Original Article

Anatomy

A study of Gross Anatomical Observations of Stomach and Changes in Body Weight of Albino Rats after Simultaneous Administration of Ibuprofen and L-Arginine

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ABSTRACT

Objective: To observe the gross anatomical changes of the stomach and the changes in the body weight in albino rats after oral administration of ibuprofen and L-Arginine

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 31.7.2008

Materials and Methods: For this study 45 albino rats of either sex between 90-120 days were taken and divided into three Groups, 'A', 'B' and 'C', containing 15 animals each, which were further sub-divided into three sub-groups containing 5 animals each according to the time of sacrifice, i.e. 4, 6, and 8 weeks respectively. Group 'A' served as control. Group 'B' received ibuprofen at the dose of 70 mg per kilogram body weight per day with feed and Group 'C' received ibuprofen at the dose of 70 mg per kilogram body weight per day with feed and L-Arginine300 mg per kilogram body weight per day with feed. Animals were weighed on Sartorius balance before and after their stipulated time period and then were fixed on a dissecting board, the abdomen was opened with a long midline incision and the stomach was removed and observed for gross anatomical changes. Stomach wasthen opened along the greater curvature with an incision extending from cardiac to the pyloric end.After removing the contents of the stomach the gastric mucosa was examined by dissecting microscope.

Results: The animals of Group 'A' were healthy and active. On gross examination no abnormality was detected. The animals of Group 'B' were weak, sluggish in activities.Their food intake was decreased when compared to control. The gross observation of the external surface of stomach was dull, slightly red and blood vessels were dilated in all subgroups. Under the dissecting microscope the mucosa was red, swollen and erosions were observed in all subgroups. There was a decrease in the final body weight of animals. In subgroup B1 it was moderately significant ($P<0.01$) and in case of B2 and B3 this decrease was significant ($P<0.05$) when compared to control. The animals in group C were looking healthy and active at the time of sacrifice.On gross examination of stomach, the surface appeared smooth, shiny and few blood vessels were observed. The mucosal surface was greyish in the cardiac part and pink in the body and pyloric part. Under the dissecting microscope, few blood vessels were observed.

Conclusion: Present study concludes that the long term use of ibuprofen can cause gastric mucosal changes and decrease in body weight in albino rats. L-Arginine supplementation can ameliorate the changes.

Key Words: Albino rats, Ibuprofen, L-Arginine..

INTRODUCTION

Acute gastritis and peptic ulceration are caused by the heavy use of the non-steroidal anti-inflammatory drugs (Bagshaw et al., 1987; Kumar et al, 2003)[1,2]. The non-steroidal anti-inflammatory drugs (NSAIDs) are the major cause of peptic ulcers in patients who do not have helicobacter pylori infection. The principal therapeutic effects of NSAIDs derive from their ability to inhibit prostaglandins production (Underwood, 2004)[3]. Mucus production is stimulated by prostaglandins, which also directly inhibit the gastric acid secretion by parietal cells (Gilman et al., 2006)[4]. Gastric ulcer disease remains widespread; a stressful lifestyle and non-steroidal anti-inflammatory drug (NSAID) make significant contributions to this pathological situation (Filaretova et al., 2007)[5]. In the USA, about 16,500 people per year die as a result of NSAID-associated gastrointestinal complications (Mizushima, 2008)[6].

Ibuprofen, the most commonly used NSAID in the United States, was the first member of the propionic acid class of NSAID to come into general use (Gilman et al., 2006)[4]. Ibuprofen produces gastric mucosal injury (Abraham et al., 2005)[7]. Ibuprofen at doses of 200 mg and 400 mg is an efficacious, cost-effective, well-tolerated, for pain of migraine headache (Codispoti et al, 2001)[8]. The recommended dose of Ibuprofen is 600mg qid. It is equivalent to 4 grams of aspirin in anti-inflammatory effects (Katzung, 2004)[9]. 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, 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)[10].

Oral L-Arginine has 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)[11,12]. Nitric oxide synthesized from L-Arginine plays an important role in the gastric mucosal integrity by interacting with endogenous prostaglandins (Takeuchi et al., 1993)[13]. Endogenous prostaglandins play a protective role on endotoxin-induced gastric mucosal micro circulatory disturbance and mucosal damage (Pique et al., 1998)[14].

Keeping in mind the effects regarding ibuprofen on the gastric mucosa we planned this study to observe the role of L-Arginine with regards to gross anatomical changes and body weight in albino rats.

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 weighed before the start of study and were divided into three Groups, A, B and C, containing 15 animals each. Animals 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)[15] and group C received ibuprofen at the dose of 70 mg per kilogram body weight per day orally with feed 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)[16].

The animals were sacrificed at the end of their respective period of treatment under the ether anaesthesia. Animal were weighed and then 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, hemorrhagic areas and the number of erosions/ulcers. After recording the readings the statistical analysis was done. The difference of various 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 of Group-A were healthy and active. On gross examination the external surface of the stomach was shinny and glistening with no dilated blood vessels,(Figure-1).The stomach in most of the animals contained the pale colored fluid, without food contents. Under the dissecting microscope the internal surface of each stomach was clearly identified into two parts by a ridge. The grayish white squamous part was continuous with esophagus, and the pink glandular part raised in folds was continuous with the duodenum. The body weights of animals are shown in table-1, and graph-1). There was a significant increase ($P<0.05$) in the weight of all subgroups when initial weight was compared with final weight.

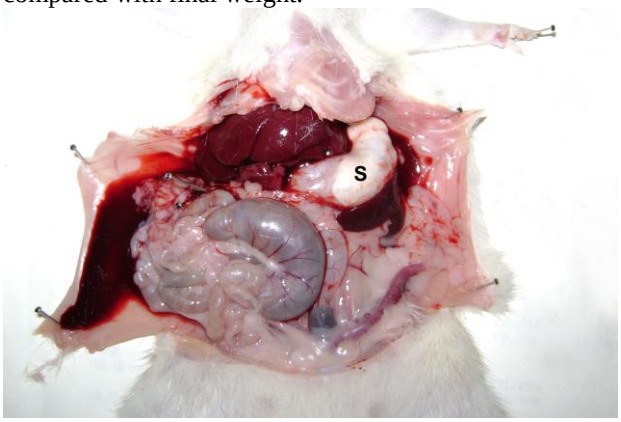


Figure No.1: Photograph of gross appearance of stomach of control albino rats, showing the normal appearance of stomach within body cavity (S) stomach.

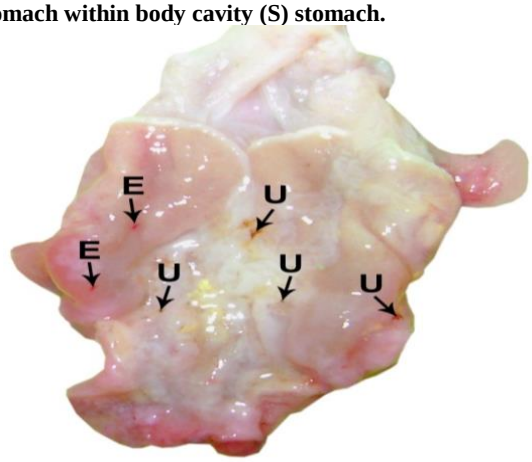
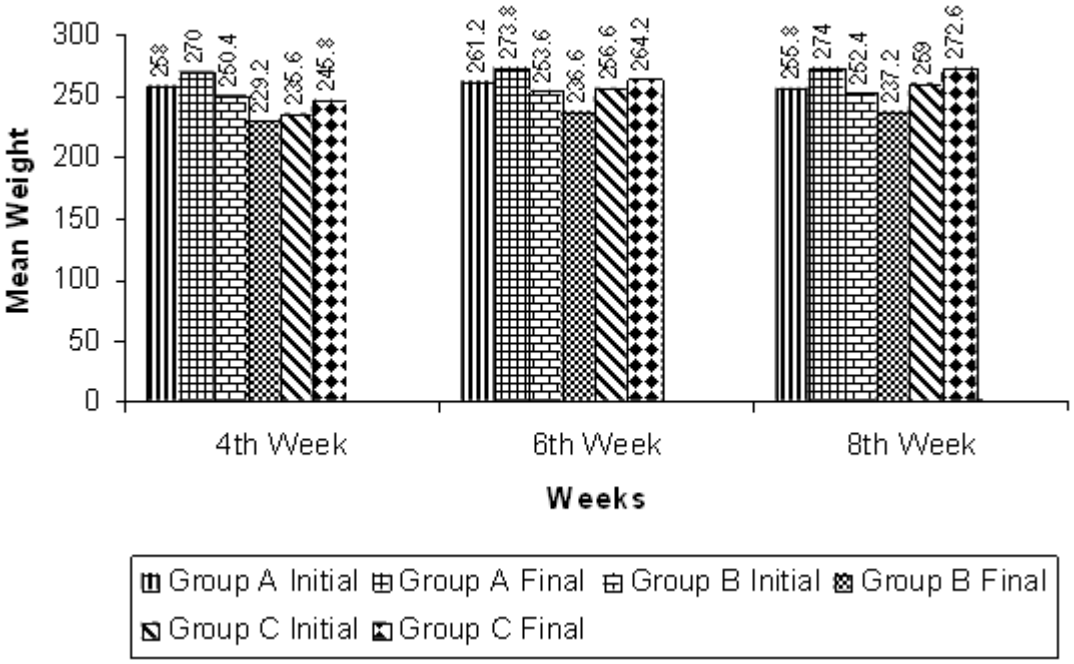


Figure No.2: Photograph of gross appearance of stomach of albino rats, showing gross mucosal changes in ibuprofen treated group (E- erosions and U- ulcers).

Table No.1: Mean* value of Body Weight (G) in Different Groups of Albino Rat

Groups	Sub-groups	Treatment Given	Initial Weights	Body Weights (G)		
				Final Weights at Sacrificial Time		
				4th Week	6th Week	8th Week
A (n=15)	A1	Control	258.00±11.38	270.00±11.66	--	--
	A2		261.20±7.24	--	273.80±8.33	--
	A3		255.80±4.60	--	--	274.00±4.50
B (n=15)	B1	Ibuprofen	250.40±4.20	229.20±3.54	--	--
	B2		253.60±2.13	--	236.60±2.13	--
	B3		252.40±3.35	--	--	237.20±3.45
C (n=15)	C1	Ibuprofen + L-Arginine	235.60±5.37	245.80±6.65	--	--
	C2		256.60±5.09	--	264.20±6.02	--
	C3		259.00±7.11	--	--	272.60±6.41

*Mean±SEM



Graph No.1: Body Weight (G) in Different Groups of Albino Rats

Group-B: The animals of Group-B were weak, sluggish in activities and their food intake was decreased compared to control.The gross observation of the external surface of stomach was dull, slightly red and blood vessels were dilated in all subgroups i.e. B1, B2 and B3 (Figure-2).Under the dissecting microscope the mucosa was red, swollen and erosions were observed in all subgroups i.e. B1, B2 and B3.

The body weights of animals are shown in table-1, and graph-1). The decrease in weight was noted which is moderately significant in subgroup B1 ($P<0.001$) and significant ($P<0.05$) in subgroups B2 and B3 when compared with each other. Amoderately significant ($P<0.001$) decrease was noted in subgroup B1 and significant ($P<0.05$) decrease was present in B2 and B3 when compared with control.

Group-C: The animals in Group-C treated with ibuprofen and L-Arginine were looking healthy and active during the time of treatment and at the time of sacrifice. On gross examination of stomach, the surface appeared smooth, shiny and few blood vessels were observed. The mucosal surface was greyish in the cardiac part and pink in the body and pyloric part under the dissecting microscope, few blood vessels were observed.

The body weights of animals are shown in table-1, and graph-1. There was increase in mean final body weight in all subgroups when compared with their initial body weight and this increase was moderately significant in Group-C1 and C3 ($P<0.001$) while it was significant in Group-C2 ($P<0.05$). There was increase in mean of final weight in all subgroups C1, C2 and C3, this increase was significant ($P<0.05$), when compared with subgroups B1, B2 and B3. There was a decrease in final body weight in all subgroups C1, C2 and C3 and this decrease was insignificant ($P>0.05$) when compared to subgroups A1, A2 and A3.

DISCUSSION

This study was planned to observe the effects of Ibuprofen and L-Arginine on the behavior, body weight of the animal and the gross changes on stomach.

Group-B animals appeared ill looking with loss of their body weight, because of the injurious effects of drug and due to loss of appetite (because of erosions/ulcers on the gastric mucosa) these findings are in agreement with Dudkiewicz (1981)[16]. He observed rat body weight in an experimental study on Ibuprofen-induced gastrointestinal changes in rats, and demonstrated that Ibuprofen caused disturbances in intestinal motor functions which might lead to the development of malabsorption syndrome. The findings of the present study are in disagreement in response to appetite but in agreement in response to body weight with the study of Esther et al (1997)[17] who observed the effects of non-steroidal drugs on glutathione S transferase of the male Wistar rat digestive tract. They noted that daily food consumption, intake of NSAIDs and gain in body weight. The animal's food consumption was increased and body weight was decreased.

The animals of Group-C appeared normal active and healthy, it appeared that their activity is more or less same as compared to Group-A. These animals put on weight, which could be explained due to increase in the appetite caused by L-Arginine and reduction to minimum of damage to stomach. The gross observations on the external surface of the stomach in Group-B showed dilatation of blood vessels but the external surface of group-C was normal in appearance as in group-A.

To observe changes in the gastric mucosa and visualize the site of lesion of the stomach the dissecting microscope was used. In Group-B animals, the numbers of the lesions were increased significantly from 1st to 8th week. These findings of ulcers over gastric mucosal surface are in agreement with the findings of Tanaka (2002)[18] who used NSAIDs (nonselective COX inhibitor), Kato (2002)[19] used indomethacin and rofecixib; and Jimenez et al (2004)[20] used Ibuprofen in their experimental studies on rats and measured the size of lesion under dissecting microscope. In Groups-C no erosions/ulcers were found. Bagshaw et al (1987)[1] studied the aspirin-induced chronic gastric ulcer in rat.

The lesion formation was time related process. Sequential observations of the changes between 4 and 8 weeks post-treatment with Ibuprofen showed that desquamation of the surface epithelial occurs after 4 weeks, while more extensive disruption and exfoliation of the surface epithelium and ulceration appeared in 8th week.

Takeuchi et al (1992)[13] reported that HCl-induced gastric injury in rats by in the surface epithelial cells and disruption of epithelial membrane. Kumar et al (2003)[2] reported that NSAIDs-induced gastric mucosal defects varied and extend from the superficial mucosal lesion down to the entire thickness of mucosa.In the light of above consideration, the net results of the study suggest that the gastric ulcer occur more frequently in people who use Ibuprofen as reported by Jimenez et al (2004)[20] and Kumar et al (2004)[2].

The body weight of all animals was changed; the animals of Group-A gained body weight between fourth and eighth weeks. The animals of Group-B lost weight than group A, this decrease in weight was due to loss of appetite as observed by Takeeda et al (2004)[21] who studied role of endogenous prostaglandin and cyclooxygenase isoenzyme in mucosal defense of inflamed rat stomach. They observed that body weight was gradually decreased depending on the duration of treatment.

L-Arginine used with ibuprofen treated animals were normal in behavior and normal in food intake and on tissue examination architecture of gastric mucosa was normal In the light of these findings it may be stated that the ibuprofen can cause cellular necrosis and gastric ulceration in a dose of 70mg per kilogram body weight, but L-Arginine protects the gastric ulceration.

CONCLUSION

Present study concludes that the long term use of ibuprofen can cause gastric mucosal changes and decrease in body weight in albino rats but L-Arginine supplementation can ameliorate the changes in gastric mucosa and have positive effects on weight.

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