Original Article

Community Medicine

Effects L-Arginine on the Gastric Mucosal Cell count when given with Ibuprofen in Albino Rats under light Microscope

1. Santosh Kumar 2. Ghulam Mujtaba Kolachi 3. Ghazala Usman

1. Asstt. Prof. of Anatomy, SMC, SMU, Karachi 2. Assoc. Prof. of Anatomy, DMC, DUHS, Karachi
3. Asstt. Prof. of Community Medicine, SMC, SMU, Karachi

ABSTRACT

Objective: To observe the protective effects of effects of L-Arginine on the gastric mucosal cells when given simultaneously with ibuprofen in albino rats under light microscope.

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 from 01.04.2008 to 31.5.2008.

Materials and Methods: This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi where 45 albino rats of either sex between 90-120 days were selected and were divided into three groups, 'A', 'B' and 'C', containing 15 animals each and were further sub-divided into three sub-groups containing 5 animals, according to time of sacrifice, i.e. 4, 6, and 8 weeks respectively. Group 'A' served as control. Group 'B' received ibuprofen, 70 mg/kg body weight and group 'C' received ibuprofen, 70mg and L-Arginine 300 mg, per kilogram body weight per day with feed. Animals were sacrificed at their respective time. The abdomen was opened, stomach was removed and opened along the greater curvature, divided into cardiac, body and pyloric parts, which in turn were fixed in Buffered neutral formalin for 24 hours. 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, Periodic Acid Schiff Orange-G and Alcian blue-Periodic Acid Schiff.

Results: Group A: No abnormality was noted in gastric mucosal cell. **Group B:** Mean number of surface mucosal cells were decreased and results were moderately significant ($P<0.001$). Mean height of surface mucous cell was increased and results were highly significant ($P< 0.0001$) in B1, moderately significant ($P<0.001$) in B2, and significant ($P< 0.05$) in B3 subgroups. The mean values of mucus neck cells count was insignificant ($P >0.05$) when compared to control group. The mean values of chief cell count were decreased results were highly significant ($P< 0.0001$) in B1 and B3 and significant ($P< 0.05$) in B2, The mean values of the parietal cells in the body were increased all these results were moderately significant ($P<0.001$) to highly significant ($P<0.0001$), when compared to control. **Group C:** No difference in results was noted when compared to group A.

Conclusion: L-Arginine protects gastric mucosal cells from damage when given with ibuprofen.

Key Words: Ibuprofen, L-Arginine, Haematoxylin and eosin.

INTRODUCTION

The gastric mucosa is constantly exposed to various stimulants, including acid, pepsin, alcohol, Helicobacter pylori, or drugs. Among the stimulants, nonsteroidal anti-inflammatory drugs, in particular, are well recognized for being responsible for causing upper gastrointestinal complications, ranging from dyspeptic symptoms to life-threatening complicated ulcers (Uno K, 2004)[1]. Acute gastritis and peptic ulceration are caused by the heavy use of the non-steroidal anti-inflammatory drugs, particularly aspirins (Bagshaw et al., 1987; Kumar et al, 2003)[2,3]. The principal therapeutic effects of nonsteroidal anti-inflammatory drugs are derived from their ability to inhibit prostaglandins production (Underwood, 2004)[4].

Ibuprofen is a nonsteroidal analgesic, antipyretic and anti-inflammatory agent that is a propionic acid derivative used for relief of pain, reduction of fever and in the treatment of osteoarthritis and rheumatoid arthritis. It acts by inhibiting the enzyme cyclooxygenase (COX) which catalyzes the conversion of the arachidonic acid to prostaglandins (Yip-Schneider et al., 2001; Takeeda et al., 2004)[5,6]. 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)[7,8]. The recommended dose of Ibuprofen is 600mg qid. It is equivalent to 4 grams of aspirin in anti-inflammatory effects (Katzung, 2004)[9]. Gastrointestinal adverse drugs reactions from ibuprofen usage include mucosal ulcers and bleeding (Abraham et al., 2005)[10]. There may be single or multiple ulcerations accompanied by gradual blood loss leading to anemia or by life threatening hemorrhage (Maricic et al., 1999)[11].

To protect the gastric mucosa, a complex defense system, which includes the production of surface mucus and bicarbonate and the regulation of gastric mucosal blood flow (GMBF), has evolved. Prostaglandins (PGs), in particular PGF2, enhance these protective mechanisms and are therefore believed to comprise a major gastric mucosal defensive factor (Tanaka et al., 2006)[12]. Endogenous prostaglandins play a protective role on endotoxin-induced gastric mucosal micro circulatory disturbance and mucosal damage (Pique et al., 1998)[13].Arginine is a semi-essential or conditionally essential amino acid in humans, is one of the most metabolically versatile amino acid and serves as a precursor for the synthesis of urea, nitric oxide, polyamines, proline, glutamate, and creatinine (Wu and Maoris, 1998)[14]. As the precursor of nitric oxide (NO) synthesis in vivo, L-arginine has been demonstrated in extensive studies to play various roles under different physiological and pathological conditions where the nitric oxide takes different effects (Yin et al., 2005)[15].

Nitric oxide synthesized from L-arginine plays an important role in the gastric mucosal integrity by interacting with endogenous prostaglandins (Takeuchi et al., 1993)[16]. Oral L-arginine was shown to have the effects to ameliorate ischemia reperfusion injury of the intestine and to protect the barrier function of the intestinal mucosa. This might be related to an increase in the nitric oxide level in intestinal mucosa resulting in maintenance of a stable Endothelin/nitric oxide ratio (Chen et al., 2005; Hung, 2006)[17]. Keeping in mind of the effects of L-Arginine, this study was designed to search for its role in ibuprofen induced gastric mucosal cell damage.

MATERIALS AND METHODS

This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi where 45 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 C, 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) and L-Arginine as "Arginine", General Nutrition Corporation, Pittsburg, USA. The dose of the L-Arginine was 300 mg per kilogram body weight per day with feed (Takeuchi et al., 1993).

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. 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.

RESULTS

Group-A: The animals were healthy and active. On gross examination the external surface of the stomach was shinny and glistening with no dilated blood vessels. All stained sections showed normal histological architecture.

The mean values of number of surface mucous cell count are shown in table 1

The mean values of height of the surface mucous cell in subgroups A1, A2 and A3 of cardiac part were 8.69±3.38µm, 8.75±2.45µm, and 8.76±3.07µm, of body of stomach were 9.36±0.12, 9.20±1.56 and 9.17±2.08, and pyloric part were 9.33±2.559, 28±4.86, and 9.30±8.00 respectively.

The mean values of mucus neck cells count of the gastric glands in the body of stomach in all subgroups A1, A2 and A3 were 28.20±1.39, 32.20±1.30 and 30.20±1.39 respectively.

The mean values of chief cell count in the body of the stomach in all sub groups A1, A2 and A3 were 82.00±0.70, 80.60±0.87, and 81.00±0.48 respectively, (Graph-1).

The mean values of the parietal cells in the body of stomach in all groups A1, A2 and A3 were 57.00±0.54, 56.00±0.44 and 57.80±0.48 respectively (Graph- 2).

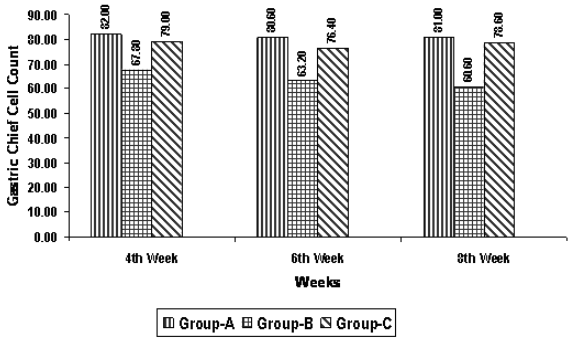
Group-B: The animals were weak, sluggish in activities and on the gross observation of the external surface of stomach were dull, slightly red and blood vessels were dilated in all subgroups. In combined

Alcian blue-PAS stained sections the surface mucus cells were less in number and elongated in appearance and enucleated and nuclei were visualized in the lumen. In the PAS orange-stained sections, the mucous neck cells were distorted in shape and the parietal cells were small in size. The chief cells were decreased in number, their shape was also distorted, and the apical part of the cells was vacuolated.

Table No.1: Mean* value of Mucus Cell Count in the Stomach in Different Groups of Albino Rats

Groups	Sub-groups	Treatment Given	Mucus cell count in the cardiac end of the 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
C	C1	Ibuprofen + L-Arginine	54.60±1.63		
	C2			54.60±0.50	
	C3				54.40±1.02
Groups	Sub-groups	Treatment Given	Mucus cell count in the body 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
C	C1	Ibuprofen + L-Arginine	54.40±1.029		
	C2			52.20±1.157	
	C3				52.40±1.749
Groups	Sub-groups	Treatment Given	Mucus cell count in the pyloric part of the 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
C	C1	Ibuprofen + L-Arginine	60.20±0.80		
	C2			60.40±0.74	
	C3				61.80±0.48

*Mean±SEM

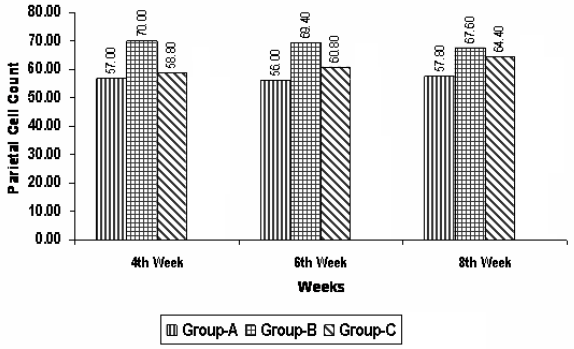


Graph No. 1: Gastric Chief Cell Count in Different Groups of Albino Rats

The mean values of surface mucous cell count are shown in table 1. Count was decreased in all subgroups. In cardiac part results are moderately significant (P<0.001) in subgroups B1 and B3, while highly significant in B2 (P< 0.0001) when compared to control group. In body of stomach in B1, results are moderately significant (P<0.001), while in B2 and B3 the results are significant (P<0.05) when compared to control groups. In the pyloric part the results are moderately significant (P<0.001) in B1 and B3 while significant (P<0.05) in B2 when compared to control.

The mean values of the height of surface mucous cell were increased in all subgroups. In cardiac part Mean values of the height of surface mucus cells were 9.08±2.80µm, 9.05±1.66µm and 9.03±6.66µm respectively. The results are moderately significant (P<0.001) in B1, highly significant (P<0.0001) in B2 and significant in subgroup B3 (P<0.05). Mean values of the height of surface mucus cells in the body of stomach in all sub groups B1, B2 and B3 were 9.56±8.86, 9.38±5.38 and 9.47±4.94 respectively. The results in B1 are insignificant (P>0.05), while in

subgroup B2 result is significant (P<0.05) and in subgroup B3 the results are moderately significant (P<0.001). The mean values of height of surface mucous cell in subgroups B1, B2 and B3 of pyloric part were 9.73±4.29µm, 9.85±1.56µm, and 9.54±1.42µm respectively. The result are highly significant (P<0.0001) in all sub groups when compared to control.



Graph No. 2: Parietal Cell Count in Different Groups of Albino Rats

The mean values of mucus neck cells count of the gastric glands in the body of stomach in all subgroups B1, B2 and B3 were 25.00±0.83, 25.60±0.73 and 22.20±0.73 respectively. The mucus cell count was decreased in all subgroups but insignificant (P >0.05) when compared to control group.

The mean values of chief cell count in the body of the stomach in all subgroups B1, B2 and B3 were 67.80±0.66, 63.20±1.98, and 60.60±1.77 respectively. The chief cell count in subgroup B1 and B3 was decreased and the results are highly significant (P<0.0001) and in B2 it is significant (P<0.05) when compared with control group (Graph 1).

The mean values of the parietal cells in the body of stomach in all groups B1, B2 and B3 were 70.00±0.54, 69.40±0.24, and 67.60±1.81 respectively. The number of parietal cells count was increased in subgroup B1 and it is moderately significant (P<0.001) whereas the increase in subgroups B2 and B3 is highly significant (P<0.0001) (Graph-2).

Group- C: The animals were healthy and active and on gross examination of stomach, the surface appeared smooth, shiny and few blood vessels were observed.

The mean value of the surface mucus cell count in sub groups of cardiac part are shown in table 1. The results are significant (P<0.05) when compared with group B. Insignificant (P>0.05) results are obtained when compared to control group. The mean values of the surface mucus cell count of the body of stomach are shown in table 1. The results are significant (P<0.05) when compared with group B and insignificant (P>0.05) when compared with control group. The mean values of the surface mucus cell count in all subgroups of the pyloric part are shown in the table 1. The results in C1 and C2 are significant (P<0.05) and in case of subgroup C3 are highly significant (P<0.0001) when compared with the subgroups B1, B2 and B3. The results are insignificant (P>0.05) in cases of subgroup C1 and C3 and are moderately significant (P<0.001) in case of C2 when compared when compared to control.

The mean values of height of the surface mucus cells in subgroups C1, C2 and C3 of the cardiac part were 8.86±4.55, 8.76±4.30 and 8.76±3.07 respectively. The height of surface mucus cells count was increased in all subgroups. The result are moderately significant (P<0.001) in C1 and in subgroups C2 and C3 the results are significant (P<0.05) when compared with group B. The result are insignificant (P>0.05) when compared with the control group. The mean value of the height of the surface mucus cell in the body of the stomach in sub groups C1, C2 and C3 were 9.16±1.69, 9.16±1.32 and 9.12±1.44 respectively. The results are significant (P<0.05) in group all subgroups when compared with subgroups B1, B2 and B3, insignificant (P>0.05) when compared with subgroups A1, A2 and A3.

The mean values of height of the surface mucus cells in sub-groups C1, C2 and C3 of the pyloric part were 9.27±2.04, 9.28±1.56 and 9.26±4.00 respectively. The results are moderately significant (P<0.0001) when compared to subgroups B1, B2 and B3 and insignificant (P>0.05) in all subgroups of control.

The mean value of number of mucus neck cells of the body of stomach in all subgroups were 26.00±0.31, 26.00±0.44 and 24.60±0.48 respectively. The cells were decreased. Insignificant (P>0.05) results are obtained in subgroup C1and C2 and significant (P<0.05) results are obtained in subgroup C3 when compared with the subgroups B1, B2 and B3. The number of mucus neck cells are decreased insignificantly (P>0.05) in subgroup C1 and significantly (P<0.05) in case of C2, C3 when compared with control.

The mean values of chief cell count in the body of the stomach in all subgroups B1, B2 and B3 were 67.80±0.66, 63.20±1.98, and 60.60±1.77 respectively. (Graph-1). The chief cell count in subgroup B1 and B3 was decreased and it was highly significant (P<0.0001), and was significant (P<0.05) when compared with control group.

The mean values of the parietal cells in the body of stomach in all groups B1, B2 and B3 were 70.00±0.54, 69.40±0.24, and 67.60±1.81 respectively. (Graph-2). The number of parietal cells count was increased in subgroup B1 and it was moderately significant where as the increase in subgroups B2 and B3 were increased and were highly significant.

DISCUSSION

Ibuprofen is a commonly used non-steroidal anti inflammatory drug that produces gastric mucosal injury and inhibition of nitric oxide synthase. (Abraham et al., 2005)[10]. Nitric oxide is produced from the amino acid L-arginine via the catalytic action of nitric oxide

synthase (Garaliene, 2006)[18]. Because of this fact our study was designed to observe the effects of L-arginine on gastric mucosal cell of albino rats when given with ibuprofen.

The present study showed that the surface mucous cell count was significantly (P< 0.05) decreased in group-B due to progressive deterioration of gastric mucosa with time. Bagshaw (1987)[2], in an experimental study of aspirin-induced chronic gastric ulceration in the rat showed that many of the heavily PAS/AB stained surface cells were lost. There was, however, no damage to the deeper layers of the mucosa. According to Takeeda et al (2004)[6] iodoacetamide treatment alone produced apparent damage in the stomach, mostly in the surface epithelial cells.

In the group-C, the surface epithelial cells were significantly (P< 0.05) increased. It might be due to the increased in blood circulation and mucosal barrier; the findings are confirmed with Kumar et al (2004). Takeeda et al (2004)[6], in their study concluded that endogenous prostaglandins derived from both COX-1 and COX-2 are involved in the mucosal defense of the inflamed stomach, partly by decreasing acid secretion and contribute to maintaining the mucosal integrity under such conditions.

The height of the surface mucus cells was significantly (P< 0.05) increased in group-B animals when compared with groups-C and A. The increase in height might be due to accumulation mucus granules within the cells. The number of mucus neck cells in the body of the stomach in group-B animals were insignificantly (P> 0.05) decreased in those sites where erosions/ulcers were not present when compared with their corresponding controls. In group-C the numbers of mucus neck cells were insignificantly (P> 0.05) increased when compared to corresponding animals of group-B and group-A. The findings of the present study might be due to the infiltration of the inflammatory cells in mucosa, which is confirmed from the statement of (Kumar et al., 2004)[3].

The present study showed that the chief cells of the gastric glands were significantly (P< 0.05) decreased in group-B, the severity of damage confirmed with the findings of Maricic et al (1999)[11], in which the mucosal injury extending beyond the surface epithelium into the region of pits and glands treated with indomethacin in rats. In group-C the results are similar to control. The L-arginine increases the prostaglandin secretions to improve the mucosal blood flow (Kumar et al., 2004)[3].

The present study showed the significant increase in the number of parietal cells in group-B, the initial mucosal injury of gastric epithelium like the intestinal epithelium have the same capacity for hyperplastic growth. The ibuprofen blocks the prostaglandin synthesis reduces cytoprotection and promotes gastric mucosal injury and increases the acid secretions of parietal cells. Because the prostaglandins favour production of mucus and bicarbonate they inhibit acid secretions by parietal cells (Kumar et al., 2004)[3]. In group-C the parietal cells count was significantly (P< 0.05) decreased when compared to the animals of group-B, this decrease in the number of the parietal cells is due to increased prostaglandins secretions which maintain the integrity of the gastric mucosa (Kumar et al., 2004)[3].

CONCLUSION

Based on present study, it is concluded that L-Arginine has protective effects on gastric mucosal cells of albino rats when given with Ibuprofen.

REFERENCES

1. Uno K, Iuchi Y, Fujii J, Sugata H, Iijima K et a. In vivo study on cross talk between inducible Nitric-Oxide synthase and cyclooxygenase in rat gastric mucosa: Effect of cyclooxygenase activity on Nitric Oxide Production. JPET 2004;309: 995-1002.
2. 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
3. Kumar V, Cotran RS, Robbins SL. Stomach. Robbins Basic Pathology. 7th ed. Philadelphia: WB Saunders; 2003.p.555-63.
4. Underwood JCE. Alimentary system. General and systematic pathology. 4th ed. Edinburgh: Churchill Livingstone; 2004.p. 369-72.
5. Yip-Schneider MT, Sweeney CJ, Jung SH, Crowell PL, Marshall MS. Cell cycle effects of non-sterodial anti-Inflammatory drugs and enhanced growth inhibition in combination with gemcitabine in pancreatic carcinoma cells. JPET 2001;298: 976-985.
6. Takeeda M, Hayashi Y, Yamato M, Murakami M, Takeuchi K. Roles of endogenous prostaglandins and cyclooxygenase isozymes in mucosal defense of inflamed rat stomach. J Physiol Pharmacol 2004; 1: 193-205.
7. Kato S, Ogawa Y, Kanatsu K, Okayama M, Watanabe T, Arakawa T, Takeuchi K. Ulcerogenic influence of selective cyclooxygenase-2 inhibitors in the rat stomach with adjuvant-induced arthritis. JPET 2002; 300: 503-509.
8. 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.
9. 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.

10. Abraham P and 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.
11. 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.
12. Tanaka A, Hase S, Miyazawa T, Takeuchi K. Up-regulation of Cyclooxygenase-2 by inhibition of cyclooxygenase-1: A key to NSAID-induced intestinal damage. JPET 2002; 300(3): 754-761.
13. Pique JM, Yonei Y, Whittle BJ, Leung FW, Guth PH. Indomethacin potentiates endotoxin-induced blood flow reduction and histological injury in rat gastric mucosa. Br J Pharmacol 1998; 93: 925-31.
14. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. J Biochem 1998;336:1-17.
15. Yin YR, Bai L, Wang F. Mechanism of L-arginine-induced sebaceous gland hyperplasia in rats. J First Mil Med Univ 2005; 25: 766-68.
16. 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.
17. Chen B, Fu JF, Yuan WH, Xu Y, Luo ZH, Wang H, Chen ZH, Li CP. Study on the mechanism of protective effect of oral L-arginine on intestine after scald injury in rats. Zhongua Shao Shang Za Zhi 2005; 21: 259-61.
18. Garaliene V. The main determinants of endothelial dysfunction. Medicina 2006; 42(5): 362-9.

Address for Corresponding Author:
Dr. Santosh Kumar
Assistant Professor
Department of Anatomy
Sindh Medical College, SMU Karachi.
E-mail: santosh.kumar@duhs.edu.pk
Cell: 03332317920