Original Article

Histomorphometric study of stomach and duodenum of aspirin treated Wistar rats

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Abstract

Introduction: Gastrointestinal ulcers and stomach bleeding are amongst the main undesirable side effects of aspirin taken by mouth. The present study examined the histological and histomorphometric changes in the stomach and duodenum, following oral administration of aspirin to Wistar rats, as well as the effects on body weight and packed cell volume (PCV). **Materials and Methods:** Twenty adult Wistar rats weighing between 160 and 210 g were randomly divided into four groups of five rats each. Group A served as control, while Group B and C received the dose equivalent of 150 mg/day and 300 mg/day in a 70 kg human respectively for 2 weeks. **Results:** The study showed a dose dependent significant reduction ($P < 0.001$) in weight of aspirin treated rats and significantly reduced ($P < 0.001$) PCV in treated rats. Histological findings in treated rats showed erosion of the epithelial lining and mucosa layer of stomach and duodenum. Histomorphometric measurements showed a significant decrease ($P < 0.05$) in thickness of the mucosa layers of the stomach and duodenum, as well as significant decrease ($P < 0.05$) in glandular layer and villi height following aspirin treatment. **Conclusion:** The present suggest that by causing gastrointestinal bleeding and ulcerative changes, oral administration of aspirin reduces the mucosa surface area of stomach and duodenum.

Key words: Aspirin, duodenum, histology, histomorphometry, stomach

INTRODUCTION

Gastrointestinal ulcers and stomach bleeding are amongst the main adverse effects of aspirin following oral administration (Macdonald 2002; Sorensen *et al.*, 2000).

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As such, aspirin is been used to experimentally induce gastric ulcers (Cryer *et al.*, 2011; Wang *et al.*, 2011).

Aspirin is a salicylate drug often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an antiinflammatory medication (Sneader 2002; Huth *et al.*, 1994). Salicylic acid, the main metabolite of aspirin, is an integral part of human and animal metabolism. While much of it is attributable to diet, a substantial part is synthesized endogenously (Paterson *et al.*, 2008).

It has been established that low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue (Julian *et al.*, 1996; Krumholz *et al.*, 1995). Many people take a daily aspirin to reduce their risk of heart attack. Aspirin is one of the most widely used medications in the world, with an estimated

40,000 tonnes of it being consumed each year (Warner *et al.*, 2002). Aspirin is part of a group of medications called nonsteroidal antiinflammatory drugs (NSAIDs), but differs from them in the mechanism of action. Though it, and others in its group called the salicylates, have similar effects (antipyretic, antiinflammatory, analgesic) to the other NSAIDs and inhibit the same enzyme cyclooxygenase (COX), aspirin (but not the other salicylates) does so in an irreversible manner and unlike others, affect more the COX-1 variant than the COX-2 variant of the enzyme (Burke *et al.*, 2006).

As a follow-up to previous findings on the effects of aspirin on the gastrointestinal mucosa, the present study examined the changes in histomorphometric parameters of the gastrointestinal mucosa following oral aspirin administration, which is yet to be carried out, hence the need for this study. The present study also determined effects of aspirin administration on body weight and packed cell volume (PCV).

MATERIALS AND METHODS

Animal Care

Twenty adult female albino Wistar rats weighing between 160 and 210 g were purchased from the Animal house in the Department of Physiology, University of Ibadan, Oyo State. The rats were housed in clean plastic cages in the Animal holdings of the Department of Anatomy, LAUTECH, Ogbomoso, and maintained under standard laboratory conditions. The rats were fed daily with normal rat pellets purchased from Ladokun livestock and feed mills, Ibadan and water was given to the rats *ad libitum*. The rats were subjected to a period of 3-week of acclimatization prior to the treatment of the rats, and the body weights of the rats were taken during this period of time. All animals were handled in accordance with the guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publication 1985).

Animal Treatment

The rats were randomly divided into three groups (A, B and C) of five animals each. Group A rats served as a control and were given distilled water during the period of the experiment. Groups B and C rats served as treated animals. A dose equivalent of 150 mg/day and 300 mg/day in 70 kg human was administered to rats in Groups B and C respectively for 2 weeks. Aspirin was administered orally using orogastric tube. The body weights of the rats were monitored weekly throughout the period of administration.

Surgical procedures, Determination of Packed Cell Volume and Histological Analysis

At the end of the treatment, blood was collected from the tail of the rats into anticoagulant bottle. It was then transferred into the capillary tube. One end of the capillary tube was sealed. It was then allowed to cool in order to avoid lyses followed by centrifugation at 15,000 revolutions per minutes for 5 min, for determination of PCV by micro-haematocrit method. The rats were euthanized by cervical dislocation. A midline incision was made through the anterior abdominal wall. The stomach and duodenum of each rat were carefully removed and fixed in 10% buffered formal-saline for routine histological paraffin embedding. Sections were stained by routine hematoxylin and eosin according to the methods of Drury and Wallington, (1980). Slides were viewed using a Leica DM750 light microscope, and digital photomicrographs were taken with an attached eyepiece camera (Leica ICC50).

Histomorphometry

Photomicrographs of hematoxylin and eosin stained sections at ×100 magnification, were imported on to OpenOffice.org™ (OOo dev 3.4.0, Oracle Corporation, USA) software for histomorphometric analysis. Measurements made from photomicrographs of stomach sections were thickness of the mucosa layer, subglandular mucosa layer, glandular mucosa layer (calculated by subtracting the subglandular mucosa from the mucosa layer), submucosa layer, muscularis propria (De Conto *et al*., 2010). Measurements made from photomicrographs of duodenum sections were muscosa layer, submucosa layer, and muscular propria. Measurements of the layers were made at four different locations in each of the stained sections and the average taken. Furthermore, five well-aligned villi and corresponding crypts of the duodenum were measured for, villi height, villi width (at the villi and crypt junction) and crypt depth, while the villi height/crypt depth ratio (villi/crypt ratio) was calculated (Alves *et al*., 2004; Burkhardt *et al*., 1998). All measurements were in μm and done at ×100 magnification.

Statistical Analysis

One-way ANOVA was used to analyze data followed by Student Newman-Keuls test for multiple comparisons. Primer for Windows (version 4.0.0.0, McGraw-Hill, USA) was the statistical package used to analyze data. Results were expressed as mean ± standard error of the mean. $P < 0.05$ was taken as accepted level of significant difference.

RESULTS

Changes in Body Weight

As shown in Figure 1, the body weight of control rats increased over the experimental period, while aspirin treated rats decreased in body weight. Final body weights at the end of 2 weeks showed that all treated group reduced significantly ($P < 0.001$) compared to control rats. Furthermore, Group C animals significantly reduced ($P < 0.001$) in body weight compared to Group B.

Effects of aspirin administration on packed cell volume

Figure 2 shows that there was significant decrease ($P < 0.001$) in PCV values of Groups B and C (48.0 ± 2.42; 51.8 ± 2.64) compared to control (65.5 ± 2.66).

Histological Findings

The layers of the stomach were well arranged and visible as mucosa layer, submucosa layer and muscularis propria layer [Figure 3].The mucosa layer of the stomach of the control rats showed normal histology with intact epithelial lining and gastric pits. Inflammation was observed in the mucosa and submucosa layers of both aspirin treated groups as well as ulceration of epithelia lining and mucosa layer in these groups. Furthermore, hemorrhages with enlarged arterioles were observed in the stomach of Group C rats. Furthermore observed, was an enlargement of the submucosa and muscularis propria layers of Group C rats.

The layers of the duodenum (muscular layer, submucosa layer, muscularis propria) were also well arranged and visible [Figure 4]. The epithelia lining of control rats were intact, while the submucosa was invested with Brunner's gland. The villi were clearly demonstrated and well oriented. The small intestine of both aspirin treated groups showed moderate to heavy mucosa inflammation. The epithelial linings were seen to be eroded in both groups, and some of the villi showed a lateral increase in size. There was no observed difference in the severity of histological alterations in both aspirin treated groups.

Histomorphometric Analysis

As shown in Figure 5, there was a significant reduction ($P < 0.001$) in thickness of the mucosa and glandular layers of aspirin treated rats compared to control. There was no significant difference in thickness of the submucosa and muscularis propria layer of control and Group B animals. The thickness in the submucosa and muscularis propria layer was significantly increased ($P < 0.001$) in Group C compared to control.

As shown in Figure 6, there was a significant decrease in the mucosa and submucosa layer thickness of aspirin treated rats when compared to control ($P < 0.05$).

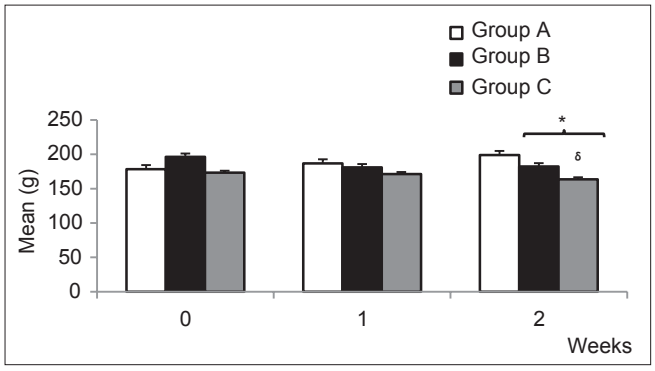

Figure 1: Effects of aspirin administration on body weight. Bars are mean ± standard error of mean, $P < 0.05$. *Significant difference compared to control. δSignificant difference compared with Group B

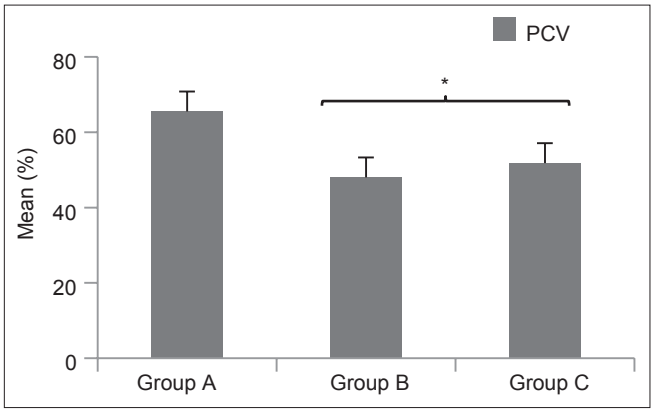

Figure 2: Effects of aspirin administration on packed cell volume. Bars are mean ± standard error of mean, $P < 0.05$. *Significant difference compared to control

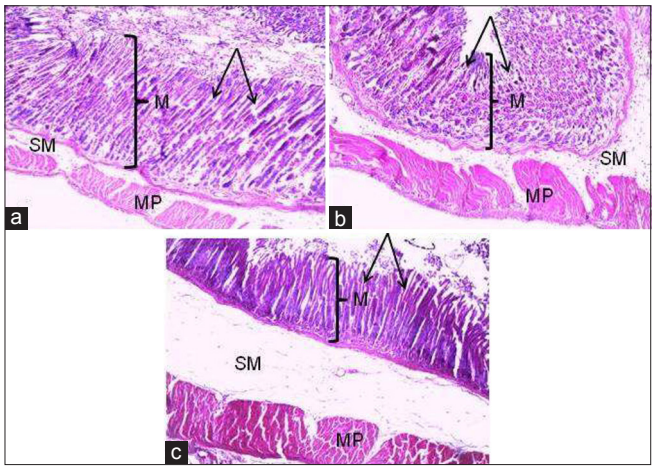

Figure 3: Micrograph of stomach of control (a) and Aspirin treated (b and c) rats. M-Muscular layer, SM - Submucosa layer, MP - Muscularis propria, Double arrows-Gastric pits (H and E, ×100)

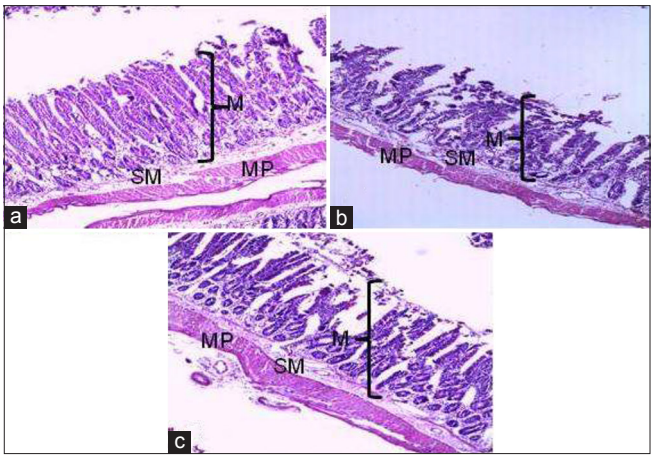

Figure 4: Micrograph of duodenum of control (a) and Aspirin treated (b and c) rats. M - Muscular layer, SM - Submucosa layer, MP - Muscularis propria (H and E, ×100)

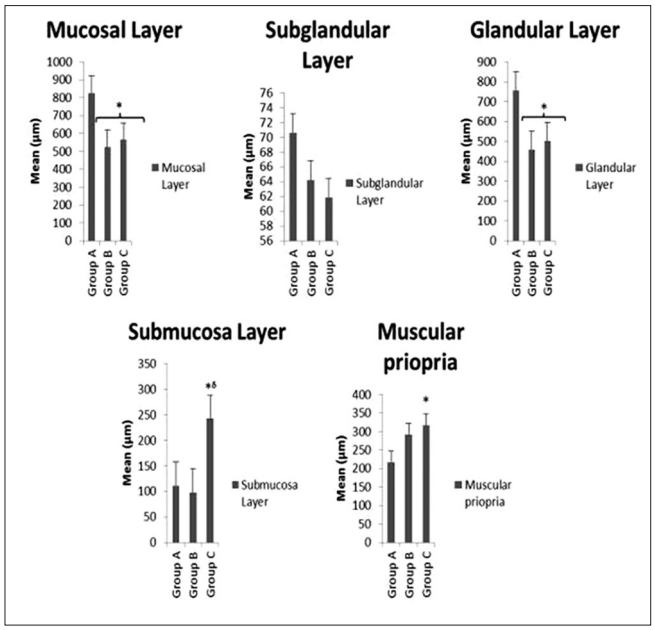

Figure 5: Histomorphometric measurements of the stomach of control (Group A) and aspirin treated (Groups B and C) rats. Bars are mean ± standard error of mean, $P < 0.05$. *Significant difference when compared to control, $^{\delta}$Significant difference between Groups B and C

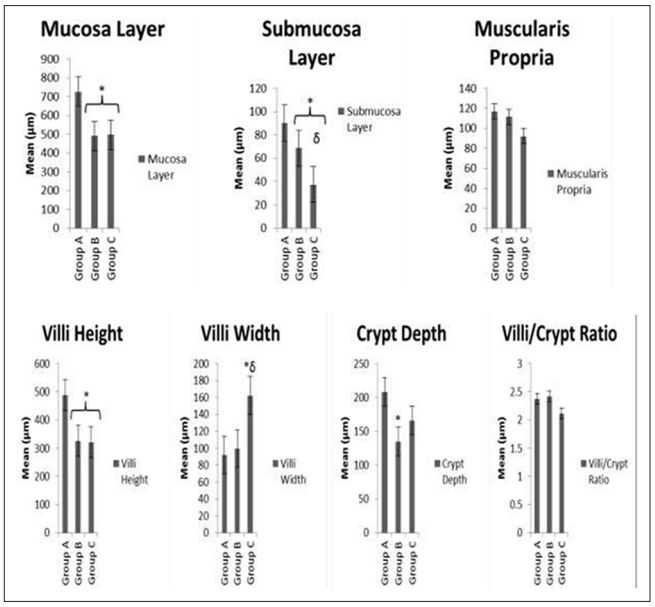

Figure **6:** Histomorphometric measurements of duodenum of control (Group A) and Aspirin treated (Groups B and C) rats. Bars are mean ± standard error of mean, $P < 0.05$. *Significant difference when compared to control, $^{\delta}$Significant difference between Groups B and C

The thickness of the submucosa layer in Group C was significantly reduced when compared to Group B ($P < 0.05$). Aspirin administration also significantly reduced villi height for both Groups B and C rats ($P < 0.05$), but only Group B rats showed a significant reduction in depth of crypts. Villi width was significantly increased in Group C rats when compared to control as well when compared with Group B ($P < 0.05$). There was no significant difference in villi/crypt ratio between treated animals and control.

DISCUSSION

Aspirin administration has been known to cause acute and chronic stomach ulcers and gastrointestinal bleeding (Sabilia *et al.*, 2004; Macdonald 2002; Sorensen *et al.*, 2000). The present findings reaffirms this as histological observation shows mucosa and submucosa inflammation accompanied by ulceration, erosion of epithelia tissues and hemorrhage within the stomach and duodenum as observed in Wistar rat treated with aspirin.

The present study also observed a dose dependent reduction in body weight as higher doses of aspirin administration reduced the body weight significantly compared to control and lower doses. The possible mechanism by which aspirin lowers body weight calls for further studies. However, we suggest that this may result in reduced digestive functions following debilitating injuries to the gastrointestinal mucosa caused by aspirin administration.

Oral aspirin administration has previously been found to significantly lower PCV as well as erythrocyte and leukocyte counts suggesting the inhibitory action of this drug on bone marrow, a speculated target site of aspirin action, thus causing blood toxicity (Merchant *et al.*, 2004). This study also showed significantly reduced PCV following aspirin administration. However, there were no dose-dependent changes on PCV as a higher dose of aspirin administration did not further lower PCV values. This reduction in PCV levels may be indicative of anemic effects and decrease in capability of blood to deliver oxygen to tissues following aspirin administration.

Aspirin use has been related to a two-fold increased risk of severe gastrointestinal events (Goldstein *et al.*, 2006). Histological findings of the present study revealed the ulceration of mucosa layer of aspirin treated groups. This was further complemented by histomorphometric measurements which showed significantly reduced mucosa and glandular layers of the stomach of aspirin treated rats. Furthermore, histological findings showed enlargement of the submucosa and muscularis propria layers in the higher doses of aspirin administration. Histomorphometric measurements further showed a dose dependent increase in the thickness of the submucosa and muscularis propria layers of the stomach aspirin treated rats. Aspirin administration also resulted in erosion of the epithelia lining and mucosa of the duodenum. This observation was also further complemented by histomorphometric analysis, which showed a decrease in the mucosa layer thickness of duodenum of aspirin treated rats, as well as a decrease in the villi height following aspirin administration.

In conclusion, the present study further confirms that oral aspirin administration causes gastrointestinal bleeding and ulcerative changes. Furthermore, aspirin administration may be associated with a dose dependent decrease in body weight, lowered PCV at low doses, and reduced thickness of mucosa layer of stomach and duodenum. These findings suggest that oral administration of aspirin reduces the mucosa surface area of stomach and duodenum. Hence, it is recommended that aspirin be administered with caution and if daily intake becomes a necessity, it will be prudent to accompany such administration with drugs that could prevent injuries to the gastrointestinal mucosa.

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