Original Article

A Morphometeric Study of the Effects of Ibuprofen on the Stomach of Albino Rats under Light Microscope

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ABSTRACT

Objective: To observe the effects of ibuprofen on the stomach of the albino rats under light microscope and its morphometeric analysis.

Study Design: A prospective experimental study.

Place and Duration of Study: This study was conducted at the Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi during 01.04.2008 to 31.05.2008.

Materials and Methods: Thirty male adult albino rats were taken for the study and were divided into two groups containing 15 animals each. Each group was divided in to three sub groups according to the time of sacrifice i.e. 4,6 and 8 weeks. Group A served as control. Group B received ibuprofen at the dose of 70 mg per kilogram body weight per day orally with feed. After completion of respective period of treatment animals were sacrificed, abdomen was opened. The stomach was removed and opened along the greater curvature, divided into cardiac, body and pyloric parts and was fixed in Buffered neutral formalin for 24 hours. After that tissues were processed in ascending strength of alcohol, cleared in xylene and infiltrated and embedded with paraffin. Five micron thick sections were made on the rotatory microtome, were stained with Haematoxylin and eosin (for general morphology and morphometric study), and with Periodic Acid Schiff Orange-G (for the mucus content of the surface mucus cells and the mucus neck cells), randomly selected every seventh stained section, in three fields were studied.

Results: In stained sections of all parts of stomach the lining epithelium was disrupted, exfoliated, and ulcers and erosions were present. The erosive areas contained red blood cells and extended deep in to lamina propria. The results of mucosal thickness and the mean values of number of surface mucous cell count was moderately significant ($P < 0.001$) to highly significant (($P < 0.0001$) in all parts of stomach when compared to control. The mucous content of the surface mucous cell in subgroups 'B'1, 'B'2 and 'B'3 of cardiac and pyloric parts were same marked (++++), while in body part of stomach it was moderate (+++) same as in control animals respectively.

Conclusion: Based on present study, it is concluded that Ibuprofen induces gastric mucosal damage.

Key Words: Ibuprofen. Hematoxylin and eosin, Periodic Acid Schiff Orange G

INTRODUCTION

Ibuprofen is the most commonly used NSAID. It was the first member of the Propionic acid class of NSAID to come into general use, (Gilman et al., 2006)[1]. Other Propionic acid derivatives include Ibuprofen, Naproxin, Fenoprofen, Ketoprofen, Flurbiprofen and Oxaprozin. These agents offer significant advantages over the aspirin and indomethacin because they are better tolerated as anti-inflammatory drugs (Kato, 2002; Hatazawa et al., 2006)[2].

It acts by inhibiting the enzyme cyclooxygenase (COX) which catalyzes the conversion of the arachidonic acid to prostaglandins. The first enzyme in the prostaglandin synthetic pathway is cyclooxygenase. This enzyme converts arachidonic acid to the unstable intermediates PPG2 and PGH2 and leads to the production of thromboxane A2 and a variety of prostaglandins (Gilman et al., 2006)[1]. The half life is roughly 2 hours. Propionic acid derivatives (Ibuprofen) are approved for use in the symptomatic treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute gouty arthritis; they also are used as analgesics, for acute tendonitis and bursitis, and for primary dysmenorrhea (Gilman et al., 2006)[1]. Ibuprofen at doses of 200 mg and 400 mg is an efficacious, cost-effective, well-tolerated, single-ingredient, treatment of migraine. In addition ibuprofen provided a beneficial effect on associated symptoms of migraine including nausea, photophobia, phonophobia, and functional disability (Codispoti et al, 2001)[4]. Ibuprofen 200mg and 400mg is effective in reducing headache intensity and rendering patients pain-free for two hours. Photophobia and phonophobia improved with 400mg dosing (Suthisisang et al., 2007)[5]. The recommended dose of Ibuprofen is 600mg qid. It is equivalent to 4 grams of aspirin in anti-inflammatory effects (Katzung, 2004)[6] Ibuprofen enhances the effect of pyrazinamide during the initial phase of tuberculosis treatment in the mouse model (Byrne et al., 2007)[7].

It has been proved that non steroidal anti-inflammatory drugs like indomethacin and ibuprofen cause the topical mucosal injury and it is the critical factor in the, development of intestinal injury (Seager et al, 2000)[8].

Gastrointestinal adverse reactions from ibuprofen usage include mucosal ulcers and bleeding (Abraham et al., 2005)[9]. Ulcerations may range from small superficial erosions to full thickness perforations of muscularis mucosa. There may be single or multiple ulcerations accompanied by gradual blood loss leading to anemia or by life threatening hemorrhage (Maricic et al., 1999)[10]. Ibuprofen is most frequent cause of aseptic meningitis induced by drugs. (Rodriguez et al, 2006)[11]. Nitric oxide-linked ibuprofen can promote resistance to mucosal injury, possibly via local synthesis of Nitric oxide (Downing et al., 2005)[12]. Ibuprofen esterified with Nitric oxide abolished irritation and significantly reduced thinning of gastric mucosa (Dudkiewicz, 1981; Downing et al., 2005)[13]. Keeping above facts in mind the present study has was designed to observe the effects of the Ibuprofen on the gastric mucosa and evaluation of the histological changes under light microscope.

MATERIALS AND METHODS

This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi where 30 healthy and active adult albino rats of either sex between 90-120 days were selected for present study. The animals were divided into three groups, A, B and containing 15 animals each and were further sub-divided into three sub-groups containing 5 animals each according to time of sacrifice, i.e. 4, 6, and 8 weeks respectively. Group 'A' served as control. Group 'B' received ibuprofen (available in the market as "BRUFEN" by Bayer Laboratories, Karachi Pakistan) at the dose of 70 mg per kilogram body weight per day orally with feed (Dokmeci et al., 2007). The animals were sacrificed at the end of their respective period of treatment under the ether anaesthesia. Their abdomen was opened with a long midline incision. The stomach was removed and opened along the greater curvature with an incision extending from cardiac end to the pyloric end and the contents of the stomach were noted for color, consistency, and blood. The stomach was stretched, fixed and cleaned and dipped in normal saline very gently. The mucosa was observed grossly for color and hemorrhagic spots and then under dissecting microscope for color, blood vessels and hemorrhagic areas and the number of erosions/ulcers.

Stomach was divided into cardiac, body and pyloric parts and was fixed in Buffered neutral formalin for 24 hours. After that tissues were processed in ascending strength of alcohol, cleared in xylene and infiltrated and embedded with paraffin

Five micron thick sections were made on the rotatory microtome and were stained with Haematoxylin and eosin (for general morphology and morphometric study which was done under the light microscope under (8x ocular and 40x objective). Sections from body were also stained with Periodic Acid Schiff Orange-G (The mucus content of the surface mucus cells and the mucus neck cells was observed in all parts of stomach under (8x ocular and 40x objective), and was graded as follows: Mild (++) (secretions in the basal part of the cells). Moderate (+++) (secretions extending upto the middle part of the cells). Marked (++++) (secretions extending upto the apical part of the cells). The cardiac and pyloric parts were also stained with combined Alcian blue-Periodic Acid Schiff technique. Randomly selected every seventh stained section, in three fields were studied for morphology and morphometery. The statistical significance of difference of various quantitative changes between the groups was evaluated by student "t" test. The difference was regarded statistically significant if the 'P' value was equal to or less than 0.05. All calculations were done by utilizing computer software SPSS version 10.

RESULTS

GROUP-A: The animals of group 'A' were healthy and active. The external surface of the stomach was shinny and glistening and the internal surface was clearly identified into two parts the grayish white part (esophagus), and the pink part which was continuous with the duodenum.

Table No.1: Mean* value of Erosions / Ulcers recorded in the Stomach of Different Groups of Albino Rats

Groups	Sub-groups	Treatment Given	Erosions of ulcers recorded in Stomach		
			4th Week	6th Week	8th Week
A	A1	Control	0		
	A2			0	
	A3				0
B	B1	Ibuprofen	6±0.31		
	B2			6±0.63	
	B3				9±0.63

*Mean±SEM

In H&E stained sections the surface lining cells were composed of simple columnar epithelium with oval to round nuclei. The lamina propria of all parts of stomach consisted of glands, connective tissue fibers and few lymphocytes. The muscularis mucosae consisted of smooth muscle fibers. The sub mucosa consisted of loose connective tissues with few blood vessels. The muscularis externa of cardiac part consisted of smooth muscle fibers which were arranged as inner circular and outer longitudinal layers but in the body and pyloric part it consisted of inner circular, middle longitudinal and outer oblique layers. The serosa was visualized normal in appearance. In Alcian blue-PAS stained sections of cardiac and pyloric parts, the surface mucus cells were present on the luminal surface of the stomach

and the mucus neck cells showed normal histological architecture.

Table No.2: Mean* value Mucosal Thickness (µm) of different parts of Stomach in Different Groups of Albino Rats

Groups	Sub-groups	Treatment Given	Measurement thickness of cardiac part of stomach		
			4th Week	6th Week	8th Week
A	A1	Control	431.40±12.36		
	A2			416.60±2.655	
	A3				418.00±2.565
B	B1	Ibuprofen	375.80±2.28		
	B2			368.60±3.14	
	B3				368.86±2.276
Groups	Sub-groups	Treatment Given	Measurement thickness of body of stomach		
A	A1	Control	560.00±1.516		
	A2			566.00±1.816	
	A3				572.00±1.414
B	B1	Ibuprofen	446.00±1.549		
	B2			441.00±1.095	
	B3				431.00±2.701
Groups	Sub-groups	Treatment Given	Measurement thickness of pyloric part of stomach		
A	A1	Control	559.20±2.49		
	A2			579.60±1.63	
	A3				580.40±2.46
B	B1	Ibuprofen	548.20±2.10		
	B2			555.00±3.22	
	B3				544.60±2.03

*Mean±SEM

Table No.3: Mean* value of Mucus Cell Count in the Cardiac Part of the Stomach in Different Groups of Albino Rats

Groups	Sub-groups	Treatment Given	Mucus cell count in the cardiac end of stomach		
			4th Week	6th Week	8th Week
A	A1	Control	66.60±0.87		
	A2			66.60±1.695	
	A3				67.40±0.50
B	B1	Ibuprofen	55.20±1.31		
	B2			51.80±1.11	
	B3				48.60±1.16
Groups	Sub-groups	Treatment Given	Mucus cell count in the body part of stomach		
A	A1	Control	54.40±1.363		
	A2			56.60±0.927	
	A3				56.20±0.663
B	B1	Ibuprofen	45.20±1.392		
	B2			40.80±0.860	
	B3				45.60±1.503
Groups	Sub-groups	Treatment Given	Mucus cell count in the pyloric part of stomach		
A	A1	Control	65±0.83		
	A2			64.20±1.11	
	A3				63.60±0.50
B	B1	Ibuprofen	55.20±1.01		
	B2			54.20±0.73	
	B3				50.40±0.75

In the PAS orange-G stained sections of the body of stomach consisted of gastric glands. The mucus neck cells were cuboidal to columnar in shape, mostly located in the neck region of the gastric glands; these cells gave irregular appearance due to compression of surrounding cells. The parietal cells were large and oval

in shape located at the base of the glands, few of them were in the neck region, the nuclei were rounded and placed in the centre of the cells. The chief cells were present on the basal part of the gland, as mostly pyramidal in shape, the nuclei were oval in shape lying in the base of the cell, where as the apical region was clear and filled with vacuolated spaces. The mean values of number of erosions/ulcers of stomach are shown in table 1. The mean value of mucosal thickness is shown in table 2. The mean values of number of surface mucous cell count are shown in table 3.

GROUP-B: Animals of group 'B' were weak, and sluggish in activities. The gross observation of the external surface of stomach was dull, under the dissecting microscope the mucosa was red, swollen and erosions were observed in all subgroups.

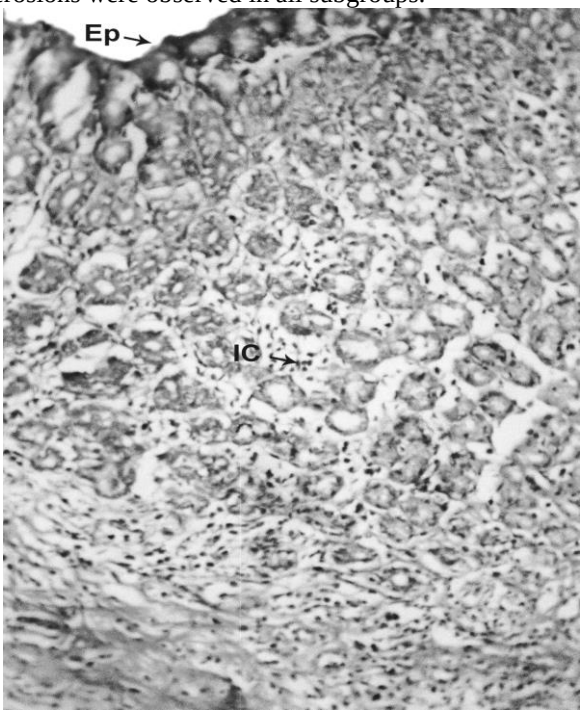

Figure No.1: H&E stained, 5µm thick sections of the body of the stomach in Ibuprofen treated albino rat, showing the (Ic) inflammatory cells in the lamina propria, (Photomicrograph x100). (6th week)

In H&E stained sections of all parts of stomach the lining epithelium was disrupted, exfoliated, and ulcers and erosions were present. The erosive areas contained red blood cells and extended deep in to lamina propria. The nuclei were small in size in between the eroded areas in subgroups 'B'1 and 'B'2. In subgroup 'B'3 the nuclei became pyknotic. The inflammatory cells were observed within and around the erosive areas, and in lamina propria. The erosions/ulcers were observed more in the pyloric part in subgroup 'B'3 while in cardiac and body parts the erosions/ ulcers were more in subgroups-'B'1 and 'B'2. The muscularis mucosae consisted of smooth muscle fibers arranged circularly in all subgroups. The submucosa showed normal appearance consisting of loose connective tissues with few blood vessels in subgroups 'B'1 and 'B'2. In subgroup 'B'3 the inflammatory cells extended in the submucosa. The muscularis externa in all subgroups consisted of two layers of smooth muscle fibers, inner circularly arranged and outer longitudinal.

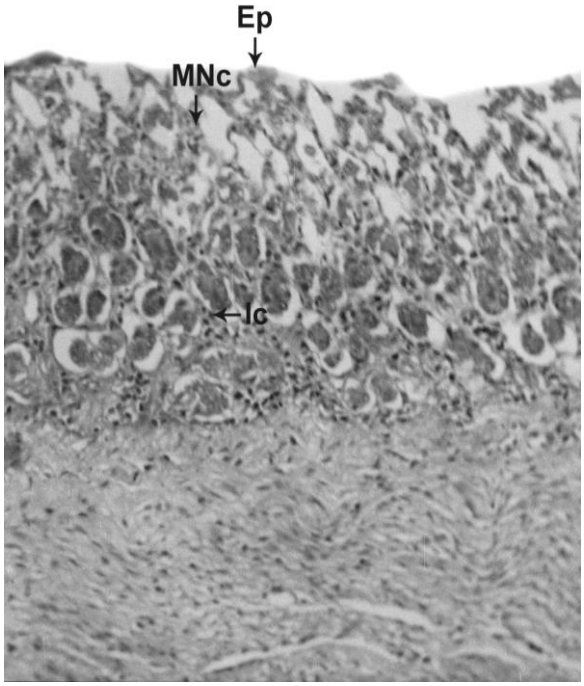

Figure No.2: Alcian blue-PAS stained, 5µm thick section of the pyloric part of the stomach in Ibuprofen treated albino rat showing the (Ep) disrupted epithelium, (MNc) distorted mucus neck cells and (Ic) inflammatory cells in the lamina propria. (Photomicrograph x100).(8th week)

The serosa was visualized as normal in appearance; connective tissue fibers and cells were loosely arranged. In Alcian blue-PAS stained sections of the cardiac and pyloric part the surface mucus cells were less in number and elongated in appearance, the gland cells were elongated and few. The cells were enucleated and nuclei were visualized in the lumen. The PAS orange-stained sections of the body of the stomach consisted of gastric glands. The mucous neck cells were distorted in shape and decreased in number. The parietal cells were more in number and small in size, their nuclei were present in the lumen of the gland. The chief cells were decreased in number, the shape was distorted, and the apical part of the cells was vacuolated. The nuclei were present in basal part of the cell. The mean values of number of erosions/ulcers of stomach are shown in table 1.

The mean value of mucosal thickness in cardiac parts of subgroups 'B'1, 'B'2 and 'B'3 the mucosal thickness was decreased in 'B'1 it was moderately significant ($P< 0.001$) while in 'B'2 and 'B'3 it was highly significant ($P<0.0001$) when compared with control. In body part

of stomach in subgroups 'B'1, 'B'2 and 'B'3 the mucosal thickness was decreased and was moderately significant (P< 0.001) when compared to control. In pyloric part in all subgroups the mucosal thickness was decreased and was moderately significant (P<0.001) when compared with control (Table 2).

The mean values of number of surface mucous cell count are shown in table 3. The number of mucous cell count was decreased in all sub groups of the cardiac part. The results were moderately significant (P<0.001) in subgroups 'B'1 and 'B'3 while highly significant (P< 0.0001) in 'B'2 when compared to control group. In the body part the surface mucous cells count was decreased in subgroup 'B'1, but was moderately significant (P<0.001), while in subgroups 'B'2 and 'B'3 the cells were decreased significantly (P<0.05) when compared to control groups. In pyloric part the number of mucous cell count was decreased in all subgroups. The results were moderately significant (P<0.001) in subgroups 'B'1 and 'B'3 while significant (P<0.05) in 'B'2 when compared to control group.

The mucous content of the surface mucous cell in subgroups 'B'1, 'B'2 and 'B'3 of cardiac and pyloric parts were same marked (++++), while in body part of stomach it was moderate (+++) same as in control animals respectively.

DISCUSSION

The present study was designed to observe the effects of on ibuprofen on the stomach of the albino rats. The animals treated with Ibuprofen in group-B appeared ill looking with loss in their body weight. Same findings were observed by the Dudkiewicz (1981)[14].

In the group-B animals, the number of ulcer was increased significantly the findings of presence of ulcers over gastric mucosal surface are in agreement with the findings of Tanaka (2002)[15] who used Indomethacin, Floribprofen, Naproxen, Kato (2002)[15] used indomethacin and rofecixib; and Jimenez et al (2004)[16] used Ibuprofen in their experimental studies on rat and measured the size of lesion under dissecting microscope.

Sequential observations of the changes between 4 and 8 weeks post-treatment with Ibuprofen showed that desquamation of the surface epithelial occurs in 4 weeks, while more extensive disruption and exfoliation of the surface epithelium and ulceration appeared in 8th week. Takeuchi et al (1992)[17] reported that HCl-induced gastric injury in rats by histological examination the apparent damage in the surface epithelial cells and disruption of epithelial membrane. Kumar et al (2003)[18] reported that NSAIDs-induced gastric mucosal defects which varied in extend from the superficial mucosal lesion down to the entire thickness of mucosa.

In the present study the decrease in mucosal thickness in group-B could be attributed to the injury caused by Ibuprofen, which resulted in demolition phase at 4th week of treatment, at 6th week extensive exfoliation of the surface epithelial cells and at 8th week damage of mucosa was more extensive with surface ulceration. Depending on the severity of injury, the mucosal response varies from vasodilatation and edema of the lamina propria, to erosion and hemorrhage. Many of their effects are probably mediated by an inhibition of prostaglandin synthesis as suggested by Underwood (2004)[19]. Hung (2006)[20] observed that necrotic cell-injury was found in both epithelial layers and lamina propria when gastric juice was present in the stomach of rats with ischemic brain.

The surface mucous cell count was significantly decreased in group-B due to progressive deterioration of gastric mucosa with time. As observed by Bagshaw (1987)[21] in an experimental study of aspirin-induced chronic gastric ulceration in the rat.

The present study demonstrates the inflammatory infiltrate is slight to marked degree in ibuprofen treated animals which confirms to the observations of Jimenez et al (2002). However at some places noticeable exfoliation accompanied by increased inflammatory infiltration in subgroup-B3 was observed. The present study also showed vaso-congestion which was concurrently increased with the extent of damage. Similar observations were confirmed by previous investigators (Kumar et al., 2004)[18].

In the present study the changes occur in mucus cells as a reaction to injury by ibuprofen in group B morphology was changed from slight to marked degree. The mucus content was increased in between the eroded/necrosis areas in group B animals, these findings are confirmed by (Kumar et al., 2004)[18].

CONCLUSION

Based on present study, it is concluded that Ibuprofen induces gastric mucosal damage.

REFERENCES

1. Goodman G. Analgesics and antipyretic agents. Goodman Gilman Pharmacology.11th ed. USA: McGraw Hill; 2006.p 671-715.
2. Kato S, Ogawa Y, Kanatsu K, Okayama M, Watanabe T, Arakawa T, et al. Ulcerogenic influence of selective cyclooxygenase-2 inhibitors in the rat stomach with adjuvant-induced arthritis. JPET 2002; 300: 503-509.
3. Hatazawa R, Ohno R, Tanigami M, Tanaka A, Takeuchi K. Roles of endogenous prostaglandins and cyclooxygenase isozymes in healing of indomethacin-induced small intestinal lesions in rats. JPET 2006;318: 691-99.
4. Codispoti JR, Prior MJ, Fu M, Harte CM, Nelson EB. Efficacy of nonprescription doses of Ibuprofen for treating migraine headache: a randomized controlled trail. Headache 2001; 41: 665-79.

5. Suthisisang C, Poolsup N, Kittikulsuth W, Pudchakan P, Wiwatpanich P. Efficacy of low-dose ibuprofen in acute migraine treatment: Systematic review and meta-analysis. Ann Pharmacother 2007.
6. Katzung BG, Trevor AJ, Masters SB. NSAIDs, acetaminophen, and drugs used in gout. Katzung and Trevor's Pharmacology. 7th ed. New York: McGraw Hill;2005.p.307-15.
7. Byrne ST, Denkin SM, Zhang Y. Aspirin and ibuprofen enhance pyrazinamide treatment of murine tuberculosis. Antimicrob Chemother 2007; 59: 313-6.
8. Seager JM, Cullen DJ, Pearson G, Holmes S, Doherty M, Wilson JV, et al. Ibuprofen versus other non-sterodial anti-inflamatory drugs: use in general practice and patient perception. Alimen Pharmacol Ther 2000; 14(20): 187-91.
9. Abraham P, Ki KD. Nitro-arginine methyl ester, a non-selective inhibitor of nitric oxide synthase reduces ibuprofen-induced gastric mucosal injury in the rats. Dig Dis Sci 2005; 50(9): 1632-40.
10. Maricic N, Ehrlich K, Gretzer B, Schuligoi R, Respondek M, Peskar BM. Selective cyclooxygenase-2 inhibitors aggravate ischemia-reperfusion injury in the rat stomach. Br J Pharmacol 1999;128:1659-66.
11. Rodriguez SC, Olguin AM, Miralles CP, Viladrich PF. Characteristics of meningitis caused by ibuprofen: report of 2 cases with recurrent episodes and review of the literature. Medicine 2006; 85: 214-20.
12. Downing JE, Madden JC, Ingram MJ, Rostron C. Gastric and thymic assay of acute oral treatment of rats with nitric oxide esters of ibuprofen or indomethacin. Biochem Biophys Res Commun 2005; 334: 646-53.
13. Dudkiewicz J. Ibuprofen-induced gastrointestinal changes. Acta Physiol Pol 1981; 32: 693-701.
14. Dokmeci D, Kanter M, Inan M, Aydogdu N, Basaran UN, Yalcin O, et al. Protective effects of ibuprofen on testicular torsion/detorsion-induced ischemia/reperfusion injury in rats. Arch Toxicol 2007; 81: 655-63.
15. Tanaka A, Hase S, Miyazawa T, Ohno R. Takeuchi K. Role of cyclooxygenase (COX)-1 and COX-2 inhibition in NSAID-induced intestinal damage in rats: relation to various pathogenic events. JPET 2002; 303: 1248-54.
16. Jimenez MD, Martin MJ, Alarcon de La Lastra C, Bruseghini L, Esteras A, Herrerias JM, et al. Role of L-arginine in Ibuprofen-induced oxidative stress ad neutrophil infiltration in gastric mucosa. Free Radic Res 2004; 38(9): 903-11.
17. Takeuchi K, Takehara K, Kato S. Effect of NG-nitro-L-arginine methyl ester, the nitric oxide synthase inhibitor, on duodenal alkaline secretion and mepirizole-induced duodenal lesions in rats. J Clin Gastroenterol 1995; 1: S66-72.
18. Kumar V, Abbas AK, Fausto N. Stomach. Robbins and Cotran Pathologic basis of disease. 7th ed. Philadelphia: Saunders; 2004.
19. Underwood JCE. Alimentary system. General and systematic pathology. 4th ed. Edinburgh: Churchill Livingstone;2004.p.369-72.
20. Hung CR. Role of gastric oxidative stress and nitric oxide in formation of hemorrhagic erosion in rats with ischemic brain. World J Gasteroenterol 2006; 12: 574-81.
21. Bagshaw PF, Munster DJ, Wilson JG. Molecular weight of gastric mucus glycoprotein is a determinant of the degree of subsequent aspirin induced chronic gastric ulceration in the rat. Gut 1987; 28: 287-293.

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