Histological changes of gastric ulcer in rabbits treated with rapebrazole

التغيرات النسجية لقرحة معدة الارانب المعالجة بدواء الربيبرازول

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Abstract

In order to study the stomach of rabbits, twelve rabbits were divided into three groups, ulcer, treatment and control group. All animals were included in this study were from the animal in house in college of Veterinary Medicine-Karbala University. This work includes anatomical and histological studies to examine for effects of the Rabeprazole during induce ulceration by 20% acetic acid. The present histological effects study revealed that the ulcer group has enumerate changes accrue in mucosa an sub – mucosa. After 4 hours we opened the gastric of ulcer group, showing the inflammation region in internal surface in fundic area but most of internal surface was normal. The main histological changes observed were damage and destroyed of epithelia cell and arrangement of parietal cell as a long cord reach in surface while the parietal cells in control and treatment group as acini-circle shape. By contrast, rabbits of all treated groups showed healing signs, such as reductions of ulcer sizes and inflammatory area, with some extent of mucosal regeneration re-epithelization, glandular organization, and proliferation of connective tissue cells granulation tissue. This study showed that densely fibrosis in sub mucosa in treatment group and less number of inflammatory aggregate cells.

المستخلص

اجريت هذه الدراسة على معدة الارانب المحلية حيث تم اخذ اثنا عشر ارنبا من البيت الحيواني في كلية الطب البيطري-جامعة كربلاء.وزعت الى ثلاثة مجاميع ،مجوعة القرحة ومجموعة المعالجة ومجموعة السيطرة خمسة حيوانات كل من مجموعة المعالجة والقرحة واثان مجموعة السيطرة.هذا العمل يحتوي على دراسات تشريحية ونسجية لتأثير دواء الرابيبرازول خلال احداث قرحة في معدة الارانب.استخدم حامض الخليك بتركيز 20% وبعد 4 ساعات من اعطاء الحامض تم فتح معدة الارانب بعد قتلها فلوحظ مناطق احتقان شديد في السطح الداخلي لمنطقة القاع بينما بقية الاجزاء كانت طبيعية.اهم التغيرات النسيجة التي لوحظت،دمج وتحطم في الطبقة المخاطية اما الخلايا الجدارية المسوؤلة عن افراز حامض الهيروكلوريك ترتبت على شكل حبال طويلة تصل الى سطح الطبقة الطلائية،بينما مجموعة السيطرة والمعالجة كان ترتيب الخلايا على شكل حويصلات دائرية.بعد المعالجة بالرايبرازول شوهد اختفاء المناطق الملتهبة والمحتقنة وتم الشفاء بالكامل.اظهرت الفحوصات النسجية لمجموعة المعالجة اعادة تكوين الطبقة الطلائية المنسلخة بشكل طبيعي،وكذلك لوحظ منطقة التحت المخاطية تليف كثيف في هذه المنطقة.

Introduction

A good number of researchers have studied the histology of the gastric mucosa of mammals. Histologically, it has been shown that the glands of mammalian stomach contain various types of cells and that these glands and their cell types are grouped into 3 distinct areas in the gastric mucosa [1, 2, 3]. The mucosa of pre-stomach, like that of the oesophagus is composed of keratinized stratified squamous epithelium and that the laminar propria has no glands [4, 5]. They also established that the mucosa of the stomach proper is lined by simple columnar epithelium. The pits of the gastric glands are lined by cells producing mucous. The isthmus contains mucous neck cells and immature undifferentiated cells. The stomach is designed anatomically into three regions; cardiac, body and pylorus [6, 7]. It has also been established that the cardiac glands contain mucous cells and parietal cells [4, 8]. The glands of the body of the stomach contain zymogenic cells, parietal cells and mucous neck cells. Like the other parts of the gastrointestinal tract, the stomach walls consist of an outer mucosa, and inner submucosa, muscularis externa, and serosa.The gastric mucosa of the stomach

consists of the epithelium and the lamina propria (composed of loose connective tissue), with a thin layer of smooth muscle called the muscularis mucosae separating it from the submucosa beneath. The submucosa lies under the mucosa and consists of fibrous connective tissue, separating the mucosa from the next layer [9, 10, 11]. The stomach plays a pivotal role in the digestion of foods that we eat. With the exception of rare cases, this organ can resist to a large variety of noxious factors, including hydrochloric acid, refluxed bile salts, with a wide range of temperatures and osmolality. This high resistance to injuries depends on a number of physiological responses elicited by the mucosal lining against potentially harmful luminal agents, as well as to the ability of rapidly repairing the mucosal damage when it does occur [12]. Nevertheless, when these protective mechanisms are overwhelmed by injurious factors, a gastric mucosal lesion may develop. Major detrimental effects on gastric mucosa are exerted by non-steroidal anti-inflammatory drugs (NSAIDs). These drugs are able not only to exert gastric injuring effects, but also to delay the healing of ulcer lesions through a variety of local and systemic mechanisms [13]. Another relevant topic, regarding the integrity of gastric mucosa, is represented by the use of proton pump inhibitors (PPIs). These drugs have been proven not only to prevent NSAID-induced upper gastrointestinal injury, but also to promote the healing process once the damage has occurred, even in the presence of a continued NSAID administration. The beneficial effects of PPIs can be largely ascribed to their ability to maintain a sustained inhibition of gastric acid secretion. However, there is also evidence to suggest that pharmacodynamic properties unrelated to acid inhibition may contribute to the therapeutic actions of these drugs [14]. Rapebrazol contains the active substance omeprazole. It belongs to a group of medicines called 'proton pump inhibitors'. They work by reducing the amount of acid that your stomach produces. Gastric ulcers were induced by applying locally acetic acid on the anterior serosal surface of the glandular stomach, as previously reported. [15,16] Briefly, 50 µL of 80% acetic acid were applied to the serosal surface of glandular portion by using a round ring of 10 mm in diameter. Twenty seconds later, the acid solution was removed, wiped with filter paper and the abdomen was closed. Thereafter, rats fed normally and received orally the treatments (vehicle, D-002, omeprazole or ranitidine) for 5 days. Chronic superficial gastritis was defined on the basis of normal mucosal thickness, superficial inflammatory and cellular changes with lymphocyte and plasma cell infiltration, and varying degrees of damage and reactive hyperplasia in the epithelium. The presence of reduced mucosal thickness, diffuse infiltration by lymphocytes and plasma cells plus a variable degree of atrophy of the epithelial elements, usually with some evidence of intestinal metaplasia, and sometimes pseudopyloric metaplasia, indicated chronic atrophic gastritis. The severity of cellular infiltration [17,18] The highest dose of omeprazole showed the greatest effect on angiogenesis. Concluding, at the doses tested, D-002 healed acetic acid-induced ulcers as effectively as omeprazole, an effect associated to the reduction of neutrophil infiltration and to the increase of restorative angiogenesis into the ulcerated areas [19]. This study is aimed for main of differences that accrue in stomach cells during treatment by Rebeprazole. The histological change that accrues in stomach during treated by Rabeprazole was conducted for the first time in Iraq.

Materials and methods:-

Animals

Twelve local rabbits were divided in to three groups (control group G1 two animals, ulcer group G2 five, treatment group G3 five animals).

Method of ulcer induce and treatment:

Ulcer group were given acetic acid 20% directly in stomach by small plastic tube, after 20 minute water administrated for acidity decrease [16]. About 4 hours were anatomy of stomach and taken many of specimens from ulcer area for histological preparations. Then the tissue specimens were sectioned from the fundic glands region. The size of the specimens were taken about 1 cm and then kept in 10% formalin for 48 hours. The samples were proceeding with routine histological technique [20]. Two type's stains were used in this work; Hematoxylin and eosin. 5 rabbits of G3

administrated by rapebrazole to 4 weeks twos day, after that were sectioned from fundic region for examination effect of rabeprazole drug.

Results:

After 4 hours we opened the gastric of ulcer group, showing the inflammation area about 1-2cm in internal surface in fundic region but most of internal surface was normal (Fig 1). After stomach application of acetic acid, lesion gastric were observed in all treatment group while the negative control rabbits did not exhibit such changes (Fig.2). By contrast, rabbits of all treated groups showed healing signs, such as reductions of ulcer sizes and inflammatory area (Fig,3). The characteristic histological pattern of acetic acid induced gastric ulcers, showing damaged mucosal epithelium, distortion of glands, the study showed that the parietal cells characteristic by polyhydral or oval in shape, have strong affinity in cytoplasm, dark nucleus and aggregation in many group and form the circles, reach in epithelial surface and lamina properia disappear severe inflammatory infiltrate, proliferation of fibroblasts and cellular debris in the ulcerated wall of stomach. These results showed congestion area located under tunica mucosa (Fig.4), we showed by ulcer enumerate of histological changes in fundic region of stomach certainly, in Tunica mucosa, epithelial surface and laminae properia and lamina mascularis. This study was observed the erosion in epithelia surface and congestion of blood vessels, gastric pits disappear but most of changes which accrue in lamina properia generally, the parietal cells characteristic by dark-pink cytoplasm arranged as radial pattern in basal part in lamina properia but the parietal cells that located in epithelial surface illustrated as circles and increase in number but chief cells less than its , which form a gastric pits near epithelial surface (Fig,5). The stomach in rabbits have lamina muscularis which separated the lamina properia and tunica submucosa, consists about 2 layers inner circular and outer longitudinal of smooth muscle fiber. It's seen oblique cut in lamina mucosa was seen due to the effect of acetic acid. The submucosa there was inflammatory response, edematous, congested blood vessels and infiltration of multinucleated cells (MNCs) (Fig, 6).

In this study, Gastro -mucosal changes at four weeks revealed complete healing in treated group with rabeprazole, this finding showed regeneration to epithelial surface and consist of normal gastric pits, reduce in congestion areas (Fig,7, the general histological appearance of parietal cell which serialized arrangement, decrease in number compared with (G1), which have pink lightly cytoplasm. The fibers in Lamina muscularis was continue without any cutting in this layer, also submucosa contains a large amount of collagen fiber (Fig,8). The stomach in G3 the entire surface of gastric mucosa was lined by a simple tall columnar epithelium with a lightly stained cytoplasm which form the surface mucous lining cells that invaginate into varying depth into the lamina propria according to the regions of stomach in rabbit forming the gastric pits that lined by the same surface epithelium and where the glands are opened in the base of it (Fig,9). The parietal cells and chief cells have lightly cytoplasm with purple nucleus and distributed randomly (Fig,10).

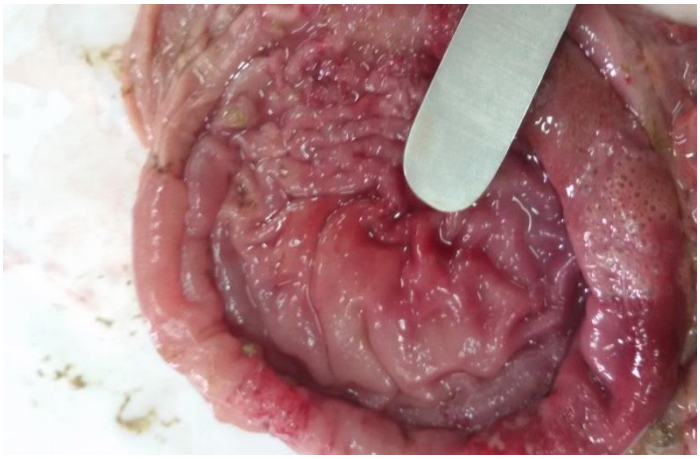


Fig (1) Ulcer group of stomach in rabbit, gastritis area.

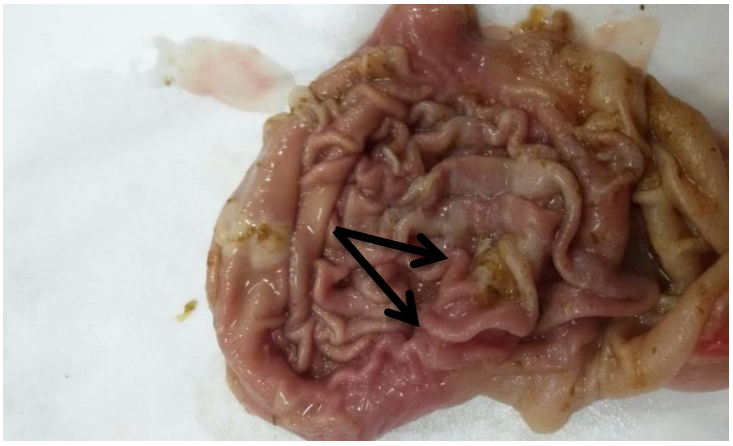


Fig (2) Treatment group of stomach in rabbit, healing area (arrows).

Fig (3) Control group of stomach in rabbit, show internal surface

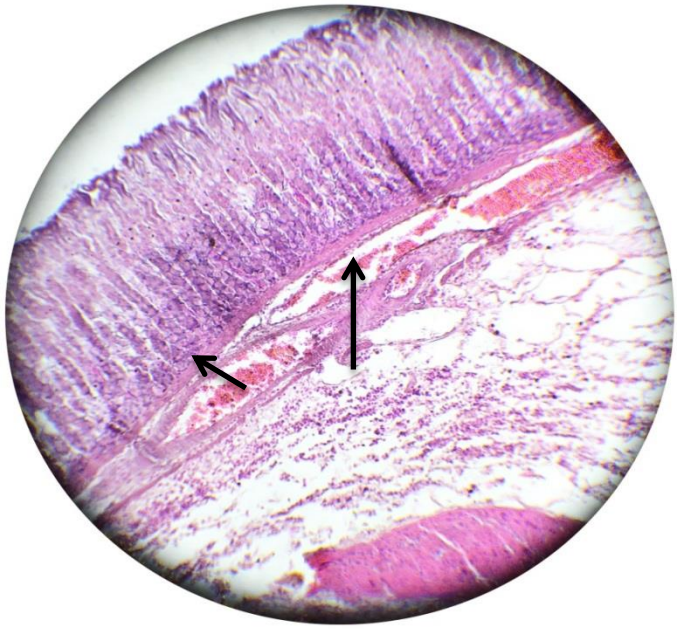
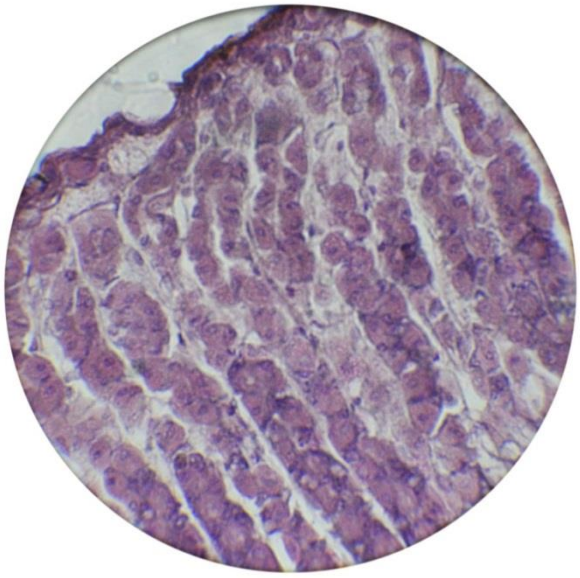


Fig (4) Stomach of ulcer group, parietal cells spread as a cord,cut in lamina muscularis(large arrow) , and congestion area(small arrow).10x.H&E stain.



Fig(5) Stomach of ulcer group, the parietal cells dark- pink cytoplasm and reach in surface of degeneration epithelia surface as a cord.40x. H&E stain.

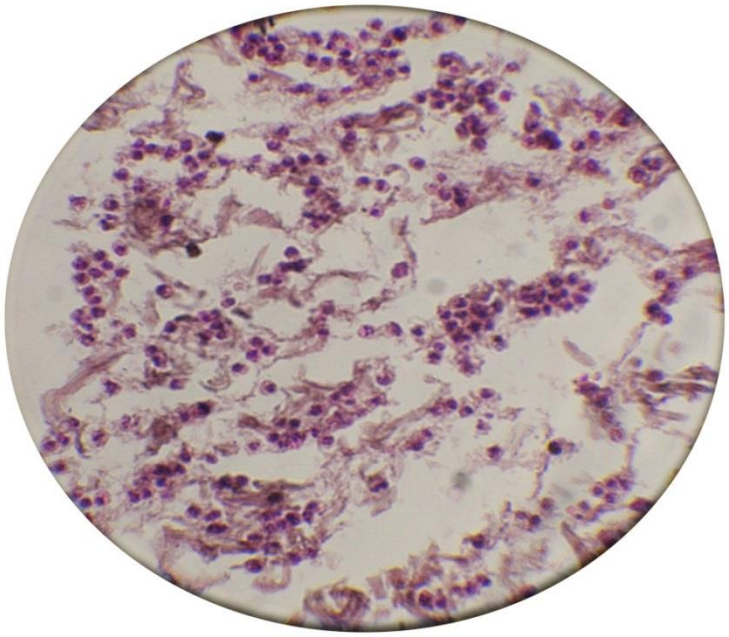


Fig (6) Stomach of ulcer group, aggregation of inflammatory cell in sub-mucosa. 40x. H&E stain.

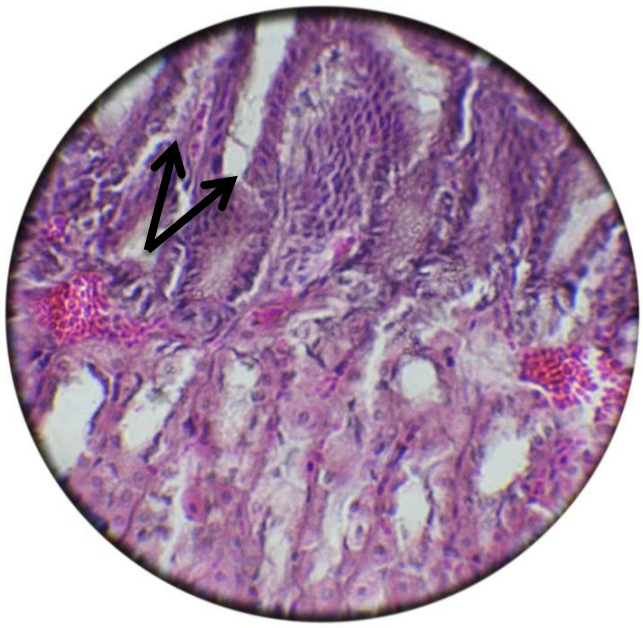


Fig (7) Stomach of treatment group show, regeneration epithelia(arrow).40x H&E stain.

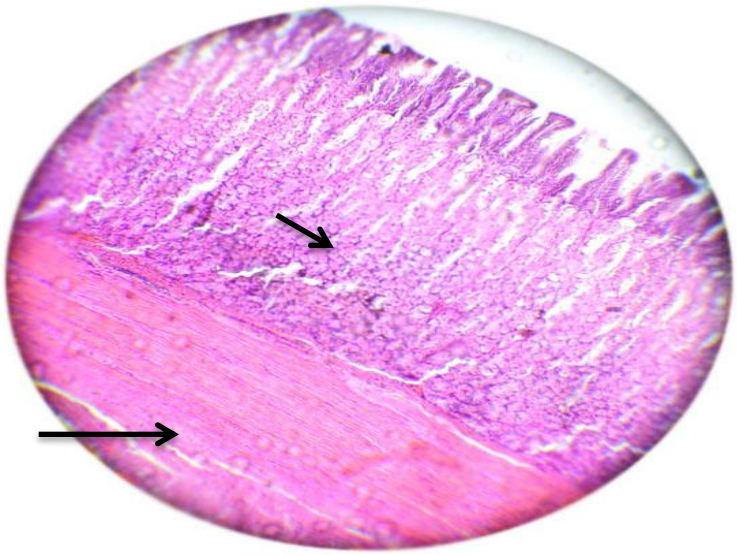


Fig (8) Stomach of treatment group show light pink cytoplasm in parietal cells (small arrow). Submucosa consist of dense connective tissue(large arrow) and inflammatory cell disappear.10x.H&E stain.

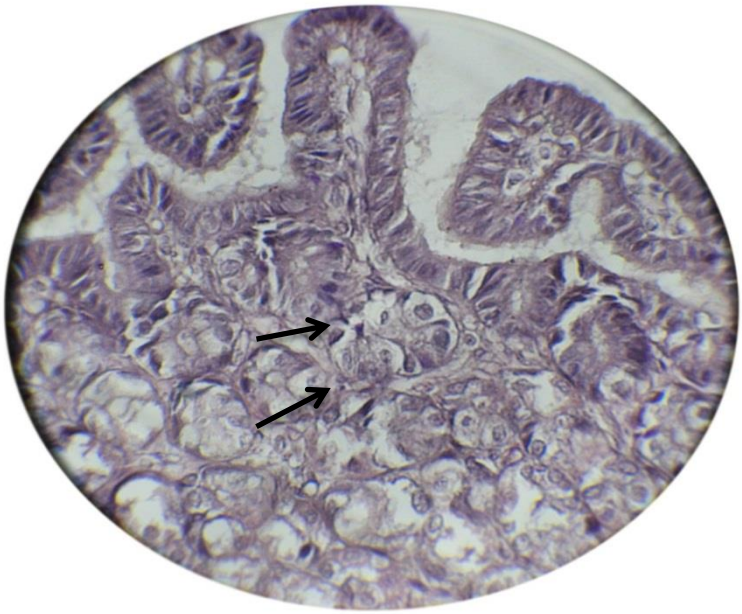


Fig (9) Stomach of control group, epithelial cell normally and parietal cells have purple little cytoplasm granules.40x.H&E stain.

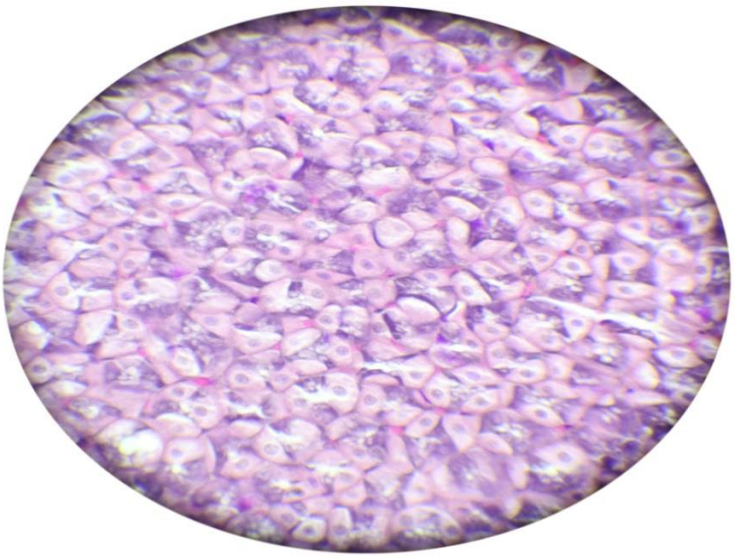


Fig (10) Stomach of control group, the parietal cell distributed randomly.40x.H&E stain.

Discussion

The present study shows that induced haemorrhagic lesions on the surface of stomach of all animals treated with acetic acid 20%, produce spot gastric hemorrhagic erosions in internal surface of stomach. Acetic acid induced both long ulcers and petechial lesions within a small duration, Ulcerative lesions of the gastrointestinal tract are one of the major side effects associated with the use of NSAIDs, alcohol, stress, and acidic acid [11-16]. In this study, the lesions were located mostly in the fundic region of the stomach, the portion of the stomach secreting acid and pepsin. No visible lesions were found in the non-secretory part of the stomach,the result of this study agrees with [21,22]. Histological results of the present study confirm the serious microscopic damages to mucosal layer of the stomach in ulcer group and cut in lamina muscularis. Also the parietal cell converted in arrangement from the circle-acini shaped to long cords shape,this state for new arrangement of parietal cells accrued because erosions in epithelial cells which indicate parietal cell act as epithelial surface instead with sloughing layer when compared to control group this results confided with[13-15]. The current study showed that the great effects of Rabeprazole for treatment the ulcer and gastritis condition in addition, this drug has highly ability and rapidly to regeneration of epithelial cells which agree with [1-5]. The parietal cells after treated with Rabeprazole appearance under regenerate epithelia and have purple cytoplasm this state indicate the Rabeprazole drug due to decrease secretion of hydrochloric acid (10). This result referred to founding a large number of inflammatory cells in sub-mucosa but during treated with Rabeprazole the inflammatory cells less in number with large amount of fibers and congestion area which disappear.

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