Effect of the Anti-Rheumatic Drug Leflunomide (Avara®) on The Pyloric Region of Stomach of Adult Male Albino Rats and The Possible Protective Effect of Ranitidine (Histological and Histochemical Study)

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Abstract: Objectives: The study was aimed to determine the histological and histochemical changes induced by leflunomideon the pyloric region of rat stomach and to clarify the possible protective effect of ranitidine. **Background:** Lefluomide (Avara®) is an immunomodulating agent and disease modifying anti rheumatic drug with anti-inflammatory and immune suppressive activity. Ranitidine (Histamine H2 receptor antagonist) is used to treat gastro-intestinal disorders. **Materials and Methods:** Fifty adult male albino rats weighting 100-150g were divided into four groups: group (1), 10 rats received 2ml distilled water by oral route for 4 weeks and served as -ve control. Group (2), 10 rats received ranitidine at a dose of 20mg/kg b.w/ day by I.m injection for 4 weeks and served as + ve control group. Group (3) 20 rats received avara at a dose of 10 mg/kg b.w/ day dissolved in 2ml distilled water by oral route for 4 weeks. Rats of this group were divided into 2 equal subgroup. Subgroup (A) was sacrificed after 4 weeks. Subgroup (B) was left for another 2 weeks without treatment and served as recovery group, Group (4), 10 rats received leflunomide and ranitidine at the same previous doses and the same route of administration for 4 weeks. At the end of the study, samples were dissected, processed for both L/M and E/M studies. Also sections were immune-stained for caspase-3 and PCNA. The area% of collagen in the submucosa, mucosal thickness, number of +ve caspase-3 cells and +ve PCNA cells were statistically analyzed. **Results:** This study revealed that avara treated group showed histological, histochemical and ultrastructural changes in pyloric mucosa.Concomitant administration of ranitidine with avararevaled good in the histological pictures. However recovery group revealed very mild improvement.**Conclusion:** Leflunomide induces histological, histochemical and ultrastructural changes in the gastric mucosa and these changes were improved with coupling therapy of leflunomide with ranitidine so ranitidine is highly recommended with leflunomide.

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Key words: avara, ranitidine, pyloric mucosa.

1.Introduction:

Rheumatoid arthritis is a common inflammatory disease characterized by progressive bone and cartilage destruction, resulting in severe functional limitations, shortened life span and increased mortality rates (1).

Leflunomide (Avara) is an immunomodulating agent and disease modifying antirheumatic drug with anti-inflammatory and immunosuppressive activity. It is used for the management of symptoms of rheumatoid arthritis to improve physical function and to retard structural damage associated with the disease in adults. (2).

Recently, it was found that treatment by leflunomide in therapeutic dose is associated with frequent gastritis reaching to ulceration at high dose. Many clinical cases of gastritis are recorded in patients with rheumatoid arthritis treated by leflunomide (3).

The mechanism of induction of gastric ulcer or just gastritis appears to depend on interaction between different factors .These factors may be changes in gastric acid output, gastric mucous barrier, mucosal blood circulation, gastric motility and mitotic activity of the mucosal lining of the stomach, antioxidants and antioxidative enzymes (4).

Ranitidine, histamine H2-receptor antagonist, is used to treat gastrointestinal disorders such as peptic or duodenal ulcers. It inhibits both basal gastric secretion and gastric acid secretion induced by histamine, pentagastrin and other secretagogues. It competes with histamine for binding at H2 receptors on gastric parietal cells (5).

Aim of the work

This wok aimed to study the histological and histochemical effects of the anti-rheumatic drug leflunomide (Avara) on the pyloric region of adult male albino rats and to verify the possible protective effect of Ranitidine.

2. Materials and Methods

This work was carried on 50 male adult albino rats weighting 100 -150g/each. They were kept under strict care and hygiene and allowed free access of food and water *ad libitium*. They were divided into four groups as follows:

Group I (control): It is comprised of 10 rats. Each rat received 2ml distilled water by oral route for 4 weeks and served as -ve control.

Group II (control): It is comprised of 10 rats. Each rat received ranitidine at a dose of 20mg/kg b.w/ day by I.m injection for 4 weeks and served as + ve control group (12).

Group III (Leflunomide treated): It is comprised of 20 rats. Each rat received 10 mg/kg b.w/ day ofleflunomideby oral route for 4 weeks (7).Rats of this group were divided into two equal subgroups:

- Group IIIA: 10 rats were sacrificed after 4 weeks.
- Group IIIB: 10 rats will be kept without treatment for another 2 weeks and served as recovery group.

Group IV (leflunomide and ranitidinetreated):

It is comprised of 10 rats. Each rat received leflunomide and ranitidine at the same previous doses and the same route of administration for 4 weeks.

Methods

At the end of each detected period, rats were sacrificed by cervical dislocation then tissue samples were obtained, rapidly fixed in 3% glutaraldehyde for 3 hours, and then processed for Electron Microscopic Study at National Tumor Institute of Cairo(8). Other tissue samples were fixed in 10% formal saline for 5-7 days. Paraffin sections of 5-6 micrometer thickness were stained with H&E for routine histological examination, Mallory trichrome stain for detection of collagen fibers of stroma and Periodic Acid Schiff stain for detection of mucopolysaccharides (9).Another sections on positive labeled slides were immune-histochemically stained for detection of caspas -3 expression (10) and for detection of proliferating cell nuclear antigen (PCNA) (11).

Ten non overlapping fields from slides of each group stained by Mallory trichrome stain were examined to measure the area percentage (%) of connective tissue fibers using an objective lens x 10.Also, the number of caspas-3 positive cells and PCNA positive cells were counted in 10 fields from the slides of each group by using an objective lens x1000. The data were statistically analyzed using SPSS (Statistical package for social science). Data was expressed as mean + SE and analyzed by using student's t- test for comparison between two groups (12). Differences were regarded as non-significant $P > 0.05$, significant $P < 0.05$ or highly significant $P < 0.01$.

3. Results

- **Light microscope**

I) Histological results

A) Haematoxyline and eosin stain

Sections of pylorus of the control rats showed numerous pyloric glands occupying the entire thickness of the mucosa. The glands were tall, branched and coiled, resting on the muscularis mucosa and covered with intact mucus coat. They opened into the bottoms of gastric pits.The entire pyloric mucosal surface was covered by the surface columnar cells, and these cells appeared columnar in shape with basal oval nuclei. The neck region was lined with mucus neck cells. These cells were cuboidal or low columnar in shape with basal flat nuclei and acidophilic cytoplasm (Figs 1, A-D).

Sections of the pyloric mucosa of rats of ranitidine treated group showed pyloric glands more or less similar to the control group with intact surface epithelium (Fig. 1, D).

Sections of the pyloric mucosa of avara treated group revealed the presence of wide areas of epithelial discontinuity with surface cellular loss (ulcer). The base of the ulcer showed distortion of the general architecture of the pyloric glands with cellular infiltration and interstitial hemorrhage in the lamina propria (Fig.1, F).There were areas of complete distortion of the pyloric glands in the middle and basal parts of the glands with detached cells .Also, some chief and parietal cells appeared degenerated and detached from their basement membrane with appearance of interstitial hemorrhage. Their nuclei appeared pyknotic or karyolitic (Fig.1, G). Atrophy and apparent decrease thickness of pyloric mucosa was noticed in some sections (Fig.1, H)

Sections of recovery group revealed no improvement of the histological picture, and the changes were nearly the same as previous group (IIIA).There were dilatation of pyloric gland, congestion of the mucosal blood vessels and presence of mononuclear cell infiltration together with exfoliation of surface epithelial cells (Fig.1,I)

Examination of the pyloric mucosa of avara and ranitidine treated group revealed intact surface epithelium, the pyloric glands appeared more or less like control group. The mucus neck cells, the parietal cells and the chief cells appeared like control group. However, there was dilated and congested blood vessels in the lamina propria (Fig.1, J).

B) Mallory's trichrome stain

Examination of sections of control group stained with Mallory trichrome stain revealed minimal amount of collagen fibers observed mainly in the lamina propria and submucosa (Fig.2,A).

Ranitidine treated group revealed nearly the same amount of collagen fibers in lamina propria and sub mucosa (Fig.2,B).However avara® treated group showed massive deposition of collagen fibers in the lamina propria and submucosa (Fig.2,C).

After two weeks recovery, there was slight decrease in amount of collagen in mucosa and sub mucosa of this group (Fig.2, D). However concomitant administration of avara® and ranitidine resulted in remarkable decrease of collagen content in mucosa and submucosa of this group (Fig.2, E).

II) Histochemical results

Sections of gastric pyloric glands of control group showed strong PAS positive reaction in the mucus coat which extends to fill gastric pits and in the mucus neck cells. While the basal parts of the glands showed negative reaction (Fig.3,A). Similarly, sections of gastric pyloric glands of ranitidine treated group showed strong PAS positive reaction more or less like control group in the mucus coat and mucus neck cells (Fig.3,B). However, Sections of gastric pyloric glands of avara treated group showed weak reaction in the mucus coat and in the upper part of the glands (Fig .3,C) .After two weeks recovery sections of gastric pyloric glands showed moderate PAS positive reaction in the mucus coat and mucus neck cells (Fig.3,D), however concomitant administration of avara and ranitidine resulted in strong PAS positive reaction in the mucus coat which extends to fill gastric pits and mucus neck cells(Fig.3,E).

III) Immunohistochemical results

Caspase -3

Sections of control group as well as ranitidine treated group revealed –ve expression of caspas-3 gene in cells lining gastric pyloric glands (Figs.4, A-B). However avara treated group showed strong +ve immunoreactivity for caspas-3 in cells lining gastric pyloric gland which appeared as dark brown dots filling the cytoplasm(Fig.4,C). After 2weeks recovery, the majority of cells showed weak to negative immunoreactivity for caspas-3, while some cells showed moderate reaction and others showed strong reaction (Fig.4,D). Concomitant administration of avara with ranitidine resulted in appearance of majority of cells with negative reaction, while few others showed +ve reaction(Fig.4,E).

Proliferating cell nuclear antigen (PCNA)

Sections of control group as well as ranitidine treated group revealed +ve immunoreactivity for PCNA gene expression in cells lining gastric pyloric glands (Figs.5, A-B). However sections of avara treated group revealed weak immunoreactivity for PCNA gene expression in cells lining gastric gland (Fig.5, C).

After two weeks recovery, moderate immunoreactivity for PCNA gene expression was noticed in sections of this group (Fig.5, D). Concomitant administration of avara with ranitidine resulted in appearance of +ve immunoreactive cells for PCNA gene expression in gastric pyloric gland (Fig.5, E).

- **Electron microscopic Results**

E/M examination of sections of control as well as ranitidine treated group revealed the normal ultrastructure of pyloric glandular cells; the mucous neck cell showed apical homogenous mucous secretory granules with flattened vesicular nuclei(Fig.6,A). Very few parietal cells were seen having the characteristic intracellular canaliculi and loaded with mitochondria in between. Also chief cells were seen among oxyntic cells. Their cytoplasm were loaded with apical zymogenic secretory granules and packed cisternae of rough endoplasmic reticulum (Fig.6, B).The enteroendocrine cells with their rounded, basally situated electron dense secretory granules and apically rounded vesicular nuclei were also seen in the section (Fig.6,C).

Sections of gastric pyloric gland of avara treated group showed ultra-structural changes in their cells lining. The parietal cells appeared with swollen mitochondria, disturbed intracellular canaliculi and multiple cytoplasmic vacuoles. The chief cells appeared with irregular nucleus, heterogeneous zymogenic granules and multiple cytoplasmic vacuoles (Fig.6, D). Numerous fibroblasts were seen surrounded by collagen fibers (Fig.6, E). Multiple dilated congested blood vessels were seen lined by intact endothelial cells (Fig.6, F).

After two weeks recovery, mild improvement of ultra-structural feature was observed mainly in mucous neck cells which appeared loaded with multiple secretory granules and having euchromatic nucleus (Fig.6, G).

Avara and ranitidine treated groupprevealed good improvement of cells lining gastric pyloric gland which showed vesicular nuclei, rough endoplasmic reticulum and zymogenic granules (Fig.6, H).

- **Quantitative results**

Area% of collagen fibers of ranitidine treated group showed non-significant change compared to control group (p>0.5) (Table 1, Fig 7,I).However, avara treated group showed significant increase of area% of collagen compared to control group (p<0.5) (Table 2, Fig.7,II) and non-significant increase compared to recovery group(p>0.5) (Table 3, Fig.7,111), and significant increase compared to avara and ranitidine treated group (p<0.5)(Table 4, Fig. 7,IV).

The mucosal thickness of ranitidine treated group showed non-significant change compared to control group (Table 1, Fig.7, I), However, it showed highly significant decrease in avara treated group compared to control group (p<0.3) (Table 2, Fig.7, II). Also highly significant decrease in avara treated group compared to recovery group (p<0.1)(Table 3, Fig.7,III) and in avara treated group compared to avara and ranitidine treated group(Table 4, Fig. 7,IV).

Caspas-3 stained cells in recovery group showed significant decrease compared to avara treated group (Table 3, Fig.7, III). It also showed significant decrease in avara and ranitidine treated group (Table 4, Fig.7, IV).

Number of PCNA cells in ranitidine treated group showed non-significant change compared to control group (Table 1, Fig.7, I). However, it showed highly significant decrease in avara treated group compared to control group (Table 2, Fig.7, II). Italso showed significant increase in recovery group compared to avara treated group (Table 3, Fig.7, III) and significant increase in avara treated group compared to avara treated group (Table 4).

Table (1): quantitative study between control and ranitidine treated group

	Control group	Ranitidine treated group.	T. test	*P*. value
	Mean±SD	Mean±SD		
Area % of collagen of different treated group	3.32±0.35	3.58±0.44944	-1.01	>0.05
muscosal thickness stained with H and E	786.35±85.13	730.82±81.22	0.720	>0.05
number of cells stained with caspase-3	0	0		
number of cells stained with PCNA	20±158	20.2±3.1145	0.038	>0.05

Table (2):quantitative study between control and Avaratreated group

	Control group	Avara treated group	T. test	*P*. value
	Mean±SD	Mean±SD		
Area % of collagen of different treated group	3.32±0.35	23.41±0.9365	-47.043	>0.05
muscosal thickness stained with H and E	786.35±85.132	263.52±57.41	11.386	>0.05
number of cells stained with caspase-3		17±1.58	-24.042	<0.05
number of cells stained with PCNA	20±1.58	2.4±1.14	20.189	>0.05

Table (3):quantitative study between recovery and Avara treated group

	Recovery group	Avara treated group	T. test	*P*. value
	Mean±SD	Mean±SD		
Area % of collagen of different treated group	10.6±1.194	23.41±0.936	-18.885	>0.05
muscosal thickness stained with H and E	415.72±185.48	263.52±57.4	1.753	>0.05
number of cells stained with caspase-3	3±1.58	17±1.58	-14.000	>0.05
number of cells stained with PCNA	9.2±1.92	2.4±1.14	6.800	>0.05

Table (4):quantitative study between Avara and Rantidine treated group and Avara treated group

	Avara and Rantidinegroup	Avaragroup	T. test	*P*. value
	Mean±SD	Mean±SD		
Area % of collagen of different treated group	9.5±2.00	23.41±0.936	-14.088	>0.05
muscosal thickness stained with H and E	591.98±58.166	263.52±57.41	8.987	>0.05
number of cells stained with caspase-3	1.6±1.14	17.00±1.58	-17.665	>0.05
number of cells stained with PCNA	14.8±1.30	2.40±1.14	16.008	>0.05

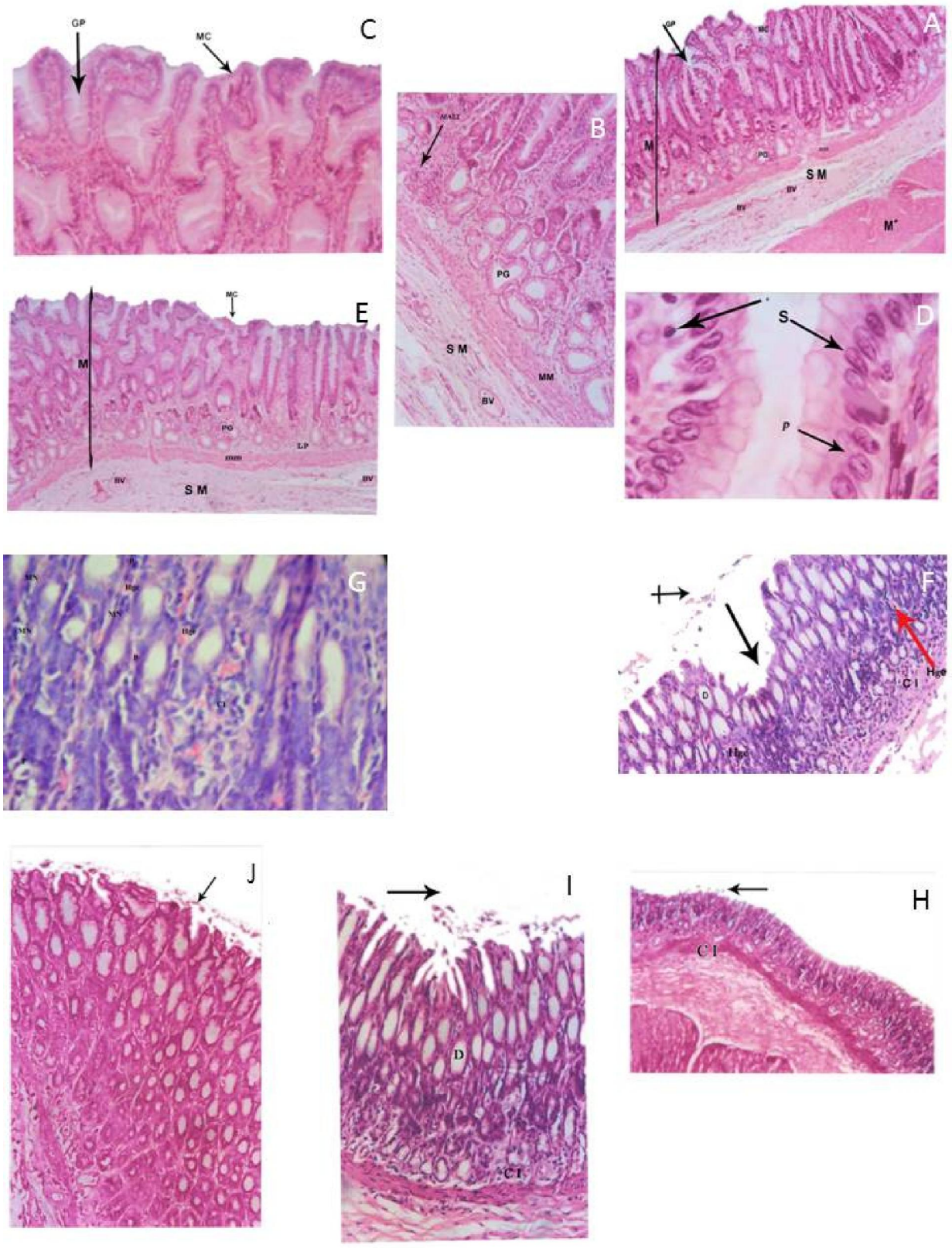

Figure 1: photomicrograph of sections of pyloric glands of H&E pictures for groups I, II, IIIA, IIIB and IV.

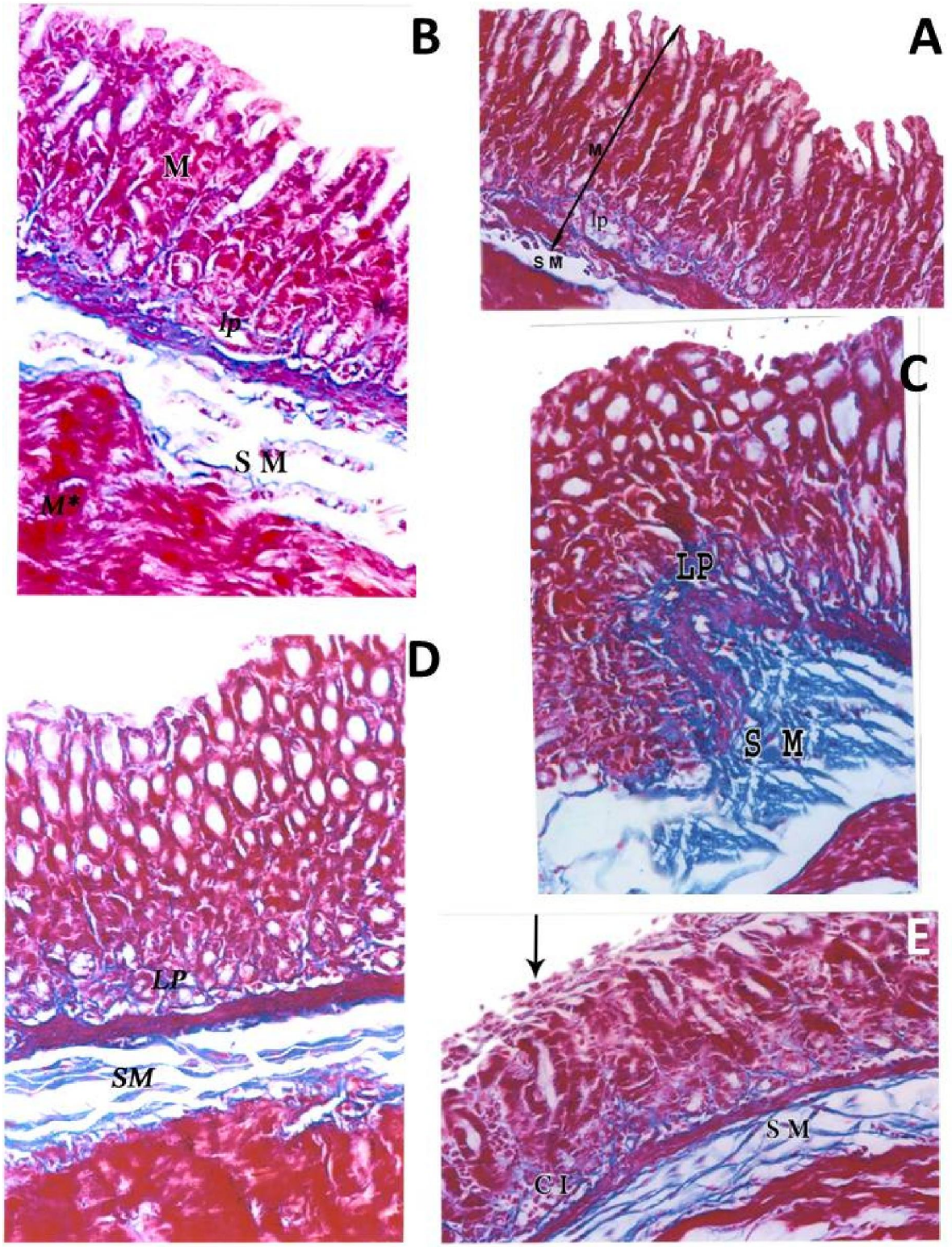

Figure 2:photomicrograph of sections of pyloric glands of Mallory trichrone stain pictures for groups I, II, IIIA, IIIB and IVshowing minimal amount of collagen fibers in groups I II IV, Moderate amount in group IIIB and massive increase collagen fiber in group IIIA.

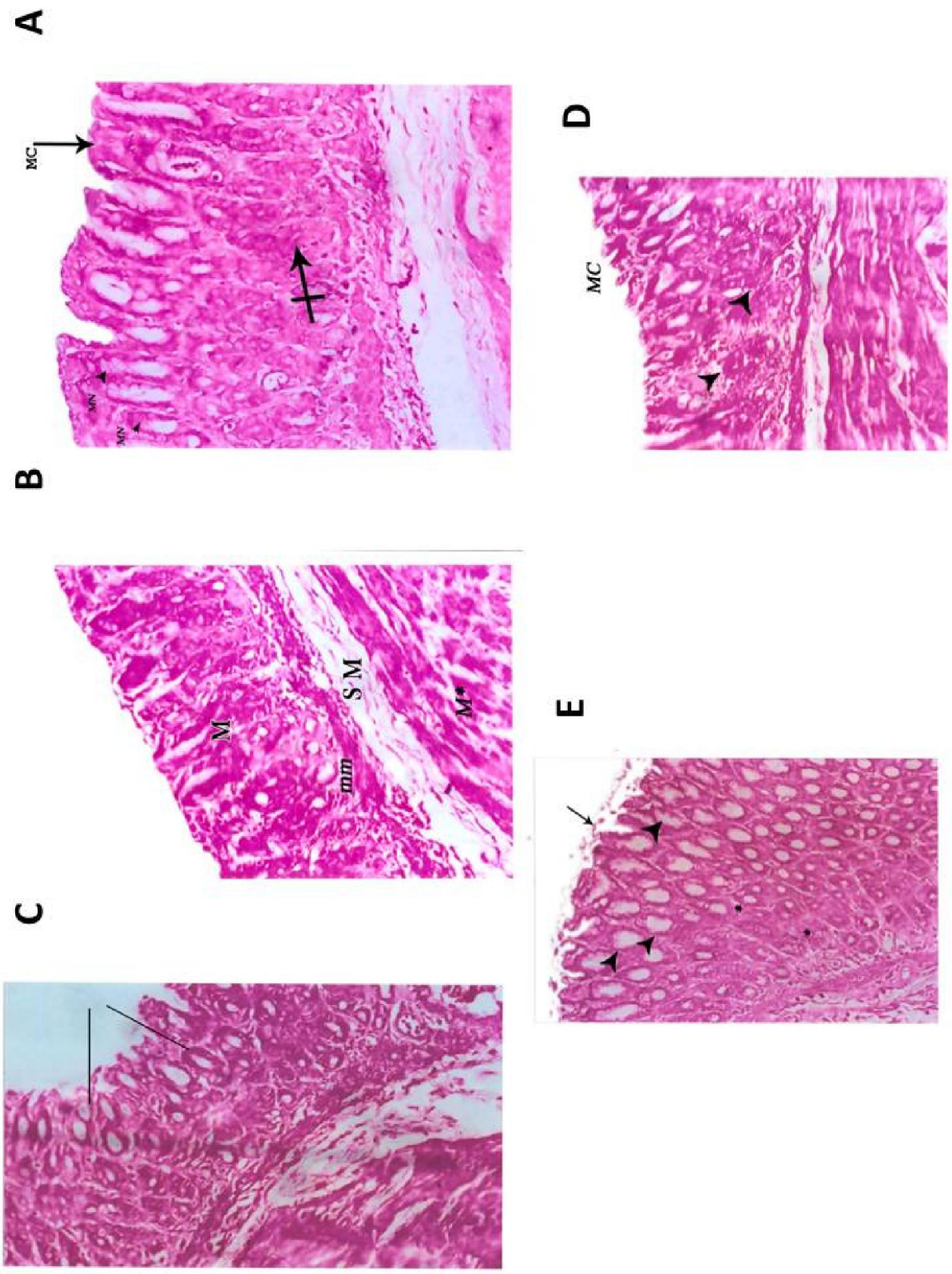

Figure 3:photomicrograph of sections of pyloric glands of PAS stain pictures for groups I, II, IIIA, IIIB and IVshowing weak reaction in group IIIA moderate reaction in group IIIB and strong reaction I, II and IV

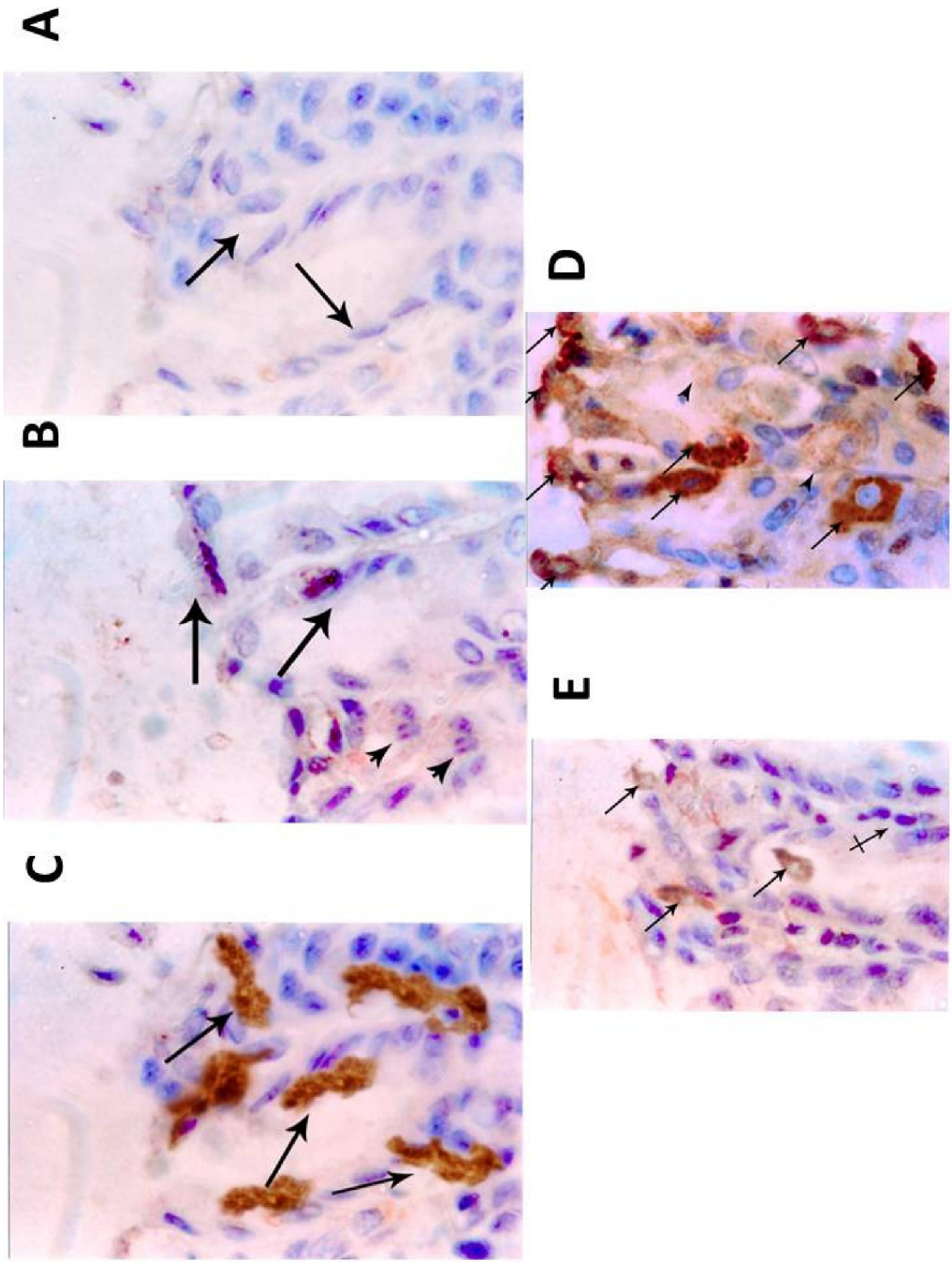

Figure 4: photomicrograph of sections of pyloric glands of Caspas-3 stain pictures for groups I, II, IIIA, IIIB and IVshowing weak reaction in group I,II and IV and strong reaction in group IIIA moderate Reaction in group IIIB.

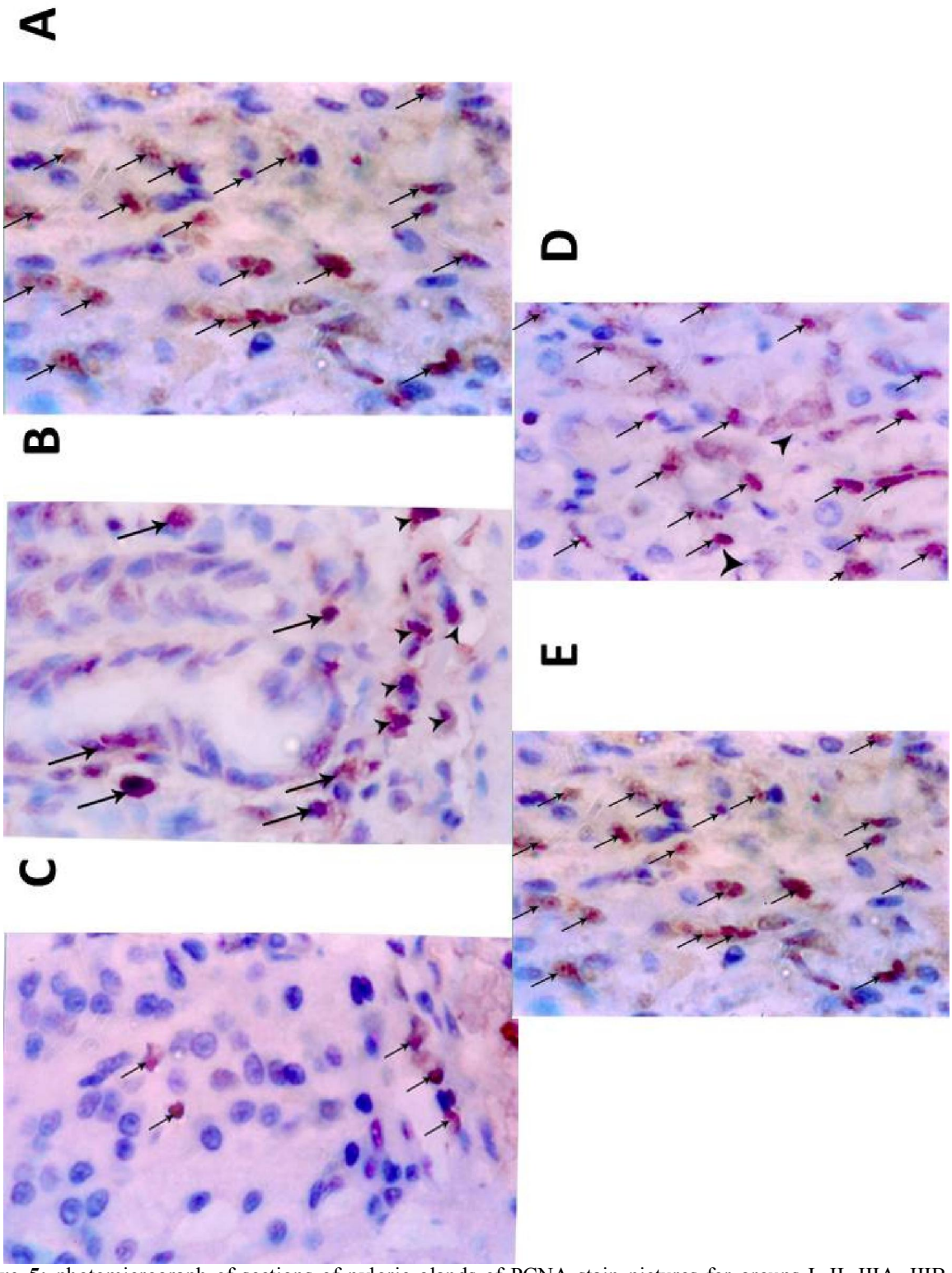

Figure 5: photomicrograph of sections of pyloric glands of PCNA stain pictures for groups I, II, IIIA, IIIB and IVshowing strong nuclear immune- reactivity in groups I, II and IV and weak immune- reactivity in group IIIA and moderate immune- reactivity IIIB.

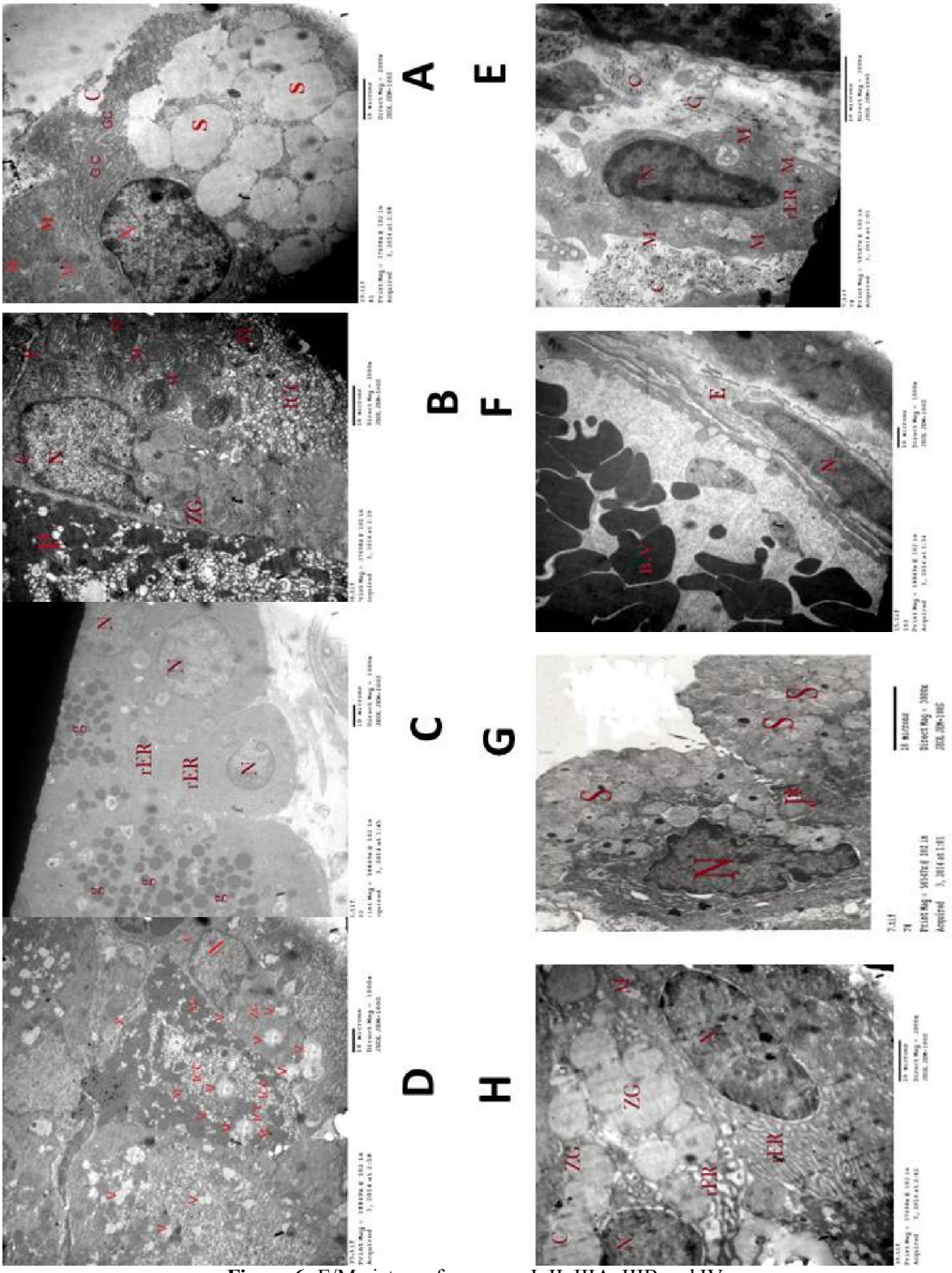

Figure 6: E/M pictures for groups I, II, IIIA, IIIB and IV.

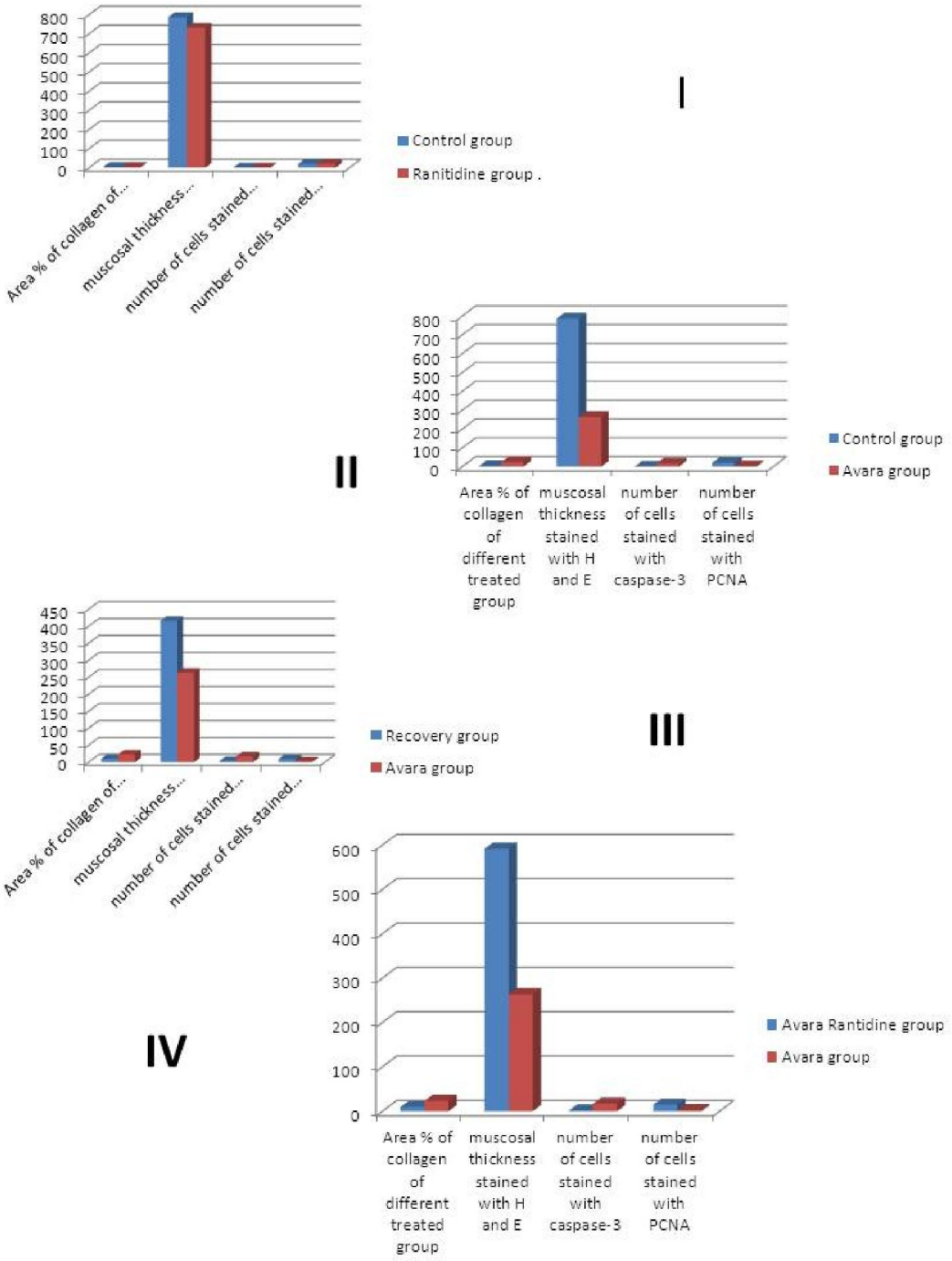

Figure 7: quantitative study.

4. Discussion

Rheumatoid Arthritis (RA) is a common autoimmune disease that clinically manifests as chronic inflammatory arthropathy (13). It is characterized by persistent synovitis that leads to cartilage destruction, bone erosions and periarticular decalcification, subsequently resulting in impaired joint function. Beside the development of long term disability and systemic complications, pain is the predominant sequelae for a patient with RA and therefore quality of life is limited. Thus RA is not only a burden for the affected individual, but also bears high socioeconomic costs (14).

An increasing number of drugs have been recognized to have deleterious effects on normal tissues when used therapeutically in man. Drugs used

to inhibit the reproduction of rapidly dividing cells as in case of cancer and RA are known to produce a number of toxic effects on organs containing self-renewing cell population such as bone marrow, skin and gastro intestinal tract (15).

Leflunomide (LEF) is an immune –modulaty agent with anti-proliferative activity that is approved for treatment of rheumatoid arthritis and marketed in USA since 1998. It improves rheumatoid arthritis symptoms such as joint swelling and tenderness, slows the progression of joint damage caused by the disease (16). It is a synthetic derivative of isoxazol and is a prodrug that is rapidly converted in the gastrointestinal tract and plasma to its active metabolite (M1), A77 1726 (16).

Gastric ulcers have also been suggested to occur due to an imbalance between the levels of defensive factors and destructive injurious by products in the gastric mucosa (17), (18). Oxidative stress, depletion of antioxidants, neutrophil accumulation, increase in inflammatory cytokines, and matrix metalloproteinase activity, and reduced blood supply to the gastric mucosa have all been implicated in the pathophysiology of gastric ulcers (19) ,(20) and (21) .

This study was aimed to clarify the effect of LEF (avara) on pyloric gastric mucosa and the possible protective effect of Ranitidine on the basis of histological, histochemical, immune histochemical and ultra-structural study.

The present study revealed that leflunomide administration resulted in various histological changes in gastric pyloric mucosa; these changes were in the form of exfoliation of the surface epithelium, area of epithelial discontinuity and ulceration. Scattered congested blood vessels together with areas of spot hemorrhage were also noticed in the damaged mucosa. These changes were in agreement with those observed by others (22) who reported that administration of therapeutic dose of LEF for four weeks resulted in marked changes in the gastric mucosal cells. The therapeutic dose chosen in this study was similar to that dose chosen by other researchers for treatment of RH (23).

The pyloric region of the stomach was chosen because it is the most common regions of gastric ulcers for patients treated with DMARDS for rheumatoid arthritis. These changes in gastric pyloric mucosa after LEF administration might be involved as defense mechanism of stomach against any potent damaging agents.

Moreover, Caselli, etal (24) noticed variable lesions in the gastric mucosal cells following treatment with therapeutic doses of leflunomide were detected. Marked ulceration of gastric mucosa detected in this study. They also found that leflunomide significantly delayed ulcer healing and inhibited mucosal regeneration.

Recently, pochetuhen,et al(12) reported that cellular infiltration and fibrosis are closely inter-related. Activated macrophages through releasing TNF- alpha stimulate the release of many chemokines from the macrophages, epithelial cells and fibroblasts. These assumptions explain clearly the associated increased deposition of collagen fibers by the activated fibroblasts which were depicted hand in hand with the cellular infiltration in leflunomide treated group of the present study. Several types of cells including neutrophils, eosinophils and plasma cells beside many other ill-defined inflammatory cells with irregular nuclei which may represent degranulated cells were seen heavily infiltrating the lamina propria and sub mucosa.

In addition, other researchers noticed that cellular infiltration is a constant feature in any inflammatory process, which in turn gives rise to generation of reactive oxygen species (ROS). ROS are one of the important factors in the pathogenesis of mucosal damage through oxidative damage in the cellular membrane and intracellular molecules (25).

The actual causes of the presence of congested blood vessels hemorrhage and extravasated blood cells in the lamina propria of pyloric mucosa of this study remained obscure. A possible explanation was that the drug had a direct toxic effect on the wall of small blood vessels leading to ischemia followed by vasodilatation and extravasations of blood from their necrotic wall. Another factor might be implicated about this finding is the destructive action of the drug on the mucosal barrier and intercellular integrity, which exposes the capillaries and venules to the harmful effect of hydrochloric acid of the gastric secretion.

The dilatation of the gastric pits and upper part of pyloric gland of LEF treated group of this study could be due to excessive production of mucous secretion by mucous neck cells, an attempt by the body to safe guard against more damage to the surface coat of the gland, or, it might be related to inhibition of prostaglandin I2 production, which is considered as a potent anti-secretory agent (26).

The present study revealed weak to moderate reaction of PAS on the surface columnar cells, mucous neck cells and cells of gastric pits of LEF treated rats. This indicates diminished mucous content which was with the histological findings i.e. degeneration and necrosis of cells covering the surface and lining the pits. The mucous secreted by these cells has an important role in protecting the gastric mucosa from acid content of the stomach (27).

Healthy gastric mucosa is always under equilibrium between cell death and cell renewal and

mucosal injury is developed when this balance is disturbed due to increase in apoptosis and/or inhibition of cell proliferation (28) and (29).

The gastric mucosal cells are continuously undergoing apoptosis and rapidly replaced by newly proliferating cells (30).

Recent studies suggest that increased apoptotic cell death and simultaneous block of mucosal cell renewal play major roles in the development of mucosal lesion (30), (31) and (32).

In this study, two immune histochemical markers were used, one was used to identify apoptosis (caspase -3 marker) and the other was used to identify cell proliferation (PCNA marker).

Caspase-3 is a member of the interleukin converting enzyme family of cysteine proteases. It is believed to be the one most commonly involved in the execution of apoptosis in various cell types (33).

In the present study, the apoptotic cells denoted by +ve caspase -3 immunoreactions were more numerous in LEF treated group mainly in the top surface and middle parts of the glands. The reaction appeared as brown dots granules filling the vicinity of the cytoplasm .These results were in agreement with other investigators who explained this reaction by activation of the intrinsic mitochondrial pathway with the release of cytochrome c and other apoptogenic factors into the cytosol with opening of the permeability transition pore that allows solutes to diffuse across the inner mitochondrial membrane, leading to depolarization, inhibition of oxidative phosphorylation and ATP depletion, resulting in apoptotic cell death (13) and (18).

According to other researches, the ulcer re-epithelialization is essential process for gastrointestinal ulcer healing and without restoration of a continuous epithelial barrier; the mucosa would be vulnerable to mechanical or chemical injury and infections thereby preventing ulcer healing. Cellular proliferation plays an essential role in maintaining the integrity of the gastric mucosa and an important proliferation marker is PCNA (proliferating cell nuclear antigen), a highly conserved 36 kDa nuclear peptide that is expressed during cell proliferation (34).

The present study revealed few cells with +ve PCNA immune reaction in the pyloric mucosa of LEF treated group; this indicates the anti-proliferating activity of the drug which means inhibition of proliferation and stem cell regeneration of the pyloric mucosa.

Electron microscopic results of LEF treated group confirmed the light microscopic findings and ultra-structural changes in the cells lining pyloric glands. The parietal cells appeared with small indented nucleus having more peripheral chromatin, swollen mitochondria with disturbed inner cristae and some cytoplasmic vacuoles. The chief cell has irregular nucleus, heterogeneous zymogenic granules and multiple cytoplasmic vacuoles. These results were in harmony with others (35) who confirmed that the early signs of parietal cell damage were disruption of their canaliculi and presence of dense bodies which were secondary lysosomes. They also detected focal chief cells disruption with rupture of their plasma membrane as well as loss of their zymogen granules.

Damiano (35) reported that disruption of the normal microenvironment of the fibroblasts may be the crucial event that triggers fibroblast activation, cell division and collagen deposition. Fibroblast proliferation and collagen deposition were found to occur when epithelial cells were severely damaged and there was a delay in the epithelial repair process.

The inflammatory response is a key component of host defense. However, excessive or persistent inflammation can contribute to the pathogenesis of disease. Inflammation is regulated, in part, by cytokines, which are small, typically glycosylated proteins that interact with membrane receptors to regulate cellular processes such as proliferation, differentiation, and secretion. There is now compelling evidence that the recruitment and subsequent activation of inflammatory cells is through the activation of stomach macrophages to release tumor necrosis factor- alpha(TNF-alpha).

TNF-alpha then acts via paracrine and autocrine pathways to stimulate cells to release other cytokines known as chemokines, which are directly chemotactic to leukocytes and other cells that participate in inflammatory and wound healing responses (36). The present results revealed that leflunomide administration for 4 weeks in rats induced pyloric ulceration with evident histological changes. On the other hand, discontinuation of the drug for another 2 weeks did not succeed in spontaneous recovery or amelioration of these changes most probably through its pharmacokinetics as regards the long half-life of elimination of the drug and the increased risk of opportunistic infection.

On the contrary to our results, other researchers (37) stated thatleflunomide over other antirheumatic drugs. They assume that, it carries lower risk of toxicity when it is used without a loading dose. However, they depended on their clinical experiences and their assumptions were not documented by histopathological studies. Moreover, long term follow up of patients treated with leflunomide are still under investigations.

In the present study, concomitant uses of ranitidine with avara drug actually protect the gastric mucosa from injurious effects of avara drug. The

surface epithelium and mucous coat of pyloric mucosa found to be mostly intact with absence of erosion. Also, the mucous neck cells, parietal and chief cells showed nearly normal appearance. However the congestion and collagen fiber deposition were still observed in this group. This was in agreement with others (38), who reported that ranitidine is H2 receptor antagonist has inhibitory effects on acid secretion and reduces mucosal blood flow to basal level. In addition to their gastro protective effect, H-2-receptor antagonists may contribute to lysosomal membrane protection in gastric injury (39 .The inhibited acid secretion stimulates the secretion of gastrin from G-cells.

Gastrin is a potent stimulator of gastric mucosal proliferation by both direct and indirect mechanisms. Firstly, it stimulates the proliferation of progenitor cells in the pyloric glandular neck zone via gastrin receptors on the cell membrane (11, 40).

Secondly, gastrin stimulates Reg protein production by ECL cells and heparin-binding epidermal growth factor-like growth factor (HB-EGF) by parietal cells (39), (41) and (42).

The produced Reg protein and HB-EGF then stimulate the proliferation of gastric mucosal progenitor cells. Thus, hypochlorhydria-induced hypergastrinaemia observed in rats with ulcers may be an important cause of increased PCNA-positive proliferating cells in the gastric mucosa.

In the present work, morphometric and statistical studies showed that there was an increase in caspase -3 stained cells in avara treated group and decrease in PCNA stained cells. Also, morphometric and statistical studies showed that there was an increase in % area of collagen fiber and decrease in the height of pyloric mucosa in avara treated group. These finding could be due to direct effect of the drug.

From the foregoing, it was demonstrated that leflunomide induces gastritis and pyloric ulcer and ranitidine minimizes this hazardous effect of leflunomide on pylorus and provides a good protective role. So, co administration of ranitidine to the patients under leflunomide treatment is highly recommended.

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