

Research Article

Effects of Clarithromycin Administration on Gastric Acid Secretion and Cytoprotection in Wistar Rats

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Summary: The incidence of peptic ulcer disease in Nigeria is 28%. Clarithromycin (CLX) is used in the treatment of peptic ulcer owing to its antibacterial effect. Whether CLX has effects on other gastrointestinal parameters that reduce peptic ulcer has not been previously investigated. Therefore, the effects of CLX on gastric acid secretion, mucus secretion and gastric ulcer scores in rats were investigated. A total of 30 albino wistar rats were used for the study. Out of this number, 10 rats each were used for gastric acid secretion, mucus secretion and ulcer scores respectively. In each of these sub groups, 5 rats served as test and were treated with CLX orally and 5 rats served as control. Standard methods were used for the estimation of these parameters of gastric function. The results showed that basal gastric acid, peak acid output following histamine stimulation and mucus secretion were significantly increased ($p < 0.001$) in CLX-treated (test) rats than in their control. Furthermore, ulcer scores were significantly reduced ($p < 0.001$) in the CLX-treated rats than control. In conclusion, Clarithromycin administration reduced gastric ulcers in rats. This may be attributable to not only its antibiotic property but also its ability to increase gastric mucus which counteracts the aggressive effect of the acid.

Keywords: clarithromycin, gastric secretion, mucus secretion, ulcer scores, cytoprotection

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INTRODUCTION

The incidence of peptic ulcer disease in Nigeria is reported to be about 28% (Ndububa and Adeyemi, 2008). Clarithromycin, a macrolide antibiotic discovered in 1980 has been used for treating various infections especially *Helicobacter pylori* infection of the stomach/duodenum which has been shown to cause peptic ulcerations (Taweesak et al., 2015). The product emerged through efforts to develop a version of the antibiotic erythromycin that did not experience acid instability in the digestive tract, causing side effects, such as nausea and abdominal ache (Greenwood, 2008). CLX is usually used in combination therapy with other drugs in treatment of peptic ulcers (Fashner, 2015). However, *H. pylori* has developed resistance to these antimicrobial agents (Masaki et al., 2020). CLX may have other anti-ulcerogenic effects such as influence on gastric acidity and gastric mucus secretion. Gastric mucus plays a significant role in physiologic defence against luminal irritants (Toshimitsu et al., 2013). Reduced Gastric mucus secretion is associated with abdominal pains due to non-steroidal drug induced gastropathy (Lijima,

2013). Gastric mucus provides physical protection of the gastric epithelium (Anna et al., 2013). There is paucity of information regarding the effect of administration of CLX on gastric acid secretion and some parameters that may contribute to cytoprotection like mucus secretion.

We therefore engaged in this study to assess effects of CLX on basal gastric acidity, peak acid output, gastric mucus secretion and emergence of gastric ulcers. We believe that if this study shows more benefits of CLX beyond its antimicrobial activities that its use as monotherapy for peptic ulcers may be studied further and encouraged in humans.

MATERIALS AND METHODS

Animals and Drugs

This study used thirty wistar rats obtained from the animal house of the Department of Physiology, University of Calabar, Cross River. The rats were kept in separate cages at $28 \pm 2^\circ\text{C}$, 12 hours light and dark cycles and had free access to water. The rats were fed standard rodent feed. Ranbaxy Pharmaceutical Company, Lagos supplied samples of CLX (Crixan)

used for this study. Dosage of 7.5mg/kg every 12 hours was the clinical dose (Periti, 1999). CLX was dissolved in normal saline.

Drug Administration: Ten Wistar rats each was used for gastric acid, mucus secretion and ulcer score studies respectively. For each experiment, 5 rats were used as control and 5 rats as test. The control group was fed with normal diet while the test group was fed normal diet and CLX solution administered orally in a dose of 7.5mg/kg body weight every 12 hours for 14days. All the animals were allowed water freely. The rats were subsequently sacrificed for the studies. After 14days, Osim modification of Gosh and Schild method was applied for the assessment of gastric acid secretion using histamine as acid secretagogue while method of Tan *et al* was adopted for mucus secretion using acid alcohol and method of Alpin and Wards for ulcer scoring.

Measurement of Gastric Acid Secretion: The method adopted for measurement of gastric acid secretion was a modification of Ghosh and Schild continuous perfusion method (Osim *et al.*, 1991). All animals were fasted for 24 hours prior to the start of the experiment to ensure that their stomachs were empty of food which may contaminate the acid been secreted. The animals were then anaesthetized by intraperitoneal administration of 6 ml/kg of 25 % (v/v) solution of urethane (Sigma, UK). The trachea was exposed and cannulated to allow for adequate air flow into the lungs. Another cannula was passed into the stomach, through the mouth and the esophagus. Both cannulae were tied firmly in place with a ligature. The abdomen was then cut open along the *linea alba* to minimize bleeding. The stomach was exposed and the pyloric end cannulated at its junction with the duodenum. Isotonic (0.9 per cent) saline was introduced gently via the esophageal cannula to wash out the stomach contents. The perfusate was allowed to flow freely after clearing the food particles. The abdominal incision was then covered with a moist cotton wool dipped in normal saline. The stomach was perfused continuously with normal saline at the rate of 1 mL/minute. The pH of the saline was maintained at 7.0 and the body temperature of the rat was maintained at 37 °C using a heating lamp. The flow was adjusted to give an effluent volume of about 1 mL per minute. The effluent was collected at 10 minutes interval and care was taken not to ligate the blood vessels as this may lead to stained perfusate or interrupt blood flow to the stomach of the rat. Each perfusate obtained after 10 minutes was titrated using two drops of phenolphthalein as indicator against 0.01 N NaOH (May and Baker, UK) to determine its total acidity. The experiments were repeated using histamine as acid secretagogue, administered subcutaneously. The dose

of histamine used was 100 mg/kg body weight. Gastric acid output in the effluent sample was measured by titrimetric analysis. The experiments were also repeated using cimetidine as a blocker at a dose of 100 mg/kg body weight to ascertain whether the pathway for acid secretion was via Histamine (H₂) receptors. Administration was via intramuscular route.

Measurement of Gastric Mucus: The adherent gastric mucus was measured by the method of (Tan *et al.*, 2006). The animals were fasted for about 18 hours prior to the experiment, after which they were sacrificed after being anaesthetized with chloroform and their stomachs removed. The stomach was then opened along the greater curvature and pinned on a flat board. Using a spatula, the gastric mucus was scraped off the surface of the mucosa and introduced into pre-weighed sterilized sample bottle containing 3 ml of distilled water. The sample bottle containing distilled water and the collected mucus was then weighed on an electronic balance. Mucus output was calculated as the difference in weights of sample bottle containing water and sample bottle containing water and mucus.

Determination of Ulcer Scores: Gastric ulcer score was assessed using the method of Alphin and Wards, (1967). The animals were anaesthetized by inhalation of 3-4 ml of 5% chloroform poured unto a cotton-wool in a small beaker placed near the nostrils of the animals. The reason for chloroform anaesthesia usage was because of its gastroprotective effects in animals with ethanol-induced gastric ulcers through antioxidant and anti-secretory effects (Zainul *et al.*, 2016). Thereafter, an abdominal incision was made through the *linea alba* and the pylorus exposed. Induction of the ulcers was done using the method by Mizui and Doteuchi, (1983). A pyloric incision was made and a cannula inserted and held in place by tying with a thread. The stomach was infused with 1.5 ml of acid alcohol to induce ulceration. The infusion was made via the pyloric incision. The animals were allowed for an hour. The stomach was then surgically removed, washed, cut open along the greater curvature and rinsed with normal saline. Pins were used to hold the tissue to the dissecting board. A magnifying lens and a Vernier caliper were used to measure the extent of ulceration. Ulcer score was done according to the grading system shown below:

Grade 0.0 - No Lesion (normal stomach)

Grade 0.5 - Pin size ulcer

Grade 1.0 - 2 or more haemorrhagic or small linear ulcers

Grade 2.0 – Ulcer spots greater than 3 mm.

The ulcer score was calculated by multiplying each grade with its frequency of occurrence. The sum of all the values formed the ulcer score for each animal (Koike *et al.*, 2001).

Statistical Analysis

The data were analyzed by unpaired t test using Microsoft excel computer program. The results were presented as mean \pm standard error of mean (SEM) and *p* value less 0.05 was considered statistically significant.

RESULTS

This study observed that treatment with CLX resulted in a rise in gastric mucus output in the test group which was significantly higher the control ($P < 0.001$). The mean gastric mucus outputs in the control and test groups were $0.22 \pm 0.04g$ and $0.28 \pm 0.02g$ respectively (Figure 1).

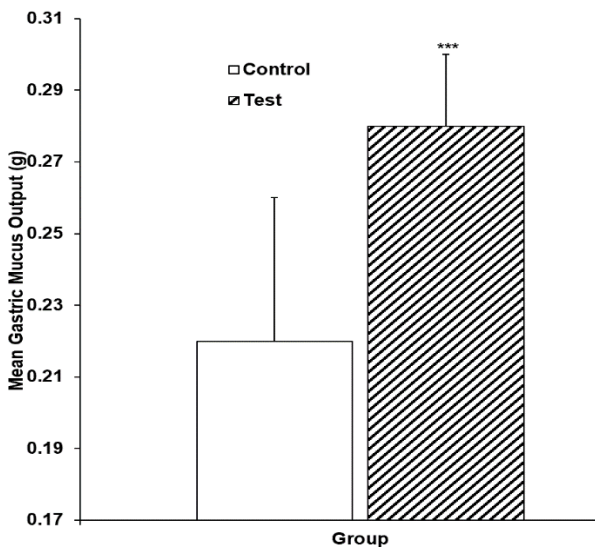


Figure. 1: Comparison of mean gastric mucus output in the different experimental groups. Values are mean \pm SEM, *n* = 5. *** = $p < 0.001$

With reference to figure 2, there was a rise in basal gastric acid secretion in the test group that was significantly different from control ($p < 0.001$) and with the administration of histamine, the peak acid output (PAO) of the test group was significantly higher than the control group ($p < 0.001$). Following the administration of cimetidine, H_2 receptor blocker, gastric acid secretion decreased to levels that were below the basal levels in the control and test groups.

DISCUSSION

Gastric ulcer, one of the most widespread diseases occurs due to an imbalance between protective and aggressive factors (Alkofahi and Atta, 1999). The gastric mucosa is continuously exposed to potentially injurious agents such as gastric acid, pepsin, bile acids,

food ingredients, bacterial agents (*Helicobacter pylori*) and drugs (Peskar and Matricic, 1998).

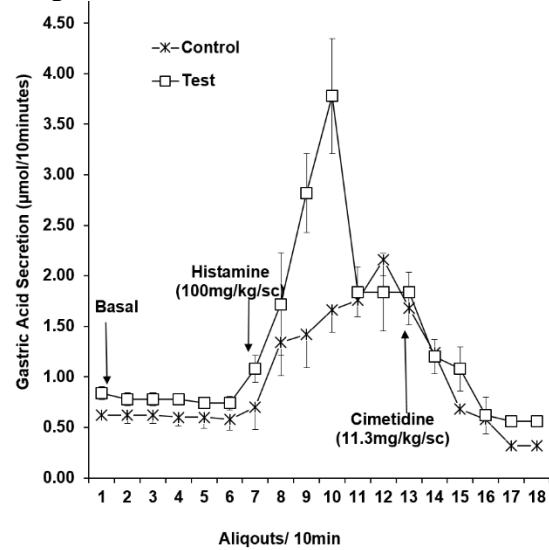


Figure 2: Gastric acid secretion in control and CLX-treated groups. Values are mean \pm SEM, *n* = 5.

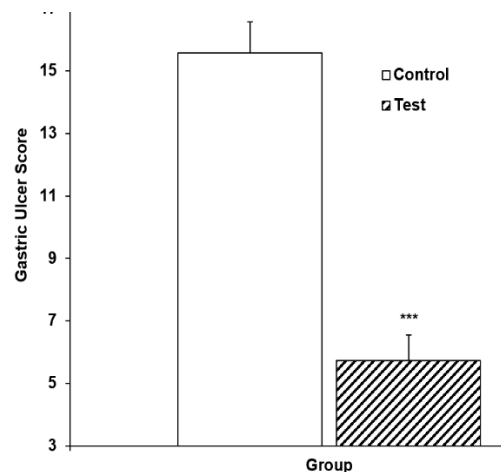
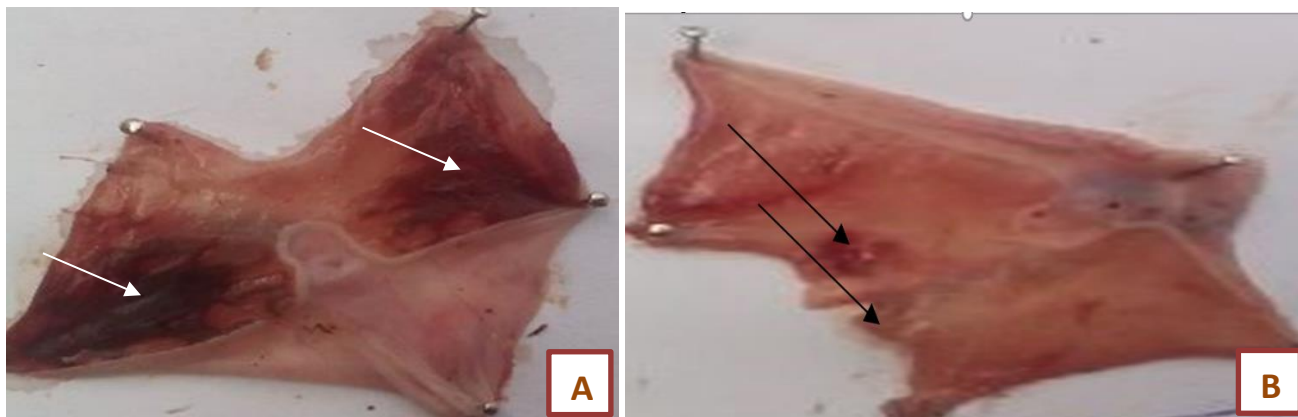


Figure. 3: Comparison of mean gastric ulcer score in the control and CLX-treated groups. Values are mean \pm SEM, *n* = 5. *** = $p < 0.001$ vs control.

These agents have been implicated in the pathogenesis of gastric ulcers including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis, inhibition of cell proliferation, increased generation of free radicals, and diminished gastric blood flow and gastric motility (Toma et al., 2005).

In this study, there was decrease in the gastric ulcer scores in the test group when compared with the control. However, there was a rise in mucus secretion in the test group which was significant when compared with the control. It is likely that this rise in the mucus output contributed to the decrease in ulcer scores in the test groups.

**Plate 1:**

Gross evaluation of the ulceration on the gastric surface of the Wistar rats

A- is the ulcer-induced group without CLX treatment (control group) showing extensive lesions to the gastric mucosa. They appear as elongated bands of hemorrhage (white arrow).

B – is the ulcer-induced group treated with CLX (test group) showing only mild injuries (black arrow) to the gastric mucosa.

(Magnification: 1.8 x) (n=5).

The decrease ulcer scores despite increase acid secretion found in this study may be explained by neutralization of hydrogen ion on the gastric mucosa by the mucus-entrapped bicarbonate ions (cytoprotection), thereby reducing the concentration of the gastric acid (Osim, 2002). In addition, the increased gastric mucus output protects the gastric epithelium from injurious effect of pepsin hence the decrease in ulcer scores.

This therefore ameliorates the ulcerative effects of the acid on the mucosa of the stomach. Similarly, this is supported by a study by Toshimitsu et al., (2013) carried out in humans which reported that increased gastric mucus secretion alleviates non-steroidal anti-inflammatory drug (NSAID)-induced abdominal pain which is likely due to gastric ulceration.

Future studies: To know if CLX alone is adequate to reduce peptic ulceration or if other drugs like Histamine-2 blockers and proton pump inhibitors are necessary.

In conclusion, administration of CLX increased gastric acid and mucus secretions but decreased ulcer scores in the rats. The increase in mucus secretion may be beneficial in the reduction of ulcer scores since presence of mucus is one of the protective factors to the gastric mucosa.

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