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Original Research Article

Gastric mucosal damage with aspirin: results of experimental models in adult albino rats

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ABSTRACT

Background: Non-steroidal anti-inflammatory drugs are the over-the-counter available medicine and are notorious for their potential in causing peptic ulcer disease. Aspirin in used to treat cardiovascular disease and are commonly prescribed medicine.
Methods: 24 healthy albino rats of either sex were purchased and kept at controlled in controlled environment. These are mainly divided into the two groups named as group A and group B having 12 rats in each group. 25 gm of the powdered henna was mixed in 250 ml distilled water. Aspirin was used in the dose of 100 mg/kg drug solution is prepared every day before administration. Animals were sacrificed from each group on day 8th and 15th. Macroscopic ulcer was identified and Fibroblast, inflammatory cells, congested blood vessels were counted. SPSS version 21 was used to statistically analyse the data. P value of ≤0.05 was considered as statistically significant.
Results: aspirin group have more ulcer, congested blood vessels, inflammatory infiltrate and increased number of fibroblasts compared to control. The values were statistically significant with p value less than 0.05.
Conclusions: gastric ulcer is quite common and it occur because of the imbalance between protecting and aggravating factors. aspirin is a common offender of gastric ulcer because of its common use.

Keywords: Aspirin, Ulcer index, Fibroblasts, Peptic ulcer

INTRODUCTION

Acetyl salicylic acid commonly named as Aspirin, a non-steroidal anti-inflammatory agent, is one of the most widely used over the counter drug for minor ailments like headache etc.[1] It has molecular formula is $C_9H_8O_4$.

Non-steroidal anti-inflammatory drugs (NSAIDs) being simple in action and few associated adverse effects, used for wide range of mild to moderate clinical indications. It is also considered to be a safe drug for clinical manifestations that require frequent administration of analgesic agents e.g., arthritis. Aspirin being a weak acid has a greater potential for developing irritation to upper gastric tract, mainly in stomach on continued use. Due to its physico-chemical characteristics, aspirin may cause injury to mucosal lining and development of peptic ulcer disease and, if complicated, it can perforate and cause local haemorrhage. A study indicated that about one fourth of patients being treated on NSAIDs for a long time period may develop peptic ulcer disease while hardly 4% of these patients will show complications like bleeding or perforation.[2,3]

Efforts are continuously being made for the development of agents that can prevent or treat NSAID associated perforation or bleeding. Parallel administration of antacids and other agents that may help in gaining gastric lining can reduce the harm to the gastric mucosa but, on the same time, it reduces the absorption of drug.[1]

In case of breakage of mucus layer, epithelial cells are also rich in bicarbonates which neutralized any acid which get its way to cells. The tight arrangement of cells also blocks the backward flow of pepsin, a gastric enzyme.[1,4]

Experimental work indicated that the process of repairing an injury starts in 30 min to 1.5 hours in rats with maximum reconstitution of damaged mucosa in 15 min only. In contrast, human takes up to 90 min to reconstitute a damaged mucosa. Stomal fibroblast present in the gastric epithelium promotes differentiation and reconstitution of epithelial cells.[5]

At therapeutic dose of 0.3 to 1 gm QID, bioavailability of aspirin is about more than 80% under equilibrium concentration of substrate. In first half hour after administration of oral dose of aspirin, degree of degradation is found to be 38% in stomach mucosa, 64% in liver cells, 86% in lungs and 10% in circulating portions in blood.[6] Major site of metabolism is liver in which it decomposes into salicylic acid and acetic acid.[7]

Gastric toxicity of a drug is depended on the bioavailability in a certain medium i.e., gastric tract. Drug dissolution at a certain condition of pH dictates the amount of drug available for its action. Aspirin being weak acid dissolved rapidly in the stomach as its pH is highly acidic due to the presence of hydrochloric acid secreted by the parietal cells. For being this physico-chemical property, aspirin is much more gastro toxic than any other NSAID drug.[8]

NSAIDs is always considered as a main causative agents for development of gastric lesions and its subsequent progression upon continued use. Mechanism involved is the inhibition of COX (cyclooxygenase) by drug which results in the depletion of prostaglandin levels, along with cytokines i.e., TNF- α, (IL)-1β, and IL-8, ultimately leads to formation of gastric lesion. Along with these factors, activated neutrophils after injury, causes microcirculation disturbances and production of free radicals which further worsen symptoms of mucosal injury.[2,3,9,10]

So, the aim of study was to observe the ulcer inducing potential of aspirin in albino rats. This will help in understanding the effect on human.

METHODS

It was an experimental trial conducted in university of health sciences, Lahore between 1st November 2017 to 20th November 2017, in which healthy albino rats of either sex were purchased from the university of veterinary and animal sciences, Lahore. Health status, average weight, complete through examination of these experimental animals was done as soon received in the experimental research laboratory and kept at controlled room temperature (22±0.5˚C), humidity (50±10%) and light cycle of 12 hours. Rats are kept on normal fed and tap water for about a week.

Fresh leaves of plant *Lawsonia inermis* were harvested from the botanical garden, department of botany, government college university, Lahore. Aspirin was a gift sample received from Reckitt and Benckiser Pakistan Ltd. F-18. S.I.TE. Karachi.

Twenty-four adult albino Wister rats of either sex was allotted numbers i.e., 1, 2, 3 and so on. These are mainly divided into two groups named as group A and group B having 12 rats in each group through balloting and further sub-divided into two groups having six rats in it. Each sub group has different days of sacrifice (Table 1).

Aspirin was used in the dose of 100 mg/kg drug solution is prepared every day before administration. Weight of animal were taken just upon receiving, before the start of drug administration and at time of animal sacrifice i.e., 8th and 15th day.

Animals were sacrificed as per schedule, animals were randomly selected, anaesthetized and dissected. Macroscopic examination was done and also with help of magnifying glass to identify ulcer. Ulcer index was calculated by method given by Ganguly.[11] For microscopic examination tissues were fixed, processed in automatic tissue processor, sectioned by using rotator microtome. Tissues were stained with haematoxylin and eosin and examined under 40 and 100x magnification.

Table 1: Division of experimental animals, dosage schedule and duration of drug.

Groups	Sub-groups	Dosage	Treatment duration (days)	Day of sacrifice
A (control group)	A-1=6	4 ml distilled water orally	7	8
	A-2=6		14	15
B	B-1=6	Oral acetyl salicylic acid at dose of 100 mg/kg in 2 ml water	7	8
	B-2=6		14	15

Fibroblast, inflammatory cells, congested blood vessels were counted in mucosal and sub-mucosal layer. 4 fields of each section were examined through 1 mm^2 graticule eyepiece. Size measurement to these counted cells (micrometry) was made by ocular graticule 30 (Leica, DM 1000) using the method as described by Culling.[12]

SPSS version 21 was used to statistically analyse the data obtained from all three main groups and six sub groups. To determine the normality among the data, Shapiro–Wilk test was applied. For quantitative variables i.e., ulcer index, inflammatory cells, fibroblasts, and congested blood vessel, mean ± SD was estimated. Statistically significant difference among the intra-group variables was determined through one-way ANOVA. For multiple comparisons, post hoc Tukey test was applied. P value of ≤0.05 was considered as statistically significant.

RESULTS

Weight of the rats were determined at the beginning of experiment and on the day of sacrifice. Application of Shapiro-Wilk test clearly demonstrate that data is normally distributed as demonstrated by p value shown in parenthesis of Table 2. Statistically, it was analysed through one-way ANOVA and found that there is no significant difference between average weight of animals before start of experiment and on day of sacrifice as evident from p value on day 8 while there was significant difference in weight between groups on day 15th.

Inflammatory cells were counted in the four regions and mean was calculated with SD. The number of inflammatory cells was observed through microscope in all subgroups are statistically significant with p value of less than 0.001 shown in Figure 1.

To compare the inter-group variations, post hoc Tukey test was used. The result of this test (Table 3) revealed that the inflammatory cells were much greater in numbers in group B1 then A1. Similarly, inflammatory cells were much greater in numbers in group B2 then A2.

The number of fibroblasts and congested blood vessels were counted in per square millimeter area with the aid of light microscope one-way ANOVA was used to statistically investigate the average among groups. A statistically significant difference was found among the sub-groups 1 and 2 as evident from p value.

To compare the inter-group variations, post hoc Tukey test was used. The result of this test revealed that the fibroblasts and congested blood vessels were much greater in numbers in groupB1 and B2 than A1 and A2 respectively and it was statistically.

Subgroup A1 was seen with no ulcer while subgroup B1 was seen with ulcer. Statistical test, one-way ANOVA was used to compare the ulcer index values. The results of ANOVA show average ulcer index between subgroups 1 and 2 which was statistically significant as (Table 4.)

The post hoc Tukey test revealed that the ulcer index was much greater in the group B1 and group B2 compared than group A1 and group A2 respectively shown in the (Table 5).

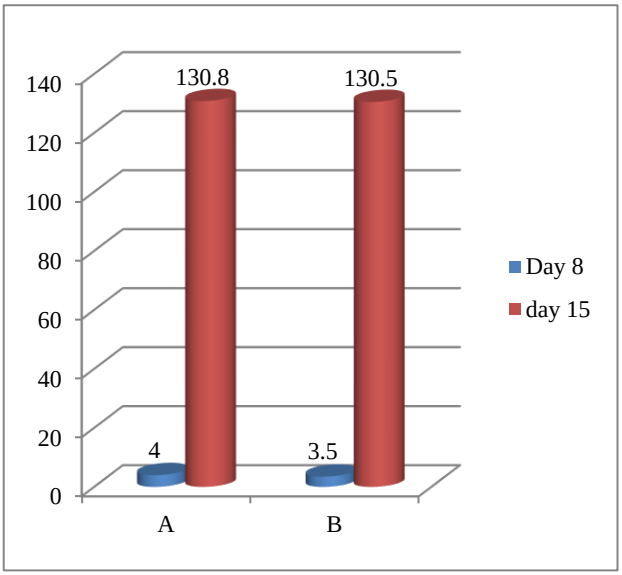

Figure 1: Number of inflammatory cells in subgroups.
*statistically significant p value (≤ 0.05)

Table 2: Average body weight of the experimental animals before and after completion of the experiment for sub-group 1 and 2, p value of Shapiro-Wilk test is shown in parenthesis.

Parameters	Groups		P
	A	B	
Weight at start of experiment (gm)	192.6±3.0 (0.057)	194.0±3.0 (0.390)	0.641
Weight on day 8th (gm)	196.5±4.1 (0.053)	192.1±4.2 (0.165)	0.112
Weight on day 15th (gm)	199.7±3.7 (0.700)	190.7±3.6 (0.340)	0.002*

Table 3: Comparative statistics for inflammatory cells in subgroup 1 and 2.

Variables	Group (1)	Group (2)	Difference (1-2)	Standard error	P value
Inflammatory cells (numbers)	A1	B1	-126.833	2.8995	<0.001*
Inflammatory cells (numbers)	A2	B2	-127.0*	1.513	<0.001*

*Statistically significant p value.

Table 4: Mean ulcer index values of subgroup 1 and 2.

Parameter	Groups		P value
	A	B	
Ulcer index on 8th day	0.00±0.00	1.01±0.00	<0.001*
Ulcer index on 15th day	0.00±0.00	1.01±0.01	<0.001*

*statistically significant p value

Table 5: Comparative statistics for ulcer index in subgroup 1 and 2.

Parameter	Group (1)	Group (2)	Difference (1-2)	Standard error	P value
Ulcer index	A1	B1	-1.00*	0.034	<0.001*
Average ulcer	A2	B2	-1.01*	0.046	<0.001*

*Statistically significant p value

Macroscopic examination of dissected stomach

Just after excision from the rat body, the stomach is spread over the thermopol. Gross anatomy of the stomach of rat was seen through naked eye. Figure 2 shows the stomach of a rat belongs to control group. This is not showing any visible signs of ulcers or damage to the mucosal membrane. Figure 3 shows the stomach of rat from group B1. Gross anatomy of these stomach clearly indicating the presence of ulcers on the inner surface i.e., mucosal membrane.

Figure 2: Gross anatomy of rat's stomach belongs to control group.

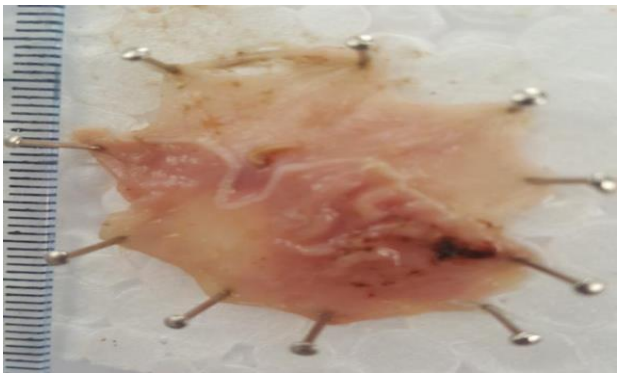

Figure 3: Gross anatomy of rat's stomach belongs to group B1, ulcer can be seen with naked eye.

DISCUSSION

Peptic ulcer disease is quite prevalent in the World due to many factors. One of the reasons is the use of NSAIDs. Among NSAIDs aspirin is used in cardiac patients as antiplatelet agent to prevent further attacks. NSAIDs are notorious for inducing gastric ulceration which it does with many mechanisms.[1,3,7] Our study showed that induced significantly more gastric ulceration compared to controls and results of study are in conformity with Jahan et al.[2] Aslam et al showed that ulcer index was higher in aspirin treated group compared to controls. These findings are same as observed in current study.[3,14,15]

Current study showed that in aspirin treated group there was more inflammatory infiltrates, congested blood vessels and increased fibroblasts compared to controls. Similar findings were suggested by Jahan et al.[2] Studies have shown that aspirin induce ulcer by increasing acid production, inhibition of prostaglandins which in turn plays role in blood flow, secretion of mucus and bicarbonate content of mucus and proliferation of epithelium.[2,13]

Macroscopic examination of inner surface of stomach reveals that subgroup B1 has relatively larger ulcer index as compared to another subgroup. These finding are in line with the observation of. [2,14,16] This typically confirms the induction of gastric injury due to aspirin as it inhibits the cyclooxygenase resulting in decrease level of prostaglandin in local tissues which in turn inhibit the production of mucus. Low level of mucus surrounding the epithelial cells results in the exposure of these cells to the locally produced acid and results in ulcer. On the other way, aspirin also penetrates to the epithelial cells and directly inhibits the prostaglandin synthesis, and increased production of ROS. ROS is responsible for gastric epithelium disruption and cell necrosis through degradation and oxidation of fatty acids and lipids of cell membrane.[17] Prostaglandins in the stomach epithelium is responsible for the optimum acid and mucus secretions and promotes epithelial regeneration.[15-18]

Limitations

The study duration was short, so long-term effects cannot be noted from this.

CONCLUSION

We can conclude from the study that gastric ulcer is quite common and it occur because of the imbalance between protecting and aggravating factors. Aspirin is a common offender of gastric ulcer because of its common use.

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