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## Light and Electron Microscopic Studies on the Effect of Tenoxicam on the Stomach of the Mouse

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**Abstract:** The effects of new nonsteroidal anti-inflammatory drug (NSAID), Tenoxicam (Epicotal), on the light and electron microscopy of the stomach of the mice were studied. Administration of daily high therapeutic dose ( $0.12 \text{ mg kg}^{-1}$  body weight) for 30 days showed obvious pathologic changes. Extensive light microscopic changes are noticed including dilation and congestion of blood vessels and blood capillaries, a decrease of the amount of lymphoid cells in the submucosa and the lamina propria and obvious changes of the smooth muscle fibers of the stomach. Significant morphological changes in gastric gland were revealed by electron microscopy. Several unusual structural features are observed in oxyntic cells including increase in the heterochromatin in nucleus, some nuclei contain dense heterochromatin, destruction of nuclear membrane, decreased and dense mitochondria, hypertrophied golgi apparatus, decrease of tubulovesicles and myofibrils in the apical cytoplasm, fragmentation of rough endoplasmic reticulum, increase of the ribosomes and vacuolation of the connective tissue in between the blood capillary and the base of oxyntic cell. Peptic cells showed no apparent changes. In addition, the administration of high therapeutic dose of the Tenoxicam for 30 days exhibited obvious histological changes in cardiac muscles including severe congestion and dilatation of blood vessels and indentation of the muscle fibers.

**Key words:** NSAID, stomach, blood vessels, electron microscopy, oxyntic cells

### Introduction

The stomach is a mixed exocrine-endocrine organ that digest food and secretes hormones. Its main functions are to continue the digestion of carbohydrates initiated in the mouth, add an acidic fluid to the ingested food, transform it by muscular activity into a viscous mass (chyme) and promote the initial digestion of proteins with enzyme pepsin. In addition, the inner layer of stomach is a protective barrier between the contents of the tract's lumen and the internal milieu of the body (Junqueira *et al.*, 1995). Arthritis and musculoskeletal disorders are common. Arthritis currently accounts for 2 to 30% of all cases of disability and the numbers are rising (Hungin and Kean, 2001). Conventional nonsteroidal anti-inflammatory drug (NSAID) is popular and commonly used to treat pain and inflammation; they are widely prescribed for the treatment of many conditions including rheumatoid arthritis, osteoarthritis, gouty arthritis, the joint and muscle discomfort associated with systemic lupus erythematosus and other musculoskeletal disorders (Raskin, 1999; Hawkins and Hanks, 2000; Buttgerit *et al.*, 2001); short-term treatment in the following conditions: post-traumatic pain, inflammation and swelling e.g. due to sprains, following dental or orthopaedic surgery, renal colic and biliary colic. Therefore, NSAIDs are among the most frequently prescribed drugs.

The gastrointestinal (GI) tract is the most common location for side effects of NSAIDs. However these are intrinsically toxic to gastroduodenal mucosa (Bjorkman, 1999). Exposure to NSAIDs is known to increase substantially the risk of upper gastrointestinal bleeding and perforation. These drugs may cause problems in any part of the GI tract from esophagus to rectum. The severity of these side effects ranges from nuisance symptoms such as dyspepsia to life-threatening ulcer complications (Wolfe, 1998). Gastrointestinal (GI) toxicity is a major limiting factor in use of NSAIDs. Because of the widespread use of these medications, the morbidity and costs associated with GI complications of NSAID use are significant (McCarthy, 1999; Bjorkmann, 1999). The volume of the side effects noted, imply the overuse of these drugs, especially in relation to the estimated prevalence of osteoarthritis, where pain relief may be considered more important than an anti-inflammatory effect (Hungin and Kean, 2001).

Several studies have shown that a person exposed to NSAIDs has three to four times the risk of upper gastrointestinal bleeding, perforation or both than a non user (Bollini *et al.*, 1992). Acutely bleeding peptic ulcers are associated with hospital mortality of 4 and 14% (Allan and Dykes, 1976; Somerville *et al.*, 1986 and Schafer, 1999). About a half of these bleeds occur in users of

NSAIDs. Garcia Rodriguez and Jick (1994) stated that for all NSAIDs together, the risk was greater for high doses than for low doses (7.0{5.2-9.6} vs 2.6 {1.8-3.8}). Hernandez and Garcia Rodrigues (2001) and Whelton (2001) stated that the use of conventional NSAIDs has been associated with acute liver injury, acute renal injury, increased blood pressure or heart failure and adverse reproductive outcome. Similarly, Griffin and Scheiman (2001) stated that traditional NSAIDs increase the risk of clinically important upper gastrointestinal ulcers and bleeds about four folds. Other risk factors for these events include advanced age, higher NSAID dose, prior ulcer or bleed, use of anticoagulants, use of corticosteroids and poor general health. Some established NSAIDs may accelerate cartilage destruction in osteoarthritis, by the inhibitory effects on cartilage proteoglycan metabolism seen with such drugs (Rainsford, 1999). It is well-established that salicylates (NSAID) inhibit the rate of synthesis of glucosaminoglycans *in vitro* and *in vivo* (Whitehouse and Bostrom, 1961). This is probably due to the inhibition of enzyme L-glutamine-D-fructose-C-phosphate amino transferase, which catalyses the synthesis of glucosamine-C-phosphate, a key intermediate. On the other hand, NSAIDs can produce a mild systemic hemostatic defect by inhibiting normal platelet function. The platelets dysfunction causing hemorrhage from various lesions including but not confined to ulcers, NSAIDs cause platelets dysfunction by inhibiting the formation of thromboxane.

Prostaglandins are well recognized as protecting the gastric mucosa and enhancing the perception of pain. Inhibition of prostaglandin synthesis by NSAIDs is the major established mechanism by which NSAIDs render the gastric mucosa vulnerable to mucosal injury (Hawkey and Rampton, 1985). Equally depression of prostaglandin synthesis, by diminishing pain perception, could at least in part account for the high proportion of NSAIDs associated ulcers that are silent (Mellum *et al.*, 1985). Prostaglandins normally stimulate mucus and bicarbonate secretion, decrease acid secretion and cause vasodilatation, thereby increasing elimination of acid that has diffused into the submucosa (Blaster, 1999).

Conventional NSAIDs are nonselective inhibitors of two isoforms of cyclo-oxygenase (COX), COX-1 and COX-2. COX-1 alone has important, necessary physiologic functions (e.g. the stomach and platelets). Inhibition of COX-1 is believed to be responsible for inducing mucosal injury primarily by impairing prostaglandin dependent mucosal protective mechanisms (Mandell, 1999 and Buttgerit *et al.*, 2001). Unfortunately, the COX-2 inhibitors still retain some of the side effects seen with traditional dual inhibitors

(NSAIDs), namely, effects on the kidney that may manifest as an increased incidence of hypertension, edema and associated clinical states. Similarly, effects on reproductive functions, endothelial function and wound healing are theoretically possible (Schnitzer, 2001). NSAIDs benefits, which are believed to be the result of their ability to inhibit cyclooxygenase-2 (COX-2), are accompanied by considerable toxicity. However, Rainsford (1999) stated that the popular view that all NSAIDs act by inhibiting the production of prostaglandins has been challenged by the discovery that they also affect a wide variety of cellular processes important for their therapeutic actions and side effects.

Unrelieved pain is dangerous and should be treated as effectively as possible in all patients for pathophysiologic as well as humanitarian reasons. Drug treatment in any patient entails multiple risk-benefit analyses and all forms of pain relief have potentially deleterious effects. The absolute risk for serious NSAID-related GI toxicity remains constant and the cumulative risk increases over time; there are no reliable warning signals >80% of patients with serious GI complications that had no prior GI symptoms (Singh, 1998). There are many different types of NSAIDs prescribed by physicians, which differ in their risk, some NSAIDs have subtle effect, while others have severe adverse effects, i.e. the severity of the adverse effects differ according to the type of the NSAID. Therefore, this work was undertaken to describe the changes in stomach by light and electron microscope and the light microscope of the heart of the mice during the treatment of high therapeutic dose of the Tenoxicam to assess the risk associated with one of new and commonly used NSAIDs.

## Materials and Methods

Female Swiss mice (*Mus musculus*) aged 2-3 months and weighing 22-25 g were used in this study. A group of mice served as control, each mouse injected intramuscularly with daily dose of 0.1 ml of distilled water for 30 days. The experimental group injected intramuscularly with daily doses of 0.12 mg kg<sup>-1</sup> body weight of epical (Tenoxicam) (dissolved in 0.1 ml distilled water) for 30 days.

Small blocks of the stomach and cardiac muscles (left ventricle) were fixed one hour in 2.5% glutaraldehyde, phosphate buffer pH 7.4, post fixed in 2% OsO<sub>4</sub> for one hour, dehydrated through graded alcohol and embedded in Epon. Thin (50 µm) sections were cut on LKB ultramicrotome. After double staining, the stomach sections with uranyl acetate and lead citrate, these were examined under a Jeol CX electron microscope. Semithin sections of the stomach and the cardiac muscles were stained with toluidin blue for light microscopic examination.

## Results

**Light microscopy of control stomach:** The stomach of the mice is surrounded by a wall made up of four principal layers: the mucosa, submucosa, muscularis and serosa (Figs. 1-3). The gastric mucosa of mice stomach consists of a surface epithelium that invaginates to varying extents into the lamina propria forming gastric pits. Emptying into the gastric pit are branched tubular glands. The lamina propria of the stomach is composed of loose connective tissue interspersed with smooth muscles and lymphoid cells. The submucosa is relatively loose, contains large blood vessels and many lymphoid cells (Figs. 1&2). The muscularis comprises the usual inner circular and outer longitudinal layer. The circular muscle fibers are with elongated nuclei (Figs. 1&2). Separating the mucosa from underlying submucosa is a layer of smooth muscle, muscularis mucosa. The epithelium covering the surface and lining the pits is simple columnar epithelium. The serosal layer which cover the peritoneal surface is thin and barely visible at the low magnification. The distribution of epithelial cells in gastric glands is not uniform (Figs. 1,2&3). The neck consists of stem and oxyntic cells; the base of the glands contains oxyntic, peptic and enteroendocrine cells. Most of the single layers of gland cells are of oxyntic cells. These large round or pyramidal cells have large centrally placed spherical nucleus of low density (Figs. 2&3).

The peptic cells are roughly cuboidal, their nuclei though indented are more or less rounded in outline, are characterized by their small dense cytoplasmic granules are recognized at all levels of the gland by the extreme density of its cytoplasm. Occasionally, the enteroendocrine cells can be identified in the sections characterized by their triangular shape.

**Light microscopy of Tenoxicam-treated stomach:** The light microscopy of the stomach of Tenoxicam-treated animals showed darkly stained tubular gland indicating the increase in the basophilic of the cells (Fig. 4). In the gastric gland cells, the changes induced by Tenoxicam administration (0.12 mg/kg b. wt. for 30 days) were confined to oxyntic cells. Oxyntic cells appeared hypertrophied with enlarged nuclei and vacuolated cytoplasm (Figs. 5&6). A pronounced change was observed in the stomach blood vessels and blood capillaries including congestion and dilation of blood vessels and blood capillaries in the lamina propria and submucosa in all treated animals (Figs. 4-6). Although, there were many lymphoid cells lies in the submucosa and lamina propria of the stomach of the control, there was an obvious decrease in the amount of lymphoid cells in the Tenoxicam-treated animals (compare Figs. 1& 2 and Figs. 4&5). The circular muscle fibers of the treated animals appeared contracted and their nuclei were oval or rounded (Figs. 4&5) (compare with the circular muscle fibers of the control animals which do not appear in a contracted state and contained highly elongated nuclei, Figs. 1&2).

## Electron microscopy of control stomach

**Oxyntic cells (acid secreting, parietal cells) of control stomach:** Oxyntic cells are distributed along the length of glands but tend to be most numerous in the upper half of gastric glands. In some oxyntic cells, the most striking features seen in the electron microscope are an abundance of mitochondria and a deep, circular invagination of the apical plasma membrane forming branching intracellular canaliculus, which extend throughout the cytoplasm and between adjacent cells (Fig. 7). The mitochondria appear to be separated into a peripheral group and a central perinuclear group by a zone of cytoplasm containing the intracellular canaliculi (Fig. 7). The matrix of the mitochondria is much dense than that of mitochondria in other cells (Fig. 7). Numerous short microvilli project into the lamina of intracellular canaliculi (Fig. 7). Oxyntic cells have a free surface with microvilli (Figs. 8&9). The pale cytoplasm results in part from the diffused nature of the granular endoplasmic reticulum. In the oxyntic cell, a number of tubulovesicular structures can be seen in the apical region of the cell just below its plasmalemma (Figs. 8&9). At this stage, the cell has few microvilli when stimulated to produce hydrochloric acid, tubulovesicles fused with the cell membrane and form more microvilli, thus providing a generous increase in the surface of cell membrane. Cytoplasmic fibrils (actin filaments) are present and dense fibrils are aggregated close to the apical cell membrane (Figs. 8&9), these cytoplasmic fibrils present between the tubulovesicles probably play a role in the interaction of these structures they appear to participate in the formation of secretion. The cytoplasm possesses a discrete golgi complex near the cell base (Fig. 10). The mucosal capillaries are in very close proximity to the oxyntic cells. The interstitial diffusion distance from capillary to oxyntic cell is very small. Therefore hindrance to the diffusion of small molecules between the capillaries and oxyntic cell is minimal (Figs. 10&11). This is important for the diffusion of metabolites to the highly active oxyntic cells (Fig. 11).

**Peptic cells (pepsin-secreting, chief cells) of control stomach:** Peptic cells are located towards the bases of gastric glands. Peptic cells are recognized by their condensed, basely located nuclei and strongly basophilic granular cytoplasm. The ultrastructural features of peptic cells are those of protein secreting (zymogenic) cells in general; these features include an extensive rough endoplasmic reticulum and membrane bound secretory vesicles (zymogen granules) crowded in the apical cytoplasm. A well

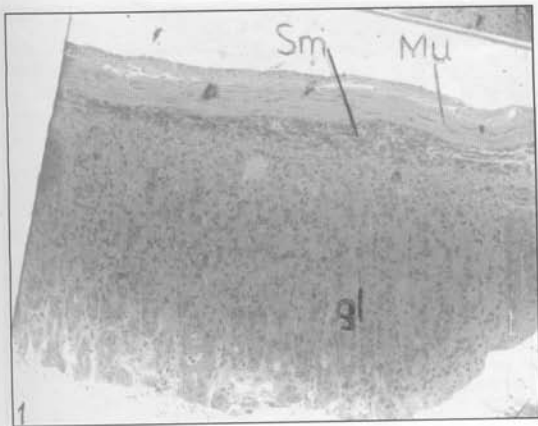


Fig. 1: Semithin section of stomach of control group showing muscularis (Mu), submucosa (Sm) contains large amount of lymphoid cells, tubular gland (gl). TB (x200).

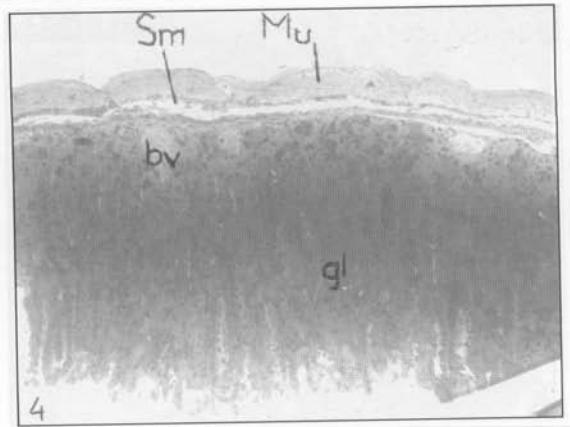


Fig. 4: Semithin section of stomach of treated group, showing contracted muscularis (Mu), submucosa (Sm) nearly devoid of lymphoid cells, congested blood vessels (bv), darkly stained tubular gland (gl) indicating the increase of the basophilia of the cells. TB (x200).

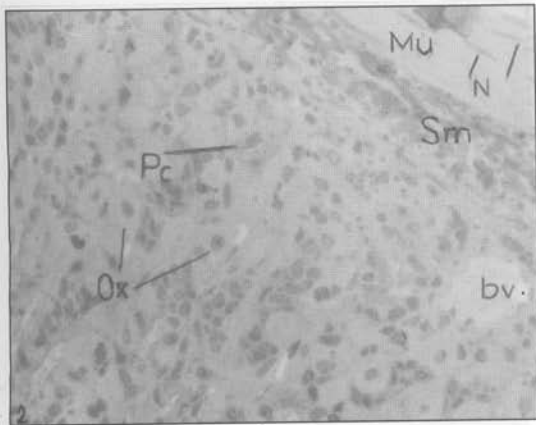


Fig. 2: Semithin section of stomach of control group showing muscularis (Mu) with elongated nuclei (N), submucosa (Sm) contains large amount of lymphoid cells, oxyntic cells (Ox), peptic cells (Pc), blood vessel (bv). TB (x400).

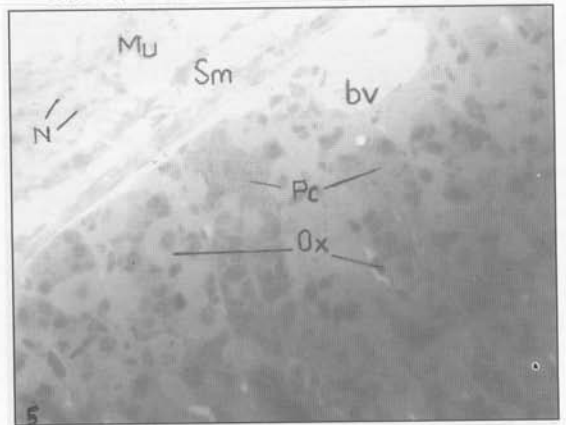


Fig. 5: Semithin section of stomach of treated group, showing muscularis (Mu) with oval nuclei (N), absence of lymphoid cells in submucosa (Sm), oxyntic cells (Ox), peptic cells (Pc), blood vessel (bv). TB (x400).

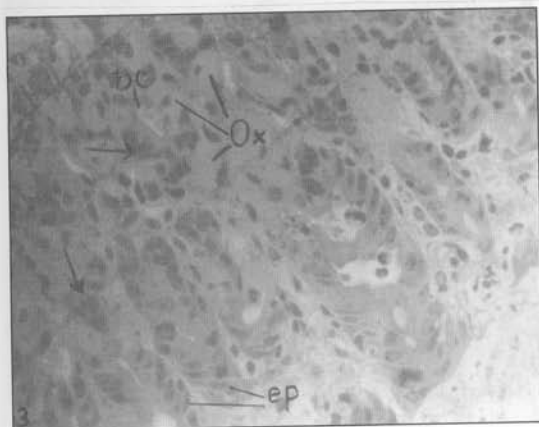


Fig. 3: Semithin section of stomach of control group, showing Oxyntic cells (Ox), undifferentiated cells (arrow), epithelium (ep), blood capillary (bc). TB (x400).

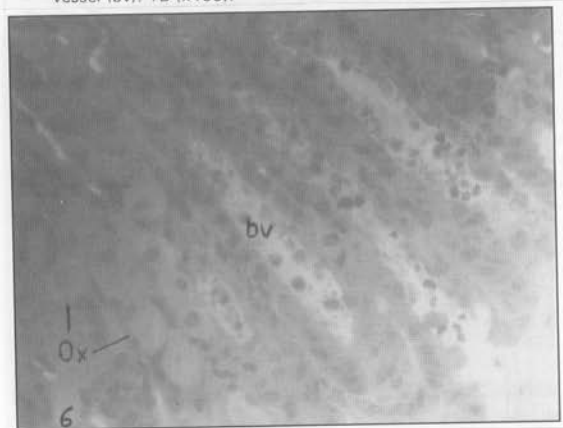


Fig. 6: Semithin section of stomach of treated group, showing mouth of tubular glands contain oxyntic cells (Ox) and some are vacuolated, dilatation of the blood vessels (bv) and some are destructed (arrow). TB (x400).

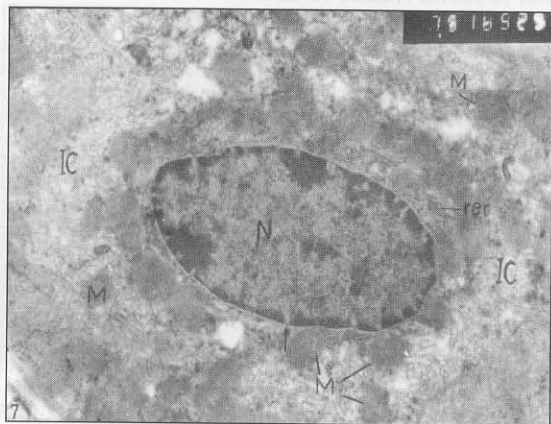


Fig. 7: Ultrathin section of stomach of control group, showing oxyntic cell, note nucleus (N), clear nuclear envelope and nuclear pores (arrow), mitochondria (M), intracellular canaliculi (IC) contained microvilli, rough endoplasmic reticulum (rer). (x7.500).

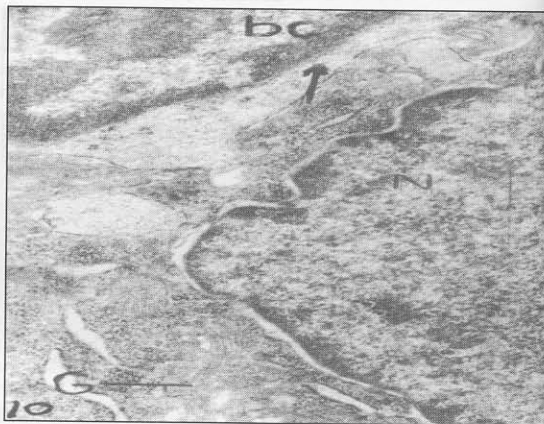


Fig. 10: Ultrathin section of stomach of control group, showing basal part of oxyntic cell, nucleus (N), the blood capillary (bc) immediately below the oxyntic cell; note minimal interstitial diffusion distance (minimal connective tissue) (arrow), Golgi apparatus (G). (x10.000).

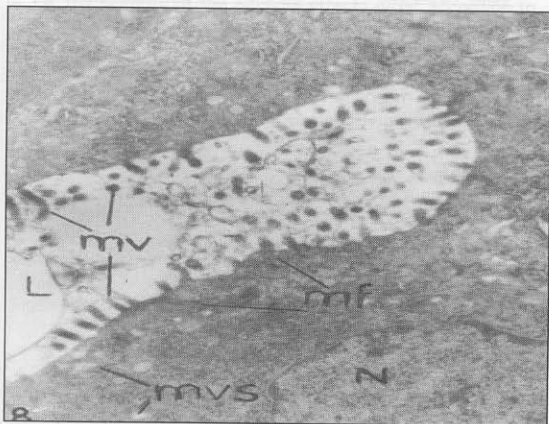


Fig. 8: Ultrathin section of stomach of control group, showing the apical cytoplasm of two oxyntic cell, note nucleus (N), micro vesicles (mv) and myofibril (mf), microvilli (mv), stomach lumen (L). (x7.500).

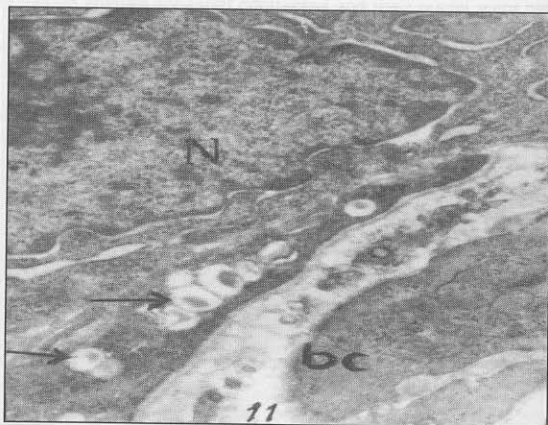


Fig. 11: Ultrathin section of stomach of control group, showing basal part of oxyntic cell, nucleus (N), blood capillary (bc) with fenestrated endothelium, the exchange of material with blood capillary (arrow). (x7.500).

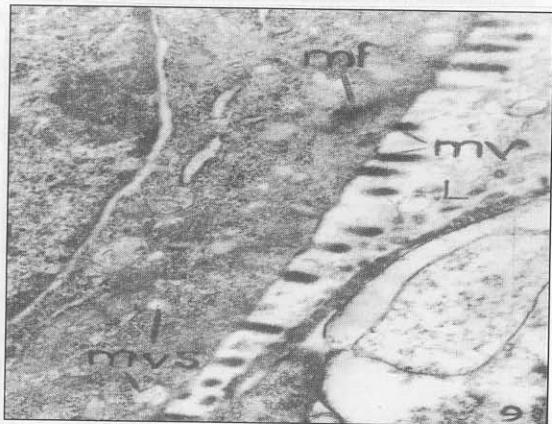


Fig. 9: Ultrathin section of stomach of control group, showing part of oxyntic cell, note nucleus (N), micro vesicles (mv) and myofibrils (mf), microvilli (mv). (x10.000).



Fig. 12: Ultrathin section of stomach of control group, showing peptic cell, the nucleus (N), rough endoplasmic reticulum (rer), zymogen granules (zg), Golgi apparatus (G), part of oxyntic cell with invagination of cell membrane which forming the intracellular canaliculi (IC). (x7.500).



Fig. 13: Ultrathin section of stomach of treated group, showing Oxyntic cell, note increased amount of heterochromatin of irregular nucleus, destructed nuclear membrane (arrow), many ribosomes(r), destructed blood capillary (bc) wall, fragmented stacks of rough endoplasmic reticulum (rer). (x10,000).

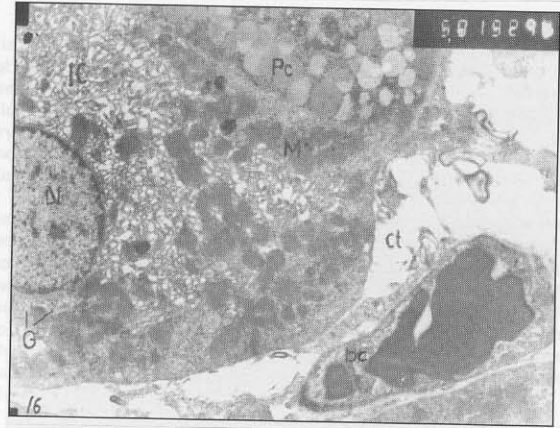


Fig. 16: Ultrathin section of stomach of treated group, showing oxyntic cell; note nucleus (N), dense mitochondria (M), dilated Golgi apparatus (G), dilated microvilli in the intracellular canaliculi (IC), blood capillary (bc), vacuolated connective tissue (ct), part of peptic cell contained the zymogen granules (zg). (x5,000)

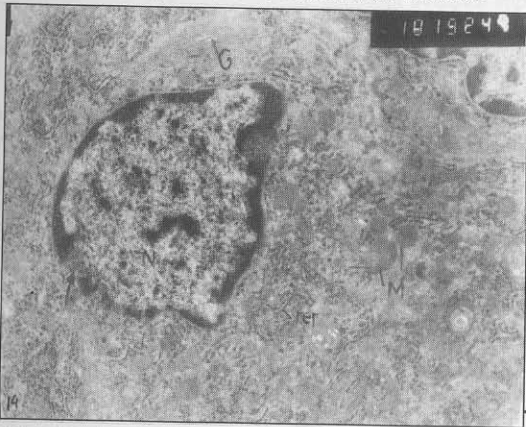


Fig. 14: Ultrathin section of stomach of treated group, showing Oxyntic cell, note small pycnotic nucleus (N) contained increased amount of electron dense heterochromatin and destructed nuclear membrane (arrow), hypertrophied Golgi apparatus (G), few and dense mitochondria (M), fragmented stacks of rough endoplasmic reticulum (rer). (x10,000).

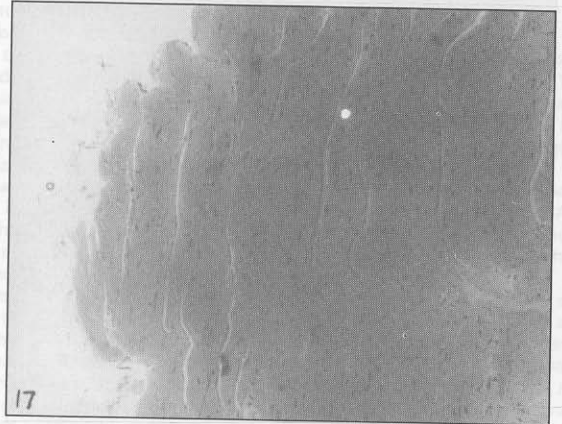


Fig. 17: Semithin section of cardiac muscles of control group, showing cardiac muscle fibers. (x200).



Fig. 15: Ultrathin section of stomach of treated group, showing Oxyntic cell, note destruction of nuclear membrane (arrow), small and dense mitochondria (M), hypertrophied Golgi apparatus (G), rough endoplasmic reticulum (rer), lysosome (Ly). (x10,000).

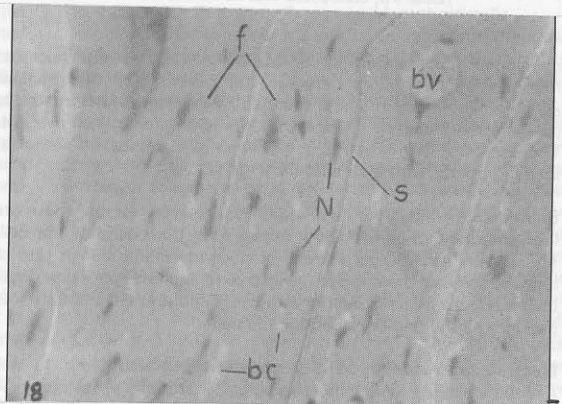
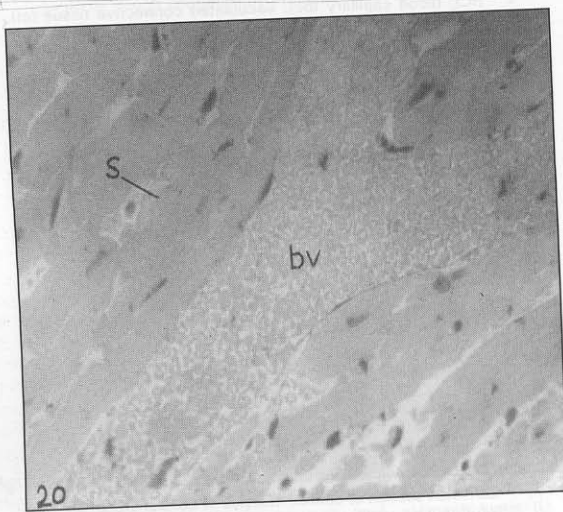
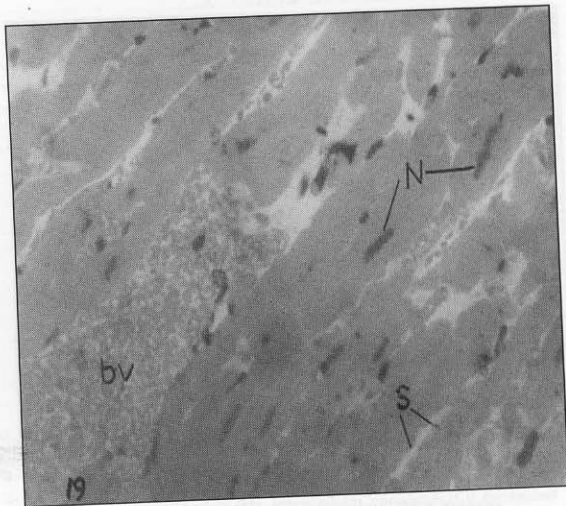


Fig. 18: Semithin section of cardiac muscles of control group, showing cardiac muscle fibers (f) nucleus (N), blood vessel (bv), blood capillary (bc), straight sarcolemma (s). TB (x400).



Figs. 19&20: Semithin section of cardiac muscles of treated group, showing dilated and congested blood vessel (bv), enlarged nuclei (N), contracted muscle fibers, indented sarcolemma (s). TB (x400).

developed golgi apparatus is above or besides the nucleus. Mitochondria are found between the cisternae of the granular endoplasmic reticulum and in the apical cytoplasm. Their matrix is pale with lead staining. Cytoplasmic fibrils are sometimes present. The secretion of the peptic cell is seen as cytoplasmic granules, staining poorly with lead acetate (Fig. 12).

**Stem cells:** Found in the neck region, but few in number these are low columnar cells with oval nuclei near the bases of the cell. These cells have large amount of ribosomes and a high rate of mitosis, some of them move upward to replace the pit cells. Other daughter cells migrate deeper into the gland and differentiate into oxyntic, peptic and enteroendocrine cells.

**Electron microscopy of Tenoxicam-treated stomach:** In the oxyntic cells significant morphological alterations were observed by electron microscope. The nuclei of some cells were irregular (Fig. 13). Most nuclei contained more peripheral heterochromatin aggregations (Fig. 13). Nuclear ultrastructural alterations observed in some oxyntic cells were more intensely heterochromatin

clumping (Fig. 14). One of the most prominent findings in the nuclear changes was the destruction of nuclear envelope (Figs. 13-15).

Tenoxicam administration for 30 days resulted in a decrease in mitochondrial numbers in the stomach oxyntic cells. In most cells the cytoplasm contained small mitochondria which were abnormally dense and usually rounded or elongated, some were vacuolated. The internal matrix and cristae were undistinguished and highly electron dense (Figs. 14&15).

Profiles of rough endoplasmic reticulum with varying sizes were noticed. In some cells the large segments of the rough endoplasmic reticulum appeared to be breaking up into smaller elements (Figs. 13-15). Free ribosomes could be seen in the cytoplasm in greater abundance than in the control (Fig. 13-15). Regarding the golgi apparatus of the oxyntic cells of treated animals, it seemed markedly hypertrophied, so that the number stalks was elevated and was dispersed over a large area of the cytoplasm (Figs. 14&15).

The numerous microvilli that project into the lumina of intracellular canaliculi appeared dilated in some oxyntic cells (Fig. 16). These microvilli were not clearly observed in some cells.

In some oxyntic cells, the lysosome were seen in the form of sac like structures enclosing dense material (Fig. 15).

The tubulovesicles and myofibrils in the apical cytoplasm of some oxyntic cells in Tenoxicam-treated stomach were not clearly observed as in the control oxyntic cells. There was a decrease in the amount of free surface microvilli of the oxyntic cells.

The connective tissue in between the blood capillary and the base of the oxyntic cell contained large vacuoles (Fig. 16). The wall of some blood capillaries appeared destructed (Fig. 13).

There were no significant ultrastructural changes in the peptic cells after Tenoxicam administration (Fig. 16). Peptic cells contained well developed organelles and many zymogen granules.

The cardiac muscle fibers show obvious histological changes following such drug treatment including congestion and dilatation of the blood vessels; the striation in the cardiac muscle fibers are not clearly observed as in the control. The muscle fibers appeared contracted. Indented sarcolemma is clearly observed in the treated cardiac muscle fibers (compare figure 17 and 18 of the control and figures 19 and 20 of the treated animals).

## Discussion

Electron microscopic studies of the stomach showed that Tenoxicam induce extensive changes in the nucleus, mitochondria, golgi apparatus, rough endoplasmic reticulum, ribosomes, tubulovesicles and myofibrils in the apical cytoplasm, intracellular canaliculi of the oxyntic cells and in the connective tissue structure. These observations are similar to those illustrated by Elewa *et al.* (1999) in liver and duodenal epithelial cells during the administration of diclofenac sodium.

The principal exocrine secretions of the stomach are pepsinogen, from the peptic cells and hydrochloric acid and intrinsic factor from the oxyntic cells. Mucus is secreted by mucus-secreting cells found amongst the surface cells throughout the gastric mucosa. Bicarbonate ions are also secreted and are trapped in the mucus, creating a gradient of pH from 1-2 in the lumen to 6-7 at the mucosal surface. The mucus and bicarbonate from an unstirred gel-like layer protecting the mucosa from the gastric juice. Alcohol and bile disrupt this layer. Locally-produced prostaglandins stimulate the secretion of both mucus and bicarbonate. Disturbance in the above secretory functions are thought to be involved in the pathogenesis of peptic ulcer and the therapy of this condition involves drugs which modify each of these factors (Raskin, 1999). The disturbance of the secretory function of the oxyntic cells which is principally demonstrated by the disappearance of tubulovesicles and myofibrils in the apical cytoplasm of the oxyntic cells in Tenoxicam-treated stomach confirm the above observations. The presence of the tubulovesicles and the myofibrils (actin filaments) in the apical cytoplasm are responsible for acid secretion of these cells (Junqueira *et al.*, 1995). (Ramadan, 2001) studied the ultrastructure of the stomach of mice treated with therapeutic

dose of indomethacin for 21 days and found that mitochondria were destructed, intracellular canaliculi were swollen and rough endoplasmic reticulum was fragmented into smaller cisternae; case of peptic cells, the drug caused conspicuous cytolysis of the cells and the cytoplasm was found to be devoid of the secretory granules. In present study similar observations were noticed in the oxyntic cells but there were no obvious changes in the peptic cells ultrastructure after the administration of Tenoxicam.

In present study, the golgi apparatus is not clearly observed in some control oxyntic cells and in other oxyntic cells it is noticed near the cell base. Golgi apparatus is clearly observed in the control and treated peptic cells and appeared hypertrophied in the Tenoxicam-treated oxyntic cells. Menzies and Fisk (1963) stated that the golgi apparatus is observed in peptic cells but not demonstrable by the light microscope in the oxyntic cells. Hally (1960) stated that golgi complex has been identified with electron microscope in a wide variety of cells, nothing conforming to this description has been found in oxyntic cells.

Cells of the gastric epithelium are responsible for secretory and protective function. To subservise these functions, an intimate relationship between these cells and the mucosal microcirculation might be expected. Physiological studies indeed show a direct relationship between acid secretion and gastric mucosal blood flow (Lanciaux and Jacobson, 1976); abundant evidence implicates a disturbed mucosal microcirculation in the pathogenesis of acute gastric ulceration (Moody, 1971). Although a quantitative study has not been made, transmission electron microscopic observation indicate that the mucosal capillaries are in very close proximity to the gastric glands (Gannon *et al.*, 1982), the parietal cells protrude from the abluminal surface of the gastric glands and the interstitial diffusion distance from capillary to parietal cell is often less than 0.3  $\mu\text{m}$ . The capillary endothelium is fenestrated and contains numerous vesicles. Therefore hindrance to the diffusion of small molecules between the capillaries and parietal cells is minimal. It has long been recognized that secretion of  $\text{H}^+$  by the parietal cell is associated with an equivalent extrusion of  $\text{HCO}_3^-$  into the interstitial fluid (Teorell, 1951). Some studies have indicated that mucosal protection against acid within the gastric lumen centers on the secretion of  $\text{HCO}_3^-$  by the surface epithelial cells into an "unperturbed layer" of gastric mucus gel (Flemstrom, 1977; Allen and Garner, 1980). A pH gradient across the mucus layer thereby established and acts as a protective barrier between the luminal acid and the gastric epithelium (Williams and Turnberg, 1981). The higher the  $\text{HCO}_3^-$  concentration in the interstitial fluid, the better able is the mucosa to cope with acid exposure (O'Brien and Bushell, 1980). Moreover, the gastric mucosa, when actively secreting acid into the lumen and therefore passing  $\text{HCO}_3^-$  into the interstitium, is better able to cope with acid exposure than is the non-secreting mucosa (O'Brien and Silen, 1976; Smith *et al.*, 1977). In the agreement of above observations, the distance between the blood capillaries and the oxyntic cell appeared highly vacuolated and some capillaries appeared destructed, leading to disturbance in the secretory process of the oxyntic cells. Gannon *et al.* (1982) stated that the blood capillaries around the base of the mucosal glands embedded in the connective tissue mixture which contained collagen fibers. The gastric mucosal microvasculature is so structured that when active acid secretion is occurring and therefore protection against luminal acid is the most required, maximal protective capacity is offered by the very organization of the mucosal microcirculation.

The previous studies suggested that salicylates (NSAID) may inhibit collagen synthesis because they inhibit procollagen proline hydroxylase in cell free system (Nagakawa and Bentley, 1969; Liu and Batnagar, 1973). McGarrity *et al.* (1981) studied the effect of sodium salicylate on the rat embryo in culture and stated that the growth inhibition produced by salicylate *in vitro* would be easily explicable in terms of the action of salicylate on RNA and DNA polymerase (Janakidevi and Smith, 1969). McGarrity *et al.* (1981) concluded that the demonstration of a specific metabolic effect of salicylates –say on proteoglycan or collagen synthesis- does not mean that other effects (e.g. nucleic acids, prostaglandin synthesis, oxidative phosphorylation, etc.) do not also contribute to overall embryopathic phenomenon.

It has also been observed that NSAID induce structural changes in the cardiac muscle fibers including congestion and dilatation in the blood vessels and indentation in muscle fibers. An association between peptic ulceration and cardiovascular disease has long been recognized (Langman, 1976).

Inhibition of renal prostaglandin by the use of NSAIDs can potentially lead to the emergence of several distinct syndromes of disturbed renal function. By blunting the homeostatic renal effects of prostaglandin, NSAIDs can adversely influence the blood pressure control. The risk of congestive heart failure is significantly increased when NSAIDs are given to patients receiving diuretic therapy who have cardiovascular risk factors (Whelton, 2001). The increased subsequent mortality from cancer, respiratory and cardiovascular disease suggests that the original admission for peptic ulcer bleeding may be a marker of disease in other systems. Another possibility is that haemorrhage from existing ulcer itself is precipitated by the presence of coexistent disease (Hudson *et al.*, 1995).

NSAIDs' untoward effects are attributed to their inhibition of constitutively expressed enzyme cyclo-oxygenase-1 (COX-1), with attendant suppression of the synthesis of Prostanoids, substances that mediate key homeostatic functions. Side effects include suppression of hemostasis through inhibition of platelet aggregation, adverse effects in patients with heart failure and cirrhosis and those with certain renal diseases, as well as complicating antihypertensive therapies, involving diuretics (Raskin, 1999). Two studies have found increased (but not significant) gastric prostaglandin E2 (PGE2) synthesis in the presence of *Helicobacter pylori* colonization (Taha *et al.*, 1990 and Avundek *et al.*, 1991), the rise in prostaglandin (PGE2) synthesis was strongly associated with intense inflammatory cell infiltration. By contrast in third smaller study claimed depressed prostaglandin (PGE2) synthesis, but surprisingly found no relation between production of prostaglandin (PGE2) and polymorphonuclear cell filtrate (Goren *et al.*, 1989). In agreement with Taha and Avundek, the administration of Tenoxicam for 30 days cause decrease in lymphoid cells in the stomach, i.e., lymphoid cells in the lamina propria and submucosa of the Tenoxicam treated stomach obviously decreased comparing with the control stomach. According to Hirasawa *et al.* (1987), diclofenac is a NSAID supported to reduce leucocytic infiltration in the inflammatory locus. In agreement with the above observations, Junqueira *et al.* (1995) stated that in the normal stomach the abundant lymphoid nodules in the lamina propria and the submucosal layers protect the organism (in association with the epithelium) from bacterial invasion. The necessity for this immunologic support is obvious since the entire digestive tract- with the exception of oral cavity, oesophagus and anal canal is lined by a simple thin and vulnerable epithelium. The lamina propria, located just below the epithelium, is a zone rich in macro phages and lymphoid cells.

For all NSAIDs together the risk of upper gastrointestinal bleeding and perforation was greater for high doses than for low doses. The estimate of the risk of multiple NSAID users was of the same magnitude as that among single NSAID users receiving a high daily dose (Garcia Rodriguez and Jick, 1994). Thrift *et al.* (1999) stated that over a 6 years period, 62 participants developed a haemorrhagic stroke. Among those taking between one and six doses of aspirin per week the relative risk was 0.76. Those taking 7 to 14 doses per week had a relative risk of 1.65. Low doses of aspirin have been shown to effectively inhibit the production of thromboxane A2, a platelet activating and vasoconstricting eicosanoid, in platelets and to be associated with less gastrointestinal toxicity than are high doses (Harker and Fuster, 1986). A similar result was found among users of NSAIDs (Schafer, 1999). In the article of McCarthy (1999), ulcers, when complicated, may either be those caused by an NSAID or "peptic" ulcers that preceded NSAID therapy (having a high prevalence in the population) and gave rise to complications resulting from NSAID effects on platelets tissues, or biologic processes, for example, healing, necrosis/apoptosis, leukocyte adherence, vasoconstriction, or generation of free radicals. Garcia Rodriguez and Jick (1994) confirmed that both short and long durations of NSAID exposure increased the risk of gastrointestinal bleeding.



Overall, there was a striking dose-response effect. Also, the increased risk with a high daily dose was independent of treatment duration. Similarly, people who had lately changed from one NSAID to another and received more than one NSAID simultaneously had more than twice the risk of individuals exposed to only one NSAID.

From the observations obtained of the present study, we can conclude that NSAIDs should not be given to patients with active peptic ulceration and in patients with a history of peptic ulcer disease. Moreover, it is prudent in all patients to start treatment at the bottom end of the dose. However NSAIDs may be intrinsically toxic to the stomach and cardiac muscles when used at high therapeutic doses.

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