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Assessment of the Effectiveness of Stepalol E in Grape Seeds Oil on the Alcohol-Related Damage of Gastric Mucous Membrane in Rats

M. Nikolaishvili, Z. Lomtatidze
The work has been done at I. Beritashvili Center of Experimental Biomedicine
Sokhumi State University Department of Microbiology

Abstract:-The carried studies showed that extraction from grape seeds oil (registered as stepalol E) is a direct correlation agent between dimensions of ulcers and indicators of oxidative stress, and moreover, the capacity of grape seeds oil has a positive effect on the dynamics of the indicated processes. The obtained data enable us to consider that the extraction from grapes seeds oil is more reasonable to be included in the complex treatment of the patients with ulcer disease who have more manifested ulcer defects with a higher degree of H. pylori infection and higher activity of POL as the medicine has the evident cytoprotective, anti-helicobacterial and antioxidative actions.

Keywords:- *Stepalol E, Alcohol, Gastric Ulcer, Rat.*

I. INTRODUCTION

Helicobacter H. Pylori exists in half of the adult population of the world. It is able to colonize chronically in gastric mucous membrane, where it is attached to epithelial cells. Although the majority of infected subjects feel no symptoms, helicobacter pylori infection causes severe gastritis and increases the risks of gastric cancer. It is considered as agents of pepticulators and gastritis and is associated with lymphoma of lymphoid tissue related to lymphoma and gastric cancer (4,6,7). H. Pylori Bacteria is one of the most spread bacteria infecting the humankind. It is detected in whole families, where it is transmitted from one to another. H. pylori bacterium occupies stomach and esophagus. It stimulates cells to produce a large amount of gastric or hydrochloric acid. Acid is sent back and up from stomach causing burning or in medical language Gastroesophageal reflux disease (*GERD*), also known as acid reflux. Acid-related diseases also involve a common pathogenetic link-acid aggression of gastric juice and the basic of its treatment is antisecretory therapy. Secretion of hydrochloric acid (HCl) by parietal cells of mucous membrane (CO) of stomach is determined by transmembrane transfer of protons, which is carried out with help of proton pump –H+/K+ ATP phase (5,11,20). The transfer of proton pump is associated with the increase in cytoplasmatic concentration of Ca2+, level of *Cyclic adenosine monophosphate* that is determined by cell receptor apparatus. Pump blockage completely excludes secretory process in parietal cell. In the regulation of the work of proton pump 3 types of receptors are participating: acetylcholinic, gastric, and histaminic. At present there are 5 types of muscarine receptors two of which participate in the secretion of HCl: MI-cholinoreceptors, localized in nervous textures of stomach and M3-on outer membrane of parietal cell. Stimulation of given receptors triggers acid formation. Three types of histaminic receptors are studied two out of which participate in the regulation of acid formation: H2 receptors are localized on a membrane of parietal cells. Their stimulation (link with histamine) leads to the activation of adenilatcyclacic system of parietal cells with farther formation of *Cyclic adenosine monophosphate. The latter one acts as secondary transmitter activating H+/K+-ATP-ase. Besides, the concentration of Ca2+ increases in cell cytosol occurs, which also activates the proton pump. H3 receptors localize on* enterochromaffinlike (***ECL***) cells secreting histamine. The stuimulation of gastric receptors slightly influence the secretion of HCI. Central and peripheral mechanisms also participate in the regulation of gastric acid formation. Central stimulation of gastric acid formation is determined by the activation of hypothalamic nuclei of vagus. (1,325). Further, along the fibers of vagus the impulse is transferred to nervous textures in stomach and through it to-parietal, pepsin forming, endocrine (G and D) enterochromaffin like cells of mucous membrane of the stomach (28-30). The mediator of vagus stimulation is acetylcholine, which, contacting with M1 and M3 cholinoreceptors, activates proton pump. Vagus effects induce the secretion of histamine by ECI cells which in turn stimulates HCI secretion. Thus, in central regulation of gastic secretion 2 mediators are participating (acetylcholine and histamine) and 3 types of receptors (M1, M3-cholinonergic and H2-histaminic). Also the role of H. pillory in the regulation of acid formation is proved. The constant formation of ammonia in the process viability of H. pillory, causes uninterrupted alkalization of antral part of stomach, destroys inhibitory mechanism of the secretion of gastrin, leading to hypersecretion of gastrin, constant stimulation of lining cells and hyperproduction of HCI; At the same time, some strains of H.pilory isolate cytotoxins, damaging CO.

Marshal and Worren were the first to have isolated H. pillory in 1983 from gastric epithelium and proved that this bacterium caused majority of gastroduodenal diseases, peptic

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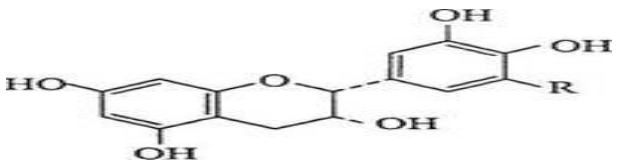
ulcers and gastric cancer. (8). H. pillory is one of the most genetically various bacteria (Helicobacter: spiral core; pillory: gate keeper), is small spiral (S-like) or curved linear gram negative movable bacillus, microaerofillic, irregular, and coccal transformation happens when it is exposed to air for approximately 2 hours. (9,13,16). Approximately 50% of the world population are infected by H. pillory, this rate could approach 80% in some developing countries and 40% in industrially developed countries and this explains why scientists consider that spreading of H. pillory could be inversely correlated with social-economical conditions and hygiene (10, 14). Ironically, in industrially developed countries relatively high spreading of gastric cancer was observed, while developing countries have low level of gastric cancer (8,14). The mechanisms of acquiring and transmission of H.pilory has not been known yet. Although it is considered that the primary means of transmission is fecal-oral and gastric-peroral from human to human, polluted food and water and some domestic animals (10,15,21,26).

Even though the treatment is usually effective, it can result in side effects, such as development of resistance to antibiotics (14) and recurrence because of low compliance. (2). Therefore the alternative methods of the treatment of H. pillory should be studied. Lots of natural plant extracts with anti-H. pillory are mentioned in studies, these extracts include: garlic, broccoli, cranberry and etc. (5,12,16,26). Pomegranates (Vitisvinifera) well known by their high levels of antioxidants and polyphenols also showed that they are new antimicrobial agents. In some studies it has been also reported about anti-H. pillory grape seeds and wines, having active chemical contents (e.g. restveratrol,) In some literature reviews the decrease in risks of gastric cancer by effective prophylactics carried out by the use of such herbal medicines as grape seed oil. It is known that fenolic compounds of grape seeds are mostly flavonoids.



Catechine and epicatechine phenol compounds contain large amount of hydroxyl groups. It enables them to reveal antioxidative features and absorb atomic oxygen. It allows us to consider poliphenols as restoning agents. Their antioxidative action is 4-5 times more than antioxidative potential of vitamin C and vitamin E. *Poliphenols*. Except for antioxidative action, grape seed poliphenols inhibit some enzymes, that catalyze the shooting the histamine into the blood,, the latter determines inflammatory and allergic reactions of the body. Phenolic compounds protect vitamins from early oxidation and enable them to perform their function where it is necessary. It is proved the joint activity of such antioxidants as vitamin C and vitamin E, selen and carotinoids (7,9). They have anti-inflammatory action, inhibit the functions of cyclooxigenease and hyperoxidase (action against the development of cancer cells). *Phytoestrogens* are biologically active herbal active compounds. They are close to female natural hormones by structure and features. There is some evidence that phytoestrogens have ability to diminish the risk of cancer development, including breast cancer. One of the mechanisms, that explains the activity of phytoestrogens, is their ability to connect and activate natural mechanisms of gastric mucincatelicidin LL-37 and O-glycan of hereditary system in host body. These mechanisms have the key role in gastric against H. pillory infection. LL-37 is proteolitic processing peptide. LL-37 enables to stop the growth of bacteria, which is carried out by neutralization of bacterial wall and by connection of specific receptors existing in host cellular membrane. hGAP18 is antimicrobial peptide, while hGAP18/LL-37 is multifunctional peptide which is activated by H. pillory at the expense of gastric epithelium by catelacidine or its derivate LL-37 believing that it will balance the existence of H. pillory in inhabitancy by the protection of mucous membrane. Cationic antibacterial peptides (GAPS), along with LL-37 are the potential source of new antibacterial molecules (27,30). All the mentioned suggest that bile acids based on antimicrobial agents oppose protolithic degradation and inhibition of mucin. Grape seed polyphenols possess specific affinity with some proteins and peptides. The reactions between protiens and polyphenols could be used for the inhibition of some enzymes. This influence can be used in the treatment of ulcers caused by H. Pillory. It is also known from the literature that procianides and proanticianides extracted from grapes seed prevent the body from the growth of cancer cells. In rats when the concentration of grape seed oil is 0.5 mg/ml, the amount of spontaneous mutations decreased by 65% while in the conditions of the same concentration nuclear mutation decreased by 92%, which is carried out by the neutralization of bacterial wall and by the connection of specific receptors existing in host cellular membrane. Hence our goal is to study the influence of Stepalol E derived from cold pressured grape seed oil on gram positive as well as gram negative bacteria (19, 22). In our opinion this medicine can be used for the prevention of H. pillory. The medicines such as aspirin and its analogues such as glucocorticoids, rezerpinetc cause the decrease in synthesis of mucin and prostaglandins, as well as the decrease in the regeneration of gastric mucous membrane. Acetylslicilic acid once penetrates gastric epithelial cells and vessels endothelium, causes capillary bleeding, severe dystrophic and erosive changes in gastric mucous membrane, decreases the proliferative activity of cambial zone cells. Consequently the decrease of mucous formation occurs and mucolytic effect is detected that is manifested by the dissociation of mucous formation structures and then by the elimination of mucous protective features. Acetilsalicilic acid causes the resistence of gastric mucous membrane with the destroy of metabolic processes: by switching off phosphorilization and oxidative processes and finally by deenrgization of cellular systems, blocks Na/K+ pump disrupting ionic homeostasis (18, 24).

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II. MATERIALS AND METHODS

We had taken of Wistar line rats of control and experiment animals with 18-18 rats in 150-170 g We had 3 groups of animals, 1 group control with weeding of water of 25 mg / 100g and we had Stepalol E, 2 group alcohol of 25 mg / 100g. And the third group of water and Alcohol + Stepalol E administration daily for 10 days. The animals were both sexes in identical conditions on standard feeding. Gastric wall was damaged by administration of alcohol per of 25mg / 100 g / kg once a day. Stepalol E was administered from the first day of alcohol administration daily for 10 days. Malonildialdehyde (MDA), ELISA Kit (#MBS9389387).

III. RESULTS OF THE RESEARCH

Gastric mucous membrane damage is given in Table 1. Based on the analysis of the received data, it is obvious that the administration of Stepalol E statistically reliably reduces the average data of erosion in experimental animals (17,8±1,45 мм2) compared with control groups (48,62±4,4мм2). According to experiments the animals which were administrated Stepalol E along with water peritoneally, the average data are reduced per 1 erosion animal than in control animals where the average number of Paul's index in case of destruction such as striped erosion and splinter hemorrhages were less than in control animals.

Index of the gastric mucosa	A group of animals after administration of alcohol and Stepalol E		
	Alcohol+water control	Alcohol+ Stepalol E animals	Stepalol E and water
Hyperemia relative unit (from 0 to 4)	3,3	3	2,6
Splinter hemorrhages	67,0 ±2,9	45,65±3,9	42,68±4,9*
The average number of dotty blood vessels in 1 animal	11,78±0,59	7,0±0,31	6,0 ±0,33*
Paul's index of dotty hemorrahages	11,7	8,0	5,0
The average size of erosion	48,62±4,4мм2	35,5±3,8 мм2	15,0±3,9* мм2
The average number of erosion in 1 animal	1,6±0,25	1,3±0,8	1,2 ±0,16
Paul's index for erosion	1,6	1,3	1,1
The average sizes of striped erosion, mm	14,68±0,20	11,56±0,22	7,67±0,20*
The average number of striped erosion in 1 animal	3,34±0,13	2,65±0,12*	2,64±0,12*
Paul's index for striped erosion	3,34	2,65	1,67

Table 1:- The impact of alcohol on the development of erosion in rats. (M ±m ; n= 6)
Note * - Authenticity in relation to controller <0,05

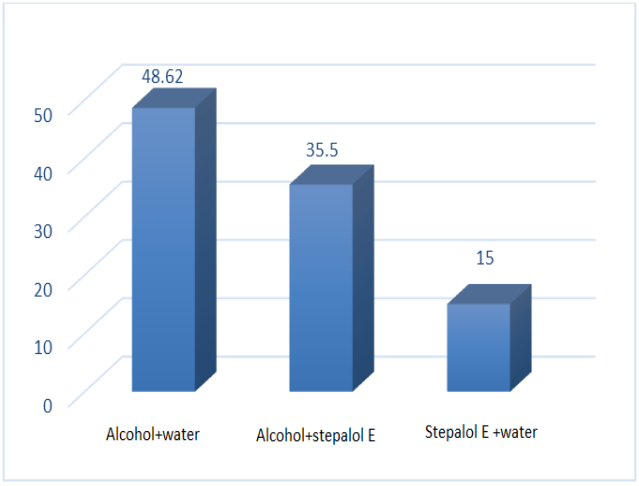

Fig 1:- The impact of Stepalol E and the decrease of ulcer in size after administration of alcohol.

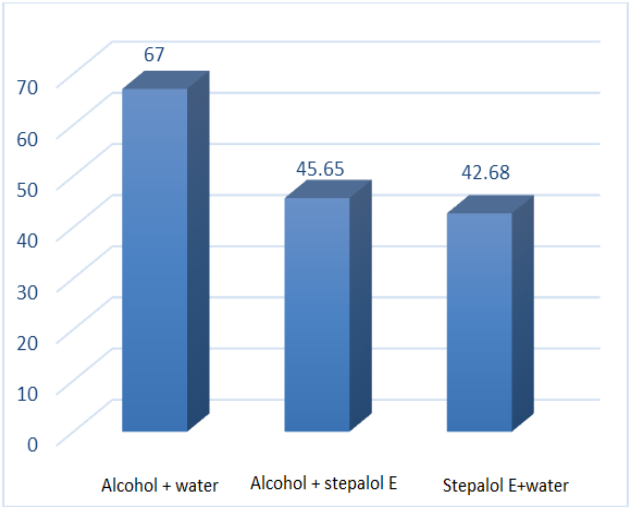

Fig 2:- The impact of Stepalol E and the decrease in size of ulcer splinter hemorrhages after administration of alcohol.

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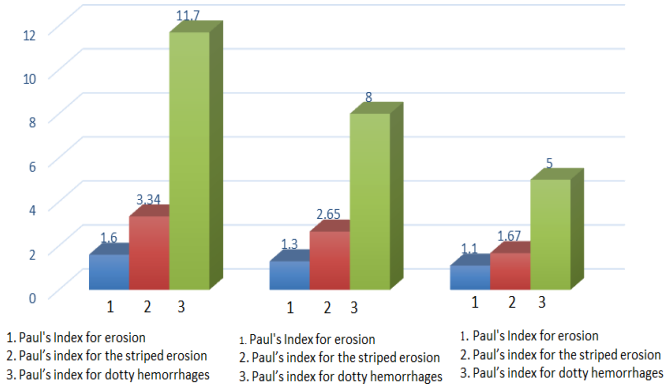

Fig 3:- The impact of Stepalol E and Paul's index for erosions, Paul's index for the striped erosion, Paul's index for dotty hemorrhages after administration of alcohol.

Hyperemia and relief smoothness are well visible in rats where alcohol and Stepalole E are observed. It should be noted that the sizes of the surface of the mucous membranes in case of splinter hemorrhages were greater in the group of rats where alcohol and water were administered by 67.0 ± 2,9 than alcohol and Stepalole E by 42,68 ± 4,9 *. Biochemical data is given in Table 2. It also shows that the free NS1 indicator was relatively more in rats taking Stepalole E + alcohol, but fewer in those rats which were administered Stepalole E with water. As for Pepsin it was alcohol+ water 487 ± 21,5, alcohol + Stepalole E 460,0 ± 9,14 and alcohol + water 478,5 ± 25.

Iindicators	Rats under appropriate procedure		
	Alcohol + water	Alcohol + Stepalol E	Stepalol E + water
Total Acidity, a titre	55,16 ±1,07	61,31 ± 0,7*	41,6 ± 2,37
Free NS1, a titre	13,14 ±1,15	15,5 ±0,7	8,05 ± 0,77
Secretion of gastric juice, ml / 100g x h	0,25±0,01	0,22±0,5	0,35± 0,02*
Pepsin mg /%	478,5± 25, 8	460,0±9,14	487±21,5

Table 2:- Biochemical indicators of gastric juice during the development of aspirin ulcer in Wistar rats (M ±m ; n= 6)
Note * - Authenticity in relation to controller <0,05

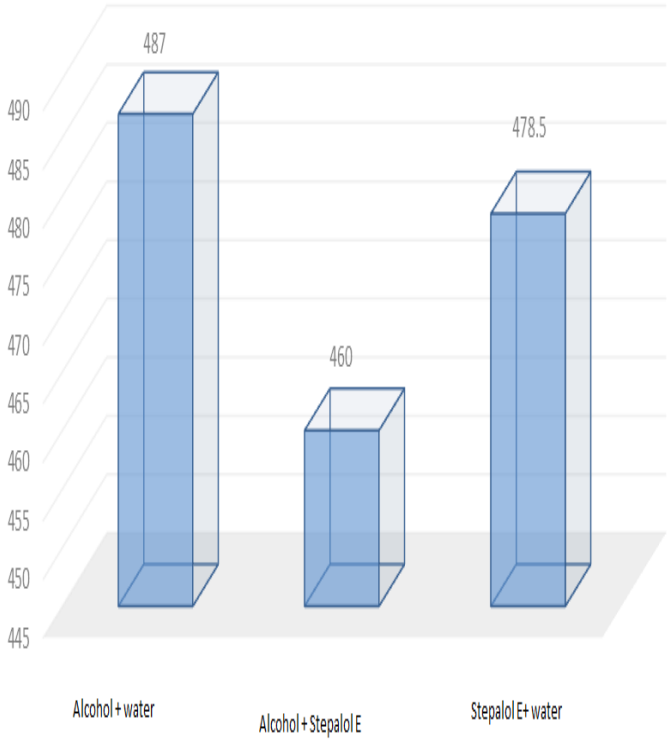

Fig 4:- Free Pepsin indicator

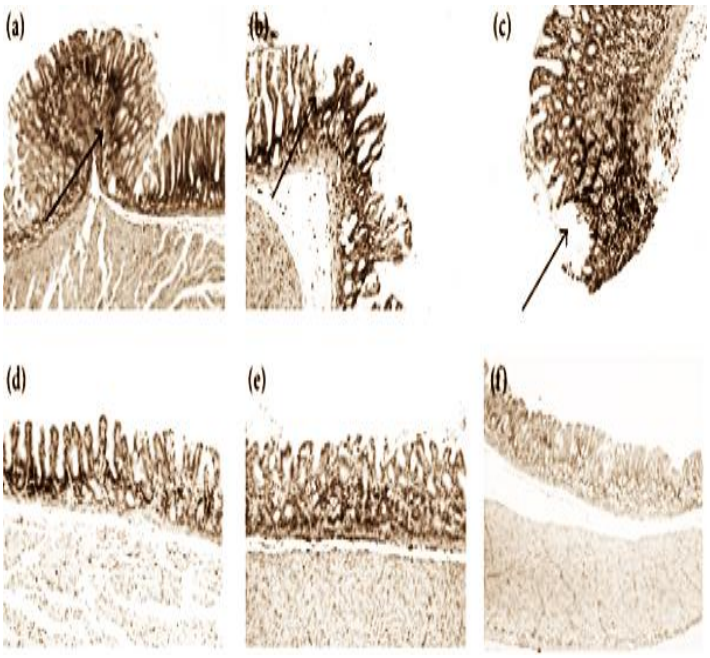

Fig 5:- Histopathological analysis of the gastricmucosain rats in fected by alcohol:
(a)Hyperemia (b) hyperplasia; (c) erosion; (d)Uninfected, antioxidant properties of Stepalol E. H&E;×40.

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Thus, we can make a conclusion that application of Stepalol E during the gastric mucosal damage causes a decrease in the destruction degree in the stomach, or in other words, it has gastroprotective effect. Biological membranes are of great importance and the focus of science is directed towards them. The important role of its function depends on the active form of oxygen. Oxidative peroxidation, according to many authors today, is the universal mechanism of damaging membrane structures during various pathological processes. Under the effect of oxidative radicals, enzymatic and non-enzymatic antioxidant systems play the protective factor of the structural and functional integrity of membrane, plays an important role in the normal functioning of the cell (24, 27).

In experimental and clinical studies, reactions of LPO were studied and are being studied in case of peptic ulcer of the stomach and duodenum. However, until now, the features of LPO in this pathology have not been fully disclosed, the question of the state of LPO at different stages of peptic ulcer has not been elucidated, which in many ways can determine the place and role of free radical reactions in the pathogenesis of this disease. We have investigated the antioxidant activity of Stepalol E on the model of gastric ulcer in white rats caused by the administration of alkohol by evaluating the inhibition of the formation of a colored malonic dialdehyde complex (MDA), the final product of LPO with thiobarbituric acid. The malonic dialdehyde concentration was calculated using the molar extinction coefficient and the result expressed in n/mol for the sample.

The contents of malongidadeide in the homogenets are 2,89, ± 0.12 nm/g and during the processes developed under the effect of Stepalol E, it reaches 4,2 ± 0,15 nm/gr. The difference between statistics is statistically significant (R <0,005), which once again approves the antioxidant properties of Stepalol E, and the membrane stabilizing effect that stimulates the regenerative processes of the damaged gastric wall.

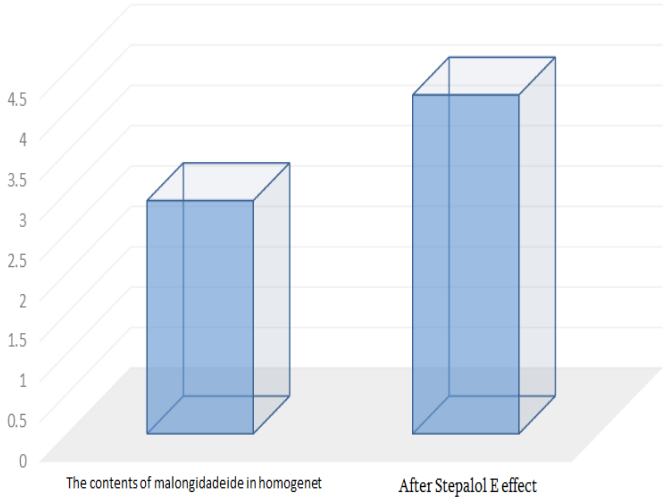

Fig 6:- Molodidaldehyde content under Stepalol E effect

The studies have shown that the inclusion of grape seeds oil (registered under the name Stepalol E) serves as the direct corrective agent between the size of the ulcer, and the indices of oxidative stress, as well as the ability of grape seeds oil to affect positively on the dynamics of these processes. Conclusions: The obtained data make it possible to consider more appropriate the inclusion of grape seeds oil (Stepalol E) in the complex treatment of patients with peptic ulcer with a more expressed ulcerative defect, with a higher degree of H. pylori infection and a higher activity of LPO, since the medicine has expressed cytoprotective, anti-Helicobacter and antioxidative actions.

IV. CONCLUSIONS

- The paper was given The scientific research is devoted to the study of the effect of oil from the bones of grapes obtained from natural raw materials on the clinical manifestations of the disease, reparative, cytoprotective and antioxidant processes in 105 patients with duodenal ulcer (mean age - 38.5-1.5 years).
- cold pressing of the grape seed oil for use in alcohol-induced gastritis promoted complete regeneration of the ulcerative defect with the formation of tender scars after 12 days of treatment in 76% -80%, while in the control groups a positive effect was noted in 66% of cases.
- Inclusion of oil from the bones of grapes, with a morphological study of biopsy specimens of the gastric mucosa, a more pronounced decrease in the inflammatory process was noted, which was manifested by a more pronounced decrease in the number of interepithelial lymphocytes and neutrophil granulocytes.
- The following tests will be carried out h.pilorit infected and cold pressed using grape seed oil antiulcer treatment stistandartuli scheme (omeprazole-clarithromycin-metronidazole and famotidine-amoxicillin-metronidazole). Our thoughts are metoidit even early and the disappearance of pain syndromes of dyspeptic rats, Who suffers from H. Pylori.

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