Original Article

Comparison of Body Weight and the Weight of Stomach after Simultaneous Administration of Ibuprofen and L-Arginine Albino Rats

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ABSTRACT

Objective: To observe the effects of ibuprofen and L-Arginine when given simultaneously on the body weight and absolute weight of stomach of albino rats and statistical analysis of the results.

Study Design: A prospective experimental study.

Place and Duration of study: This study was conducted at Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi during 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, which 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 at the dose of 70 mg per kilogram body weight per day with feed and L-Arginine 300 mg per kilogram body weight per day with feed. Animals were weighed on sartorius balance before and after their stipulated time period. Animals were fixed on a dissecting board, the abdomen was opened with a long midline incision and the stomach was removed and opened along the greater curvature with an incision extending from cardiac to the pyloric end. After removing the contents of the stomach the absolute weight of the stomach was recorded on a Sartorius balance.

Results: Observations on body weight (G) in Group-'A' There was a significant increase ($P<0.05$) in the weight of all subgroups. In Group-'B' their was moderate decrease in weight subgroup 'B'1 and significant decrease in subgroups 'B'2 B''3 when compared to control. No difference was observed in Group C when compared with control. Observations of absolute weight of stomach (G) the weight was increased significantly ($P<0.05$) when compared with control. No difference in weight was noted between Group 'C' and control group.

Conclusion: The effects of ibuprofen on the body weight and absolute weight of the stomach could be minimized when given simultaneously with L-Arginine**.**

Key Words: L-Arginine, ibuprofen, albino rats.

INTRODUCTION

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)[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 magnitude of gastro-duodenal toxicity caused by these agents is quite large; risk factors for these drugs are increasing age, higher doses and prolonged use. In the USA, about 16,500 people per year die as a result of NSAID-associated gastrointestinal complications (Mizushima, 2008)[3]. Drug-induced injury of the gastrointestinal tract is increasingly common but generally under-recognized. Acute gastritis and peptic ulceration are also caused by the heavy use of the non-steroidal anti-inflammatory drugs, particularly aspirins (Bagshaw et al., 1987; Kumar et al, 2003)[4-5].The principal therapeutic effects of NSAIDs derive from their ability to inhibit prostaglandins production (Underwood, 2004)[6].

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)[7]. Gastrointestinal adverse drugs reactions from ibuprofen usage include mucosal ulcers and bleeding (Abraham et al., 2005)[8]. According to Gilman et al (2006)[9] the most common symptoms associated with Ibuprofen are anorexia, nausea, dyspepsia, abdominal pain, and diarrhea. These symptoms may be related to the induction of gastric or intestinal ulcers, which is estimated to occur in 15% to 30% of regular users. 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].

L-arginine is an essential amino acid in children, which participates in many important biochemical reactions associated to the normal physiology of the organism (Jimenez et al., 2004)[11]. It is an essential amino acid for the infants and the children and it is semi essential in the adults. Although the arginine is made by the liver as a step in the synthesis of the urea, children cannot produce arginine rapidly enough to support growth requirements (Nakaki, 1994). 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]. Arginine supplementation did not affect plasma glucose levels in non diabetic rats. Thus, dietary L-arginine supplementation stimulates endothelial Nitric oxide synthesis by increasing BH provision, which is beneficial for vascular function and glucose, homeostasis in diabetic subjects (Kohli et al., 2004)[15]. Adding L-arginine to diet of the mice intoxicated with mercury, restoration to normal homeostatic conditions were achieved (Santarelli et al., 2007)[16]. L-arginine augments myotube formation and increased nitric oxide production in a process limited by cellular L-arginine uptake (Long et al., 2006)[17]. Keeping in mind the effects of ibuprofen and L-Arginine on the gastrointestinal tract, we decided to study simultaneous use of above drugs and find their effects on the body weight and the absolute weight of stomach.

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 available in the market 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). At the end of respective time period the animals were weighed on Sartorius balance and then anaesthetized with ether in a glass container and sacrificed. Animals were fixed on a dissecting board, the abdomen was opened with a long midline incision and the stomach was removed and opened along the greater curvature with an incision extending from cardiac to the pyloric end. After removing the contents of the stomach the absolute weight of the stomach was recorded on a Sartorius balance. 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 16.

RESULTS

Observations on Body Weight (G)

Group-A: The body weight of animals in all subgroups was increased during their respective period of time. The mean initial weights in 'A'1, 258.00±11.38; 'A'2, 261.20±7.24 and 'A' 3, 255.80±4.60 were observed. The mean final weight of subgroups 'A'1, 'A' 2 and 'A' 3 were 270±11.66, 273.80±8.33 and 274.00±4.50 respectively. There was a significant increase ($P<0.05$) in the weight of all subgroups (table-1 and graph-1).

Group-B: The mean initial body weight in 'B'1, 'B'2 and 'B'3 were observed as 250.40±4.20; 253.60±2.13, and 252.40±3.35 respectively while the mean of final weight observed in same subgroups were 229.20±3.54, 236.60±2.13, and 237.20±3.45 respectively, this decrease was moderately significant in subgroup 'B'1 ($P<0.001$) while it was significantly decreased ($P<0.05$) in subgroups 'B'2 and 'B'3. There was a decrease in the final body weight in subgroup 'B'1 was moderately significant when compared with 'A'1, while in subgroups 'B'2 and 'B'3 this decrease was significant ($P<0.05$) when compared with subgroups 'A'2 and 'A'3 (table-1 and graph-1).

Group-C: The initial body weight of animals in subgroups 'C'1, 'C'2 and 'C'3 recorded was 235.60±5.37, 256.60±5.09 and 259.00±7.11 respectively. While the final weight recorded in subgroups 'C'1, 'C'2 and 'C'3 were 245.80±6.65, 264.20±6.02 and 272.60±6.41. 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 'C'1 and 'C'3 ($P<0.001$) while it was significant in group 'C'2 ($P<0.05$). There was increase in mean of final weight in all subgroups 'C'1, 'C'2 and 'C'3, this increase was significant ($P<0.05$), when compared with subgroups 'B'1, 'B'2 and 'B'3. There was a decrease in final body weight in all subgroups 'C'1, 'C'2 and 'C'3 and this decrease was insignificant ($P>0.05$) when compared to subgroups 'A'1, 'A'2 and 'A'3 (table-1 and graph-1).

Observations of Absolute Weight 0f Stomach (G)

Group-A: The mean weight of stomach in subgroups 'A'1, 'A'2 and 'A'3 was 1.85±2.33; 1.90±9.24 and 1.85±2.18 respectively (table 2 and graph 2)

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

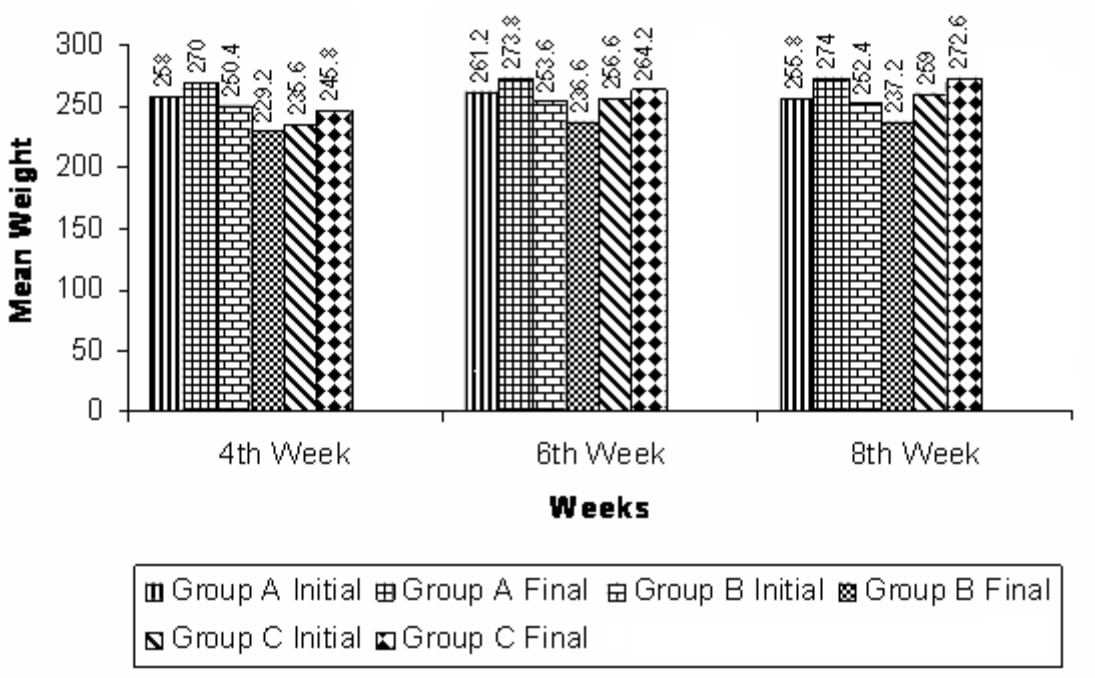
Groups	Sub-groups	Treatment Given	Initial Weights	Body Weights (G)		
				Final Weights at Sacrificial Time		
				4th Week	6th Week	8th Week
A (n=15)	A1	Control	258.00±11.38	270.00±11.66	--	--
	A2		261.20±7.24	--	273.80±8.33	--
	A3		255.80±4.60	--	--	274.00±4.50
B (n=15)	B1	Ibuprofen	250.40±4.20	229.20±3.54	--	--
	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

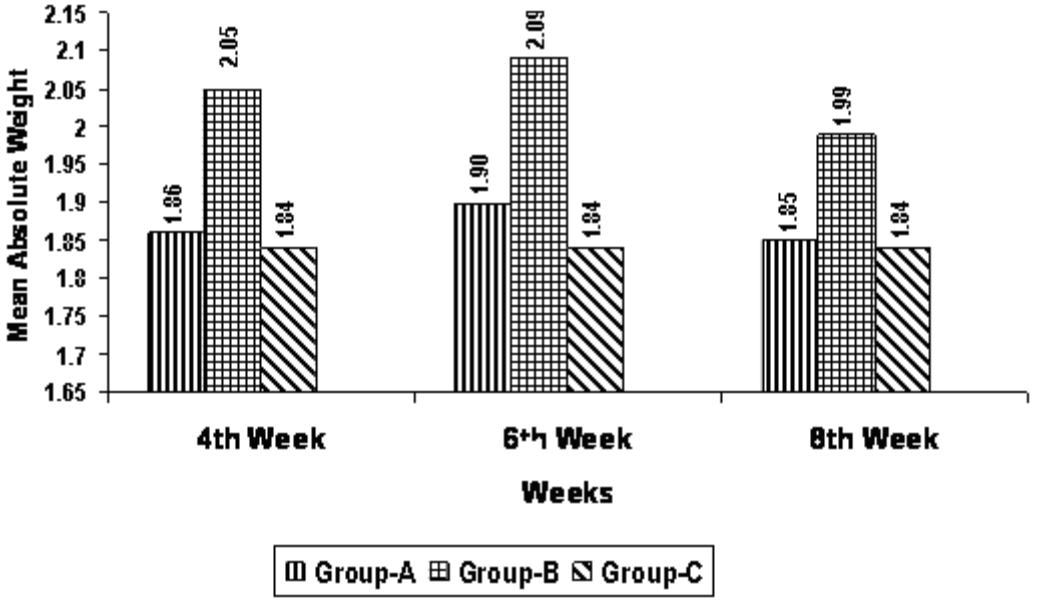
Table No. 2: Mean* value of Absolute Weight (G) of stomach in Different Groups of Albino Rats

Groups	Sub-groups	Treatment Given	Absolute weight (G) of stomach at the time of sacrifice		
			4th Week	6th Week	8th Week
A	A1	Control	1.858±2.33	--	--
	A2		--	1.90±9.24	--
	A3		--	--	1.85±2.18
B	B1	Ibuprofen	2.05±5.22	--	--
	B2		--	2.09±3.80	--
	B3		--	--	1.99±1.88
C	C1	Ibuprofen + L-Arginine	1.84±1.45	--	--
	C2		--	1.84±1.46	--
	C3		--	--	1.84±1.87

*Mean±SEM



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



Graph No. 2: Absolute Weight (G) of Stomach in Different Groups of Albino Rats

Group-B: The mean weight of the stomach in subgroups 'B'1, 'B'2 and 'B'3 was 2.05±5.22; 2.09±3.80 and 1.99±1.88 respectively. The weight was increased and significant (P<0.05) when compared with subgroups 'A'1, 'A'2 and 'A'3 respectively (table 2 and graph 2).

Group-C: The mean weight of the stomach in subgroups 'C'1, 'C'2 and 'C'3 observed was 1.84±1.45, 1.84±1.46, and 1.84±1.87 respectively. The mean weight of the stomach was decreased and significant (P<0.05) in subgroups 'C'1 and 'C'3 when compared with subgroups B1 and B3, while this decrease was moderately significant (P<0.05) in subgroup C2 when compared with B2. There was a decrease and insignificant (P>0.05) in absolute stomach weight in all subgroups 'C'1, 'C'2 and 'C'3 when compared with control subgroups 'A'1, 'A'2 and 'A'3 (table 2 and graph 2).

DISCUSSION

Ibuprofen is a commonly used non-steroidal anti-inflammatory drug that produces gastric mucosal injury and that inhibition of nitric oxide synthase, reduces gastric mucosal injury (Abraham et al., 2005). Nitric oxide synthesized from L-arginine plays an important role in the gastric mucosal integrity by interacting with endogenous prostaglandins (Takeuchi et al., 1993).

The animals treated with Ibuprofen in group-'B' appeared ill looking with loss in their body weight because of the injurious effects of Ibuprofen due to loss of appetite (because of erosions/ulcers on the gastric mucosa) and it is agreement with same Dudkiewicz (1981) who observed 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) who observed the effects of non-steroidal drugs on glutathione S transferase of the male Wistar rat digestive tract. They noted that the animals' 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; it is in agreement to Mahmoud et al (2002).

The absolute weight of the stomach in group-'B' animals showed an initial increase in subgroups-'B'1 and 'B'2, as compared to corresponding controls. The increase in weight may also be due to inflammatory infiltration in lamina propria and submucosa and dilatation of glands, these finding are same as of Kumar et al (2004), that in active ulcers with on-going necrosis beneath the superficial necrotic debris a zone of inflammatory infiltrate was present. The increase in weight might be due to edema and accumulation of the mucus content in surface epithelial cells and mucus neck cells as compared to control. In subgroup-'B'3 the absolute tissue weight was less than from groups-'B'1 and 'B'2, but slightly increased as compared to corresponding control.

In group-'C' animals the absolute weight of the stomach was significantly decreased when compared to

their corresponding group-'B' animals, while the weight was insignificantly decreased when compared with group-'A'.

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) and Kumar et al (2004). The body weights of all animals were changed, the animals of group 'A' gained body weight between four and eight weeks. The animals of group 'B' loss weight than group 'A', this decrease in weight was due to loss of appetite as observed by Takeeda et al (2004) who studied role of endogenous prostaglandin and cyclooxygenase isoenzyme in mucosal defence of inflamed rat stomach. They observed that body weight gradually decreased depending on the duration of treatment. In relation to body weight the absolute weight of the stomach of ibuprofen treated rats showed a significant increase in weight which may be attributed to mucosal edema.

CONCLUSION

The effects of ibuprofen on the body weight and absolute weight of the stomach could be minimized when given simultaneously with L-Arginine

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