of neutophil-lymphocyte ratio to assess rate of healing in ulcer studies.

2.5. Reactive Oxygen Species

Oxygen is vital for aerobic life processes. However, about 5% or more of the inhaled O_2 is converted to reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) (Harman, 1993). Thus cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect. When the balance between ROS production and antioxidant defenses is lost, 'oxidative stress' results which through a series of events deregulates the cellular functions leading to various pathological

conditions including: cardiovascular dysfunction, neurodegenerative diseases, gastroduodenal pathogenesis, metabolic dysfunction of almost all the vital organs, cancer, and premature aging (Thomas and Kalyanaraman, 1997). The free-radical-mediated oxidative stress results in oxidation of membrane lipoproteins, glycoxidation, and oxidation of DNA; subsequently cell death results.

Several reactive oxygen species are known. Reactive oxygen species are defined as any species capable of independent existence that contains one or more unpaired electrons (those which occupy an atomic or molecular orbital by themselves). They are formed either by the loss of a single electron from a non-radical or by the gain of a single electron by a non-radical. Among them, the most frequently studied are given below:

1 - Superoxide radical ($O_2^{\cdot -}$)

This ROS are produced in the mitochondria of cell during biological oxidation. Oxygen is required for energy generating combustion involving endogenous substrates and to detoxify xenobiotics. Oxidation reaction ensures that the molecular oxygen is completely reduced to water. During this process, oxygen acts as a terminal 4-electron acceptor and is eventually converted to stable chemical compound, water. However the reduction of oxygen is frequently incomplete, even under normal conditions or some time, univalent reduction occurs with electrons added one at a time and a series of chemical intermediates are produced which are highly reactive and obnoxious to living systems (Pillai and Pillai, 2002).

$$O_2 + e^- \rightarrow O_2^{\cdot -}$$ (source, Pillai and Pillai, 2002)

The production of superoxide radicals at the membrane level is initiated in specialized cells (oxidative burst) with phagocytic functions (macrophages) and contributes to their

bactericide action. Superoxide radical has a limited reactivity with lipids, raising questions about its true toxicity. Thus, its action is frequently considered to result from a secondary production of the far-more reactive ·OH species by the iron-catalyzed Haber-Weiss reaction.

2 - Hydrogen peroxide (H_2O_2)

A "two electron reduction" of oxygen would produce hydrogen peroxide. The electron deficiency of superoxide radical may be made up by the addition of a second electron to give the peroxyl anion; afterward, peroxyl anion interacts with two hydrogen ions to form hydrogen peroxide:

$O_2^{\cdot -} + e^- \rightarrow O_2^{2-}$

$O_2^{2-} + 2H^+ \rightarrow H_2O_2$ Source: en.wikipedia.org/wiki/reactive_oxygen_species

Alternatively hydrogen peroxide may also be generated in biological systems via superoxide generating systems (a so called "dismutation reaction" in which free radical reactants give non free radical products):

$2O_2^{2-} + 2H^+ \rightarrow H_2O_2 + O_2$ Source: en.wikipedia.org/wiki/reactive_oxygen_species

Hydrogen peroxide is mainly produced by enzymatic reactions. For the production of H_2O_2, peroxisomal oxidases and flavoproteins, as well as D-amino acid oxidase, L-hydroxy acid oxidase, and fatty acyl oxidase participate (Bast *et al.*, 1991). Cytochrome P-450, P-450 reductase and cytochrome b-5 reductase in the endoplasmic reticulum under certain conditions generate $O_2^{\cdot -}$ and H_2O_2 during their catalytic cycles (Turrens and Boveris, 1980). The phagocytic cells, such as neutrophils, when activated during phagocytosis, generate $O_2^{\cdot -}$ and H_2O_2 through activation of NADPH oxidase. Neutrophil accumulation in inflammated tissue is one of the major reasons of oxidative damage due to generation of ROS.

3 - Hydroxyl radical (·OH)

In the presence of Fe^{2+} or copper, H_2O_2 produces the very active species ·OH by the Fenton reaction:

$Fe^{2+} + H_2O_2$ ----> $Fe^{3+} + {\cdot}OH + OH^-$ (Source: Kehrer, 2000)

This iron-catalyzed decomposition of hydrogen peroxide is considered the most prevalent reaction in biological systems and the source of various deleterious lipid peroxidation products (Kehrer, 2000).

2.5.1. Lipid Peroxidation

Lipids in the cell membrane undergo degradation to form hydroperoxides. Polyunsaturated fatty acids, PUFA, are especially liable to lipid peroxidation. Lipid hydroperoxides decompose to form a variety of products including malondialdehyde (MDA). Malondialdehyde was used as an indicator to assess oxidative damage of cells and tissues (Bonnes-Taourel *et al*., 1992). It is now well admitted that lipids are non-enzymatically peroxidized through three types of reaction: autoxidation, photo-oxygenation, enzymatic peroxidation.

1. Autoxidation

This is a radical-chain process involving 3 sequences: initiation, propagation and termination.

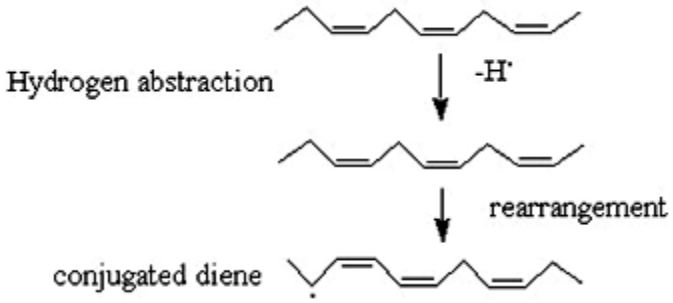
1.1 - Initiation

In a peroxide-free lipid system, the initiation of a peroxidation sequence refers to the attack of a ROS able to abstract a hydrogen atom from a methylene group (- CH_2-), this

hydrogen having very high mobility. This attack generates easily free radicals from polyunsaturated fatty acids. Hydroxyl radical is the most efficient ROS to do that attack, whereas $O_2^{\cdot -}$ is insufficiently reactive (Porter, 1986).

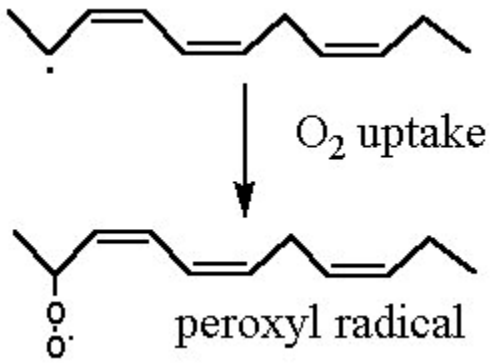
(Source: www.cyberlipid.org)

The presence of a double bond in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bond and so makes H removal easier. The carbon radical tends to be stabilized by a molecular rearrangement to form a conjugated diene.



(Source: www.cyberlipid.org)

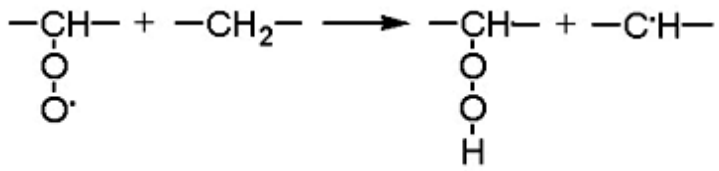
Under aerobic conditions conjugated dienes are able to combine with O_2 to give a peroxyl (or peroxy) radical, ROO·



(Source: www.cyberlipid.org)

1.2 – Propagation

As a peroxyl radical is able to abstract H from another lipid molecule (adjacent fatty acid), especially in the presence of metals such as copper or iron, thus causing an autocatalytic chain reaction. The peroxyl radical combines with H to give a lipid hydroperoxide (or peroxide). This reaction characterizes the propagation stage (Porter, 1986).



(Source: www.cyberlipid.org)

1.3 - Termination

Termination (formation of a hydroperoxide) is most often achieved by reaction of a peroxyl radical with a-tocoplerol which is the main lipophilic "chain-breaking molecule" in the cell membranes. Furthermore, any kind of alkyl radicals (lipid free radicals) $L^{\cdot}$ can react with a lipid peroxide $LOO^{\cdot}$ to give non-initiating and non-propagating species such as the relatively stable dimers LOOL or two peroxide molecules combining to form hydroxylated derivatives (LOH). Some bonds between lipid peroxides and membrane proteins are also possible (Porter, 1986).

2 - Photo-oxidation

As singlet oxygen ($^1O_2$) is highly electrophilic, it can react rapidly with unsaturated lipids but by a different mechanism from free radical autoxidation. In the presence of sensitizers (chlorophyll, porphyrins, myoglobin, riboflavin, bilirubin, erythrosine, rose bengal, methylene blue) (Wagner *et al.*, 1988), a double bond interacts with singlet oxygen produced from O_2 by light. Oxygen is added at either end carbon of a double bond which takes the trans configuration.

Thus, one possible reaction of singlet O_2 with a double bond between C12 and C13 of one fatty acid is to produce 12- and 13-hydroperoxides (Wagner *et al.*, 1988).

3 - Enzymatic peroxidation

Lipoxygenase enzymes (from plants or animals) catalyze reactions between O_2 and polyunsaturated fatty acids, such as arachidonic acid (20:4 n-6), containing methylene interrupted double bonds. When 20:4 n-6 is the substrate, these hydroperoxides are known as HpETEs which can be transformed into hydroxy products (HETEs). These HETEs are also formed directly via cytochrome P450 induced reactions (mono-oxygenases) and sometimes also via cyclooxygenase enzymes (Yamamoto, 1992).

Cyclooxygenase enzymes (in plants and animals) catalyze the addition of molecular oxygen to various polyunsaturated fatty acids, they are thus converted into biologically active molecules called endoperoxides (PGG, PGH), intermediates in the transformation of fatty acids to prostaglandins (Yamamoto, 1992).

2.5.2. Antioxidants

Halliwell (1997) suggested that an antioxidant is any substance that when present at low concentrations compared to those of an oxidisable substate significantly delays or prevent oxidation of that substrate. Organism welfare depends on the activity of efficient defense system against oxidative damage induced by free radicals/reactive oxygen species. Antioxidants include glutathione, vitamin C, vitamin E as well as enzymes such as catalase (Pompella *et al*., 2003; Brown and Jones, 1996; Chelikan, *et al.*, 2004), superoxide dismutase and various peroxidases.

Others include albumin, ceruloplasmin, and transferin. Low levels of antioxidants or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity (German, 1999). Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamin A, C and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Knight, 1998).

A functional classification of antioxidant system based on the way they act (Niki, 1996) appears to be the more useful. On this basis, antioxidant defense system *in vivo* are mainly three kinds: preventive antioxidants, radical scavengers and finally de novo enzymes.

Classification of antioxidant Systems Based on Their Mode of Action

i. Preventive antioxidants

Mode of Action - Suppress the formation of free radical:

(a) non-radical decomposition of H_2O_2

Examples: Catalase, glutathione peroxidase and S transferase

(b) sequestration of metal by Chelation.

Examples: Transferin, ceruloplasmin, albumin, haptoglobulin

(c) quenching of active $O_2^{\cdot}$

Examples: Superoxide dismutase, carotenoids (Niki, 1996)

ii. Radical-scavenging antioxidants

Mode of Action - Scavenge radical to inhibit chain initiation and break chain propagation.

Examples: Lipophilic ubiquinol, vitamin A, vitamin E. carotenoids, hydrophilic uric acid, albumin, bilirubin (Niki, 1996).

iii. Repair and de novo enzymes

Mode of Action - Repair the damage and constitute membranes.

Examples: DNA repair enzymes, proteases, transferase, lipase (Niki, 1996).

The activities of some antioxidants are discussed below

1. Superoxide Dismutases (SOD)

Different types of SOD exist. All catalyse the reaction below, accelerating it up to 10 000 times (Zelko *et al.*, 2002).

$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$ (Source: Zelko *et al.*, 2002)

The different types of SOD are differently distributed among organisms as well as among subcellular compartments and they contain metals (copper, zinc, manganese or iron) essential for their catalytic function. Since SOD speed H_2O_2 production, they have to work in conjunction with the enzyme that destroys H_2O_2, because accumulation of such species that yields $OH^{\cdot}$ is even more dangerous than $O_2^{\cdot -}$ (Zelko *et al.*, 2002).

2. Catalase

Catalase was first noticed as a substance in 1818 when Louis Jacques Thenard, who discovered H_2O_2 (hydrogen peroxide), suggested that its breakdown is caused by a substance. In 1900, Oscar Loew was the first to give it the name catalase, and found its presence in many plants and animals (Loew, 1900). In 1937 catalase from beef liver was crystallised by James B. Sumner and the molecular weight was worked out in 1938 (Sumner and Gralén, 1938).

Catalase is an enzyme found in nearly all living organisms that are exposed to oxygen, where it catalyse the decomposition of hydrogen peroxide to water and oxygen (Chelikan, *et al.*, 2004). Catalase has one of the highest turnover number of all enzymes; one catalase molecule can convert 40 million molecules of hydrogen peroxide to water and oxygen each second (Goodsell, 2004). Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide.

The reaction of catalase in the decomposition of hydrogen peroxide is:

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$ (Source: Goodsell, 2004)

Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition.

Catalase is usually located in the peroxisome. Pathogens that are catalase-positive, such as *Mycobacterium tuberculosis, Legionella pneumophila* and *Campylobacter jejuni* make catalase in order to deactivate the peroxide radicals, thus allowing them to survive unharmed within the host (Srinivasa Rao *et al.*, 2003). All known animals use catalase in every organ, with particularly high concentrations occurring in the liver.

According to recent scientific studies, low levels of catalase may play a role in the greying process of human hair. Hydrogen peroxide is naturally produced by the body and catalase breaks it down. If there is a dip in catalase levels, hydrogen peroxide cannot be broken down. This causes the hydrogen peroxide to bleach the hair from the inside out. This finding may someday be incorporated into anti-greying treatments for aging hair (Wood *et al.*, 2009).

3. Glutathione, Glutathione peroxidase, Gluthatione Reductase, Glutathione Transferase

Glutathione is not an essential nutrient, since it can be synthesized in the body from the amino acids L-cysteine, L-glutamic acid and glycine. The sulfhydryl (thiol) group (SH) of cysteine serves as a proton donor and is responsible for the biological activity of glutathione (Pompella *et al*., 2003).

Glutathione exists in reduced (GSH) and oxidized (GSSG) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules, such as reactive oxygen species. In donating an electron, glutathione itself becomes

reactive, but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG). Such a reaction is possible due to the relatively high concentration of glutathione in cells (up to 5 mM in the liver). Glutathione can be regenerated from GSSG by the enzyme glutathione reductase. In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress (Pompella *et al.*, 2003).

Glutathione is known as a substrate in reduction reactions, catalyzed by glutathione S-transferase enzymes in cytosol, microsomes, and mitochondria. Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isozymes in animals (Brigelius-Flohé, 1999). Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. Surprisingly, glutathione peroxidase 1 is dispensable, as mice lacking this enzyme have normal lifespans (Brigelius-Flohé, 1999), but they are hypersensitive to induced oxidative stress. In addition, the glutathione *S*-transferases show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism (Hayes *et al.*, 2005).

4. Flavonoids

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine (Middleton, 1998). These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids

have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves. Research on flavonoids received an added impulse with the discovery of the French paradox, ie, the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption and a high saturated fat intake. The flavonoids in red wine are responsible, at least in part, for this effect (Formica and Regelson, 1995). Furthermore, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease (de Groot and Rauen, 1998).

Falvonoids can be divided into four classes i). Flavones e.g. apigenin, rutin, sibelin, quercetin ii). Flavanones e.g. fisetin, narigin, hesperetin , iii). catechins e.g. catechin, epicatechin and iv). anthcyanins e.g cyanidine, malvidine, petundin.

Flavonoids can directly scavenge free radicals. Flavonoids stabilize reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of flavonoids, radicals are made inactive, according to the equation below (Korkina and Afanas'ev, 1997):

Flavonoid(OH) + R• > flavonoid(O•) + RH (Source: Korkina and Afanas'ev, 1997)

where R• is a free radical and O• is an oxygen free radical. Selected flavonoids can directly scavenge superoxides, others, peroxynitrite.

Several flavonoids, including quercetin, result in a reduction in ischemia-reperfusion injury by interfering with inducible nitric-oxide synthase activity (Shoskes, 1998). Although the early release of nitric oxide through the activity of constitutive nitric-oxide synthase is important in maintaining the dilation of blood vessels, the much higher concentrations of nitric oxide

produced by inducible nitric-oxide synthase in macrophages can result in oxidative damage. In these circumstances, activated macrophages greatly increase their simultaneous production of both nitric oxide and superoxide anions. Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite (Shoskes, 1998). Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize LDLs, resulting in irreversible damage to the cell membrane. When flavonoids are used as antioxidants, free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage.

The xanthine oxidase pathway has been implicated as an important route in the oxidative injury to tissues, especially after ischemia-reperfusion (Sanhueza *et al*., 1992). Both xanthine dehydrogenase and xanthine oxidase are involved in the metabolism of xanthine to uric acid. Xanthine dehydrogenase is the form of the enzyme present under physiologic conditions, but its configuration is changed to xanthine oxidase during ischemic conditions. Xanthine oxidase is a source of oxygen free radicals. In the reperfusion phase (ie, reoxygenation), xanthine oxidase reacts with molecular oxygen, thereby releasing superoxide free radicals. At least 2 flavonoids, quercetin and silibin, inhibit xanthine oxidase activity, thereby resulting in decreased oxidative injury (Shoskes, 1998). When reactive oxygen species are in the presence of iron, lipid peroxidation results. Specific flavonoids are known to chelate iron, thereby removing a causal factor for the development of free radicals.

5. Uric Acid

Uric acid is produced by the oxidation of hypoxanthine and xanthine by xanthine oxidase. In human tissues, because of a lack of urate oxidase (which catalyzes the oxidation of uric acid to

allantoin), therefore, uric acid accumulates as the end product of purine metabolism. In vitro, uric acid could inhibit transition metal-ion dependent $^{\cdot}OH$ generation, is a powerful quencher and /or scavenger of singlet O_2 and might trap peroxyl radical in an aqueous phase more effectively than ascorbic acid (Halliwell, 1996).

6. Tocopherols and Tocotrienols (vitamin E)

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties (Herrera and Barbas, 2001). Of these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolising this form. It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrera and Barbas, 2001). This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol(Wang and Quinn, 1999).

Vitamin C

Ascorbic acid is the only endogenous antioxidant in plasma that can completely protect against peroxidative damage induced by aqueous peroxyl radicals and the oxidants released from activated neutrophils (Brown and Jones, 1996). There would appear to exist a concerted action of vitamin E and C, in which ascorbic acid regenerates vitamin E thus maintaining the serum value at a constant level. When ascorbic acid is depleted, no generation of vitamin E is possible and a

decrease in its concentration is observed. Chemically, ascorbic acid is an excellent reducing agent (Buettner and Lirkiewicz, 1996), and most of its antioxidant properties are ascribed to this feature. Unfortunately, it is also able to reduce iron and copper ions. If H_2O_2 is also present, ascorbate can drastically accelerate $^{\cdot}OH$ formation, thus producing another pro-oxidant. The pro-oxidant effects of ascorbic acid do not usually occur in vivo simply because in the healthy state, free ion and copper are not available in the ECF. Finally Ascorbic acid is required for the conversion of the procollagen to collagen by oxidizing proline residues to hydroxyproline.

2.5.3. Effects of Hyperthyroid and Hypothyroid State on Oxidative Stress and Antioxidant Status

The development of a hyperthyroid state in vertebrates leads to an enhancement in basal metabolic rate (BMR) due to an increase in the rate of O_2 consumption in most tissues excluding the spleen, testis, and adult brain (Baker and Klitgaard, 1952). There is increased synthesis of enzymes involved in energy metabolism and the components of the respiratory chain apparatus, leading to a higher capacity of oxidative phosphorylation (Videla, 2000). Accompanying this increase cellular respiration is an increase in redox imbalance, leading to generation of reactive oxygen species (Videla, 2000).

Studies on the effect of hyperthyroid state on oxidative stress shows that hyperthyroidism leads to increase generation of superoxide radical and hydrogen peroxide (Fernandez and Videla, 1993), with resultant increase in lipid peroxidation in the liver and testis of rats (Chandra *et al.*, 2010). Erdamar *et al.*, **(**2008**)** also reported increase level of malondialdehyde in fasting blood samples of patients with hyperthyroidism.

However, contrary reports exist about the effect of thyroxine treatment on antioxidant status. Wynn (1968) reported that pharmacologic, nonphysiologic doses thyroxine serves as an antioxidant to lipid peroxidation *in vivo*. Chandra *et al.*, (2010) reported that thyroxine treatment increased SOD and catalase activities in response to oxidative stress. Other studies reported that thyroxine treatment or hyperthyroidism significantly increased SOD, catalase, glutathione peroxidase and glutathione in erythrocyte (Sal'nikova and Dubinina, 1985; Seven *et al.*, 1996), revealing that antioxidant status is enhanced in hyperthyroid state. On that other hand, Fernandez *et al*., (1988) reported that hyperthyroid state reduces the activities of SOD, catalase and GSH in the hepatocytes and kupffer cells.

Hypothyroidism also increases oxidative stress by inducing dysfunction of the respiratory chain in the mitochondria leading to accelerated production of free radicals (i.e., superoxide anion, hydrogen peroxide, and hydroxyl radical), which consequently leads to oxidative stress (Yilmaz *et al.,* 2003; Resch *et al*., 2002). Chattopadhyay *et al*., (2003) found out that lipid peroxidation was elevated in the heart tissue in hypothyroid state but reduced upon T_3 supplementation. Erdamar *et al*., (2008) also reported increase level of malondialdehyde in fasting blood samples of patients with hypothyroidism.

Hypothyroidism has been reported to decrease the activity of antioxidant defense system. Hypothyroidism significantly decreased hepatic SOD, gluthathione peroxidase and catalase activity (Pasupathi and Latha, 2008). Chattopadhyay *et al*., (2003) reported that mitochondrial SOD activity was reduced in the heart of hypothyroid rats

2.5.4. Reactive Oxygen Species and Peptic Ulcer

Reactive oxygen species has been implicated in gastrointestinal disorders, related to gastric hyper secretion and gastrointestinal mucosal damage (Halliwell and Gutteridge, 1986). Tandon *et al.*, (2006) reported a high free radical activity in patients of peptic ulcer and gastric carcinoma and a decline in the enzymatic activity of superoxide dismutase along with catalase. Reactive oxygen species have been reported to be involved in stress (Das *et al.*, 1997), nonsteroidal antiinflammatory drugs (Langman *et al.*, 1991), and *H. pylori* (Konturek *et al.*, 1999) ulcer.

2.5.5. Lipid Peroxidation and Ulcer

Lipid peroxidation, an important parameter for ˙OH-induced oxidative damage of membrane. It is increased in gastric lesions caused by ethanol (Pihan et al, 1987), indomethacin (Yoshikawa et al, 1993), and water immersion stress (Yoshikawa et al, 1986).

2.6.1. Apoptosis

Apoptosis, or programmed cell death, has been used to describe a form of cell death in an active and inherently controlled manner that eliminates no longer wanted cells (Kerr *et al.*, 1972). Cell and nuclear shrinkage, chromatin condensation, formation of apoptotic bodies and phagocytosis by neighboring cells characterize the main morphological changes of the apoptosis process (Kerr *et al.*, 1972). Cleavage of chromosomal DNA into oligonucleosomal size fragments is a biochemical hallmark of apoptosis (Wyllie, 1980). The extensive morphological and biochemical changes during apoptosis are likely to ensure that dying cells would leave minimum impact to neighboring tissues in vivo. These features of apoptosis contrast those of

necrosis, another form of cell death in response to noxious stimuli or injury. Necrosis is accompanied by membrane rupture and leakage of cellular contents, and it thus often causes tissue inflammation. During normal development and tissue homeostasis, majority of the cells are believed to die through apoptosis. Moreover, dysregulation of apoptosis seems to be directly linked to human diseases including cancer, autoimmune diseases, and neuronal degeneration (Thompson, 1995; Nijhawan *et al.*, 2000).

Apoptosis is a complex process that can be divided into the following four basic steps: induction, detection, effector, and removal (Au *et al.*, 1997). In the induction step, the cell receives an apoptotic signal. Various external stimuli can trigger apoptosis, including nutrient deprivation, cytokine depletion, ionizing radiation, and oxidative stress (Teraki and Shiohara, 1999; Yang and Korsmeyer, 1996). The cell then integrates numerous signals derived from signal transduction pathways to decide whether to commit to apoptosis. Once the cell commits to apoptosis, the signal to activate death machinery is detected and transduced to downstream effectors. Various regulators then carry out the apoptotic response (Hale *et al.*, 1996; Schwartzman and Cidlowski, 1994) and finally the cell is removed by phagocytosis.

Each step of the apoptosis process requires the concerted effort of many molecules, and among the most influential ones are the caspases, the Bcl-2 family of proteins, and p53.

At least 14 caspases have been identified. All caspases have in common three domains: a large subunit (20 kD), a small subunit (10 kD), and a NH2-terminus. Initially zymogens, caspases become activated by selectively cleaving aspartic acid and assembling into heterotetramers. In general, they can be categorized into three groups. The first group (caspase-1, caspase-4, and caspase-5) plays a role in inflammatory response (Salvesen and Dixit, 1997). Members of the second group are initial transducers (caspase-2, caspase-8, caspase-9, caspase-

10), and members of the third group are effectors (caspase-3, caspase-6, and caspase-7) (Roth, 2001; Clerk *et al.*, 2003). Caspases -12 through -14 have not yet been categorized due to insufficient information.

One family of cellular proteins that regulates caspase activity is the inhibitor of apoptosis (IAP) (Hay, 2000). Inhibitor of apoptosis(s) are the first class of endogenous cellular inhibitors of caspases to be found in mammalian species (Deveraux and Reed,1999). These proteins have in common a baculovirus IAP repeat (BIR), a ~70-residue binding domain (Miller, 1999). At least eight members of the IAP family have been identified, including X-chromosome-linked IAP (XIAP), cellular IAP1 (cIAP1), and cellular IAP2 (cIAP2) (Salvesen and Duckett, 2002). Originally discovered in baculoviruses, IAPs suppress host cell death response (Clem and Miller, 1994). It has been shown by ectopic expression that some IAPs block apoptosis in mammalian cells (Liston *et al.*, 1996). Although it is unclear how IAPs suppress apoptosis, some studies suggest that XIAP, cIAP1, and cIAP2 bind and inhibit caspases -3, -7, and -9 (Deveraux *et al.*, 1997).

Bcl-2 family.

The Bcl-2 gene was originally identified to be translocated in human follicular lymphoma. Since this discovery, at least 18 members of the Bcl-2 family of proteins have been discovered (Deveraux *et al.*, 2001). As effectors of the apoptosis pathway, the Bcl-2 family of proteins either promotes or prohibits apoptosis. Pro-apoptotic proteins include Bax and Bak, while Bcl-2 and Bcl-xL are anti-apoptotic proteins (Chao and Korsmeyer, 1997; Cameron and Feuer, 2000).

Genome p53.

Often regarded as the guardian of the genome, p53 controls the fate of damaged cells by detecting and arresting the cell cycle (Lane, 1992). It consists of a N-terminal transcriptional domain, a C-terminal regulatory domain, and a central DNA binding domain (Picksley and Lane, 1994). Normally, the supply of p53 is continually replenished because of its interaction with mouse of double minute 2 homologue (MDM2). Mouse of double minute 2 homologue binds to p53 in a negative feedback loop in which p53 stimulates the production of MDM2, which then degrades p53 (Wu *et al.*, 1993). However, when there is DNA damage, p53 becomes stabilized and targets genes like p21WAF1/CIP1 to inhibit cyclin-dependent kinases, which leads to cell cycle arrest at the G1 and G2 stages (Rouault *et al.*, 1996). p53 also regulates other transcriptional targets that arrest the cell cycle, including GADD45, wildtype p53-induced phosphatase 1 (Wip1), 14-3-3 sigma, and BTG2 (Chang, 2002; Sheikh *et al.*, 2000). At the same time, p53 is regulated by proteins like ataxia telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK). When DNA damage cannot be repaired, p53 activates the apoptosis machinery (Miyashita and Reed, 1995).

2.6.1.1 Apoptosis Pathways

Multiple pathways are known to induce apoptosis: the Fas-mediated pathway, mitochondria-mediated pathway, and the p53-dependent pathway as explained below (Zimmermann and Green, 2001):

Fas-mediated pathway

Fas (APO-1/CD95), the death receptor, is a 48 kD membrane protein from the tumor necrosis factor receptor (TNFR) family (Itoh *et al.*, 1991). It contains an intracellular domain of 80 amino acids known as the death domain (DD). The Fas ligand (FasL), a member of the tumor necrosis family (TNF), is a 40 kD membrane protein that produces a soluble homotrimer. The binding of three Fas molecules to a FasL homotrimer leads to subsequent binding of Fas-associated DD (FADD) and procaspase-8. The formation of this complex, known as the death-inducing signaling complex (DISC), triggers a cascade of caspase activation, including caspase-3, leading to cell death (Kischkel *et al*., 1995). Besides the recruitment of FADD, Fas-induced apoptosis pathway can also be mediated by a receptor-interacting protein (RIP), RIP associated ICH/CED-3-homologous protein with a DD (RAIDD), and procaspase-2 (Duan and Dixit, 1997).

Mitochondria-dependent pathway

In contrast to death receptor-mediated apoptosis, mitochondria-dependent apoptosis is characterized by the mitochondrial permeability transition (MPT), in which the permeability of the inner mitochondrial membrane increases for small solutes (Zoratti and Szabo, 1995). The MPT causes membrane swelling, the collapse of the mitochondrial voltage (Dym), formation of reactive oxygen species, and the release of matrix Ca^{2+} (Blatt andGlick, 2001). These events trigger cytochrome c release from the intermembrane space. Binding of cytochrome c with APAF-1 leads to the activation of caspase-9 and then caspase-3. In this pathway, the Bcl-2 family of proteins plays an important role (Zimmermann and Green, 2001).

p53-dependent pathway.

Responding primarily to DNA damage, activated p53 serves as a transcription factor that modulates the transcription of several apoptosis-related genes. For example, p53 upregulates the transcription of Bax while downregulating that of Bcl-2, thus favoring mitochondria-dependent apoptosis (Miyashita and Reed, 1995). In addition, it upregulates transcription of Fas to support Fas-mediated apoptosis.

2.6.1.2. Detection Methods

Numerous methods to detect apoptotic cells have been established based on the morphological and biochemical events of apoptosis. Morphological features are the definitive standard for detecting apoptotic cells, but such changes may be difficult to observe in large samples. Biochemical techniques may be easier methods for detection, but they often do not distinguish between apoptotic and necrotic pathways (Sgonc and Wick, 1994). Together, however, morphological and biochemical methods can provide meaningful information about apoptosis. On the cellular level, microscopy of dyes is an indicator of cell viability. Using light microscopy, viable cells can be detected by their rejection of Trypan blue, while apoptotic cells whose plasma membranes are permeable will take up the dye (Sgonc and Wick, 1994). Ethidium bromide and propidium iodide both stain apoptotic cells red under fluorescent microscopy. For cell surface changes, annexin V, a phospholipid-binding protein, preferentially binds to phosphatidylserine. Since the externalization of phosphatidylserine occurs in the early stages of apoptosis, this assay is capable of detecting early apoptosis using flow cytometry (Ormerod *et al.*, 1992). On the nuclear level, fluorescent dyes, such as ethidium bromide and propidium iodide, can detect DNA strand breaks associated with apoptosis by flow cytometry or laser

scanning microscopy. Another nuclear detection method called terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) takes advantage of an apoptosis hallmark in which DNA cleaves into 180 to 200 bp fragments, generating 3'-OH groups. The terminal deoxynucleotidyl transferase (Tdt) enzyme recognizes and attaches to these hydroxy groups, thus labeling apoptotic cells. Finally, immunohistochemical staining can be performed to analyze the expression of apoptosis-related markers, such as caspase-3, Bcl-2, and APAF-1 (Schmidt-Kastner *et al*., 2000; Telford *et al*., 2002).

2.6.2. Apoptosis in Normal Wound Healing

Apoptosis is vital to normal wound healing, especially in the removal of inflammatory cells and scar formation. As cell populations rapidly proliferate during tissue reconstruction, cell growth is balanced by apoptosis. Inflammatory cells, for example, must be removed in order to begin the next stage of wound healing. Otherwise, persistent inflammation can lead to nonhealing wounds. Similarly, the granulation tissue must decrease in cellularity to evolve into a scar (Greenhalgh, 1998).

Research by Brown, *et al.*, (1997), supports that inflammatory cells undergo apoptosis. In comparative studies of full-thickness wounds in diabetic and nondiabetic mice, they observed that apoptosis was detected in the inflammatory cells as early as 12 hours in nondiabetic mice. The apoptosis level peaked at the fifth day after wounding and slowly decreased afterwards. Apoptosis was consistently observed in the inflammatory cells beneath the leading edge of the migrating epithelium. This may be an indication that apoptosis signals the end of the inflammatory phase of healing. In comparison to nondiabetic mice, diabetic mice were

characterized by delayed apoptosis, but the trend was reversed when topical PDGF and insulin-like growth factor II (IGF-II) treatment was applied to the wound.

Kane and Greenhalgh, (2000) further explored the role of apoptosis-related markers during inflammation at the leading edge of the epithelium. They compared the expression of p53, Bcl-2, and apoptosis between normal and diabetic mice for 42 days. Their results suggest that normal mice exhibit an inverse relationship between Bcl-2 and p53 over time. Upon injury, Bcl-2 expression increased while that of p53 decreased, in order to allow for cellular proliferation to occur. As the inflammation process declined, p53 levels increased while Bcl-2 levels decreased. The rate of apoptosis appeared to parallel the rate of p53. For diabetic mice, there was no inverse relationship, and p53 expression was consistently higher than that of Bcl-2. The levels of both p53 and Bcl-2 decreased over the days 21 to 42, and the peak of apoptosis activity did not occur until day 14 (Kane and Greenhalgh, 2000).

2.6.3. Apoptosis in Abnormal Wound Healing

Since the regulation of apoptosis is crucial to normal wound healing, altered apoptotic behavior results in a number of pathologic processes, of which the most notable types are hypertrophic scars and keloids. Hypertrophic scars and keloids, two forms of hyperproliferative healing, are characterized by hypervascularity and hypercellularity (Wassermann *et al.*, 1998).They are characterized by excessive scarring, inflammation, and an overproduction of extracellular matrix components, such as collagen I and proteoglycans. Keloids are clinically similar to hypertrophic scars, with the exception that keloids expand beyond the original boundaries (Tredget *et al.*, 1997).

2.6.4. Apoptosis in Gastric Ulceration

Recent studies also indicate that programmed cell death or apoptosis plays a significant role in gastric ulceration. Gastric mucosal lesions caused by stress, indomethacin, ethanol, and *H. pylori* are also due to increased cell death by apoptosis (Konturek *et al.*,1999). Apoptosis is promoted because of an imbalance between the Bcl-2 family of antiapoptotic protein and apoptotic Bax protein in stress ulcer (Konturek *et al.,* 1999). Induction of tumor necrosis factor-α and release of cytochrome *c* to activate caspase-3-like protease are involved in apoptotic cell death in indomethacin ulcer (Fuji *et al.,* 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Animals

Male albino Wistar rats (160 -200g) were obtained from animal house, College of Medicine, University of Ibadan, Nigeria. They were randomly divided into four groups with adequate matching of weight. They were kept in wire meshed cages with beddings which were adequately changed throughout the period of work. Each animal was allowed to acclimatise to the laboratory conditions for at least two weeks before being used. Animals were fed with standard diet of commercial rat chow and tap water *ad libitum.*

3.2. Drugs Used

Table 3.1: Sources of Some of the Drugs Used in This Study.

S/No	Drug	Source
1.	Levothyroxine	Octavis, Barnstaple, UK,
2.	Procaine penicillin	China Medical Medicines, Guorui Pharmaceuticals Co. Ltd.
3.	Histamine acid phosphate	BDH Chemicals, UK
4.	Sodium thiopentone	Rotex Medica, Trittau, Germany
5	Trichloroacetic acid	Sigma chemical company, USA
6	Thiobarbituric acid	Sigma chemical company, USA
7	Tris	Vicker Laboratory, Burnley-in-Warfedale West Yorks, England

3.2.1. Preparation of Hormones and Drugs

i. **Thyroxine:** Levothyroxine tablets (100 µg) was used. A tablet was dissolved in 1ml of distilled water to make a concentration of 100 µg/1ml. A dose of 0.1ml was given per 100g of animal in order to give 100 µg/kg. Fresh drug solution was prepared daily and given to animals orally.

ii. **Histamine Acid Phosphate.** Histamine for injection was prepared by dissolving 5mg of histamine acid phosphate in 10mls of distilled water to give a concentration of 0.5mg/1ml. Therefore a dose of 0.1ml/100g was injected intramuscularly to give a dose of 0.5mg/kg.

iii. **Sodium thiopentone:** Sodium thiopentone (1g) was dissolved in 20mls of water for injection. A dose of 0.1mls was given per 100g of animals in order to give a dose of 50mg/kg

iv. **Normal Saline (0.**9%): 4.5g of NaCl was dissolved in 5 litres of distilled water.

v. **Urethane (Ethyl carbamate):** It was used as an anaesthetic agent in gastric acid secretion study. It was prepared by dissolving 25g of ethyl carbamate in 100ml normal saline to give 25% stock solution. Animals were anaesthetized with a dose of 0.6mls per 100g.

vi. **0.05 M phosphate buffer:** 6.97g of diphosphate K_2HPO_4 and 1.36 g of KH_2PO_4 were dissolved in little quantity of water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 10.2

vii. **30% Trichloroacetic acid (TCA) solution:** 9g of TCA was dissolved in 30ml of water.

viii. **0.75% Thiobarbituric acid (TBA) solution:** 0.225 g of TBA was dissolved in 30ml 0.1 m HCl, shaking in hot water.

ix. **Digestion buffer (pH 8.0):** 10mM NaCl, 10mM Tris (pH 8.0), 10mM EDTA (pH 8.0), 0.5% SDS.

x. **Sodium acetate 3M (pH 5.2):** 20.41 g of Na acetate, 32 mL of distilled water were mixed. Glacial acetic acid was added to 50 mL. The mixture was sterilize by autoclaving (121^{o}C, 15 min) and stored at 4^{o}C

xi. **0.5M EDTA Buffer, pH 8.0 (50 mL):** 9.305 g of Na_2EDTA, 1 g of NaOH, was added to 30 mL of distilled water and made up to 50mL with distilled water. The mixture was sterilised by autoclaving (121^{o}C, 15 min) and stored at 4^{o}C.

xii. **TBE Buffer (1 Liter):** 108g Tris base, 55g Boric acid, 40mls 0.5M EDTA (pH 8.0) was made up to 1 liter with distilled water and autoclaved for 20 min.

xiii. **5 M Sodium Chloride (50 mL):** 14.6 g of NaCl, 30 mL of distilled water, mixed and made up to 50 mL with distilled water. It was then stored at 4^{0}C.

xiv. **1 M Tris-HCl (50 mL):** 6.06 g of Tris-base, 40 mL of distilled water, 2.1 mL of concentrated HCl were mixed. The mixture was made up to 50mls with distilled water, sterilized by autoclaving (121^{o}C, 15 min) and stored at 4^{o}C

xv. **Ethidium Bromide (10 mg/mL):** 10 mg Ethidium bromide dye was added to 1 ml of sterile distilled water and mixed gently in a brown bottle or foiled Eppendorf tube. It was then store at room temperature.

xvi. **Bromophenol Blue Loading Buffer:** 25 mg bromophenol blue, 4 g sucrose was added to sterile water (10 mL) and mixed gently. The buffer was stored at 4^{0}C.

3.3. Animal Grouping

Table 3.2: Animals Grouping in the Study

S/No	Groups	Treatment
1.	Control	Sham operated euthyroid animals
2.	Thyroidectomised	Untreated thyroidectomised animals
3.	Thyroidectomised + T_4	Thyroidectomised animals given levothyroxine (100ug/Kg/day per oral for 35 days) supplementation
4.	Sham operated + T_4	Sham operated animals given levothyroxine (100ug/Kg/day per oral for 35 days) supplementation

3.4. Surgical Procedure and Treatment Schedule

i. Thyroidectomy:

The animals were anaesthetized using thiopentone sodium (50mg/kg). A midline incision was made in the neck region, the skin was bilaterally retracted, the fascia and the muscle covering the thyroid gland were carefully removed in order to expose the thyroid gland. The thyroid gland was then made free, the thyroid arteries were ligated to prevent loss of blood. The gland was then extirpated from the underlining tissues, with adequate care taken so that the parathyroid glands are not removed. The incision was sutured back, dabbed with procain penicillin and the animals were returned to standard diet and tap water.

ii. Sham operation

The animals were anaesthetised using 50mg/kg thiopentone sodium. A midline incision was made in the neck region, the thyroid gland was exposed, but the thyroid gland was left intact. The incision was sutured back, dabbed with procain penicillin and the animals were returned to standard diet and tap water.

iii. Thyroxine Treatment

A chronic thyroxine treatment was used as previously described by Olaleye (1999). A dose of 100µg/day p.o was given for 35 days using esophageal cannula.

iv. Ulcer Induction

After 35 days post surgery and of drug treatment, ulcer was induced in the stomach of each animal. The rats were fasted for 18 h before ulcer induction. Gastric ulcers were produced by the method of Wang *et al.*, (1989) with slight modifications. Animals were anaesthetised using 50mg/kg thiopentone sodium. Laparatomy was performed and stomach was exposed. Acetic acid (0.5 ml, 80% vol/vol) was applied to the serosal surface of glandular portion of the stomach for 1 minute through a 2ml syringe barrel that has been cut and smoothed. The syringe containing the acid was removed from the stomach and the area of stomach that had contact with acid was cleaned with cotton wool soaked in normal saline. The abdomen was sutured. Thereafter, the animals were returned to standard diet of laboratory chow and tap water.

3.5. Experimental Procedures

The experiments were divided into 6 studies as follows:

3.5.1. Study 1 - Preliminary study on the efficacy of the surgical and treatment methods.

a. Measurement of plasma T4, T3 and TSH

b. Measurement of body weight of the animals weekly

3.5.2. Study 2 – Measurement of ulcer dimensions

a. By planimetry

b. By histomorphometry

3.5.3. Study 3 – Assessment of the characteristics and quality of healing

a. By histological method

3.5.4. Study 4 – Assessment of gastric acid secretion during ulcer healing

a. By measuring the basal and histamine stimulated gastric acid secretion

3.5.5. Study 5 – Assessment of the effects of blood cells during ulcer healing

a. Estimation of Haematological Indices

3.5.6. Study 6 – Assessment of cellular activities during ulcer healing

a. By biochemical study (Lipid peroxidation)

b. By assessment of Index of Apoptosis - DNA Fragmentation

3.5.1. Study 1: Preliminary study on the efficacy of the surgical and treatment methods

a. **Measurement of Plasma Thyroxine T_4, Triiodothyronin T_3 and Thyroid Stimulating Hormone (TSH)**

After 35 days post-surgery and of levothyroxine treatment, plasma sample were collected from animals. Levels of T_4, T_3 and TSH were analysed using radioimmunoassay technique. Analysis was performed at WHO, HRP Collaborating Center, Reference Unit, Department of Obstetrics and Gynaecology, College of Medicine, University of Lagos, Lagos.

b. Measurement of Animal weight

Each animal was weighed weekly for the total period of study using digital weighing scale (Citizen Model MP 2000)

3.5.2. Study 2: Measurement of ulcer dimensions

a. By Planimetry

On days 3, 7 and 10 after ulcer induction, five animals were randomly picked from each group, blood was collected from each rat into EDTA containing sample bottle, via the orbital artery, for blood cell count. Red and white blood cell count was done using a method previously described by Lewis *et al*., (2006).

Each animal was then killed by cervical dislocation and the stomach was removed, opened along greater curvature, rinsed with normal saline and pinned on a wax block. Transparent paper was placed over ulcer area and the ulcer area was traced out. The area of ulceration was converted to units of square millimeters using 1mm by 1mm paper grid. The rate of healing per day on day 7 was calculated as:

(Ulcer area on day 7 – ulcer area on day 3)/7.

While rate of healing per day on day 10 was calculated as:

(Ulcer area on day 10 - ulcer area on day 3)/10.

b. Histomorphometry

A graticle having 1 to 100µm calibration was attached to a microscope, using the histological slides prepared, ulcer depth and width were measured, the area of mucosa eroded was then calculated.

3.5.3: Study 3: Assessment of the characteristics and quality of healing

a. Histological Study

On day 3, 7 and 10 five animals were randomly picked from each group for histological study. Histological studies were performed according to the methods previously described by Ogihara and Okabe (1993). Stomachs were fixed with 10% formalin. At autopsy, small pieces of tissue, including ulcers, were embedded in paraffin and sectioned at 5µm in an automated microtome. Haematoxylin and eosin staining was done. Evidence of tissue inflammation, exudates, formation of granulation tissue, regeneration of the ulcerated mucosa and the different types of cells on days 3, 7 and 10 after ulcer induction were noted.

3.5.4. Study 4: Measurement of gastric acid secretion during ulcer healing

a. Measurement of Basal and Histamine Stimulated Gastric Acid Secretion at Different Stages of Healing.

a. Surgical Preparation of Animals

Each animal was weighed. They were then anaesthetised with 25% urethane injected intraperitoneally at a dose of 0.6mls/100g body weight. The animal limbs were tied to a dissecting board. A blunt dissection was done in the neck region, the trachea was exposed, the esophagus was separated from the trachea by using a curved forceps to free them from each other. Threads were passed between the two structures. A small cut was made in the upper part

of the trachea and a 2.5cm long polythene size 3 cannula was inserted into it. The thread below the trachea was then tied lightly round the inserted cannula. The significance of trachea cannulation is to avoid any respiratory difficulties, bye-passing the nasal passage to the larynx. Also with the cannula in place, any fluid accumulating in the trachea can be readily aspirated. Also the essence of exposing the esophagus before cannulating the trachea is to prevent puncturing the esophagus during the passage of esophageal cannula from the mouth. To prevent drying up of the exposed tissue, saline soaked cotton wool was placed over the dissected area.

Another size 3 polythene cannula from a Lagendorff apparatus was passes into the esophagus to reach the cardia region of the stomach.

The linear alba was cleared of hair, a midline incision was made through the skin and muscle of the abdomen. The stomach was brought out, a small cut was made about an inch distal to the pyloroduodenal junction, the perfusion fluid maintained at 37^0C was rushed from the Lagendorff apparatus tap to wash out all food debris from the stomach of the animal. With the perfusion fluid still running, the early part of the duodenum was cannulated and the stomach was packed back into the peritoneum. The muscle was sutured back before the skin. It was ensured that the perfusion fluid was running out of the stomach. After collecting consistent basal output 0.5mg/kg histamine acid phosphate was injected into each animal intramuscularly.

b. Collection of Samples

The rate of flow of the perfusing fluid was manually adjusted such that 10mls of effluent (which contains gastric juice from the stomach) was collected at 15 minutes intervals. The first few effluents (4-5) were collected to establish a consistent basal output.

c. Titration Procedure

After each sample collection, 5 mls of the 10mls collected was measured into a cornical flask and two drops of phenolphthalein reagent was added. 0.0025M NaOH was run dropwise into the cornical flask, shaking the flask in the process until the first appearance of a pink colour. After this, the titration was stopped and the end point recorded.

d. Calculation of Acidity

The reaction between gastric acid and the titrating base can be represented by the equation below:

$NaOH + HCl \quad NaCl + H_2O$ ……………………………………………(1)

At the end-point of titration

$$N_A V_A = N_B V_B$$

i.e. $N_A = \frac{N_B . V_B}{V_A}$ ………………………………(2)

But normality (N) = Concentration (C) in gm/litre

Gm. Eq. Wt (G) i.e C = N x G

(Acid concentration is gm/litre (C) = N_A x G

Substituting for N_A in equation 2,

$$C = \frac{N_B . V_B . G}{V_A} \text{ gm/litre}$$

$$C = \frac{N_B . V_B . G}{V_A} \times 100 \text{ mg/100ml} \quad \text{……………………………………(3)}$$

But acid concentration is meq/litre – Concentration in mg/litre (m. Eq. Wt. (G)

Therefore, Acid concentration in Meq/litre

$= \underline{N_B . V_B . G}$ x 100……………………………………………(4)

V_A. G

But N_B = 1/800

Substituting for N_B in (equation 3)

Acid concentration in Meq/litre = 1/800 x V_B 1000 = 5/4 V_B.

Since the gastric effluent sample titrated =VA = 5ml.

The acid concentration titrated sample (Meq/litre) = $\frac{V_B}{2}$ ……..(5)

But the 5ml is only ½ of the total effluent sample

Therefore, total acidity for each 10ml sample = $\frac{V_B \text{ x } 2}{4}$ = $\frac{V_B}{2}$

3.5.5. Study 5: Estimation of blood cells during ulcer healing

a. Estimation of Haematological Indices

Packed Cell Volume Estimation

A plane capillary tube was filled with blood to about three-quarter its length. The end of capillary tube free of blood was sealed off with plasticine. The tube was spinned in a haematocrit centrifuge for 20 minutes at 3000 rpm. The height of the column of packed red cells was read and the result was expressed as the percentage of volume of red cells to whole column of blood.

Red Blood Cell Count

Blood was sucked into red blood cell pipette up to 0.5 mark, holding the pipette more or less horizontal during the process. Hayem's solution was drawn to the 101 mark, care was taken not to over shoot and rotating the pipette while doing so. The rubber tubing was detached, mixed thoroughly for 1minute by vigorous agitation of the pipette held between the thumb and middle

finger. The tip of pipette was brought in contact with exposed part of the counting chamber, the pipette was raised towards vertical position until diluted blood flowed under the coverslip. The counting chamber was placed on the horizontal stage of the microscope, the corpuscles were allowed to settle for 2 minutes.

Using x 40" objective, the number of corpuscles in each of 80 small squares i.e. 5 sets of small squares was counted. All corpuscles overlapping the top and left hand sides of the square were counted while those overlapping the bottom and right side were not counted to avoid counting twice.

Calculation:

Volume of diluted blood over each small square 1/4000 cumm (since depth of the chamber is 1/10mm and area is 1/400 sq.mm). Volume of diluted blood over 80 small squares 80/4000 cu.mm. Let X be the number of corpuscles lying over 80 small squares, then X corpuscles were present in 80/4000 cu.mm of diluted blood. Blood is diluted 1 to 200.

Hence 80/4000 cu.mm of the (undiluted) blood contains 4000/80 corpuscles and 1 cu.mm of the (undiluted) blood contained.

$$\frac{4000X \times 200}{80} = 10000N \text{ corpuscle}$$

White Blood Cells Count

From the blood collected, 20ul of blood was added to 0.38ml of Tuerk's solution in a test tube, to give a 1:20 dilution. After mixing, a pasture pipette was used to draw the mixture and the counting chamber was charged. The counting chamber was placed on the horizontal stage of a microscope, the corpuscles were allowed to settle for 2 minutes.

Using x 10" objective, the number of corpuscles in the four large squares was counted. All corpuscles overlapping the top and left hand sides of the square were counted while those overlapping the bottom and right side were not counted to avoid counting twice.

Calculation

N X 50cells/mm^3

Differential WBC count

A drop of blood was added to one end of a slide, the slide was quickly placed on the bench, holding it in position with the thumb and the index finger on the left hand. The narrow end of the second slide is then placed in the drop and held there until the blood has spread across it is then drawn slowly over the whole length of the first slide, being held meanwhile at an angle of 45^0. After the blood has spread, it was dried by waving the slide rapidly in air to prevent undue shrinkage of the cells.

The film was stained with Leishman stain for 10 minutes, washed in a gentle stream of water. Dried with filter paper and examine under low and high power. With a properly stained film, the red cells are pink, the cytoplasm of white cells faintly blue. The small neutrophil granules are brick red and the basophil dark blue.

A table was made headed with the different varieties of white cells. Blood cells (200) were counted, marking each as it was recognized in the appropriate column of the table. The percentage of each type present was calculated.

3.6.6: Study 6: Assessment of cellular activities during ulcer healing

a. Biochemical study (Lipid peroxidation)

Assessment of lipid peroxidation was carried out following the procedure described by Varshney and Kale (1990). It is based on the reaction of malondialdehyde (MDA) produced during lipid peroxidation with thiobarbituric acid (TBA) forming a pink coloured MDA-TBA adduct that absorbs strongly at 532nm.

The animals were sacrificed, laparatomy was perfomed and the stomach was brought out. The stomach was cut open along the greater curvature, rinsed with normal saline, the mucosa of the ulcerated area was scraped and homogenised in phosphate buffer (tissue to buffer ratio - 1:3) using homogenizer (Model SM-FSH2, Surgifield Medical, England), 0.2ml of test sample was added to 0.8ml of Tris-KCl. The solution was quenched with 0.25ml of TCA. 0.25ml of TBA was then added and the solution was then incubated for 45minutes at 80^0C. A pink coloured reaction mixture was formed. The reaction mixture was then centrifuged at 1400 rpm for 15 minutes. The absorbance of the supernatant was read at 532 nm.

Calculations:

$$\text{MDA (Units/mg protein)} = \frac{\text{Absorbance X Volume of mixture}}{\text{E532 x volume of sample x mg of protein}}$$

Where E532 = 1.56 x 10-5

b. Assessment of Index of Apoptosis - DNA Fragmentation

The gastric mucosa was scraped and homogenized by maceration in PBSE (phosphate buffer solution and EDTA in the ratio of I EDTA: 24 PBS. Stomach tissue (~0.05 g in solution) was digested in a solution containing proteinase K (5 ul) added to 1mL of digestion buffer (final

0.5 mg/ml), it was then incubated at 65^0C for 2 hours. It was mixed by vortexing, then centrifuged at 13,000 rpm for 15 min (Iwalokun *et al.*, 2001). Supernatant was transferred into new tube. Protein and cell debris was precipitated by adding 1/10 volume of sodium acetate, 3M pH 5.2 (final 0.3M), it was inverted to mix and then incubated at -20^oC for 20 minutes. After this it was centrifuged at 13,000 rpm for 20 minutes. Supernatant was then transferred into another tube. Nucleic acid was precipitated out by adding 98% ethanol (twice vol of supernatant). It was inverted to mix and incubated at -20^0C for 15 min and then centrifuged at 13,000 rpm for 15 min. Pellets were washed twice with 70% ethanol by spinning at I3,000rpm for 5mins, allowed to air dry and then re-suspended in Tris EDTA buffer (Iwalokun *et al.*, 2001).

Agarose gel (0.8%) was prepared by dissolving 0.8g of agarose powder in 100 mL of x1 TBE (prepared by 1:10 dilution of x10 TBE stock with distilled water). The gel was boiled and allowed to cool to 50^0C before adding 50 uL of 1 mg/mL ethidium bromide. After this, the ethidium bromide-stained gel was poured into the gel casting tray with comb inserted for well creation. DNA sample was loaded (8 uL of DNA sample + 2 uL Loading buffer) into each well of the gel submerged in x1 TBE (pH 8.3) buffer in the electrophoretic tank. The circuit was closed and run at 10V/cm for 45 min (Itoh *et al*., 1995).

DNA bands were visualized under UV light using a UV transilluminator. Photograph of DNA bands was taken using a digital camera. The size of DNA was extrapolated based on mobilities and sizes of the DNA markers co-electrophoresed with the sample.

3.6. Statistical Analysis

Results were expressed as Mean ± SEM, the difference between the means was determined using independent sample students t-test. The level of statistical significance was $p<0.05$.

CHAPTER FOUR

RESULTS

4.1. Study 1: Preliminary study on the efficacy of the surgical and treatment methods

4.1.2. Measurement of plasma thyroxine T_4, triiodothyronin T_3 and thyroid stimulating hormone (TSH)

After 35 days of drug treatment, radioimmunoassay study showed that thyroidectomy significantly ($p< 0.01$, 0.01) reduced the plasma level of T_3 and T_4 respectively. Thyroxine treatment (Sham + T_4) significantly ($p< 0.001$, 0.001) increased the plasma level of T_3 and T_4 respectively. Thyroidectomy also significantly ($p< 0.01$) increased the level of TSH in plasma, while thyroxine treatment significantly ($p< 0.05$) decreased the TSH level as shown in Table 4.1. There was no significant ($p> 0.05$) difference in the level of T_3, T_4 and TSH between control and thyroidectomised animals given thyroxine treatment (Table 4.1).

Table 4.1: Plasma level of thyroxine, triiodothyronine and thyroid stimulating hormone

Groups	T_3 (µg/dl)	T_4 (µg/dl)	TSH(µu/ml)
Control	3.9 ± 0.2	3.8 ± 1.0	0.96 ± 0.006
Thyroidectomised	1.0 ± 0.1xx	1.1±0.1xx	2.12 ± 0.012xx
Thyroidectomised +T_4	3.7 ± 0.4	4.3 ± 0.7	0.86 ± 0.005
Thyroxine treated (Sham + T_4)	13.4 ± 1.05xxx	11.5 ± 1.1xxx	0.620 ± 0.080x

N= 5, value are mean ± SEM

x= significant compared with control group at $P < 0.05$

xx= significant compared with control group at $P < 0.01$

xxx = significant compared with control group at $P < 0.001$

4.1.2. Measurement of total body weight

Result showed that after 35 days of drug treatment, all groups of animal had weight gain. Control animals had 22.7 ± 1.7% increase in total body weight. Thyroidectomy significantly ($p < 0.05$) increased the percentage weight gain by 29.1 ± 1.8% compared with control, thyroxine treatment significantly ($p < 0.05$) decreased the percentage weight gain by 18.4 ± 1.2% as compared with control, while there was no significant ($p > 0.05$) difference in the weight gain between thyroidectomised animals given thyroxine supplementation and control animals (Figure 4.1.).

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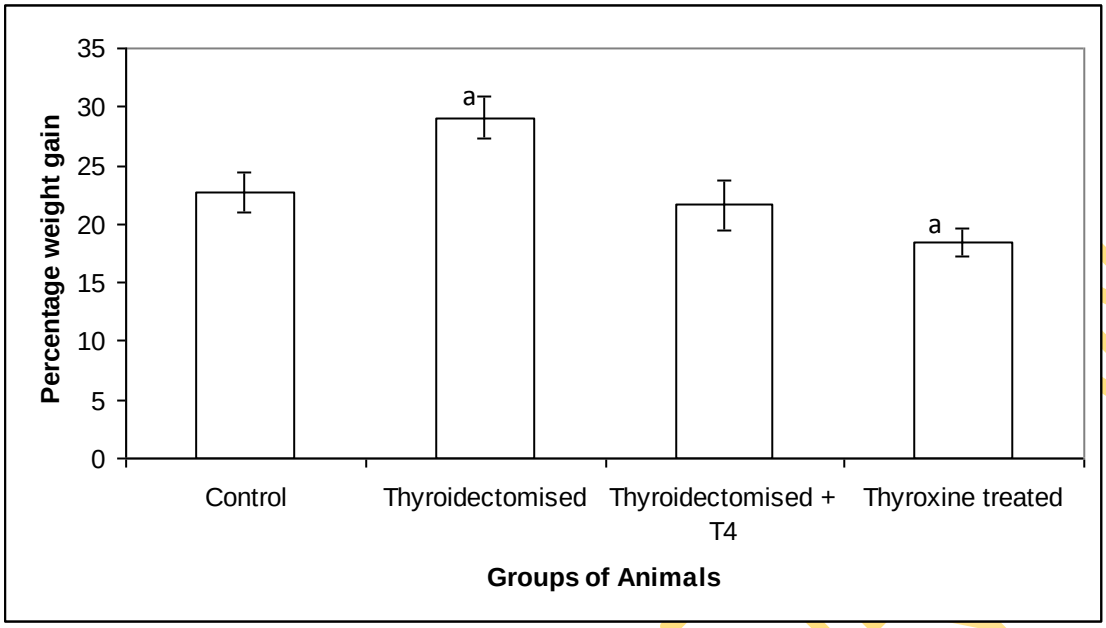

Fig 4:1 Percentage weight change in animals groups after 35 days post surgery and of drug treatment.

N= 15, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

4.2. Study 2 – Measurement of ulcer dimensions

4.2.1. Measurement of Ulcer Area by Planimetry

On day 3 after ulcer induction, ulcer area was significantly ($p< 0.01, 0.01$) higher in thyroxine treated rats ($13.5 \pm 0.2mm^2$) as compared with that in control animals ($8.0 \pm 0.4\ mm^2$) and thyroidectomised animals ($8.5 \pm 0.4mm^2$) respectively. However by day7, thyroxine treatment significantly ($p< 0.0001, 0.0001$) reduced the ulcer area in both thyroxine treated (sham + T_4) and thyroidectomised + T_4, ulcer area was also significantly ($p < 0.001$) reduced in control animals, but the reduction was not significant ($p> 0.05$) in thyroidectomised animals. On day 10 post ulcer induction, ulcer area was significantly ($p < 0.05$) reduced in all groups of animals (Table 4.2).

Figure 4.2 showed that on day 7, ulcer area was reduced by $40.2 \pm 2.1\%$ in control animals. Thyroidectomy significantly ($p< 0.01$) reduced the percentage area healed ($17.8 \pm 4.5\%$), when compared with control animals, while there was no significant ($p> 0.05$) difference between reductions in thyroxine treated and control animals. On day 10, ulcer area was reduced by $57.6 \pm 1.3\%$ in control animals, thyroidectomy significantly ($p< 0.01$) reduced the percentage area healed ($32.9 \pm 2.5\%$), while thyroxine treatment significantly ($p< 0.05$) increased the percentage area healed ($62.3 \pm 1.4\%$) as compared with control. There was no significant ($p> 0.05$) difference in the percentage area of ulcer healed between control and thyroidectomised animal given thyroxine supplementation by day 10.

The rate of ulcer healing in control animals was $0.5 \pm 0.0\ mm^2$/day on day 7. Thyroidectomy significantly ($p< 0.01$) reduced rate of healing ($0.2 \pm 0.1mm^2$/day) while thyroxine treatment significantly ($p < 0.001$) increased the healing rate ($0.8 \pm 0.0mm^2$/day). By day 10 after ulcer induction, the rate of healing in control animals was $0.5 \pm 0.1\ mm^2$/day,

thyroidectomy significantly ($p < 0.01$) delayed the healing rate ($0.3 \pm 0.1 mm^2$/day) while thyroxine treatment significantly ($p< 0.001$) accelerated the rate of healing ($0.8 \pm 0.1 mm^2$/day) (Figure 4.3).

Table 4.2: Ulcer area in thyroxine treated and thyroidectomised animals after ulcer induction

Groups	Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)
Control	8.0 ± 0.4	4.8 ± 0.4xx	3.4 ± 0.2xxx
Thyroidectomised	8.5 ± 0.4	7.0 ± 0.5	5.7 ± 0.4x
Thyroidectomised +T_4	13.7 ± 0.2aa	7.8 ± 0.4xxx	6.1 ± 0.3xxx
Thyroxine treated (Sham + T_4)	13.5 ± 0.2aa	7.6 ± 0.7xxx	5.1 ± 0.2xxx

N= 5, value are mean ± SEM

aa= significant compared with control on day 3 at $p < 0.01$

x= significant compared with animals in same group on day 3 at $p < 0.01$

xx= significant compared with animals in same group on day 3 at $p < 0.001$

xxx= significant compared with animals in same group on day 3 at $p < 0.0001$

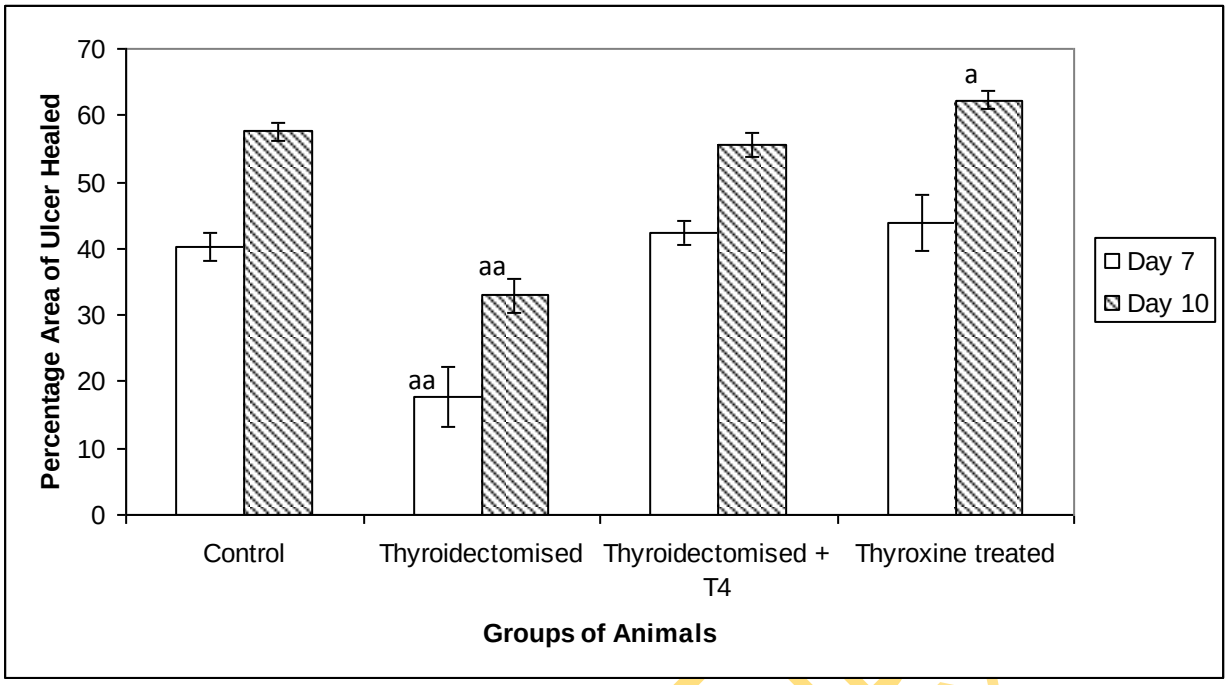

Figure 4.2: Percentage reduction in ulcer area in thyroxine treated and thyroidectomised animals after ulcer induction as compared with day 3.

N= 5, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

aa= significant compared with animals in Control group on same day at $p < 0.01$

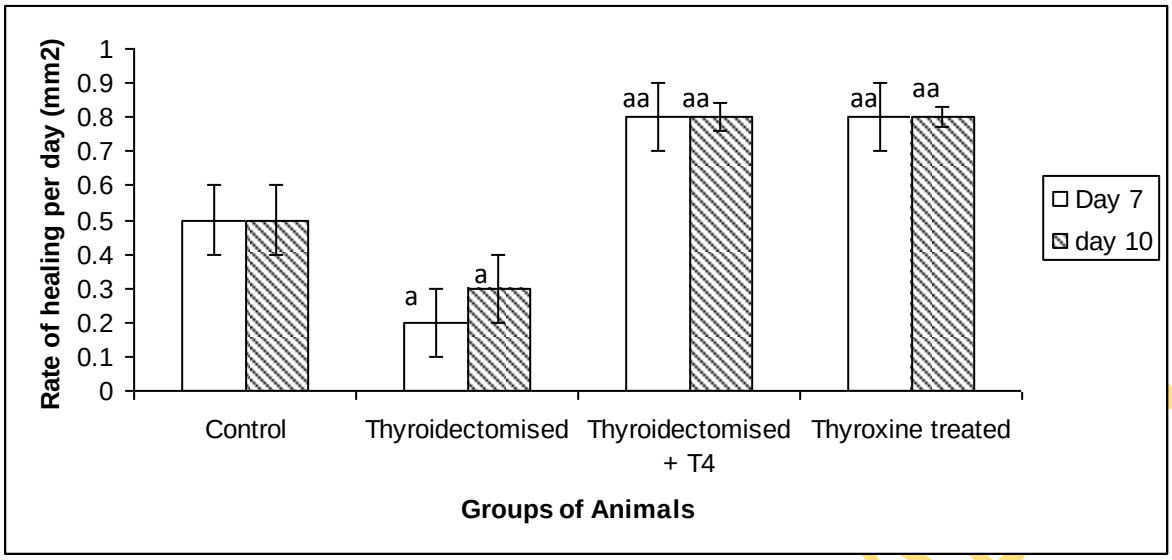

Figure 4.3: Rate of healing of gastric ulcer in thyroidectomised and thyroxine treated rats

N= 5, value are mean ± SEM.

a= significant compared with animals in control group on same day at $p < 0.01$

aa= significant compared with animals in control group on same day at $p < 0.001$

4.2.2. Histomorphometry

Histomorphometry showed that ulcer width was significantly($p< 0.05, 0.01, 0.01$) higher in thyroidectomised (126.0 ± 4.0 µm), thyroidectomised animals given thyroxine supplementation (135.0 ± 6.3 µm) and thyroxine treated rats (176.0 ± 1.9 µm) as compared with ulcer width in control animals (106.2 ± 5.3 µm) on day 3 post ulcer induction (Table 4.3). However, there was significant ($p< 0.05$) reduction in ulcer width on days 7 and 10 in all groups of animals. Figure 4.4 showed that on day 7, thyroxine treatment significantly ($p< 0.01$) decreased the ulcer width by 58.5 ± 3.7% as compared with control animals (21.3 ± 4.6%), while the reduction in ulcer width in thyroidectomised animals (13.6 ± 1.7%) was significantly ($p< 0.05$) low as compared with control. By day 10, likewise as on day 7, thyroxine treatment significantly ($p< 0.01$) decreased the ulcer width by 69.3 ± 1.5% as compared with control (40.5 ± 2.2%). The reduction was significantly ($p< 0.05$) less in hypothyroid animals (34.1 ± 0.5%) as compared with control animals. There was also a significantly higher reduction of ulcer width in thyroidectomised animals given thyroxine supplementation on day 7 and 10 ($p< 0.05$) than that in control animals (Figure 4.4).

Table 4.4 showed that the ulcer depth in control animals was 34.0 ± 1.9 µm on day 3. The ulcer depth was significantly ($p< 0.05$) higher in thyroxine treated rats (42.0 ± 1.2 µm). By day10, ulcer depth significantly ($P< 0.05$) decreased in all groups of animals. However on day 7, thyroxine treatment significantly ($p< 0.05$) reduced the ulcer depth by 47.0 ± 1.2% as compared with control (38.0 ± 2.1%), there was also significant ($p< 0.05$) reduction in the ulcer depth of thyroidectomised animals given thyroxine treatment as compared with control, but there was no significant difference in the reduction of ulcer depth between control and thyroidectomised animals (Figure 4.5). By day 10, thyroxine treatment also significantly reduced the ulcer depth

by 65.7 ± 1.4% ($p< 0.01$) as compared with control (53.9 ± 1.6%). The reduction in ulcer depth was significantly ($p< 0.01$) higher in thyroidectomised animal given thyroxine supplementation than in control, while the reduction was significantly ($p< 0.05$) less in hypothyroid animals (35.6 ± 7.5%) as compared control (Figure 4.5).

Knowing the ulcer width and depth, the area of mucosa eroded was calculated. The pattern of healing is as observed in ulcer depth healing (Table 4.5). On day 10, thyroxine treatment significantly ($p< 0.001$) reduced the mucosa area eroded (89.4 ± 0.9%) as compared with control (72.4 ± 1.8%), while the reduction in area of mucosa eroded was significantly ($P< 0.05$) less in thyroidectomised animals (59.1 ± 3.9%). Thyroidectomy + T_4 also significantly ($P< 0.01$) reduced the ulcer area eroded on day 10 by 81.9 ± 1.4% as compared with control (Figure 4.6).

Table 4.3: Ulcer width in thyroxine treated and thyroidectomised animals after ulcer induction

Groups	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)
Control	106.2 ± 5.3	83.0 ± 4.3xx	62.8 ± 1.2xxx
Thyroidectomised	126.0 ± 4.0a	109.0 ± 4.6x	83.0 ± 2.5xx
Thyroidectomised + T_4	135.0 ± 6.3aa	84.0 ± 1.9xxx	69.0 ± 1.9xxx
Thyroxine treated (Sham + T_4)	176.0 ± 1.9aa	73.0 ± 6.2 xxx	54.0 ± 2.4xxx

N= 5, value are mean ± SEM

a= significant compared with control on day 3 at $p < 0.05$

aa= significant compared with control on day 3 at $p < 0.01$

x= significant compared with animals in same group on day 3 at $p < 0.05$

xx= significant compared with animals in same group on day 3 at $p < 0.01$

xxx= significant compared with animals in same group on day 3 at $p < 0.001$

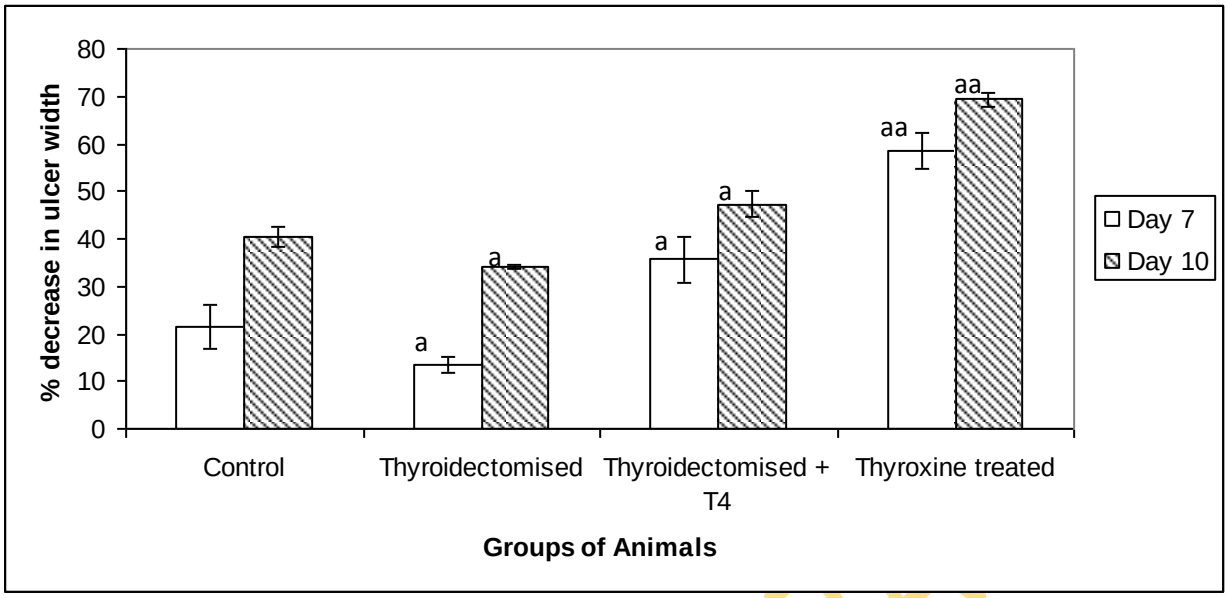

Figure 4.4: Percentage decrease in ulcer width in thyroxine treated and thyroidectomised animals after ulcer induction as compared with day 3.

N= 5, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

aa= significant compared with animals in Control group on same day at $p < 0.01$

Table 4.4: Ulcer depth in thyroxine treated and thyroidectomised animals after ulcer induction

Groups	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)
Control	34.0 ± 1.9	21.0 ± 1.0xx	15.6± 0.6xx
Thyroidectomised	40.0 ± 4.4	27.0 ± 2.5x	24.4 ± 0.2xx
Thyroidectomised + T_4	54.0 ± 2.4aa	28.0 ± 2.0xxx	18.8 ± 0.5xxx
Thyroxine treated (Sham + T_4)	42.0 ± 1.2a	22.3 ± 1.1xxx	14.4 ± 0.6xxx

N= 5, value are mean ± SEM

a= significant compared with control on day 3 at $p < 0.05$

aa= significant compared with control on day 3 at $p < 0.01$

x= significant compared with animals in same group on day 3 at $p < 0.05$

xx= significant compared with animals in same group on day 3 at $p < 0.01$

xxx= significant compared with animals in same group on day 3 at $p < 0.001$

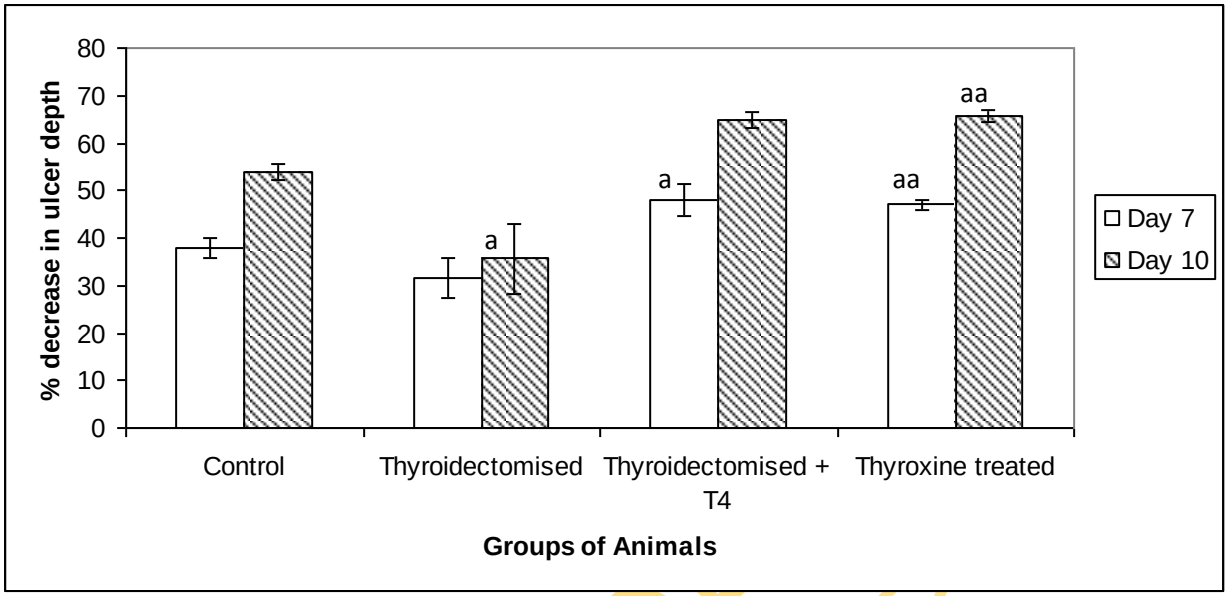

Figure 4.5: Percentage decrease in ulcer depth in thyroxine treated and thyroidectomised animals after ulcer induction compared with day 3.

N= 5, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

aa= significant compared with animals in Control group on same day at $p < 0.01$

Table 4.5: Area eroded in thyroxine treated and thyroidectomised animals after ulcer induction

Groups	Day 3 (μm^2)	Day 7 (μm^2)	Day 10 (μm^2)
Control	3648.0 ± 383.6	1740 ± 107.0xx	981.0 ± 49.4xx
Thyroidectomised	5080.0 ± 493.3	2945.0 ± 296.6xx	2001.0 ± 65.8xx
Thyroidectomised + T4	7300.0 ± 511.6aa	2345.0 ± 145.0xx	1300 ± 64.9xxx
Thyroxine treated (Sham + T_4)	7390.0 ± 212.2aa	1640.0 ± 191.6xx	780.0 ± 55.8xxx

N= 5, value are mean ± SEM

a= significant compared with control on day 3 at $p < 0.05$

aa= significant compared with control on day 3 at $p < 0.01$

x= significant compared with animals in same group on day 3 at $p < 0.05$

xx= significant compared with animals in same group on day 3 at $p < 0.01$

xxx= significant compared with animals in same group on day 3 at $p < 0.001$

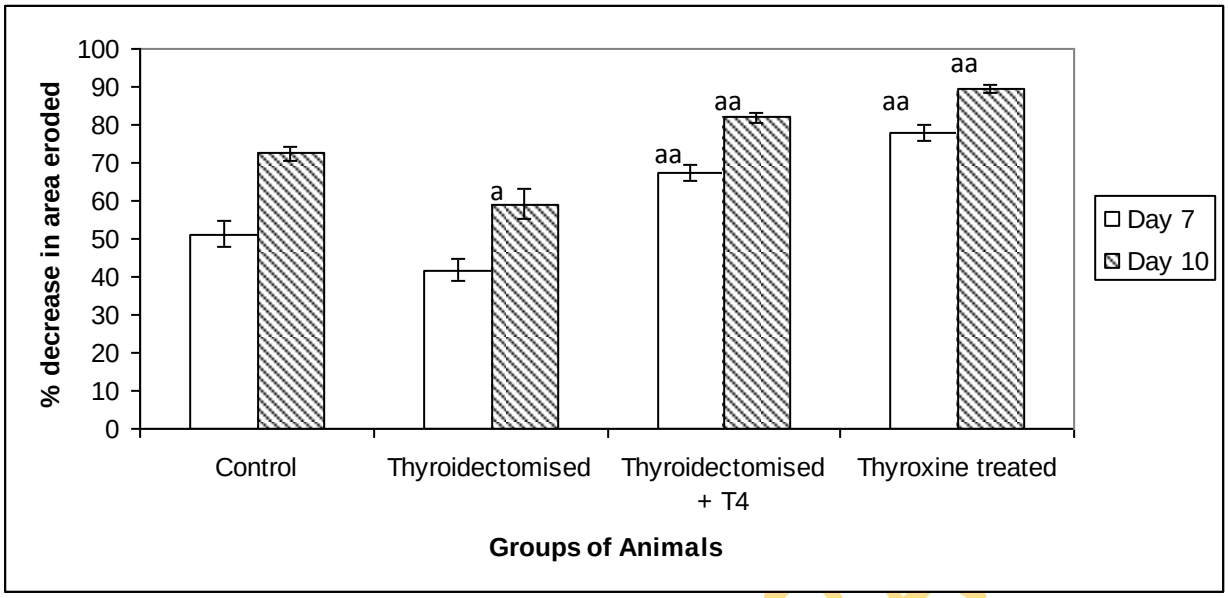

Figure 4.6: Percentage decrease in area of mucosa eroded in thyroxine treated and thyroidectomised animals after ulcer induction as compared with day 3.

N= 5, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

aa= significant compared with animals in Control group on same day at $p < 0.01$

aaa= significant compared with animals in Control group on same day at $p < 0.001$

4.5. Study 3 – Assessment of the characteristics and quality of healing

4.5.1. Histology Study

By day 3 after ulcer induction, ulcer was observed in the gastric mucosa of all groups of animal. In control animals, histology showed that there were abundant neutrophils (both intact and degenerate) with some macrophages with hemosiderin, congested blood vessels, swollen epithelial cells at the margin, hemorrhage and neovascularization at the serosal surface (Figure 4.7a, 4.7b). Inflammatory cells were observed to be more abundant in thyroidectomised animals (Figure 4.8a, 4.8b, 4.8c), while inflammatory cells were observed to be less in thyroxine treated animals as compared with thyroidectomised animals (Figure 4.10a, 4.10b, 4.10c). Neutophils were also abundant in ulcer bed of thyroidectomised animals given thyroxine supplementation (Figure 4.9a, 4.9b, 4.9c).

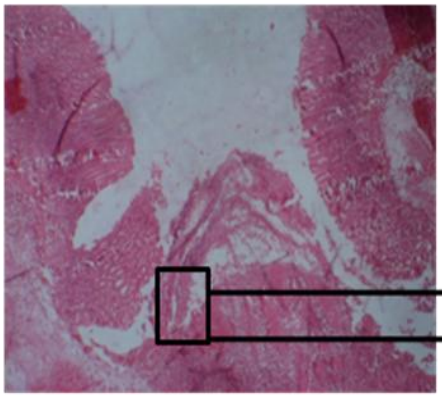

Fig 4.7a: Ulcerated gastric mucosa on Day 3 after ulcer induction in Control Animals X40

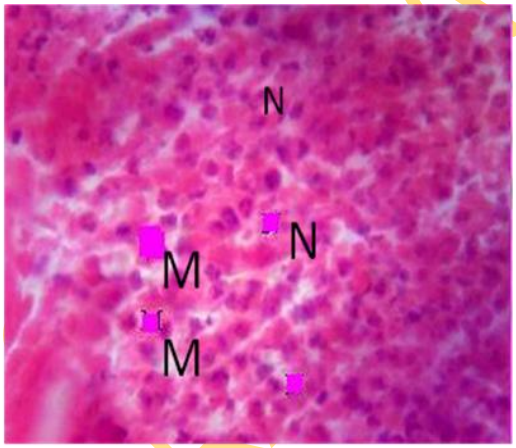

Fig 4.7b. Day 3 after ulcer induction in Control Animals X 400 N = Neutrophil, M = Macrophage

The abundant neutrophils and macrophages seen in the ulcer bed figure 4.7a and b reveal that ulcer in the control animals is in the inflammatory phase of healing on day 3.

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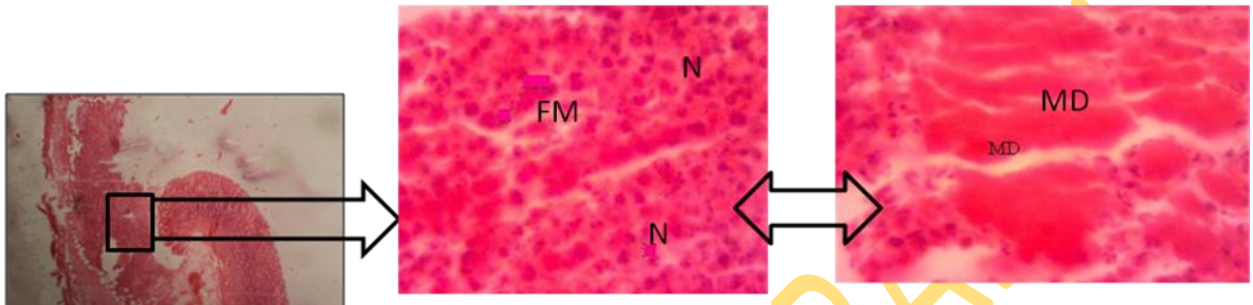

Fig 4.8a: Ulcerated gastric mucosa on Day 3 after ulcer induction in thyroidectomised Animals X40

Fig 4.8b: Day 3 after ulcer induction in thyroidectomised Animals X400 N = Neutrophil, FM = Fibrin Mesh

Fig 4.8c: Day 3 after ulcer induction in thyroidectomised Animals X400 MD = Muscle Degeneration.

The abundant neutrophils and macrophages seen in the the ulcer bed figure 4.8a, b and c reveal that ulcer in the thyroidectomised animals was in the inflammatory phase of healing on day 3. The muscle degeneration shows that there is damage in the muscularis mucosa.

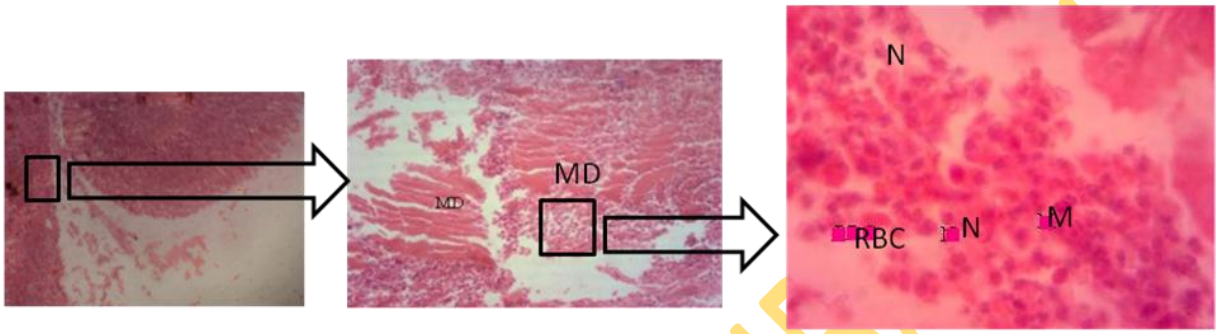

Fig. 4.9a: Ulcerated gastric mucosa on Day 3 after ulcer induction in Thyroidectomised + T4 Animals X40

Fig 4.9b: Day 3 after ulcer induction in Thyroidectomised + T4 Animals X100
MD = Muscle Degeneration

Fig. 4.9c: Day 3 after ulcer induction in Thyroidectomised + T4 Animals X400
N = Neutrophil, M = Macrophage, RBC = Red Blood Cells

The abundant neutrophils and macrophages seen in the the ulcer bed figure 4.9a, b and c reveal that ulcer in the thyroidectomised animals given thyroxine supplementation was in the inflammatory phase of healing on day 3. The muscle degeneration shows that there is damage in the muscularis mucosa.

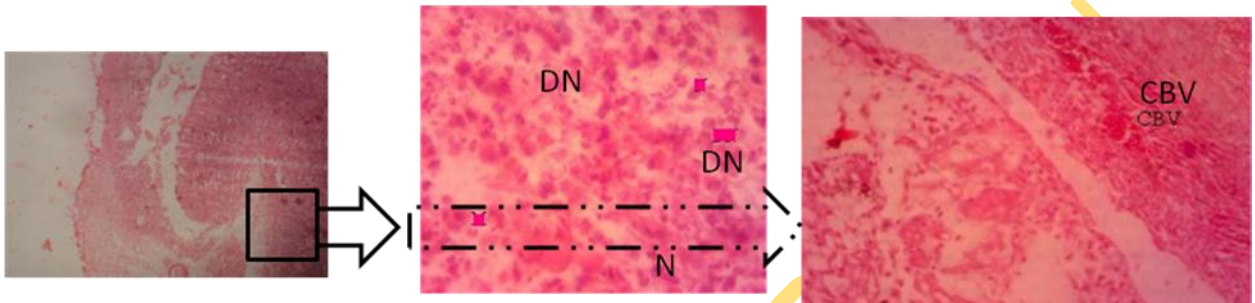

Fig. 4.10a: Ulcerated gastric mucosa on day 3 after ulcer induction in thyroxine treated animals X40

Fig. 4.10b: Day 3 after ulcer induction in thyroxine treated animals X400
N = Neutrophil, DN = Degenerate neutrophil

Fig. 4.10c: Day 3 after ulcer induction in thyroxine treated animals X100
CBV = Congested Blood Vessels

The abundant neutrophils and macrophages seen in the the ulcer bed figure 4.10a, b and c reveal that ulcer in the thyroxine treated animals was in the inflammatory phase of healing on day 3. The degenerate neutrophils seen reveal that inflammatory cells are getting eliminated, giving way to the proliferative phase of healing.

On day 7, neutrophils and macrophages were still present in ulcer bed of control animals, but not as much as on day 3, fibroblasts were young with plump and large nucleus with few plasma cells, fibrous connective tissues were haphazard (Figure 4.11a, 4.11b, 4.11c). In thyroidectomised animals by day 7, there was purulent exudate, there was still massive neutrophils infiltrating the muscularis mucosa, abundant macrophages, fibrin trapping lymphocytic cell, abundant lymphocytic cells, few plasma cells and gut associated lymphoid tissue (nodular) with few fibroblasts (Figure 4.12a, 4.12b). In thyroxine treated animals, few neutrophils were observed in ulcer bed, there were hyperplastic epithelial cells, neovascularization at the epithelial surface, fibrous connective tissues present, but not well organized, blood vessels were forming (Figure 4.14a, 4.14b).

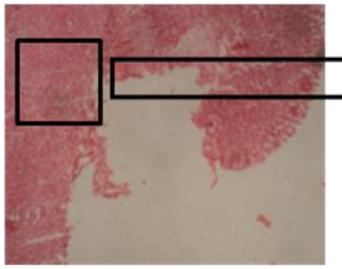

Fig. 4. 11a: Ulcerated gastric mucosa on Day 7 after ulcer induction in Control Animals X 40

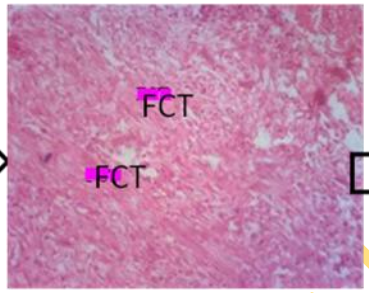

Fig. 4.11b: Day 7 after ulcer induction in Control Animals X 100
FCT = Fibrous Connective Tissue

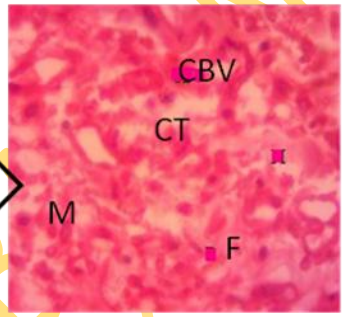

Fig4.11c: Day 7 after ulcer induction in Control Animals X 400
F = Fibroblast, CBV =Congested blood vessels, CT = Connective Tissue, M = Macrophage

The few neutrophils and macrophages seen in Fig 4.11a, b, c, with fibroblast and connective tissue reveal that proliferative phase has began, while some inflammatory cells are yet to be cleared from ulcer site. The congested blood vessel might be as a result of angiogenesis which occur during the proliferative phase.

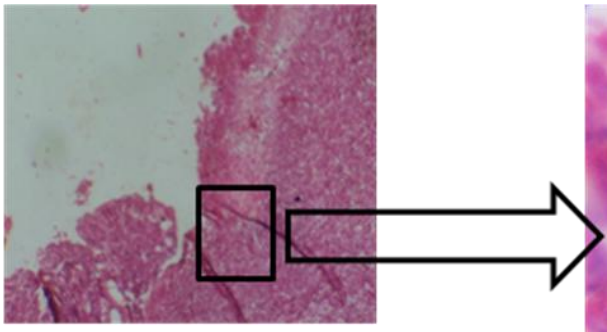

Fig. 4.12a: Ulcerated gastric mucosa on Day 7 after ulcer induction in thyroidectomised animals X 40

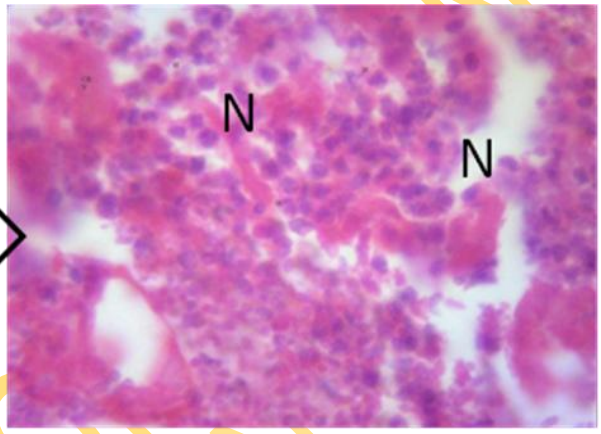

Fig. 4.12b: Day 7 after ulcer induction in thyroidectomised animals X 400. N = Neutrophil

The abundant neutrophils present at the ulcer bed in Fig 4.12a and b above reveal that healing was still at the inflammatory phase in thyroidectomised animals on day 7.

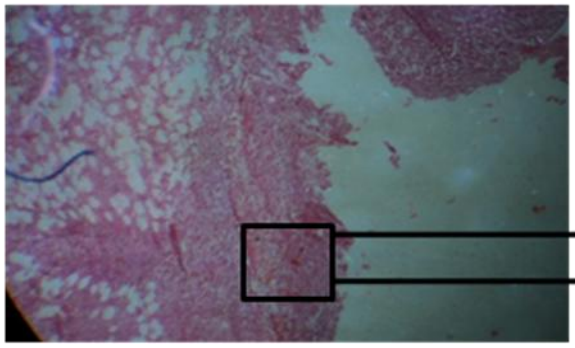

Fig. 4.13a: Ulcerated gastric mucosa on Day 7 after ulcer induction in Thyroidectomised + T4 Animals X 40

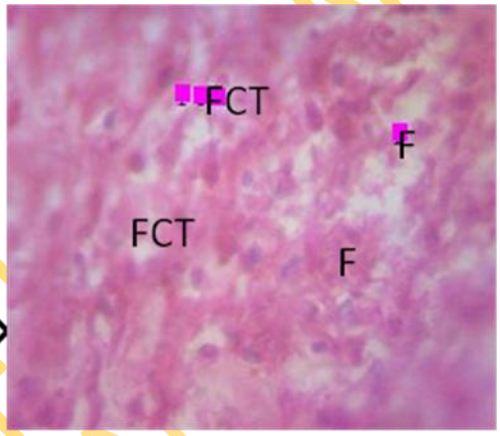

Fig. 4.13b: Day 7 after ulcer induction in Thyroidectomised + T4 Animals X 400
F = Fibroblast, FCT = Fibrous Connective Tissue

The fibroblast and connective tissue in Fig 4.13a and b above reveal that healing is at the proliferative phase in thyroidectomised given thyroxine supplementation on day 7.

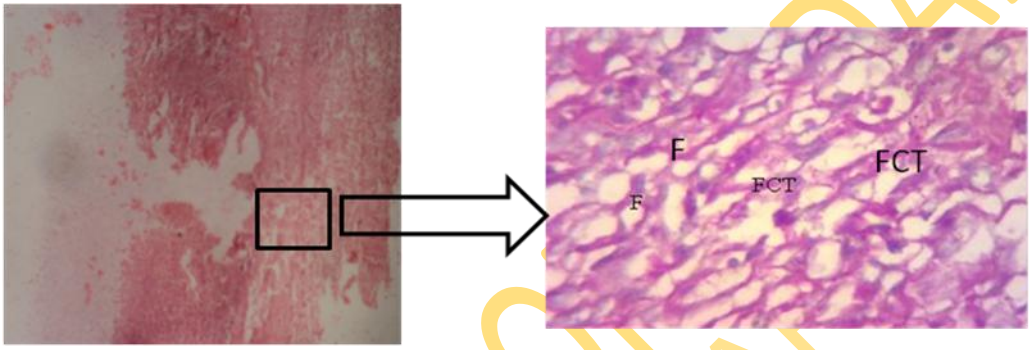

Fig. 4.14a: Ulcerated gastric mucosa on Day 7 after ulcer induction in thyroxine treated animals X 40

Fig. 4.14b: Day 7 after ulcer induction in thyroxine treated animals X 400. F = Fibroblast, FCT =Fibrous Connective Tissue

The fibroblast and connective tissue in Fig 4.14a and b above reveal that healing is at the proliferative phase in thyroxine treated animals on day 7.

By day 10, Figure 4.15a and 4.15b showed that inflammatory cells were still present although not massive in control animals. Fibroblasts were present, but not on the mucosal area, debris had not been cleared. In thyroidectomised animals, there were still abundant inflammatory cells in the gastric mucosa, however submucosa layer filled with fibrous connective tissues (Figure 4.16a, 4.16b, 4.16c). Result showed macrophages, fibroblasts with fibrous connective tissues in thyroidectomised animals given thyroxine supplementation (Fig 4.17a, b and c), while figure 4.18a, 4.18b and 4.18c showed that in thyroxine treated animals, there was marked regeneration of epithelial cells, focus of degenerate neutrophils at the epithelial junction with the gastric lumen, hyperplastic cells, and abundant fibroblasts.

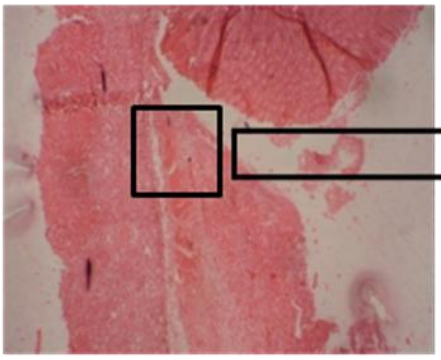

Fig. 4:15a: Ulcerated gastric mucosa on Day 10 after ulcer induction in control Animals X 40

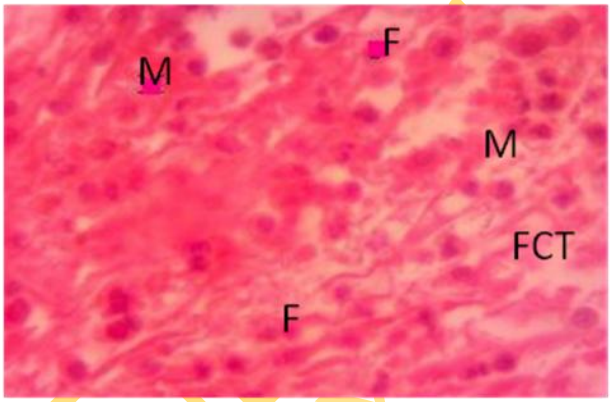

Fig. 4.15b: Day 10 after ulcer induction in control Animals X 400
M = Macrophage, F = Fibroblast, FCT = Fibrous connective tissue

The connective tissue lay down at the ulcer bed (Fig 4.15a and b) reveals that the wound site is getting filled up with granulation tissue; this implies that healing is taking place in this animal.

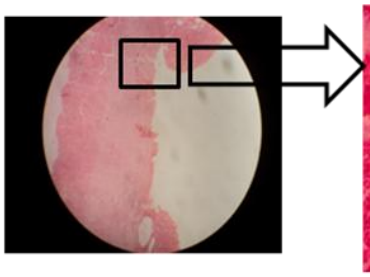

Fig. 4.16a: Ulcerated gastric mucosa on Day 10 after ulcer induction in thyroidectomised animals X 40

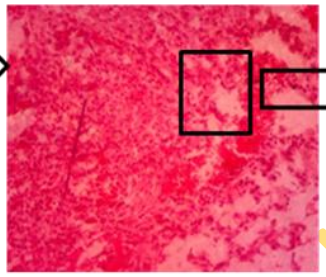

Fig. 4.16b: Day 10 after ulcer induction in thyroidectomised animals X 100

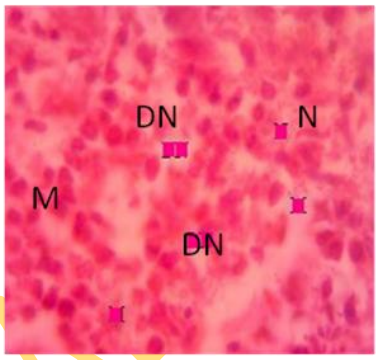

Fig. 4.16c: Day 10 after ulcer induction in thyroidectomised animals X 400. M = Macrophage, N = Neutrophil, DN = Degenerate Neutrophil

The slide above (Fig 4.16a, b and c) reveal that presence of connective tissues, and degenerate neutrophils and macrophage. This implies that proliferative phase of healing was beginning to set in.

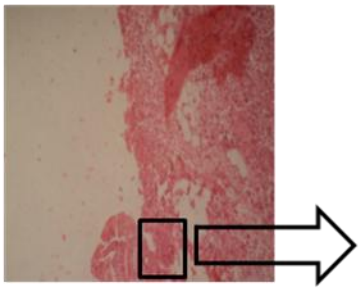

Fig. 4.17a: Ulcerated gastric mucosa on Day 10 after ulcer induction in Thyroidectomised + T4 Animals X 40

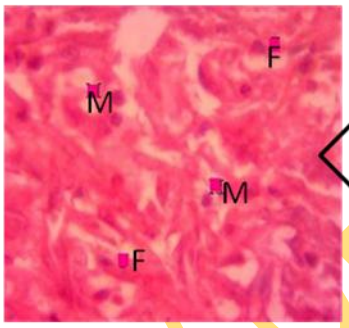

Fig. 4. 17b: Day 10 after ulcer induction in Thyroidectomised + T4 Animals X 400. F = Fibroblast, M = Macrophage

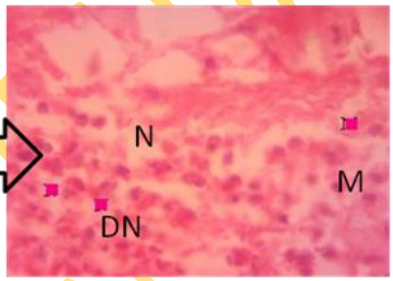

Fig. 4.17c: Day 10 after ulcer induction in Thyroidectomised + T4 Animals X 400
N = Neutrophil, M = Macrophage, DN = Degenerate Neutrophil

Fig 4.17a, b and c shows fibroblast, connective tissues with few degerate neutrophils and macrophages. This reveals that the ulcer site is being filled up with granulation tissue, the healing was still at the proliferative phase.

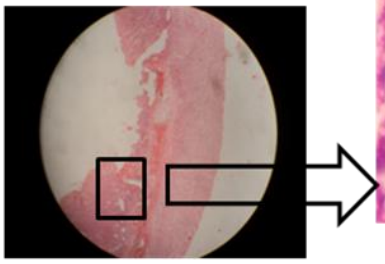

Fig.4. 18a: Ulcerated gastric mucosa on Day 10 after ulcer induction in thyroxine treated animals X 40

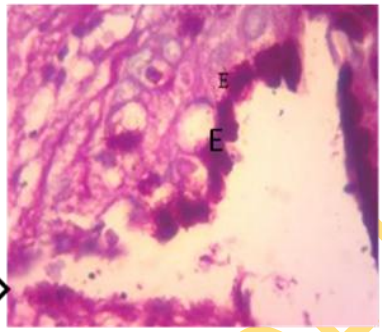

Fig. 4.18b: Day 10 after ulcer induction in thyroxine treated animals X 400
E = Epithelial Cell

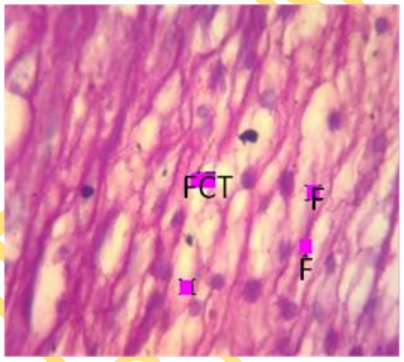

Fig. 4.18c: Day 10 after ulcer induction in thyroxine treated animals X 400
M = Macrophage

Epithelial cell has started to migrate in the ulcer site to cover up the wound. The phases of healing are observed to be faster in this group than any other group. Connective tissue are also well laid down in the wound site in the thyroxine treated rats on day 10 (Fig. 4.18a, b and c).

4.6. Study 4 - Measurement of gastric acid secretion during ulcer healing

4.4.1. Basal gastric acid output during healing

On day 3, gastric acid secretion was 4.0 ± 0.00 Meq/L (x1000) in control animals. Thyroxine treatment significantly ($p< 0.01$) increased basal gastric acid secretion (8.4 ± 0.0 Meq/L [x1000]) while thyroidectomy significantly ($p< 0.05$) reduced acid secretion (3.0 ± 0.00 Meq/L [x1000]) (Table 4.6) Basal acid secretion was also significantly ($p< 0.05$) higher in thyroidectomised animals given thyroxine supplementation than in control on day 3. As healing progressed on day 7 and 10 respectively, all groups of animals had significantly ($p< 0.05$) decreased gastric acid secretion. However, Figure 4.19 showed that by day 7, thyroidectomy and thyroxine treatment significantly($p< 0.01, 0.05$) reduced gastric acid secretion by 49.4 ± 1.2% and 48.7 ± 4.2% respectively as compared with the reduction in control animals (34.7 ± 2.2%). By day 10, gastric acid secretion had reduced by 65.0 ± 0.0% in control animals. Thyroxine treatment significantly ($p< 0.01$) reduced the acid secretion by 77.4 ± 2.6%, while the decrease in thyroidectomised animals (51.5 ±3.3%) was significantly ($p< 0.01$) lower as compared with control animals. There was however no significant ($p> 0.05$) difference in the reduction in acid secretion between contol animal and thyroidectomised given thyroxine supplementation by day 7 and 10.

Table 4.6: Basal gastric acid output after ulcer induction in thyroxine treated and thyroidectomised rats

Groups	Day 3 (Meq/L x 1000)	Day 7 (Meq/L x 1000)	Day 10 (Meq/L x 1000)
Control	4.0 ± 0.00	2.6 ± 0.1xx	1.4± 0.0xx
Thyroidectomised	3.0 ± 0.00a	1.5 ± 0.1aaxx	1.4 ± 0.0xxx
Thyroidectomised + T4	4.9 ± 0.00a	3.0 ± 0.2xx	1.5 ± 0.1xx
Thyroxine treated (Sham + T4)	8.4 ± 0.0aabb	4.0 ± 0.1axx	1.92 ± 0.00xxx

N= 5, value are mean ± SEM

a= significant compared with control on day 3 at $p < 0.05$

aa= significant compared with control on day 3 at $p < 0.01$

bb= significant compared Hypothyroid on day 3 at $p < 0.01$

x= significant compared with animals in same group on day 3 at $p < 0.05$

xx= significant compared with animals in same group on day 3 at $p < 0.01$

xxx= significant compared with animals in same group on day 3 at $p < 0.001$

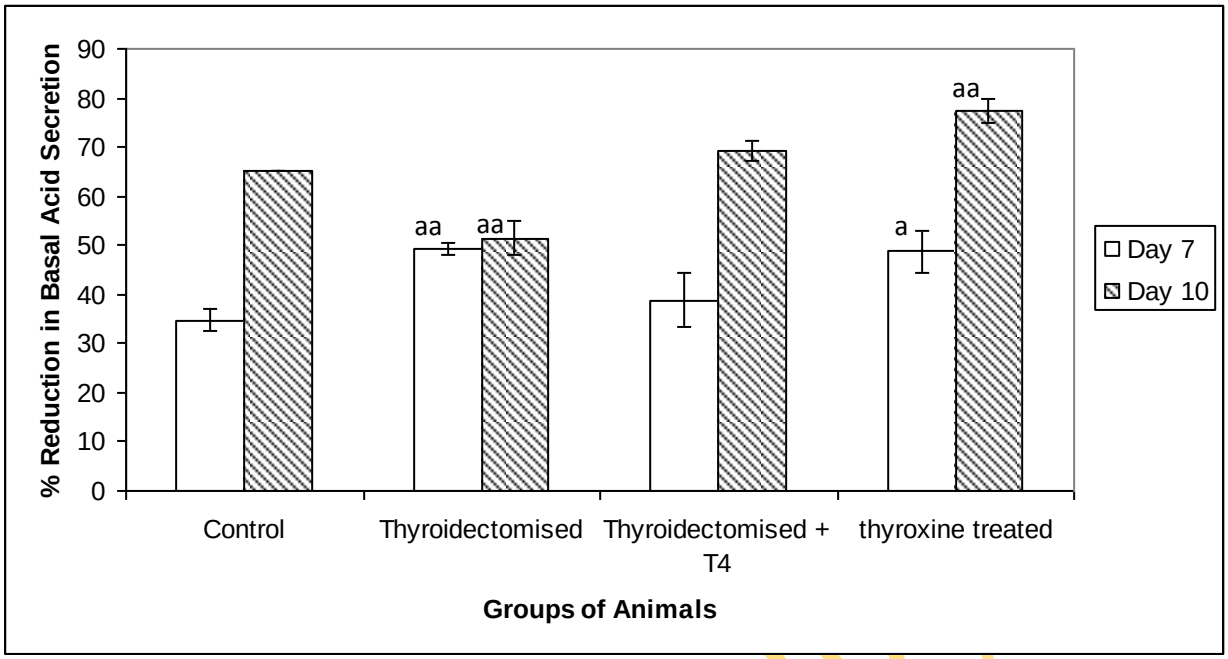

Figure 4.19: Percentage reduction in basal acid secretion after ulcer induction in thyroxine treated and thyroidectomised rats

N= 5, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

aa= significant compared with animals in Control group on same day at $p < 0.01$

4.4.2. Histamine Stimulated Gastric Acid Output during Healing

Table 4.7 showed that thyroidectomy significantly ($p< 0.05$) reduced peak histamine stimulated gastric output (8.5 ± 0.3 Meq/L [x1000]), while thyroxine treatment significantly ($p< 0.001$) increased acid output (21.0 ± 0.6 Meq/L [x1000]) as compared with control (9.8 ± 0.3 Meq/L [x1000]) by day 3. On day 7 and 10, all groups of animals had a significant ($p< 0.05$) decrease in histamine stimulated acid output. However, Figure 4.20 showed that by day 7 after ulcer induction, the percentage reduction in gastric acid secretion was significantly ($p< 0.01$) higher in thyroxine treated animals (36.6 ± 5.2%) than in control (17.9 ± 8.0%). By day 10, the percentage decrease in acid secretion in thyroidectomised animals (19.6 ± 3.4%) was significantly ($p< 0.05$) less than in control (30.4 ± 1.9), while percentage reduction in acid secretion was significantly ($p<0.01$) higher in thyroxine treated animals (48.3 ± 3.4%). There is however no significant ($p> 0.05$) difference in the reduction of stimulated acid output between control and thyroidectomised animals given thyroxine treatment (Figure 4.20).

Table 4.7: Peak histamine stimulated gastric acid output after ulcer induction in thyroxine treated and thyroidectomised rats

Groups	Day 3 (Meq/L x 1000)	Day 7 (Meq/L x 1000)	Day 10 (Meq/L x 1000)
Control	9.8 ± 0.3	7.1± 0.8x	6.8 ± 0.0xx
Thyroidectomised	8.5 ± 0.3a	7.08 ± 0.3x	6.8 ± 0.6x
Thyroidectomised + T4	10.5 ± 0.6	8.2 ± 0.4x	7.4 ± 0.5x
Thyroxine treated (Sham + T4)	21.0 ± 0.6aab	13.2 ± 0.7abxx	10.8 ± 0.56abxx

N= 5, value are mean ± SEM

a= significant compared with control on day 3 at $p < 0.05$

aa= significant compared with control on day 3 at $p < 0.01$

bb= significant compared thyroidectomised on day 3 at $p < 0.01$

x= significant compared with animals in same group on day 3 at $p < 0.05$

xx= significant compared with animals in same group on day 3 at $p < 0.01$

xxx= significant compared with animals in same group on day 3 at $p < 0.001$

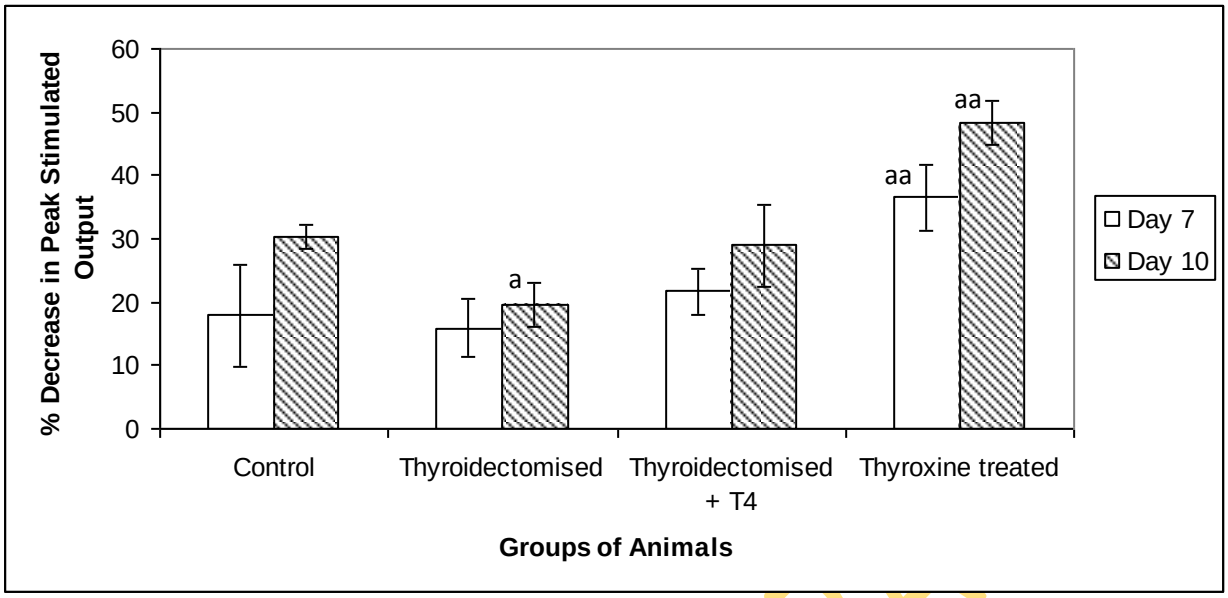

Figure 4.20: Percentage decrease in peak histamine stimulated gastric acid output after ulcer induction.

N= 5, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

aa= significant compared with animals in Control group on same day at $p < 0.01$

4.5. Estimation of blood cells during ulcer healing

4.5.1. Effect of thyroidectomy and thyroxine treatment on Packed Cell Volume (PCV) after Ulcer Induction

Result showed that the control group PCV was 40.4 ± 1.0% by day 3. There was no significant change on day 7, but by day 10, PCV (50.2 ± 0.9%) significantly ($P< 0.001$; 0.001) increased compared with value on days 7 and 3 respectively. Thyroxine treatment significantly ($p< 0.01$, 0.001) increased packed cell volume by day 7 (44.2 ± 0.6%) and 10 (47.0 ± 0.5%) compared with the value on day 3 (38.0 ± 1.6%). Thyroidectomy significantly ($P< 0.05$) increased PCV only on day 10 (47.4 ± 0.4%) compared with value on day 3 (43.2 ± 1.2%). Thyroxine replacement therapy also increased PCV count as in the thyroxine treated animal; packed cell volume in this group was significantly ($P< 0.05$, 0.01) increased on days 7 and 10 respectively (Figure 4.21).

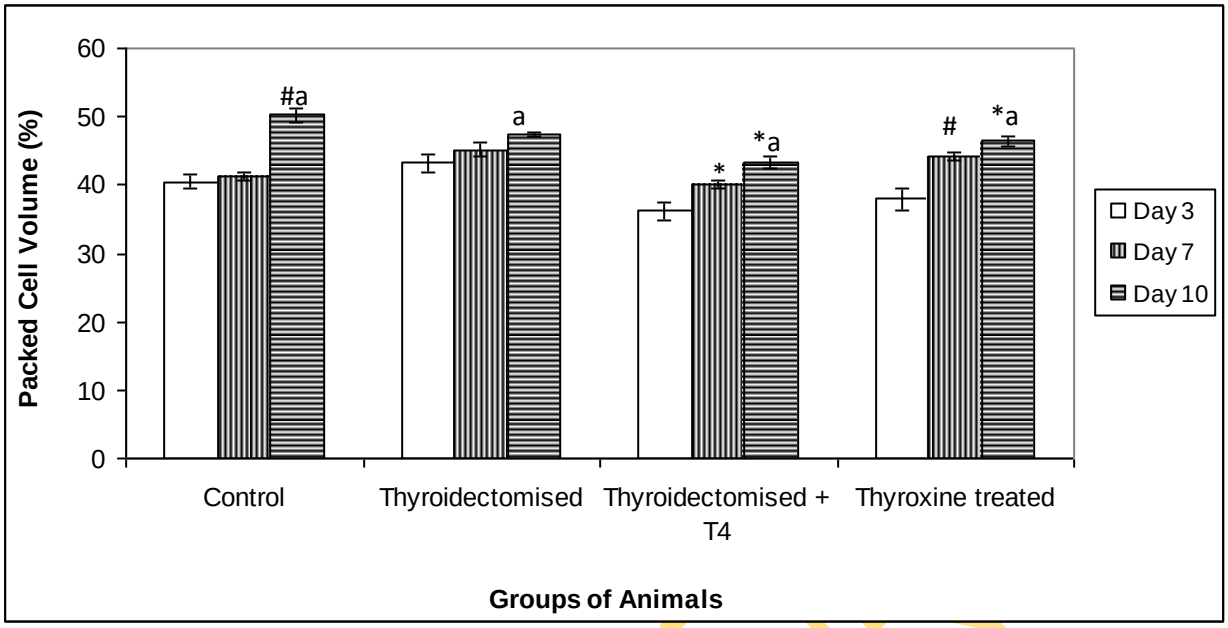

Figure 4.21: Packed cell volume in animals after ulcer induction in thyroxine treated and thyroidectomised rats

N = 5, Values are mean ± SEM

*= significant compared with animals in same group the previous day of assessment at $p < 0.05$

#= significant compared with animals in same group the previous day of assessment at $p < 0.01$

a= significant compared with animals in same group on day 3 at $p < 0.01$

4.5.2. Effect of Thyroidectomy and Thyroxine Treatment on Red Blood Cell Count (RBC) after Ulcer Induction

In control animals, RBC count was significantly increased ($p< 0.001$, 0.001) on day 10 as compared with value on days 7 and 3 respectively. Thyroxine treatment significantly ($p< 0.01$, 0.001) increased RBC count on days 7 and 10 respectively. Thyroidectomy also caused a significant ($p< 0.01$) increase in RBC count only on day 10 compared with day 3. Thyroxine replacement therapy also caused significant ($p< 0.01$) increase in RBC count only on day 10 compared with day 3 (Figure 4.22).

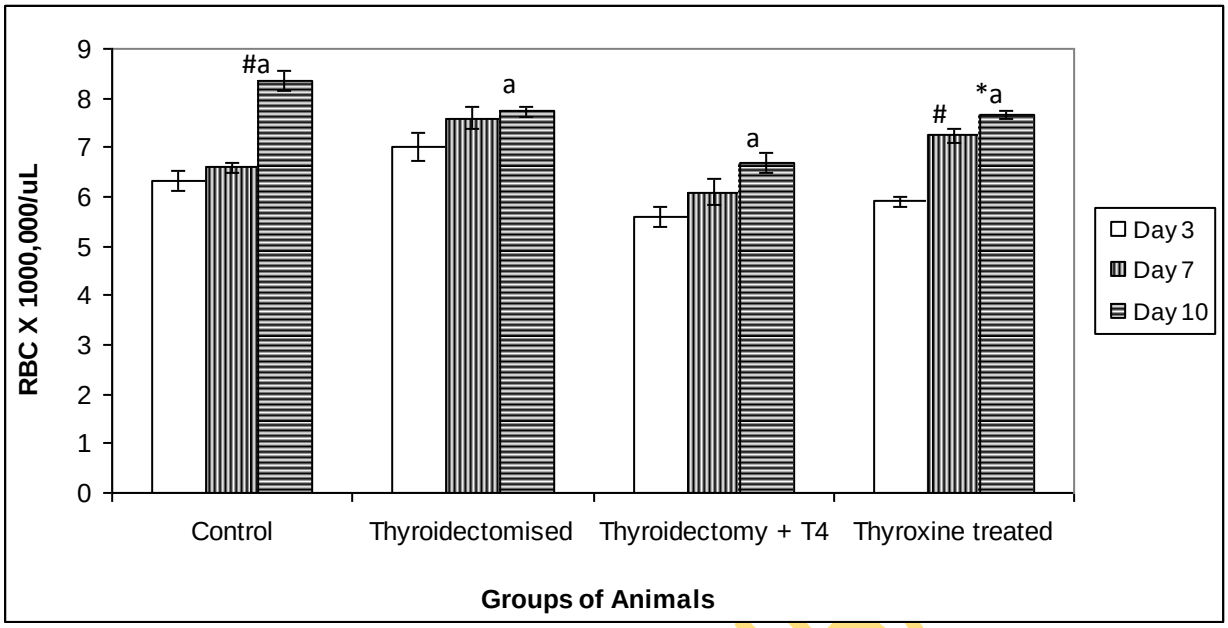

Figure 4.22: Red blood cells in animals after ulcer induction in thyroxine treated and thyroidectomised rats

N = 5, Values are mean ± SEM

*= significant compared with animals in same group the previous day of assessment at $p < 0.05$

#= significant compared with animals in same group the previous day of assessment at $p < 0.01$

a= significant compared with animals in same group on day 3 at $p < 0.01$

4.5.3. Effect of thyroidectomy and thyroxine treatment on white blood cell count after ulcer induction:

Thyroxine treatment significantly ($p<0.0001$) increased white blood cells (WBC) count on day 7 after ulcer induction as compared with value on day 3, but on day 10, WBC count significantly ($p < 0.0001$) dropped as compared with value on day 7, to a value not significantly ($p > 0.05$) different from the value on day on day 3. White blood cells also increased significantly ($p < 0.0001$) in control animals on day 7, but also dropped significantly ($p < 0.001$) on day 10 as compared with day 7 to a level still higher ($p < 0.01$) than the value on day 3. In thyroidectomised animals there was no significant ($p > 0.05$) increase in WBC count on day 7, but value significantly ($p < 0.01$) increased on day 10. Thyroxine replacement therapy also significantly ($p< 0.001, 0.001$) increased WBC count on day 7 and 10 respectively as compared with day 3 (Figure 4.23)

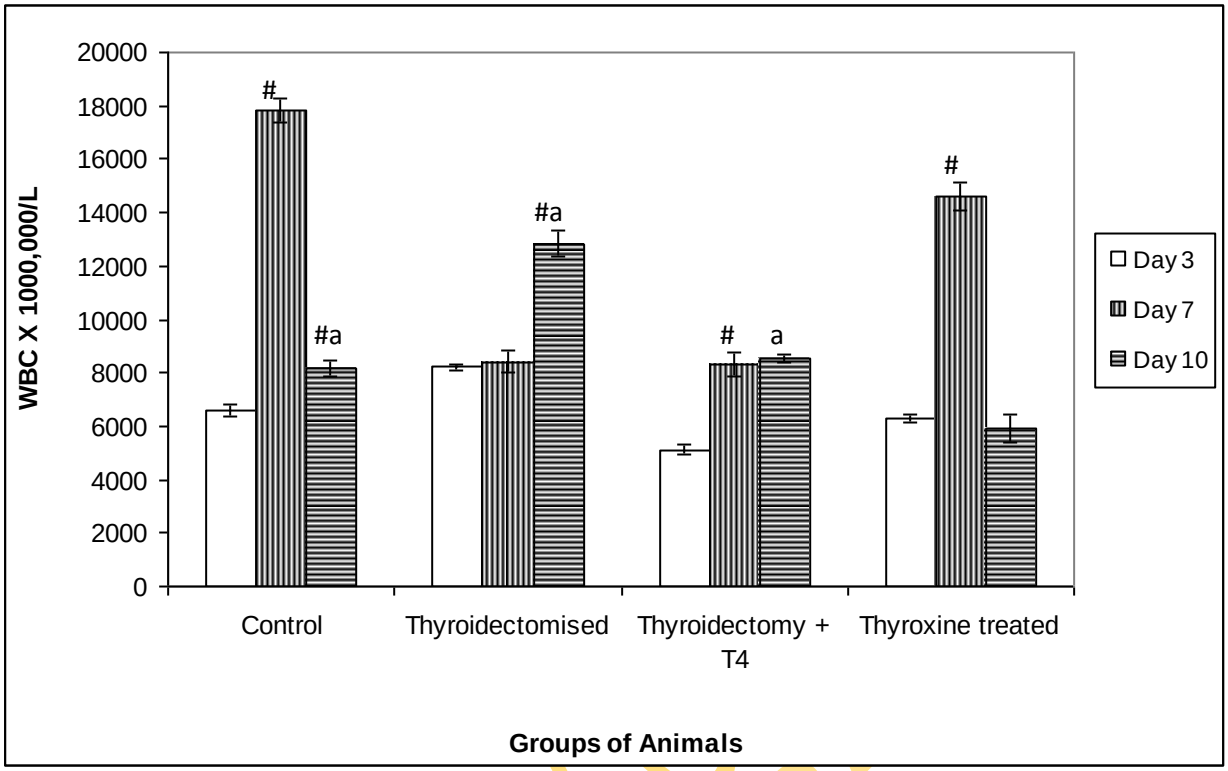

Figure 4.23: White blood cells in animals after ulcer induction in thyroxine treated and thyroidectomised rats

N = 5, Values are mean ± SEM

#= significant compared with animals in same group the previous day of assessment at $p < 0.0001$

a= significant compared with animals in same group on day 3 at $p < 0.01$

4.5.4. Effect of Thyroidectomy and Thyroxine Treatment on Neutrophil Lymphocyte Ratio after Ulcer Induction

Table 4.8 showed that the neutrophil lymphocyte ratio (NLR) was significantly ($p < 0.05$) lower in control animals on day 3 compared with the other groups. Neutrophil lymphocyte ratio significantly ($p < 0.05$) reduced in all groups of animals by day 10 after ulcer induction. However, Figure 4.24 showed that on day 10, the NLR significantly ($p < 0.01$) reduced in thyroxine treated animals by 65.0 ± 2.5% compared with control (28.3 ± 2.8%). Thyroxine replacement therapy also caused a significant ($p < 0.01$) decrease in NLR, while the reduction was significantly ($p < 0.05$) lower in thyroidectomised animals (20.1 ± 1.7%) compared with control animals.

Table 4.8: Neutrophil lymphocyte ratio after ulcer induction in in thyroidectomised and thyroxine treated rats

Groups	NLR (Day 3)	NLR (Day 7)	NLR (Day 10)
Control	0.6 ± 0.0	0.5 ± 0.0xx	0.4 ± 0.0xxx
Thyroidectomised	0.7 ± 0.0a	0.6 ± 0.0	0.6 ± 0.0xx
Thyroidectomised + T_4	0.8 ± 0.0a	0.5 ± 0.1xx	0.4 ± 0.0xxx
Thyroxine treated (Sham + T_4)	0.9 ± 0.1a	0.5 ± 0.0xxx	0.3 ± 0.0xxx

N= 5, value are mean ± SEM

aa= significant compared with control on day 3 at $p < 0.01$

xx= significant compared with animals in same group on day 3 at $p < 0.01$

xxx= significant compared with animals in same group on day 3 at $p < 0.001$

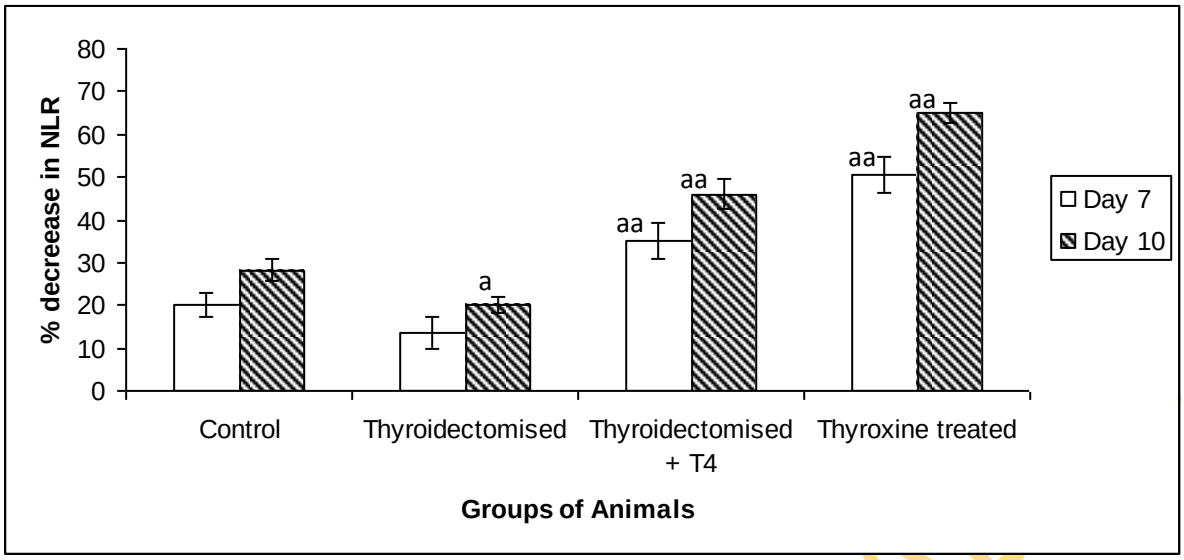

Figure 4.24: Percentage decrease in neutrophil lymphocyte ratio after ulcer induction in thyroxine treated and thyroidectomised rats

N= 5, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

aa= significant compared with animals in Control group on same day at $p < 0.01$

4.6. Study 6 – Assessment of cellular activities during ulcer healing

4.6.1. Biochemical study (Lipid Peroxidation)

On day 3 after ulcer induction, lipid peroxidation was significantly ($p< 0.001, 0.001$) higher in thyroxine treated (271.6 ± 9.9 nmolMDA/mg protein) than in thyroidectomised animals (181.3 ± 6.8 nmolMDA/mg protein) and control (171.4 ± 6.5 nmolMDA/mg protein) respectively. By day 10, contol, thyroxine treated and thyroidectomised animals given thyroxine supplementation had a significant ($P< 0.01, 0.01, 0.001$) reduction in lipid peroxidation respectively, but there was no significant ($p> 0.05$) difference in thyroidectomised animals (Table 4.9). Figure 4.25 showed that on day 7, thyroxine treatment and thyroxine replacement therapy significantly ($p< 0.01, 0.01$) reduced peroxidised lipid by 35.6 ± 2.4 and 15.4 ± 0.7% respectively compared with control (12.4 ± 0.5%). On day 10, thyroxine treatment and replacement therapy further decreased ($p< 0.001, 0.05$) lipid peroxidation by 57.0 ± 0.5% and 23.7 ± 0.6% respectively compared with control animals (19.6 ± 1.6%). The reduction in lipid peroxidation was significantly ($p< 0.05$) less in thyroidectomised animals 15.7 ± 1.6% compared with control.

Table 4.9: Lipid peroxidation after Ulcer Induction in thyroxine treated and thyroidectomised rats

Groups	Day 3 nmolMDA/mg protein	Day 7 nmolMDA/mg protein	Day 10 nmolMDA/mg protein
Control	171.4 ± 6.5	151.6 ± 4.0x	140.1 ± 1.3xx
Thyroidectomised	181.3 ± 6.8	171.7 ± 7.5	166.6 ± 5.4
Thyroidectomised + T_4	170.3 ± 6.3	158.2 ± 7.3	136.0 ± 1.7xx
Thyroxine treated (Sham + T_4)	271.6 ± 9.9a	160.71 ± 8.3xxx	120.0 ± 13.9xxx

N= 5, value are mean ± SEM

a= significant compared with control on day 3 at $p < 0.001$

x= significant compared with animals in same group on day 3 at $p < 0.05$

xx= significant compared with animals in same group on day 3 at $p < 0.01$

xxx= significant compared with animals in same group on day 3 at $p < 0.001$

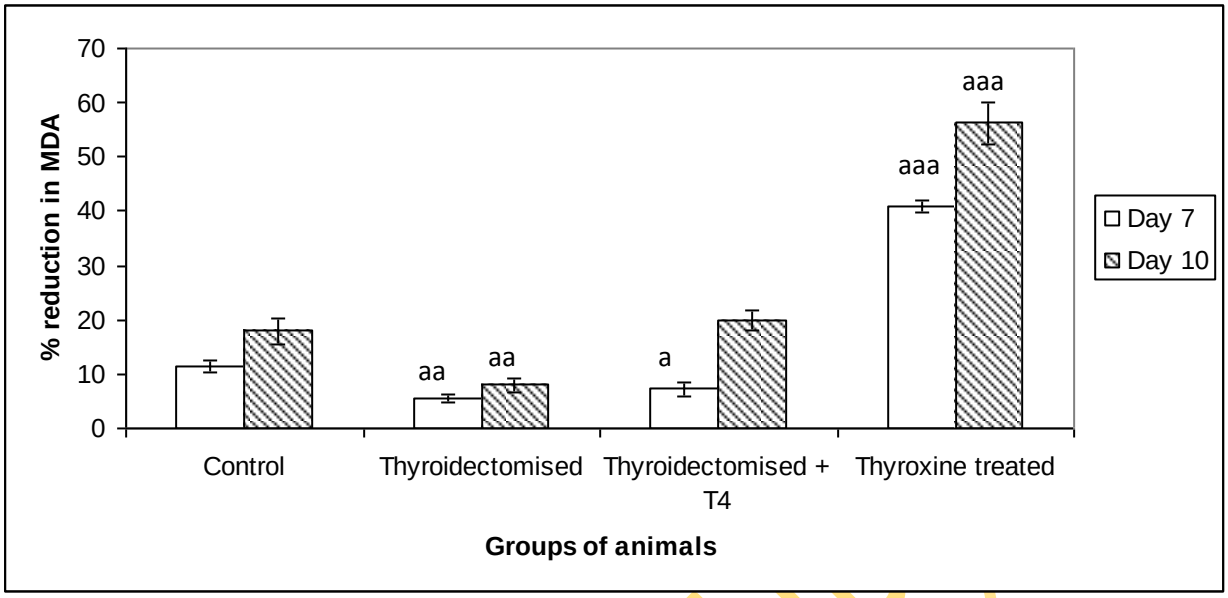

Figure 4.25: Percentage reduction in lipid peroxidation after ulcer induction in thyroxine treated and thyroidectomised rats

N= 5, value are mean ± SEM

a= significant compared with animals in Control group on same day at $p < 0.05$

aa= significant compared with animals in Control group on same day at $p < 0.01$

aaa= significant compared with animals in Control group on same day at $p < 0.001$

4.6.2. Assessment of Index of Apoptosis (DNA Fragmentation)

Figure 4.26 shows that there was apoptosis in the ulcerated gastric mucosa of rats on day 3. Apoptosis was least in control animals and highest in thyroxine treated animals on day 3 after ulcer induction. Apoptosis increased in all groups of animals on day 7 (Figure 4.27). By day 10, DNA fragmentation reduced in all groups as compared with that on day 7. However, apoptosis was higher thyroxine treated than thyroidectomised animals by day 10 (Figure 4.28).

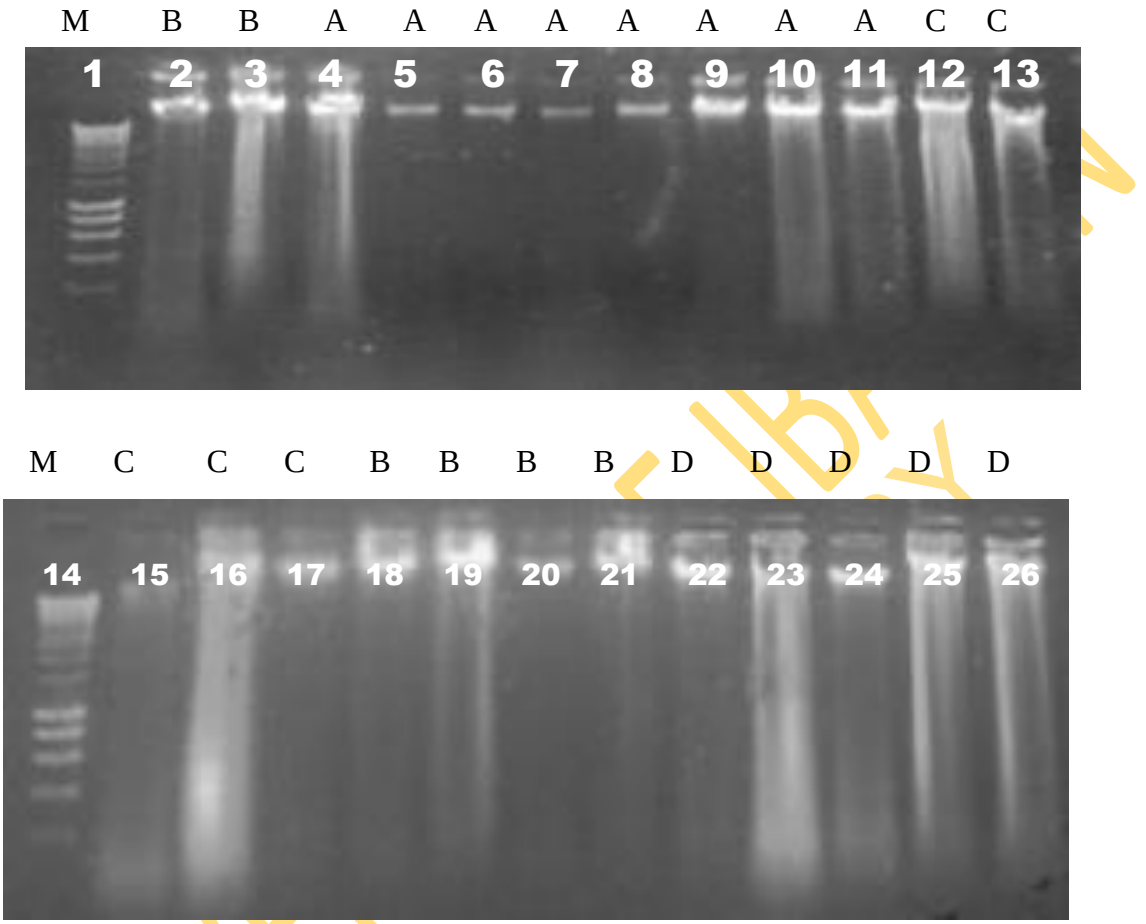

Figure 4.26: Agarose gel electrophoresis of chromosomal DNAs from gastric mucosa cells of acetic acid–induced ulcer in rats on day 3 after ulcer induction in thyroidctomised and thyroxine treated rats.

M = 50 bp DNA ladder (Bioline), A = Control B = Thyroidectomised C = Thyroidectomised + T4, D = Thyroxine treated

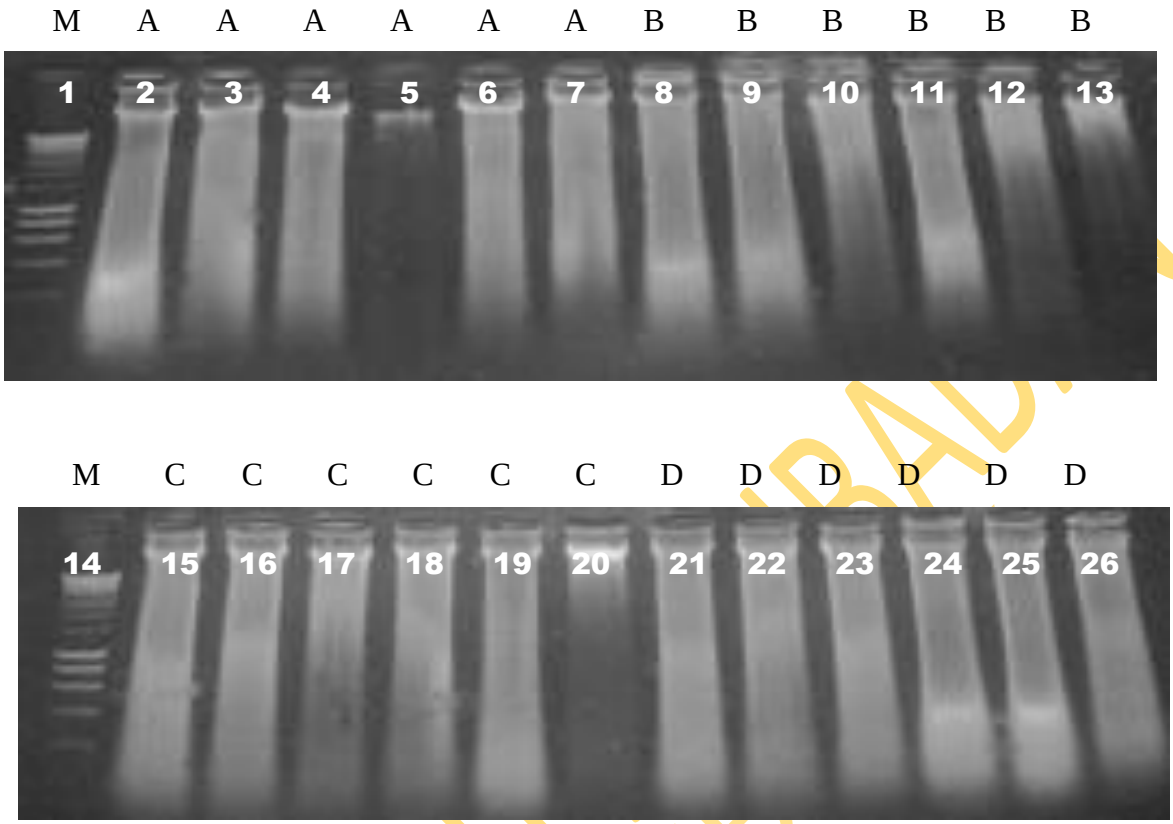

Figure 4.27: Agarose gel electrophoresis of chromosomal DNAs from gastric mucosa cells of acetic acid–induced ulcer in rats on day 7 after ulcer induction in thyroidctomised and thyroxine treated rats.

M = 50 bp DNA ladder (Bioline), A = Control B = Thyroidectomised C = Thyroidectomised + T4, D = Thyroxine treated

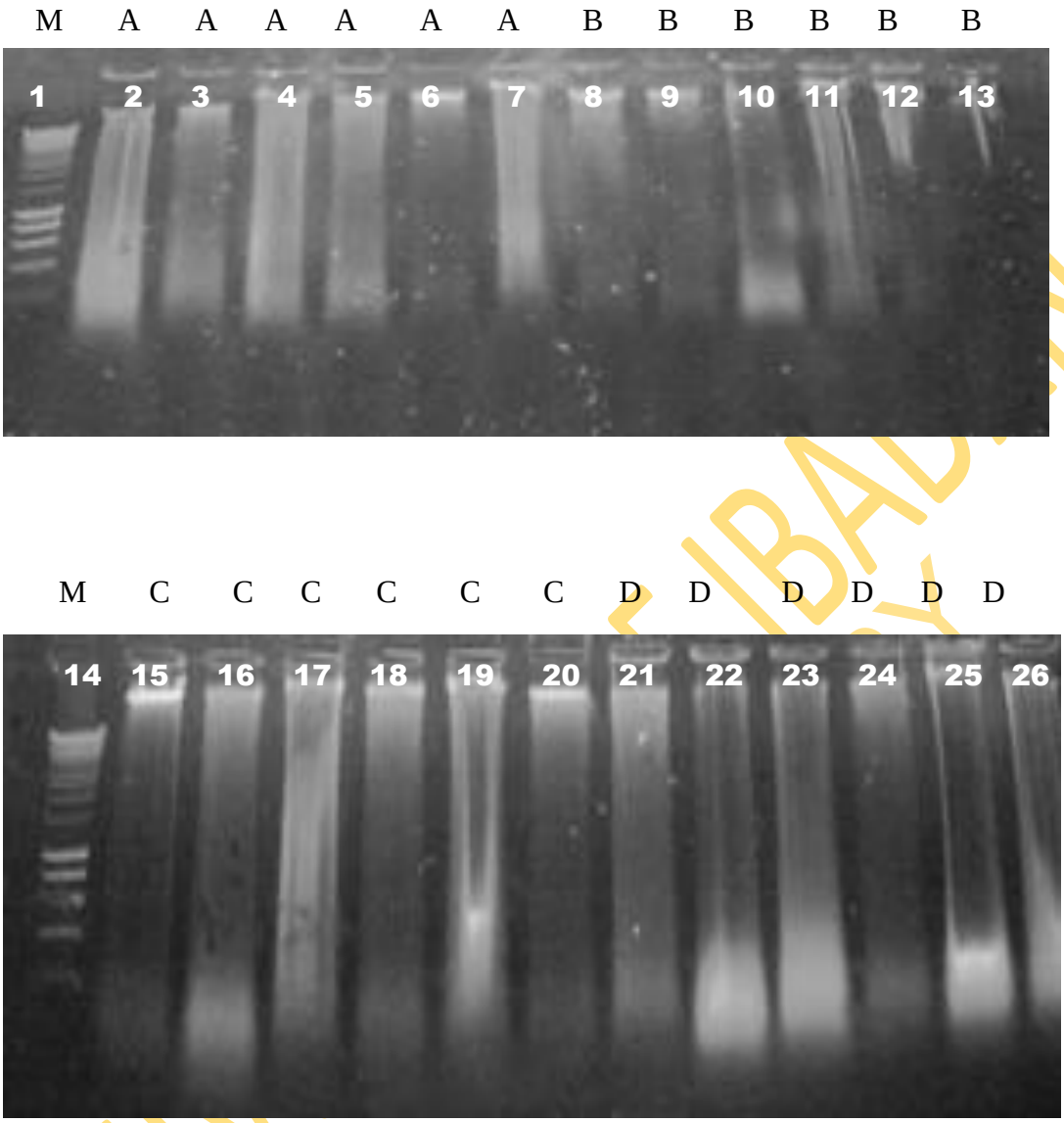

Figure 4.28: Agarose gel electrophoresis of chromosomal DNAs from gastric mucosa cells of acetic acid–induced ulcer in rats on day 10 after ulcer induction in thyroidctomised and thyroxine treated rats.

M = 50 bp DNA ladder (Bioline), A = Control B = Thyroidectomised C = Thyroidectomised + T4, D = Thyroxine treated

CHAPTER 5

DISCUSSION

In this present study, the effects of thyroidectomy and thyroxine treatment on acetic acid induced gastric ulceration in rats was investigated for the first time. This was accomplished by removing the thyroid gland of a group of rats, thereby making them thyroid deficient and giving another set of euthyroid rats thyroxine treatment. Ulcer was induced in animals and healing processes were observed on days 3, 7 and 10 in control animals. The results were compared with that in thyroidectomised animals, thyroxine treated and thyroidectomised animals given thyroxine supplemetation.

Rats were used in this research to study, because this model of animal has been used by several researchers to study the process of healing in some tissues of the body; on the heart (Kranz *et al.,* 1976), skin (Safer *et al.,* 2003; Lennox and Johnston, 1973) radiation-induced neck fistulae (Talmi *et al*., 1989, Alexander *et al*., 1982). Furthermore rats have been used in previous studies to study ulcer healing processes (Dharmani *et al*., 2003, Luo *et al*., 2003, Ma *et al*., 2000).

The dose of thyroxine used was as previously described by Olaleye (1999). A chronic treatment of 35 days was used in this study. This dose was previously used by Isreal *et al*., (1975), but administered for 21 days. Adeniyi *et al.,* (1993) used a dose of 6-8 micrograms/100 g body wt/day (60-80 µg/kg/day) for 35 days.

In order reduce the plasma thyroxine level, thyroidectomy was performed in anaesthetised animals. Likewise, sham surgery was performed on animals in groups of animals that would not require thyroidectomy in order to ensure that all animals passed through the same experimental (surgical) conditions.

Acetic acid induced gastric ulceration model was used in this study because (1) The ulcer induction procedure is quite simple, readily resulting in ulcers of consistent size and severity at an incidence of 100%. (2) The ulcer models highly resemble human ulcers in terms of both pathological features and healing mechanisms. Spontaneous relapse of healed ulcers is frequently observed, just as in peptic ulcer patients. (3) The ulcers respond well to various anti-ulcer drugs, such as acid pump inhibitors, sucralfate, and several growth factors (Okabe and Amagase, 2005).

Study 1: Preliminary study on the efficacy of the surgical and treatment methods

The preliminary result showed that the thyroxine treatment significantly increased the plasma T_3 and T_4, therefore the drug treatment successfully increased the thyroid hormone level after 35 days of treatment. The low plasma level of thyroid stimulating hormone (TSH) revealed that excess thyroid hormones inhibited the secretion of TSH through a negative feedback mechanism. Result also showed that plasma level of T_3 and T_4 were significantly low in thyroidectomised rats as compared with that of control. This revealed that the thyroid glands were successfully removed during thyroidectomy. The increased level of TSH in the thyroidectomised animals might be due to a low plasma level of T_3 and T_4, which caused the stimulation and release of TSH through a negative feedback mechanism. The effects effects of thyroxine treatment and thyroidectomy on body weight gain are as previously reported (Guyton and Hall, 2002)

Study 2 – Measurement of ulcer dimensions

The result of this study revealed that thyroxine treatment accelerated the rate and processes involved in gastric ulcer healing while thyroidectomy delayed gastric ulcer healing.

This result is consistent with previous works in other tissues (organs) of the body, that thyroid hormone administration accelerated the healing of myocardial infarction (Kranz *et al*., 1976), laryngeal fistula in post operative hypothyroid patients (Talmi *et al.*, 1989), and skin (Lennox and Johnston, 1973, Safer, 2005), while hypothyroidism retarded wound healing on the skin 2-fold relative to euthyroidism (Safer *et al*., 2004, Lennox and Johnston, 1973).

Result showed the presence of ulceration in the gastric mucosa of animals on day 3 after inducing ulcer using acetic acid. Measurement of ulcer area on day 3 revealed that ulcer area was significantly larger in thyroxine treated animals than that in control; all animals had a significant reduction in ulcer area on day 10. However, the rate of healing was significantly higher in thyroxine treated rats as compared with control animals, while thyroidectomy significantly reduced the rate of healing. The initial larger ulcer area seen in thyroxine treated animals might be due to the ability of thyroxine to increase oxidative stress in animal (Videla, 2000; Chandra *et al.*, 2010), but healing was faster in this group. Contrary reports however exist about the effects of thyroid hormones on healing; Pirk et al. (1974) noted no change in wound healing with euthyroid hamsters receiving thyroxine (ip). Cannon (1994) reported that hypothyroidism did not diminish wound strength in pigs, and Ladenson et al. (1984) failed to detect wound healing deficits in hypothyroid humans after surgery.

Histomorphormetry result revealed that thyroxine treatment significantly decreased ulcer depth and width as compared with control animals. This effect on ulcer depth is an important healing effect of thyroxine as "depth" is an attribute that actually characterises ulcer - penetrating the muscularis mucosa (Tarnaswski, 2000). This effect of thyroxine might be due to the ability of thyroxine to stimulate the biochemical process involved in the cells growth and increases the mitotic activity of cells in the digestive system of rat as previously reported by Adeniyi and

Olowokorun (1989). Thyroidectomy on the other hand slowed down the rate of decrease in ulcer depth and width.

Study 3 - Assessment of the characteristics and quality of healing

Ulcer healing is an active process that involves various stages: inflammatory, proliferative and scar formation. In this research, thyroid hormone treatment accelerated the healing processes observed in each stage of healing, while thyroidectomy slowed down the processes. There was faster rate of clearing of inflammatory cells in thyroxine treated animals. Fibroblast proliferation and collagen deposition and epithelial cell proliferation (proliferative phase) began earlier in thyroxine treated animals than in control animals, while thyroidectomy prolonged the inflammatory phase with less collagen synthesis - there was still massive inflammatory cells in thyroidectomised animals on day 10. This delay of healing in thyroidectomised animals corresponds with the report of Zimmermann *et al*., (2009)**.** The presence of debris and necrotic tissue slows down rate of healing (Ekmektzoglou and Zografos, 2006). Ulcer healing requires interactions between different tissues and cell types to achieve restoration of the normal mucosal architecture. Studies have indicated a pivotal role for the extracellular matrix (ECM) in wound repair (Mikami *et al.*, 1994), not only by providing the support for the regenerating cells (Domschke *et al.*, 1993), but also by creating the environment necessary for cellular interactions (Zern *et al.*, 1993). Fibrillar collagens, in particular, are important for the generation of wound strength (Shahina *et al*, 1997). Therefore the faster rate of healing observed in thyroxine treated animals might be due to the ability of thyroid hormones to increase the formation of connective tissue, which increased the strength of healing ulcer and also create an enabling environment for healing to occur. The rapid proliferation of cells

observed in thyroxine treated rats might also be due to the ability of thyroxine to increase mitotic activity and stimulate growth (Adeniyi and Olwookorun, 1989). The faster rate of healing observed in thyroxine treated animals might also be due to the ability of thyroxine to increase blood flow and angiogenesis (Davis *et al.*, 2004, Davis *et al.*, 2009). The increase blood flow is important in supplying oxygen and nutrient to the healing mucosa (Tarnawski 2001, Guo *et al.*, 2002). While the slower rate of healing in thyroidectomised animals might be due to decrease blood flow to the gastric mucosa. Previous studies reported that thyroidectomy decreased blood vessel density in rat brain (Schlenker *et al.*, 2008) and heart (Liu *et al.*, 2008), therefore there might be a slower rate of delivery of nutrients and growth factors to the ulcerated gastric mucosa of thyroidectomised rats. The faster rate of healing observed in thyroxine treated animals might be due to increase in metabolic activity of cells involved in healing (Bhagavan, 1992), while thyroidectomy decreased metabolic activity of cells in the body. The quality of healing observed in thyroidectomised rats given thyroxine supplementation is similar to that in control animal. This reveals that physiologic level of thyroxine is important for gastric ulcer healing to take place.

Study 4 – Assessment of the effects of gastric acid secretion during ulcer healing

In an attempt to protect the gastric mucosa from gastric acid, enhance ulcer healing, and prevent ulcer recurrence, pharmacological control of gastric acid secretion has long represented a desirable goal (Aihara *et al.*, 2003; Tuorkey and bdul-Aziz, 2009; Tuorkey and Karolin, 2009). Luminal acid interferes with the process of restitution, resulting in the conversion of superficial injury to deeper mucosal lesion and inactivates the acid-labile growth factors important for maintenance of mucosal integrity and repair of superficial injury (Wallace and Muscara, 2001).

Brzozowski *et al.*, (2000) noticed that the suppression of gastric acid secretion by omeprazole or ranitidine prevents the progression of gastric erosions into ulcers, and the addition of exogenous acid restores the progression of the acute lesions into gastric ulcers, therefore increase secretion of acid aggravates ulcers that are already formed. The result of this study showed that thyroxine treatment significantly increased basal gastric acid secretion on day 3 after ulcer induction, while thyroidectomy significantly decreased basal gastric acid secretion as compared with control animals. This result agrees with previous studies on the effects of thyroxine and thyroidectomy on gastric acid secretion (Adeniyi and Olowookorun, 1989; Rafsanjani *et al.*, 2003). However, basal gastric acid secretion decreased in all groups of animals on days 7 and 10 respectively, this reduction in acid secretion is important for healing to take place (acid is an aggressive factor) This result agrees with reports that effective acid reduction by a variety of antisecretory drugs is associated with a significant acceleration of duodenal and gastric ulcer healing in controlled clinical trials (Chiverton and Hunt, 1989, Aihara *et al.*, 2003). Table 4.19 however showed that on day 10 after ulcer induction, thyroxine reatment caused a significantly reduced basal acid secretion, while thyroidectomy caused a significantly less reduction in acid secretion as compared with control.

On day 3, thyroxine treatment also increased peak histamine gastric acid output, while thyroidectomy decreased secretagogue stimulated acid output as compared with control. Table 4.7 also showed that on day 10, thyroxine treatment significantly decreased histamine stimulated acid output, while the decrease was less in thyroidectomised animals as compared with control. The result of this research shows that reduction in acid secretion is important for gastric ulcer healing to occur. The highest healing rate on day 10 in thyroxine treated animals correlated with

the highest percentage reduction in acid secretion, while the least healing rate in thyroidectomised animals correlated with the lowest percentage reduction in acid secretion

Study 5 - Assessment of the effects of blood cells during ulcer healing

Thyroxine treatment significantly increased packed cell volume and red blood cell count on days 7 and 10 as compared with day 3 after surgery and ulcer induction. This in agreement with previous studies that thyroxine stimulates erythropoiesis via a direct, beta 2-adrenergic receptor-mediated stimulation of red cell precursors, and an indirect, erythropoietin-mediated mechanism (Sullivan and McDonald, 1992). An increase in red blood cells might result in increased delivery of oxygen to healing ulcer site, which is important for healing to take place. The increase in PCV and RBC count in thyroidectomised animals was only significant on day 10, thus a slower response of erythropoiesis. This might be as a result of low metabolic rate in the bone marrow. Low red blood cells might result in inadequate delivery of oxygen to the healing tissue, hence causing slow rate of healing. Result of this study showed that there was a significant boost in white blood cell count in control and thyroxine treated rats on day 7 as compared with day 3, but on day 7 the change in WBC in thyroidectomised animals was not significant. The increase in WBC count in control and thyroxine treated animals might be responsible for the faster rate of healing in these groups of animal as compared with thyroidectomised animals. White blood cells are responsible for helping the body to heal. They do this by ingesting materials that need to be removed from the body. These materials include: old red blood cells, debris from body tissues and dead cells in the body. However, if the white blood cells in the body are low, they might likely be unable to remove materials from the body that could be toxic if left to remain, promoting the possibility of complications for the patient

who is trying to heal. It is known that the process of tissue repair involves a biological response whereby the body's cellular defense mechanisms are recruited to the damaged area with accompanying vascular and neural responses (Mann *et al.*, 1995). Moreover, white blood cell WBC counts have been used as an indicator by clinicians to monitor progress of healing in patients (Haffor, 2010). On day 10 after ulcer induction WBC significantly dropped in both control and thyroxine treated animals, showing that these cells had accomplished their functions, while on day 10, WBC count had just significantly increased in thyroidectomised animals, showing a slower immune response in this group.

The neutrophil-lymphocyte ratio (NLR) provides an indicator of inflammatory status (Halazun *et al.*, 2008). The physiologic immune response of circulating white blood cells to various stressful events as tissue injury, severe trauma, major surgery, burns, sepsis syndrome, is characterized by elevation of neutrophils and decline in lymphocyte counts. The result of this study revealed that on day 3 thyroxine treatment and thyroidectomy significantly increased NLR, indicating that inflammation was more severe in these groups than in control animals. However, all groups of animals except thyroidectomised animals had a significant ($p< 0.05$) decrease NLR on the 7^{th} day and on day 10 all groups of animals had a significant ($p< 0.01$) decrease in NLR. The percentage decrease in NLR was significantly higher in thyroxine treated animals as compared with control, while it was significantly lower in thyroidectomised animals as compared with control. Zahorec (2001) found out that the improvement of the clinical status following major surgery, severe infection, systemic inflammation, sepsis, injury or trauma coincides with a gradual increase in lymphocyte counts (more than 1.000/mm3 or higher than 10 % of WBC population) and concomitant decrease in neutrophil counts (less than 8.000/mm3 or less than 80 % of WBC) (reduced NLR). Therefore the highest reduction in NLR observed in

thyroxine treated animals might imply the most rapid improvement after surgery and inflammation and the least reduction observed in thyroidectomised animals might be responsible for the slower rate of healing observed in the group

Study 6 – Assessment of cellular activities during ulcer healing

The result of this research revealed that there was lipid peroxidation in all groups of animals on day 3, and this shows that free oxygen radicals are involved in the aetiology of acetic acid induced gastric ulceration, as previously reported (Olaleye *et al.*, 2007, Demir *et al.*, 2003). The result revealed that there was greater reduction in peroxidised lipid in thyroxine treated rats as compared with control, while there was no change in lipid peroxidation by day 10 in thyroidectomised animals. Experimental and clinical studies suggested that the reactive oxygen species have an important role in the aetio-pathogenesis of the inflammation and ulceration of the digestive tract (Perry *et al*., 1986, Olaleye *et al.*, 2007). This is evidenced by the increased oxidative stress by pro-ulcerative factors in the gut such as *H pylori* (Janulaityte-Gunther *et al.*, 2003), use of non-steroidal anti-inflammatory drugs(Rostom *et al.*, 2000), smoking (Ma *et al.*, 2000), psychological stress, corticosteroid use (Levenstein, 1999), and loss of sleep (Guo *et al.*, 2005).

The significantly higher lipid peroxidation on day 3 in thyroxine treated animals might be due to the ability of thyroxine to increase cellular respiration and thus increase the production of reactive oxygen species (Videla, 2000; Chandra *et al.*, 2010). The increased oxidative stress then increase; size of ulcer and lipid peroxidation. However, the accelerated healing found in hyperthyroid animals might be due to the antioxidant effect of thyroxine (Wynn, 1968). Hence, there was greater inhibition of lipid peroxidation in thyroxine treated animals by day 7 and 10.

Authors reported that thyroxine increased the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (Sal'nikova and Dubinina, 1985; Seven *et al.,* 1996). Chandra *et al.,* (2010) reported that thyroxine administration developed oxidative stress; the organism defends itself against the effects of oxidative stress by increasing SOD and catalase activities as a protective mechanism. Teprenone, which promote the healing of acetic acid-induced chronic gastric ulcers in rats was reported to inhibit lipid peroxidation (Kobayashi *et al.,* 2001). The lack of reduction in lipid peroxidation in thyroidectomised animals might be due to the decrease antioxidant defense system (Pasupathi and Latha, 2008; Chattopadhyay *et al*., 2003), hence there was slower rate of healing in this group.

As discussed earlier, after the inflammatory phase of wound healing, fibroblasts migrate, proliferate, and synthesize extracellular matrix components, participating in the formation of granulation tissue. It has been discussed that this rapid increase in cell proliferation is allowed by an initial decrease of apoptosis. Later, when the inflammatory process begins to shut down with wound closure and scar evolution, there is a dramatic decrease of cellularity, which has been clearly shown to be mediated by an increase of apoptotic cell death (Desmouliere *et al*., 1995; 1997).

The result of this research confirms the report that apoptosis plays a significant role in gastric ulceration (Konturek *et al.,* 1999; Fuji *et al.,* 2000). By day 3, apoptosis occured in the gastric mucosa of all groups of animals. Animals in control group showed less DNA fragmentation as compared with thyroidectomised and thyroxine treated animals. By day 7, apoptosis increased in all groups of animals, while on day 10, DNA fragmentation reduced in all groups of animals. However DNA fragmentation was higher in thyroxine treated animals than in thyroidectomised animals on day 10. The initial decrease in apoptosis was important for

proliferation to occur. In wound healing when healing proceses had advanced in granulation tissue containing mainly small vessels, inflammatory cells, fibroblasts and myofibroblasts are removed by apoptosis at the same time cell proliferation and reepithelisation is taking place (Desmouliere *et al.*,1997). Thus the increase in apoptosis on day 10 in thyroxine treated animals might be important for faster healing process (Desmouliere *et al*, 1997). Brown, *et al*., (1997) reported that apoptosis might signal the end of the inflammatory phase of healing. Kane and Greenhalgh (2000) also reported that as the inflammation process declined, p53 levels increased while Bcl-2 levels decreased. Cellular activity is lower in thyroidectomised animals and healing is slow in the group, this might be as a result of the reduced apoptosis in the group by day 10. Sanchez-Fidalgo *et al.*, (2004) reported that agents that reduced apoptosis slowed down gastric ulcer. Desmouliere *et al.,* (1997) suggested that in cutaneous wounds as well results of other laboratories (particularly in lungs and kidney), apoptosis is the mechanism responsible for the evolution of granulation tissue into a scar.

CONCLUSION

In conclusion, this research reveals the thyroxine treatment accelerates gastric ulcer healing by:

i. Accelerating the reduction the ulcer area, width and depth
ii. anti- inflammatory effect, acceleration of fibroblast, connective tissue growth and epithelial cells proliferation
iii. increase in white blood cell count,
iv. reduce gastric acid output during healing,
v. reduced lipid peroxidation and apoptosis during healing,

while thyroidectomy delayed ulcer healing by:

i. Delaying reduction in dimensions (area, width and depth)
ii. by prolonging the inflammatory phase of healing, slower collagen deposion and epithelial cell proliferation,
iii. slower increase in white blood cells associated with healing,
iv. lower reduction in acid secretion during ulcer healing
v. Increase oxidative damage and apoptosis during healing.

SIGNIFICANCE OF STUDY

i. In this study, the pattern of changes in gastric cell function in altered thyroid states was followed after ulcer induction on 3, 7 and 10 post-acetic acid induced ulcer. This is the first available observation in this area of gut-thyroid interrelationships.
ii. The study further buttresses the fact the physiological levels of thyroid hormones are needed for the maintenance of the integrity of the gastric mucosal cells
iii. Reduction in acid secretion is still required for the healing processes to be effective. This is evidenced by the reduction in the rate of acid secretion as healing progressed. The highest reduction rate in the thyroxine treated rats on day 10 corresponds with the highest healing rate
iv. Neutrophil-lymphocyte ratio (NLR) was used to assess state of inflammation during ulcer healing for the first time. Rapid reduction in NLR corresponds with faster healing in thyroxine treated animals

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APPENDICES

Body Weight of Animals during Period of Study in Control Animals in Kg

Animals	wk1	wk2	wk3	wk4	wk5	diff	% Diff.

1	248.0	236.0	240.0	256.0	272.0	24.0	9.7
2	154.0	156.0	178.0	190.0	205.0	51.0	33.1
3	175.0	169.0	181.0	192.0	205.0	30.0	17.1
4	154.0	167.0	175.0	182.0	200.0	46.0	29.9
5	146.0	152.0	162.0	172.0	183.0	37.0	25.3
6	154.0	161.0	171.0	181.0	194.0	40.0	26.0
7	170.0	174.0	184.0	194.0	208.0	38.0	22.4
8	250.0	247.0	268.0	281.0	292.0	42.0	16.8
9	174.0	184.0	197.0	208.0	215.0	41.0	23.6
10	153.0	163.0	178.0	161.0	202.0	49.0	32.0
11	146.0	155.0	167.0	178.0	187.0	41.0	28.1
12	196.0	204.0	211.0	220.0	231.0	35.0	17.9
13	226.0	236.0	244.0	253.0	261.0	35.0	15.5
14	160.0	156.0	170.0	181.0	193.0	33.0	20.6
15	174.0	180.0	194.0	105.0	212.0	38.0	21.8
Mean	**178.7**	**182.7**	**194.7**	**196.9**	**217.3**	**38.7**	**22.7**
SD	**35.4**	**32.5**	**32.1**	**43.0**	**32.6**	**7.0**	**6.6**
SEM	**9.1**	**8.4**	**8.3**	**11.1**	**8.4**	**1.8**	**1.7**

Body Weight of Animals during Period of Study in Hypothyroid Animals in Kg

Animals	wk1	wk2	wk3	wk4	wk5	diff	% Diff.
1	180.0	187.0	199.0	210.0	225.0	45.0	25.0

2	238.0	240.0	252.0	270.0	290.0	52.0	21.8
3	151.0	145.0	160.0	172.0	192.0	41.0	27.2
4	169.0	170.0	185.0	198.0	218.0	49.0	29.0
5	200.0	212.0	222.0	234.0	248.0	48.0	24.0
6	162.0	170.0	185.0	200.0	218.0	56.0	34.6
7	250.0	255.0	264.0	275.0	302.0	52.0	20.8
8	133.0	145.0	156.0	172.0	192.0	59.0	44.4
9	161.0	239.0	174.0	199.0	211.0	50.0	31.1
10	240.0	245.0	255.0	269.0	285.0	45.0	18.8
11	155.0	170.0	182.0	196.0	212.0	57.0	36.8
12	163.0	172.0	185.0	195.0	220.0	57.0	35.0
13	144.0	155.0	167.0	180.0	196.0	52.0	36.1
14	164.0	159.0	175.0	185.0	210.0	46.0	28.0
15	182.0	177.0	194.0	200.0	225.0	43.0	23.6
Mean	**179.5**	**189.4**	**197.0**	**210.3**	**229.6**	**50.1**	**29.1**
SD	**36.4**	**38.3**	**35.0**	**35.0**	**35.6**	**5.5**	**7.1**
SEM	**9.4**	**9.9**	**9.0**	**9.1**	**9.2**	**1.4**	**1.8**

Body Weight of Animals during Period of Study in Thyroidectomy + T_4 Animals in Kg

Animals	wk1	wk2	wk3	wk4	wk5	diff	% Diff.
1	171.0	176.0	204.0	207.0	222.0	51.0	29.8
2	206.0	215.0	222.0	234.0	254.0	48.0	23.3
3	143.0	148.0	157.0	168.0	180.0	37.0	25.9
4	146.0	151.0	164.0	168.0	175.0	29.0	19.9
5	158.0	154.0	168.0	164.0	182.0	24.0	15.2
6	172.0	186.0	206.0	197.0	208.0	36.0	20.9
7	163.0	165.0	182.0	184.0	196.0	33.0	20.2
8	236.0	236.0	240.0	246.0	264.0	28.0	11.9
9	163.0	165.0	183.0	186.0	204.0	41.0	25.2
10	155.0	154.0	170.0	170.0	189.0	34.0	21.9
11	131.0	137.0	155.0	167.0	189.0	58.0	44.3
12	241.0	240.0	245.0	251.0	270.0	29.0	12.0
13	168.0	161.0	177.0	186.0	200.0	32.0	19.0
14	175.0	179.0	193.0	196.0	205.0	30.0	17.1
15	180.0	184.0	200.0	201.0	212.0	32.0	17.8
Mean	**173.9**	**176.7**	**191.1**	**195.0**	**210.0**	**36.1**	**21.6**
SD	**31.5**	**31.3**	**28.3**	**28.7**	**30.2**	**9.5**	**8.0**
SEM	**8.1**	**8.1**	**7.3**	**7.4**	**7.8**	**2.5**	**2.1**

Body Weight of Animals during Period of Study in Hyperthyroid Animals in Kg

Animals	wk1	wk2	wk3	wk4	wk5	diff	% Diff.
1	155.0	164.0	179.0	184.0	189.0	34.0	21.9

2	244.0	259.0	268.0	267.0	270.0	26.0	10.7
3	133.0	144.0	154.0	156.0	164.0	31.0	23.3
4	153.0	160.0	171.0	180.0	186.0	33.0	21.6
5	177.0	192.0	204.0	201.0	209.0	32.0	18.1
6	154.0	167.0	178.0	180.0	185.0	31.0	20.1
7	123.0	115.0	139.0	141.0	154.0	31.0	25.2
8	155.0	161.0	177.0	183.0	189.0	34.0	21.9
9	148.0	142.0	165.0	165.0	178.0	30.0	20.3
10	192.0	186.0	207.0	213.0	220.0	28.0	14.6
11	208.0	207.0	229.0	226.0	230.0	22.0	10.6
12	167.0	168.0	189.0	188.0	195.0	28.0	16.8
13	139.0	138.0	159.0	158.0	170.0	31.0	22.3
14	149.0	153.0	161.0	160.0	170.0	21.0	14.1
15	163.0	169.0	175.0	176.0	186.0	23.0	14.1
Mean	**164.0**	**168.3**	**183.7**	**185.2**	**193.0**	**29.0**	**18.4**
SD	**31.0**	**33.8**	**32.6**	**31.8**	**29.4**	**4.2**	**4.6**
SEM	**8.0**	**8.7**	**8.4**	**8.2**	**7.6**	**1.1**	**1.2**

MEASUREMENT OF ULCER AREA

Ulcer Area in Control Animals after Ulcer Induction

	Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)	Diff (day 3&7)	Diff (day 3&10)	% reduction (Day 7)	% reduction (Day 10)
	7.0	4.0	3.0	0.43	0.40	42.86	57.14
	8.0	5.0	3.0	0.43	0.50	37.50	62.50
	7.0	4.0	3.0	0.43	0.40	42.86	57.14
	9.0	6.0	4.0	0.43	0.50	33.33	55.56
	9.0	5.0	4.0	0.57	0.50	44.44	55.56
mean	**8.0**	**4.8**	**3.4**	**0.46**	**0.46**	**40.20**	**57.58**
SD	**1.00**	**0.84**	**0.55**	**0.06**	**0.05**	**4.65**	**2.86**
SEM	**0.45**	**0.37**	**0.24**	**0.03**	**0.02**	**2.08**	**1.28**

Ulcer Area in Hypothyroid Animals after Ulcer Induction

	Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)	Diff (day 3&7)	Diff (day 3&10)	% reduction (Day 7)	% reduction (Day 10)
	10.0	8.0	7.0	0.29	0.30	20.00	30.00
	8.0	7.5	6.0	0.07	0.20	6.25	25.00
	7.5	5.0	5.0	0.36	0.25	33.33	33.33
	8.0	7.0	5.0	0.14	0.30	12.50	37.50
	9.0	7.5	5.5	0.21	0.35	16.67	38.89
mean	**8.5**	**7.0**	**5.7**	**0.21**	**0.28**	**17.75**	**32.94**
SD	**1.00**	**1.17**	**0.84**	**0.11**	**0.06**	**10.11**	**5.66**
SEM	**0.45**	**0.52**	**0.37**	**0.05**	**0.03**	**4.51**	**2.53**

Ulcer Area in Thyroidectomised + T_4 Animals after Ulcer Induction

	Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)	Diff (day 3&7)	Diff (day 3&10)	% reduction (Day 7)	% reduction (Day 10)
	14.0	9.0	7.0	0.71	0.70	35.71	50.00
	13.0	7.0	5.0	0.86	0.80	46.15	61.54
	14.0	8.0	6.5	0.86	0.75	42.86	53.57
	14.0	8.0	6.0	0.86	0.80	42.86	57.14
	13.5	7.5	6.0	0.86	0.75	44.44	55.56
mean	**13.7**	**7.9**	**6.1**	**0.83**	**0.76**	**42.41**	**55.56**
SD	**0.45**	**0.74**	**0.74**	**0.06**	**0.04**	**3.98**	**4.28**
SEM	**0.20**	**0.33**	**0.33**	**0.03**	**0.02**	**1.78**	**1.91**

Ulcer Area in Hyperthyroid Animals after Ulcer Induction

Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)	Diff (day 3&7)	Diff (day 3&10)	% reduction	% reduction

						(Day 7)	(Day 10)
	13.0	6.0	5.0	1.00	0.80	53.85	61.54
	14.0	9.0	5.0	0.71	0.90	35.71	64.29
	13.0	6.0	4.5	1.00	0.85	53.85	65.38
	13.5	8.5	5.0	0.71	0.85	37.04	62.96
	14.0	8.5	6.0	0.79	0.80	39.29	57.14
mean	**13.5**	**7.6**	**5.1**	**0.84**	**0.84**	**43.95**	**62.26**
SD	**0.50**	**1.47**	**0.55**	**0.15**	**0.04**	**9.13**	**3.20**
SEM	**0.22**	**0.66**	**0.24**	**0.07**	**0.02**	**4.07**	**1.43**

RATE OF HEALING

Rate of Healing of Ulcer Area per Day in Control Animals after Ulcer Induction

	Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)	Rate of reduction (Day 7)	Rate of reduction (Day 10)
	7.0	5.0	4.0	0.3	0.3
	8.0	5.0	3.0	0.4	0.5
	7.0	4.0	3.0	0.4	0.4
	9.0	6.0	4.0	0.4	0.5
	9.0	4.0	3.0	0.7	0.6
mean				**0.5**	**0.5**
SD				**0.2**	**0.1**
SEM				**0.1**	**0.1**

Rate of Healing of Ulcer Area per Day in Hypothyroid Animals after Ulcer Induction

	Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)	Rate of reduction (Day 7)	Rate of reduction (Day 10)
	10	8	5.5	0.3	0.45
	8	7.5	6	0.1	0.2
	7.5	5	5	0.4	0.25
	8	7.5	5	0.1	0.3
	9	7	7	0.3	0.2
mean				**0.2**	**0.3**
SD				**0.1**	**0.1**
SEM				**0.1**	**0.0**

Rate of Healing of Ulcer Area per Day in Thyroidectomised + T_4 Animals after Ulcer Induction

	Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)	Rate of reduction (Day 7)	Rate of reduction (Day 10)
	14	8	5	0.9	0.9
	13	7.5	7	0.8	0.6
	14	6.5	6	1.1	0.8
	14	8	6.5	0.9	0.75
	13.5	9	6	0.6	0.75
mean				**0.8**	**0.8**
SD				**0.2**	**0.1**
SEM				**0.1**	**0.0**

Rate of Healing of Ulcer Area per Day in Hyperthyroid Animals after Ulcer Induction

	Day 3 (mm^2)	Day 7 (mm^2)	Day 10 (mm^2)	Rate of reduction (Day 7)	Rate of reduction (Day 10)
	13	8.5	5	0.6	0.8
	14	9	6	0.7	0.8
	13	6	5	1.0	0.8
	13.5	6	6	1.1	0.75
	14	8.5	5	0.8	0.9
mean				0.8	0.8
SD				**0.2**	**0.1**
SEM				**0.1**	**0.0**

HISTOMORPHOMETRY

Ulcer Width in Control Animals after Ulcer Induction

	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
	94.0	70.0	60.0	25.53	36.17
	100.0	90.0	64.0	10.00	36.00
	125.0	95.0	65.0	24.00	48.00
	110.0	80.0	65.0	27.27	40.91
	102.0	80.0	60.0	21.57	41.18
mean	**106.2**	**83.0**	**62.8**	**21.67**	**40.45**
SD	**11.97**	**9.75**	**2.59**	**6.85**	**4.90**
SEM	**5.32**	**4.33**	**1.15**	**3.05**	**2.18**

Ulcer Width in Hypothyroid Animals after Ulcer Induction

	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
	120.0	100.0	80.0	16.67	33.33
	120.0	100.0	80.0	16.67	33.33
	130.0	110.0	85.0	15.38	34.62
	140.0	125.0	90.0	10.71	35.71
	120.0	110.0	80.0	8.33	33.33
mean	**126.0**	**109.0**	**83.0**	**13.55**	**34.07**
SD	**8.94**	**10.25**	**4.47**	**3.81**	**1.08**
SEM	**3.98**	**4.55**	**1.99**	**1.69**	**0.48**

Ulcer Width in Thyroidectomised + T_4 Animals after Ulcer Induction

	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
	150.0	80.0	65.0	46.67	56.67
	130.0	85.0	65.0	34.62	50.00
	125.0	90.0	70.0	28.00	44.00
	120.0	85.0	70.0	29.17	41.67
	150.0	80.0	75.0	46.67	50.00
mean	**135.0**	**84.0**	**69.0**	**37.0**	**48.5**
SD	**14.14**	**4.18**	**4.18**	**9.15**	**5.88**
SEM	**6.29**	**1.86**	**1.86**	**4.07**	**2.61**

Ulcer Width in Hyperthyroid Animals after Ulcer Induction

	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
	180.0	80.0	50.0	55.56	72.22
	175.0	50.0	60.0	71.43	65.71
	180.0	70.0	50.0	61.11	72.22
	175.0	80.0	60.0	54.29	65.71
	170.0	85.0	50.0	50.00	70.59
mean	**176.0**	**73.0**	**54.0**	**58.48**	**69.29**
SD	**4.18**	**13.96**	**5.48**	**8.26**	**3.33**
SEM	**1.86**	**6.21**	**2.43**	**3.67**	**1.48**

Ulcer Depth in Control Animals after Ulcer Induction

Animals	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
1	30.0	20.0	15.0	33.33	50.00
2	30.0	20.0	15.0	33.33	50.00
3	40.0	25.0	18.0	37.50	55.00
4	35.0	20.0	15.0	42.86	57.14
5	35.0	20.0	15.0	42.86	57.14
mean	**34.0**	**21.0**	**15.6**	**37.98**	**53.86**
SD	**4.18**	**2.24**	**1.34**	**4.77**	**3.63**
SEM	**1.86**	**0.99**	**0.60**	**2.12**	**1.61**

Ulcer Depth in Hypothyroid Animals after Ulcer Induction

Animals	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
1	50.0	30.0	24.0	40.00	52.00
2	30.0	20.0	24.0	33.33	20.00
3	30.0	25.0	25.0	16.67	16.67
4	40.0	25.0	25.0	37.50	37.50
5	50.0	35.0	24.0	30.00	52.00
mean	**40.0**	**27.0**	**24.4**	**31.50**	**35.63**
SD	**10.00**	**5.70**	**0.55**	**9.14**	**16.91**
SEM	**4.44**	**2.53**	**0.24**	**4.06**	**7.51**

Ulcer Depth in Thyroidectomised + T_4 Animals after Ulcer Induction

Animals	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
1	60.0	35.0	18.0	41.67	70.00
2	50.0	30.0	18.0	40.00	64.00
3	60.0	25.0	20.0	58.33	66.67
4	50.0	25.0	18.0	50.00	64.00
5	50.0	25.0	20.0	50.00	60.00
mean	**54.0**	**28.0**	**18.8**	**48.00**	**64.93**
SD	**5.48**	**4.47**	**1.10**	**7.40**	**3.70**
SEM	**2.43**	**1.99**	**0.49**	**3.29**	**1.64**

Ulcer Depth in Hyperthyroid Animals after Ulcer Induction

Animals	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
1	45.0	25.0	15.0	44.44	66.67
2	40.0	21.5	15.0	46.25	62.50
3	40.0	20.0	12.0	50.00	70.00
4	40.0	20.0	15.0	50.00	62.50
5	45.0	25.0	15.0	44.44	66.67
mean	**42.0**	**22.3**	**14.4**	**47.03**	**65.67**
SD	**2.74**	**2.54**	**1.34**	**2.81**	**3.20**
SEM	**1.22**	**1.13**	**0.60**	**1.25**	**1.42**

Ulcer Area Eroded in Control Animals after Ulcer Induction

Animals	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
1	2820.0	1400.0	900.0	50.35	68.09
2	3000.0	1800.0	960.0	40.00	68.00
3	5000.0	2375.0	1170.0	52.50	76.60
4	3850.0	1600.0	975.0	58.44	74.68
5	3570.0	1600.0	900.0	55.18	74.79
mean	**3648.0**	**1755.0**	**981.0**	**51.3**	**72.4**
SD	**863.17**	**374.33**	**111.04**	**7.00**	**4.08**
SEM	**383.63**	**166.37**	**49.35**	**3.11**	**1.81**

Ulcer Area Eroded in Hypothyroid Animals after Ulcer Induction

Animals	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
1	6000.0	3000.0	1920.0	50.0	68.0
2	3600.0	2000.0	1920.0	44.4	46.7
3	3900.0	2750.0	2125.0	29.5	45.5
4	5600.0	3125.0	2250.0	44.2	59.8
5	6000.0	3850.0	1920.0	35.8	68.0
mean	**5020.0**	**2945.0**	**2027.0**	**40.8**	**57.6**
SD	**1175.58**	**668.11**	**153.04**	**8.09**	**11.03**
SEM	**522.48**	**296.94**	**68.02**	**3.60**	**4.90**

Ulcer Area Eroded in Thyroidectomised + T_4 Animals after Ulcer Induction

Animals	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
1	9000.0	2800.0	1170.0	68.9	87.0
2	6500.0	2550.0	1170.0	60.8	82.0
3	7500.0	2250.0	1400.0	70.0	81.3
4	6000.0	2125.0	1260.0	64.6	79.0
5	7500.0	2000.0	1500.0	73.3	80.0
mean	**7300.0**	**2345.0**	**1300.0**	**67.5**	**81.9**
SD	**1151.09**	**326.15**	**146.12**	**4.90**	**3.10**
SEM	**511.59**	**144.96**	**64.94**	**2.18**	**1.38**

Ulcer Area Eroded in Thyroidectomised + T_4 Animals after Ulcer Induction

Animals	Day 3 (µm)	Day 7 (µm)	Day 10 (µm)	% reduction (Day 7)	% reduction (Day 10)
1	8100.0	2000.0	750.0	75.3	90.7
2	7000.0	1075.0	900.0	84.6	87.1
3	7200.0	1400.0	600.0	80.6	91.7
4	7000.0	1600.0	900.0	77.1	87.1
5	7650.0	2125.0	750.0	72.2	90.2
mean	**7390.0**	**1640.0**	**780.0**	**78.0**	**89.4**
SD	**477.49**	**431.06**	**125.50**	**4.80**	**2.11**
SEM	**212.22**	**191.58**	**55.78**	**2.13**	**0.94**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 3 after Ulcer Induction in Control Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X1000
15	0.004	0.004	0.004	0.004	0.004	
30	0.004	0.004	0.004	0.004	0.004	
45	0.004	0.004	0.004	0.004	0.004	
Mean Basal	**0.004**	**0.004**	**0.004**	**0.004**	**0.004**	**4.0**
60	0.004	0.0055	0.004	0.004	0.0055	
75	0.0055	0.0068	0.0068	0.0055	0.0068	
90	0.0068	0.0082	0.0082	0.0082	0.0082	
105	0.0095	0.0095	0.011	0.0095	0.0095	
120	0.0095	0.0075	0.0095	0.0095	0.0095	
135	0.0082	0.0082	0.0068	0.0055	0.0082	
150	0.0055	0.0055	0.0055	0.004	0.0055	
165	0.004	0.0027	0.004	0.004	0.004	
180	0.0027	0.0027	0.004	0.004	0.004	
Peak Hist	**0.0095**	**0.0095**	**0.011**	**0.0095**	**0.0095**	**9.8**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 3 after Ulcer Induction in Hypothyroid Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0027	0.0027	0.0027	0.0027	0.004	
30	0.0027	0.0027	0.0027	0.0027	0.004	
45	0.0027	0.0027	0.0027	0.0027	0.004	
Mean Basal	**0.0027**	**0.0027**	**0.0027**	**0.0027**	**0.004**	**3.0**
60	0.0027	0.0027	0.0027	0.0027	0.004	
75	0.0055	0.0055	0.0055	0.004	0.0055	
90	0.0068	0.0068	0.0068	0.0055	0.0082	
105	0.0082	0.0095	0.0082	0.0082	0.0082	
120	0.0055	0.0068	0.0055	0.0082	0.0068	
135	0.0055	0.0055	0.004	0.0068	0.0055	
150	0.004	0.004	0.0027	0.0055	0.004	
165	0.0027	0.004	0.0027	0.004	0.004	
180	0.0027	0.004	0.0027	0.0027	0.004	
Peak Hist	**0.0082**	**0.0095**	**0.0082**	**0.0082**	**0.0082**	**8.5**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 3 after Ulcer Induction in Thyroidectomised + T_4 Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X1000
15	0.004	0.0055	0.004	0.0055	0.0055	
30	0.004	0.0055	0.004	0.0055	0.0055	
45	0.004	0.0055	0.004	0.0055	0.0055	
Mean Basal	**0.004**	**0.0055**	**0.004**	**0.0055**	**0.0055**	**4.9**
60	0.004	0.0055	0.004	0.0068	0.0055	
75	0.0068	0.0068	0.0055	0.0082	0.0082	
90	0.0095	0.0082	0.0082	0.0095	0.011	
105	0.012	0.0095	0.0095	0.0095	0.012	
120	0.012	0.0095	0.0082	0.0082	0.012	
135	0.011	0.0068	0.0068	0.0068	0.0082	
150	0.0082	0.0055	0.0055	0.0055	0.0068	
165	0.0055	0.0055	0.004	0.004	0.0055	
180	0.004	0.0055	0.004	0.004	0.0055	
Peak Hist	**0.012**	**0.0095**	**0.0095**	**0.0095**	**0.012**	**10.5**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 3 after Ulcer Induction in Hyperthyroid Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X1000
15	0.0095	0.0068	0.0095	0.0068	0.0095	
30	0.0095	0.0068	0.0095	0.0068	0.0095	
45	0.0095	0.0068	0.0095	0.0068	0.0095	
Mean Basal	**0.0095**	**0.0068**	**0.0095**	**0.0068**	**0.0095**	**8.4**
60	0.012	0.0095	0.011	0.0068	0.0095	
75	0.014	0.012	0.014	0.0095	0.012	
90	0.016	0.015	0.019	0.014	0.016	
105	0.018	0.018	0.022	0.016	0.018	
120	0.022	0.02	0.022	0.019	0.022	
135	0.02	0.019	0.019	0.018	0.022	
150	0.018	0.016	0.016	0.016	0.019	
165	0.014	0.0095	0.014	0.012	0.012	
180	0.0095	0.0068	0.0095	0.0068	0.0095	
Peak Hist	**0.022**	**0.02**	**0.022**	**0.019**	**0.022**	**21.0**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 7 after Ulcer Induction in Control Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0027	0.0027	0.0027	0.0027	0.0027	
30	0.0027	0.0027	0.0027	0.0027	0.0027	
45	0.0027	0.0027	0.0027	0.0027	0.0014	
Mean Basal	**0.0027**	**0.0027**	**0.0027**	**0.0027**	**0.002267**	**2.6**
60	0.004	0.004	0.004	0.0055	0.004	
75	0.0068	0.0055	0.0068	0.0055	0.005	
90	0.0068	0.0068	0.0068	0.0068	0.0068	
105	0.0055	0.0082	0.0055	0.0082	0.0082	
120	0.0055	0.0082	0.0027	0.0082	0.0082	
135	0.0027	0.004	0.0027	0.004	0.004	
150	0.0027	0.004	0.0027	0.004	0.004	
165	0.0027	0.0027	0.0027	0.0027	0.0027	
180	0.0027	0.0027	0.0027	0.0027	0.0027	
Peak Hist	**0.0055**	**0.0082**	**0.0055**	**0.0082**	**0.0082**	**7.12**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 7 after Ulcer Induction in Hypothyroid Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0014	0.0014	0.0014	0.0014	0.0014	
30	0.0014	0.0014	0.0014	0.0014	0.0014	
45	0.0014	0.0014	0.0014	0.0014	0.0027	
Mean Basal	**0.0014**	**0.0014**	**0.0014**	**0.0014**	**0.0018**	**1.5**
60	0.004	0.004	0.0027	0.0027	0.004	
75	0.0055	0.0055	0.0055	0.0055	0.0055	
90	0.0068	0.0068	0.0068	0.0068	0.0082	
105	0.0055	0.0068	0.0055	0.0055	0.0068	
120	0.0055	0.004	0.0055	0.0027	0.004	
135	0.0027	0.0027	0.0027	0.0014	0.0027	
150	0.0014	0.0027	0.0014	0.0014	0.0027	
165	0.0014	0.0014	0.0014	0.0014	0.0014	
180	0.0014	0.0014	0.0014	0.0014	0.0014	
Peak Hist	**0.0068**	**0.0068**	**0.0068**	**0.0068**	**0.0082**	**7.1**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 7 after Ulcer Induction in Thyroidectomised + T_4 Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0027	0.0027	0.0027	0.0027	0.004	
30	0.0027	0.0027	0.0027	0.0027	0.004	
45	0.0027	0.0014	0.0027	0.004	0.004	
Mean Basal	**0.0027**	**0.0023**	**0.0027**	**0.0031**	**0.004**	**3.0**
60	0.0055	0.0027	0.0055	0.0055	0.0068	
75	0.0068	0.0068	0.0068	0.0068	0.0082	
90	0.0082	0.0082	0.0082	0.0082	0.0082	
105	0.0082	0.0082	0.0082	0.0082	0.0095	
120	0.0068	0.0068	0.0068	0.0095	0.0082	
135	0.0068	0.0068	0.0068	0.0068	0.0068	
150	0.0055	0.0055	0.0055	0.0055	0.0055	
165	0.004	0.0014	0.004	0.004	0.004	
180	0.004	0.0014	0.004	0.004	0.004	
Peak Hist	**0.0082**	**0.0068**	**0.0082**	**0.0082**	**0.0095**	8.2

Basal and Histamine Stimulated Gastric Acid Secretion on Day 7 after Ulcer Induction in Hyperthyroid Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0055	0.0027	0.0055	0.004	0.004	
30	0.0055	0.0027	0.0055	0.004	0.004	
45	0.0055	0.0027	0.0055	0.004	0.004	
Mean Basal	**0.0055**	**0.0027**	**0.0055**	**0.004**	**0.004**	**4.3**
60	0.0055	0.004	0.0055	0.0055	0.004	
75	0.0082	0.0095	0.011	0.011	0.0095	
90	0.012	0.014	0.014	0.015	0.011	
105	0.014	0.015	0.0095	0.011	0.015	
120	0.011	0.011	0.0082	0.011	0.011	
135	0.0068	0.0055	0.0068	0.0082	0.011	
150	0.0055	0.004	0.0055	0.0055	0.0055	
165	0.0055	0.004	0.0055	0.004	0.004	
180	0.0055	0.004	0.0055	0.004	0.004	
Peak Hist	**0.012**	**0.014**	**0.014**	**0.015**	**0.011**	**13.2**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 10 after Ulcer Induction in Control Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0014	0.0014	0.0014	0.0014	0.0014	
30	0.0014	0.0014	0.0014	0.0014	0.0014	
45	0.0014	0.0014	0.0014	0.0014	0.0014	
Mean Basal	**0.0014**	**0.0014**	**0.0014**	**0.0014**	**0.0014**	**1.4**
60	0.0014	0.0014	0.0014	0.0027	0.0014	
75	0.0047	0.0027	0.0027	0.0047	0.0027	
90	0.0055	0.004	0.0055	0.0055	0.0055	
105	0.0068	0.0068	0.0068	0.0068	0.0068	
120	0.0068	0.0055	0.0082	0.0068	0.01	
135	0.0055	0.004	0.0068	0.0055	0.0055	
150	0.004	0.0027	0.0055	0.004	0.0027	
165	0.0027	0.0014	0.004	0.0027	0.0014	
180	0.0014	0.0014	0.0014	0.0014	0.0014	
Peak Hist	**0.0068**	**0.0068**	**0.0068**	**0.0068**	**0.0068**	**6.8**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 10 after Ulcer Induction in Hypothyroid Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0014	0.0014	0.0014	0.0014	0.0014	
30	0.0014	0.0014	0.0014	0.0014	0.0014	
45	0.0014	0.0014	0.0014	0.0014	0.0014	
Mean Basal	**0.0014**	**0.0014**	**0.0014**	**0.0014**	**0.0014**	**1.4**
60	0.0014	0.0014	0.0014	0.0027	0.0014	
75	0.0027	0.004	0.0034	0.0068	0.0027	
90	0.0055	0.0068	0.0068	0.0068	0.004	
105	0.0068	0.0082	0.0068	0.0055	0.0068	
120	0.0055	0.0068	0.0095	0.004	0.004	
135	0.004	0.004	0.0095	0.0027	0.0027	
150	0.0034	0.002	0.0068	0.0014	0.0014	
165	0.0027	0.0014	0.0034	0.0014	0.0014	
180	0.0014	0.0014	0.0014	0.0014	0.0014	
Peak Hist	**0.0068**	**0.0082**	**0.0068**	**0.0055**	**0.0068**	**6.8**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 10 after Ulcer Induction in Thyroidectomised + T_4 Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0014	0.0027	0.0014	0.0014	0.0014	
30	0.0014	0.0014	0.0014	0.0014	0.0014	
45	0.0014	0.0014	0.0014	0.0014	0.0014	
Mean Basal	**0.0014**	**0.001833**	**0.0014**	**0.0014**	**0.0014**	**1.5**
60	0.0014	0.0014	0.0014	0.0014	0.0014	
75	0.004	0.0027	0.004	0.004	0.004	
90	0.0055	0.0055	0.0055	0.0055	0.0055	
105	0.0068	0.0068	0.0068	0.0082	0.0068	
120	0.0082	0.0082	0.0055	0.0082	0.0068	
135	0.0055	0.0068	0.004	0.0068	0.0055	
150	0.0027	0.0055	0.0027	0.004	0.004	
165	0.0014	0.0027	0.0027	0.0027	0.0027	
180	0.0014	0.0014	0.0027	0.0014	0.0014	
Peak Hist	**0.0082**	**0.0082**	**0.0055**	**0.0082**	**0.0068**	**7.4**

Basal and Histamine Stimulated Gastric Acid Secretion on Day 10 after Ulcer Induction in Hyperthyroid Animals (Meq/L)

Time (min)	1	2	3	4	5	Mean X 1000
15	0.0014	0.0014	0.0027	0.0014	0.0027	
30	0.0014	0.0014	0.0027	0.0014	0.0027	
45	0.0014	0.0014	0.0027	0.0014	0.0027	
Mean Basal	**0.0014**	**0.0014**	**0.0027**	**0.0014**	**0.0027**	**1.9**
60	0.0014	0.0014	0.0027	0.0014	0.0027	
75	0.004	0.0027	0.0082	0.004	0.0055	
90	0.0068	0.0055	0.0082	0.0068	0.0082	
105	0.012	0.0095	0.0095	0.012	0.011	
120	0.0011	0.0095	0.0082	0.011	0.011	
135	0.0095	0.0082	0.0055	0.0095	0.0082	
150	0.0082	0.0055	0.004	0.0082	0.0055	
165	0.0055	0.0027	0.0027	0.0055	0.0027	
180	0.0014	0.0014	0.0027	0.0014	0.0014	
Peak Hist	**0.012**	**0.0095**	**0.0095**	**0.012**	**0.011**	**10.8**

Haematological Parameters in Control Animals on Day 3 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	38.0	7000.0	5.6	36.0	64.0	0.6
2	41.0	6000.0	6.5	40.0	60.0	0.7
3	43.0	6000.0	6.5	40.0	60.0	0.7
4	38.0	7200.0	6.6	40.0	60.0	0.7
5	42.0	6800.0	6.4	36.0	64.0	0.6
mean	**40.4**	**6600.0**	**6.3**	**38.4**	**61.6**	**0.6**
SD	**2.30**	**565.69**	**0.41**	**2.2**	**2.2**	**0.1**
SEM	**1.03**	**252.54**	**0.18**	**1.0**	**1.0**	**0.0**

Haematological Parameters in Hypothyroid Animals on Day 3 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	40	8500	6.5	45.0	55.0	0.8
2	44	8000	7.1	40.0	60.0	0.7
3	46	8100	7.1	44.0	56.0	0.8
4	45	8500	7.5	42.0	58.0	0.7
5	41	8000	6.5	43.0	57.0	0.8
mean	**43.2**	**8220**	**6.94**	**42.8**	**57.2**	**0.7**
SD	**2.588436**	**258.8436**	**0.43359**	**1.9**	**1.9**	**0.1**
SEM	**1.155552**	**115.5552**	**0.193567**	**0.9**	**0.9**	**0.0**

Haematological Parameters in Thyroidectomised + T_4 Animals on Day 3 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	35.0	4500.0	5.5	47.0	53.0	0.9
2	41.0	5700.0	6.3	40.0	60.0	0.7
3	35.0	5200.0	5.3	48.0	52.0	0.9
4	37.0	5300.0	5.7	47.0	53.0	0.9
5	33.0	4900.0	5.3	45.0	55.0	0.8
mean	**36.2**	**5120.0**	**5.6**	**45.4**	**54.6**	**0.8**
SD	**3.03**	**449.44**	**0.41**	**3.2**	**3.2**	**0.1**
SEM	**1.35**	**200.64**	**0.19**	**1.4**	**1.4**	**0.0**

Haematological Parameters in Hyperthyroid Animals on Day 3 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	37.0	6500.0	5.8	49.0	51.0	1.0
2	34.0	6500.0	5.5	52.0	48.0	1.1
3	43.0	6000.0	6.0	42.0	58.0	0.7
4	40.0	6000.0	5.9	48.0	52.0	0.9
5	36.0	6500.0	6.3	50.0	50.0	1.0
mean	**38.0**	**6300.0**	**5.9**	**48.2**	**51.8**	**0.9**
SD	**3.54**	**273.86**	**0.29**	**3.8**	**3.8**	**0.1**
SEM	**1.58**	**122.26**	**0.13**	**1.7**	**1.7**	**0.1**

Haematological Parameters in Control Animals on Day 7 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	43.0	18000.0	7.0	30.0	70.0	0.4
2	40.0	16000.0	6.4	32.0	68.0	0.5
3	41.0	18600.0	6.4	36.0	64.0	0.6
4	42.0	18000.0	6.4	36.0	64.0	0.6
5	40.0	18500.0	6.8	32.0	68.0	0.5
mean	**41.2**	**17820.0**	**6.6**	**33.2**	**66.8**	**0.5**
SD	**1.30**	**1054.51**	**0.28**	**2.7**	**2.7**	**0.1**
SEM	**0.58**	**470.77**	**0.13**	**1.2**	**1.2**	**0.0**

Haematological Parameters in Hypothyroid Animals on Day 7 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	46.0	8300.0	7.5	45.0	55.0	0.8
2	48.0	8500.0	7.9	37.0	63.0	0.6
3	42.0	8000.0	6.8	40.0	60.0	0.7
4	46.0	8500.0	7.5	37.0	63.0	0.6
5	44.0	8300.0	7.7	37.0	63.0	0.6
mean	**45.2**	**8320.0**	**7.5**	**39.2**	**60.8**	**0.6**
SD	**2.28**	**204.94**	**0.41**	**3.5**	**3.5**	**0.1**
SEM	**1.02**	**91.49**	**0.19**	**1.6**	**1.6**	**0.0**

Haematological Parameters in Thyroidectomised + T_4 Animals on Day 7 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	42.0	8000.0	6.8	35.0	65.0	0.5
2	39.0	9600.0	5.5	30.0	70.0	0.4
3	40.0	9000.0	6.0	40.0	60.0	0.7
4	41.0	7000.0	6.5	40.0	60.0	0.7
5	39.0	8000.0	5.5	30.0	70.0	0.4
mean	**40.2**	**8320.0**	**6.1**	**35.0**	**65.0**	**0.5**
SD	**1.30**	**1005.98**	**0.59**	**5.0**	**5.0**	**0.1**
SEM	**0.58**	**449.10**	**0.26**	**2.2**	**2.2**	**0.1**

Haematological Parameters in Hyperthyroid Animals on Day 7 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	43.0	16700.0	6.9	32.0	68.0	0.5
2	44.0	14300.0	7.3	30.0	70.0	0.4
3	46.0	14000.0	7.7	32.0	68.0	0.5
4	45.0	14000.0	7.4	30.0	70.0	0.4
5	43.0	14000.0	6.9	32.0	68.0	0.5
mean	**44.2**	**14600.0**	**7.2**	**31.2**	**68.8**	**0.5**
SD	**1.30**	**1181.10**	**0.34**	**1.1**	**1.1**	**0.0**
SEM	**0.58**	**527.28**	**0.15**	**0.5**	**0.5**	**0.0**

Haematological Parameters in Control Animals on Day 10 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	50.0	7500.0	8.3	30.0	70.0	0.4
2	53.0	7500.0	8.8	31.0	69.0	0.4
3	48.0	8000.0	8.0	30.0	70.0	0.4
4	51.0	9000.0	8.0	32.0	68.0	0.5
5	49.0	8800.0	8.6	31.0	69.0	0.4
mean	**50.2**	**8160.0**	**8.3**	**30.8**	**69.2**	**0.4**
SD	**1.92**	**709.22**	**0.36**	**0.8**	**0.8**	**0.0**
SEM	**0.86**	**316.62**	**0.16**	**0.4**	**0.4**	**0.0**

Haematological Parameters in Hypothyroid Animals on Day 10 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	46.0	8300.0	7.5	40.0	60.0	0.7
2	48.0	8500.0	7.9	35.0	65.0	0.5
3	42.0	8000.0	6.8	40.0	60.0	0.7
4	46.0	8500.0	7.5	36.0	64.0	0.6
5	44.0	8300.0	7.7	36.0	64.0	0.6
mean	**45.2**	**8320.0**	**7.5**	**37.4**	**62.6**	**0.6**
SD	**2.28**	**204.94**	**0.41**	**2.4**	**2.4**	**0.1**
SEM	**1.02**	**91.49**	**0.19**	**1.1**	**1.1**	**0.0**

Haematological Parameters in Thyroidectomised + T_4 Animals on Day 10 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	43.0	8900.0	6.3	30.0	70.0	0.4
2	46.0	8000.0	7.5	31.0	69.0	0.4
3	41.0	8500.0	6.3	32.0	68.0	0.5
4	44.0	8500.0	6.9	30.0	70.0	0.4
5	42.0	8800.0	6.5	31.0	69.0	0.4
mean	**43.2**	**8540.0**	**6.7**	**30.8**	**69.2**	**0.4**
SD	**1.92**	**350.71**	**0.51**	**0.8**	**0.8**	**0.0**
SEM	**0.86**	**156.57**	**0.23**	**0.4**	**0.4**	**0.0**

Haematological Parameters in Hyperthyroid Animals on Day 10 after Ulcer Induction

Animals	PCV	WBC X10^6/L	RBC X 10^{12}/L	Neutrophil (%)	Lymphocyte (%)	NLR
1	48.0	5000.0	7.9	20.0	80.0	0.3
2	48.0	5000.0	7.8	29.0	71.0	0.4
3	47.0	7900.0	7.5	20.0	80.0	0.3
4	45.0	6000.0	7.7	25.0	75.0	0.3
5	47.0	5800.0	7.5	29.0	71.0	0.4
mean	**47.0**	**5940.0**	**7.7**	**24.6**	**75.4**	**0.3**
SD	**1.22**	**1186.59**	**0.18**	**4.5**	**4.5**	**0.1**
SEM	**0.55**	**529.73**	**0.08**	**2.0**	**2.0**	**0.0**

Lipid Peroxidation (nmolMDA/mg protein) on Day 3 after Ulcer Induction

Animals	Control	Hypothyroid	Thyroidectomised + T_4	Hyperthyroid
1	158.0	166.2	192.3	271.6
2	160.7	206.0	153.8	244.5
3	195.1	171.7	164.8	281.6
4	171.7	181.3	170.3	289.8
5	171.7	181.3	170.3	270.6
Mean	**171.4**	**181.3**	**170.3**	**271.6**
SD	**14.6**	**15.3**	**14.0**	**19.7**
SEM	**6.5**	**6.8**	**6.3**	**9.9**

Lipid Peroxidation (nmolMDA/mg protein) on Day 7 after Ulcer Induction

Animals	Control	Hypothyroid	Thyroidectomised + T_4	Hyperthyroid
1	142.9	158.0	184.1	160.7
2	145.6	199.2	144.2	137.4
3	166.2	158.0	144.2	171.7
4	151.6	171.7	160.7	173.1
5	151.6	171.7	158.0	160.7
Mean	**151.6**	**171.7**	**158.2**	**160.7**
SD	**9.0**	**16.8**	**16.3**	**16.5**
SEM	**4.0**	**7.5**	**7.3**	**8.3**

Lipid Peroxidation (nmolMDA/mg protein) on Day 10 after Ulcer Induction

Animals	Control	Hypothyroid	Thyroidectomised + T_4	Hyperthyroid
1	136.0	151.8	140.8	120.0
2	140.1	181.3	130.5	83.8
3	144.2	158.0	135.3	136.7
4	140.1	175.1	136.0	146.3
5	140.1	166.6	137.4	113.3
Mean	**140.1**	**166.6**	**136.0**	**120.0**
SD	**2.9**	**12.1**	**3.7**	**27.8**
SEM	**1.3**	**5.4**	**1.7**	**13.9**