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Research Article

Chloroquine Delays Healing of Acetic Acid-Induced Mucosal Damage in the Rat Stomach

Salami A.T[1], Odukanmi A.O[1], Duduyemi B.M[1] and Ajeigbe K.O[2]

[1]*Gastrointestinal Secretion and Inflammation Research Unit, Department of Physiology, College of Medicine, University of Ibadan Ibadan, Nigeria;*

[2]*Department of Physiology, School of Basic Medical Sciences, Igbinedion University, Benin City, Nigeria*

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Abstract

Chloroquine (CQ) is a 4-aminoquinoline derivative, most widely used in treating malaria. Although previous studies have reported the gastric acid enhancing and pro ulcerogenic effect of CQ, its role in resolution of gastric injury is relatively unknown. In this study, the effect of CQ on acetic acid induced gastric ulceration was investigated. Chronic gastric ulcers were induced in male Wistar rats by serosal application of acetic acid for 60 seconds. Subsequently, the animals were given either 0.1ml normal saline (Group B) or 3mg/kg Chloroquine sulphate (Group C) for three days starting 24 hours after ulcer induction. A control (sham-operated) group of animals (Group A) did not have any ulcer induced. The animals were sacrificed by days 7, 14 and 21 post ulceration for gastric ulcer score. Homogenised gastric tissues were also analysed colorimetrically for malodialdehyde (MDA) estimation. In another study, basal and stimulated gastric acid secretion was measured using the continuous perfusion technique, after which small sections of the gastric tissue were fixed in formalin for histological evaluation. Statistical analysis was by ANOVA and Bonferoni post-hoc test at p-0.05. Acetic acid produced visible and measurable ulcers in all animals in groups B and C while no visible ulcers were seen in the unulcerated control rats. Ulcer diameter and ulcer index were significantly increased in the Chloroquine-treated group on the 7th, 14th and 21st day post-ulcer induction. Reduction in ulcer diameter (noticeable in all the animals by day 21) was significantly reduced in the chloroquine treated rats. These observations were confirmed by results from microscopy. Compared with the control, basal gastric acid secretion was increased by chloroquine but decreased with days after ulcer induction. There was no significant increase in the percentage change of the histamine stimulated gastric acid secretion between the control and chloroquine treated groups (12.5%, and 15.52%) compared with the basal gastric acid secretion (45.34% and 24.55%) on days 14 and 21 respectively. It is concluded that chloroquine prolongs the natural healing of acetic acid induced ulcer in Wistar rat.

Keywords: *Chloroquine, stomach, healing, inflammation*

INTRODUCTION

Peptic ulcer, a circumscribed complete loss of gut epithelium in parts of the digestive tract exposed to hydrochloric acid and pepsin secretion, occurs as a result of an imbalance between the defensive and aggressive forces at play in the mucosal lining of the stomach, combined with superimposed environmental or immunological injury (Tulassay and Herszenyi, 2010). In addition to the pain caused by the ulcer itself, gastric ulcers give rise to such complications as haemorrhage from the erosion of a major blood vessel, perforation of the wall of the stomach, with resultant peritonitis or obstruction of the gastrointestinal tract because of spasm or swelling in the area of the ulcer (Bertleff and Lange, 2010). Formation of gastric ulcers is characterized by the imbalance between gastric offensive factors (like gastric acid, pepsin secretion, lipid peroxidation, nitric oxide) and defensive mucosal factors (like glycoprotein proliferation, and antioxidants like catalase, superoxide dismutase and glutathione level) (Prabha et al., 2009). On the other hand, the healing of gastric ulcers involves a complex process of restoring the mucosal to its normal state through proliferation of epithelial and endothelial cells and the concerted actions of a wide range of growth factors to correcting the already altered imbalance between the aggressive and defensive factors in the stomach (Perini et al., 2003; Tarnawski 2000, 2001).

With sustained increase in the prevalence of gastric ulcer in Africa (Nwokediuko et al., 2012), attempts to investigate this trend show certain factors as being responsible. Such factors include dietary and occupational exposure and/or socio-economic status of the patient (Nneli et al., 2007). Besides, some pharmaceutical and medicinal drugs have equally been implicated, either through appropriate use or misuse. For example, antimalarials have been shown to affect gastric acid secretion (Barth et al., 1975; Etimita et al., 2005; Olaleye et al., 2012), which plays a very crucial role in both pathogenesis and healing of peptic ulcer. Gastric acid secretion is influenced by the interplay of stimulatory and inhibitory factors arising from both the central nervous system and within the gastrointestinal system (Yao and Forte, 2003).

**Author for Correspondence:* +2348023351570
E-mail: adeolathabitha@yahoo.com

The acid, facilitated by the activity of H+ K+- ATPase in the apical membrane (Davies, 1951; Geibel, 2005), is finely regulated by overlapping neural, hormonal, paracrine pathways (Schubert, 2005), and when levels of the acid and proteolytic enzymes overwhelm the mucosal defence mechanisms, ulcers occur. Studies have also reiterated the beneficial role of acid suppression in the healing process of gastroduodenal mucosal injury (Douglas et al., 1990; Dent, 1998; Al Mofleh, 2010).

Despite introduction of combination therapies to combat uncomplicated malaria, Chloroquine, a 4-aminoquinoline derivative, still remains a popular antimalaria drug in the tropics. This may be due to its affordability, efficacy and availability regardless of the well documented side effects. Chloroquine inhibits parasitic growth (Wellems, 1992) and blocking of production of cytokines and cell proliferation in monocytes, macrophages and lymphocytes (Picot et al., 1993; Landewe et al., 1995). Although the relative popularity of chloroquine is being compromised by resistance from the parasites (Ursos and Roepe, 2002), the drug is still commonly used as antimalarial due to its relatively low cost (Goodman and Coleman, 2000, Ogungbamigbe et al., 2008; Wellems and Plowe, 2001; Happi et al., 2004; Laufer et al., 2006;). Chloroquine, in therapeutic doses, has been shown to interfere with a number of physiological processes (Ogunkunle et al., 2011; Falade, 2014). These include increased inhibition of cytochrome P450-mediated oxidation reactions (Farombi et al., 2000) and the induction of oxidative stress in animals (Magwere et al., 1997) and man (Toler, 2004). The mutagenicity and genotoxicity of chloroquine have been reported in a number of cells (Obaseki et al., 1986; Chen et al., 2005; Farombi et al., 2006).

Chloroquine has been shown to increase gastric acid secretion via histaminergic and cholinergic system of acid production by the parietal cells (Ajeigbe et al., 2012). Earlier reports even showed that it can cause gastric mucosal cell damage (Iskander et al., 1990) and potentiation of mucosal cell injury induced by indomethacin and acidified ethanol (Ajeigbe et al., 2008). It is however not known if chloroquine would interfere with gastric ulcer healing process. In this study, the effect of Chloroquine on acetic acid induced wound healing in the rat stomach was investigated.

MATERIAL AND METHODS

Animals: Male Wistar rats (100 – 140g) were used in the study. The animals were obtained from the Central Animal House, University of Ibadan, Nigeria. They were kept in wire meshed cages and fed with standard commercial rat pellets (Ladokun Feeds Limited, Nigeria) and allowed water ad libitum prior to the start of the experiment. All animals received humane care and procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and use of animals, (WMA, APS., 2002).

Acetic acid-induced ulceration: Gastric kissing ulcers were produced in fasted rats following the method described by Wang et al., (1989) with slight modifications. Briefly, food (but not water) was withheld 24 hours prior to gastric ulcer induction and a brief laparotomy was performed and the stomach exposed in a sodium thiopental – anesthetized rat. The anterior and posterior walls of the stomach were held using forceps and acetic acid (0.5ml, 80%vol) was instilled into a plastic writing pen (Biro®) barrel (diameter 10mm) which had been cut at the end and had been stuffed with little cotton wool just enough to soak up the acetic acid such that there would be no spillage of the acid into the rat's abdomen. The open end of the barrel was then placed on the wall of the rat's stomach for a minute. The surface of the stomach was then washed with saline to ensure that the acid did not touch other parts of the abdomen. The abdomen was sutured, and rats were allowed to recover

Experimental groups and treatments: The animals were randomly grouped and treated into three as follows:
Group A: Animals in this group served as overall negative control. Ulcers were not induced in this group of animals but the abdomen was opened under anaesthesia and sutured back.
Group B: Twenty-four hours after induction of ulcer, the animals in this group were given 0.1ml of normal saline (vehicle) for three days.
Group C: Twenty-four hours after induction of ulcer 3mg/kg, the animals in this group were given Chloroquine sulphate (Group C) for three days.

The animals were sacrificed on days 7, 14 and 21 after ulcer induction and gastric lesions evaluated by examination of the inner gastric surface with a dissecting magnifying glass. The diameter (mm2) of the ulcer area was determined by planimetry by two independent observers. Ulcer index was calculated as

Ulcer Index = 10/x

where x = Total Gastric mucosal area /Gastric mucosal lesion area

The reduction in gastric mucosal lesions was calculated as:

$$\frac{\text{ulcer index (control)} - \text{Ulcer index (test)}}{\text{ulcer index (control)}}$$

Determination of lipid peroxidation status: Assessment of lipid peroxidation was carried out following the procedure described by Varshney and Kale (1990). It is based on the reaction of malondialdehyde (MDA) produced during lipid peroxidation with thiobarbituric acid (TBA) forming a pink coloured MDA-TBA adduct that absorbs strongly at 532nm. Animals were sacrificed after the experimental period, and the stomach of each rat was dissected. Stomach from each animal was cut open along the greater curvature, rinsed with normal saline, the mucosa of the ulcerated area was scraped and homogenised in phosphate buffer (tissue to buffer ratio - 1:3), 0.2mLs of test sample was added to 0.8ml of Tris-KCl. The solution was quenched with 0.25ml of TCA. 0.25ml of TBA was then added and the solution was then incubated for 45minutes at 80%C. A pink coloured reaction mixture was formed. The reaction mixture was then centrifuged at 1400 rpm for 15 minutes. The absorbance of the supernatant was read at 532 nm. MDA was calculated for each sample as described in a previous report by Adeniyi et al., (2014).

Gastric Acid Secretion.: The changes in gastric acid secretion during ulcer healing was studied in a separate set of rats with acetic acid-induced peptic ulcers randomly assigned to chloroquine (test) and normal saline (control) groups. Acid secretion was studied 21 days after ulcer induction using the he continuous perfusion technique, described by Ghosh and Schild (1958). Briefly, animals were anesthetized with urethane (0.6ml/100g b.w). The animal was then placed on the dissecting board and the limbs tied to the board. The trachea was located and isolated around the neck; it was cannulated and slightly opened to aid breathing. The stomach was then located and a cannula placed in it from the duodenal end of the stomach. Normal was then passed to the stomach from the mouth through the aid of a cannula. The gastric content of the stomach was collected after every 10 minutes. This was for the basal recording of gastric acid. After about three different recordings, histamine was injected into the animal's system via the hepatic portal vein. The gastric content was also collected after every 10 minutes and three readings were collected for the stimulated acid secretion.

The acidity of each 10-minute effluent collected was assayed by titration against sodium hydroxide. After gastric secretory studies, rats were sacrificed through cervical dislocation and dissected. The stomachs were collected for histological studies.

Histological processing and examination: Sections of stomach were taken from two distinct areas from each stomach and placed in 10% formalin for histological examination. The stomach was fixed, cut into 5 µm sections, stained with hematoxylin and eosin. The stained sections were assessed for any inflammatory/other pathologic changes including infiltration of cells, necrosis or damage to nucleus or tissue structures

Statistical analysis: Results were expressed as mean ± standard error of mean. One-way analysis of variance (ANOVA) complemented with Student's t-test or Bonferroni post-hoc tests using GraphPad Prism for windows (Version 6) for differences between means was used to detect any significant difference ($p < 0.05$) between the groups.

RESULTS

Ulcer area after acetic acid-induced ulcer: In all rats with acetic acid treatment, uniform ulceration was produced similar to those reported in earlier studies. Table 1 shows the mean ulcer area, ulcer index and lipid peroxidation in the three groups of animals used. In both ulcerated groups of animals, ulcer areas increased significantly 7 days after ulcer induction as evidenced by the higher ulcer area in groups B and C (88.19 ± 2.32mm2 and 245.50 ± 9.96mm2 respectively) when compared to 78.57mm2 area of ulcer at induction. However, evidence of healing were observed by day 14 in the normal saline group (5.52%) but not in the chloroquine treated animals where ulcer area (110.70 ± 6.05mm2) was still higher than the initial value. At day 21, healing rate in the normal saline group (98.31%) was higher than those observed for the chloroquine-treated group (82.95%) as shown in Figure 1.

Lipid peroxidation: As shown in Table 1 also, seven days after ulcer induction, lipid peroxidation was significantly higher in both normal saline treated (3.42 ± 0.61µmolMDA/mg protein; p< 0.001) and chloroquine treated (5.84 ± 1.49µmolMDA/mg protein; p< 0.001) groups than the animals without ulcer (0.37 ± 0.09 µmolMDA/mg protein). By day 14, significant reduction in lipid peroxidation was observed in the normal saline (2.55 ± 0.72 µmolMDA/mg protein; p< 0.05) and chloroquine-treated (3.92 ± 1.01 µmolMDA/mg protein; P< 0.05) respectively. However, the reduction in lipid peroxidation was significantly lower in chloroquine-treated rats when compared with the normal saline group.

Parietal cell count: Parietal cell counts are also shown in Table 1. While significant increases in cell counts were observed as healing progressed in the normal saline group, chloroquine treatment significantly reduced parietal cell levels 14 and 21 days after ulcer induction, when compared with the values on day 7.

Gastric acid secretion in control and chloroquine-treated rats 7, 14 and 21 days after ulcer acetic acid induced ulcer: As shown in figure 2, the basal gastric acid secretion in the animals without ulcer was 0.42±0.125mmol/10mins and did not change significantly with values observed for days 14 and 21. In the animals with acetic acid induced ulcer given normal saline, basal acid secretion was 1.6067±0.25mmol/10mins while in the chloroquine administered rats, basal acid secretion was 2.37±0.485mmol/10mins (p<0.05) this value was significantly higher than control. Histamine significantly increased acid secretion in all the animals. However, change in acid secretion by histamine over the basal values (normalised as 100%) in the normal saline group (161.94%).

Histological assessment

Histological examination of the stomachs removed from animals (days 7, 14 and 21) in all the experimental groups: control, ulcer and normal saline treated (group B) as well as ulcer and chloroquine treated rats (group C) are presented in plates 1 - 3.

Table 1:
Effect of chloroquine on ulcer area on healing of acetic acid-induced ulcer

Grp	Treatment	Ulcer area (mm^2)			Ulcer index			Malondialdehyde (µm/g)			Parietal Cell Count		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
A	Saline (no ulcer)	0	0	0	-	-	-	0.37 ± 0.09	0.40 ± 0.12	0.36 ± 0.10	N/A	N/A	N/A
B	Ulcer +Saline	88.19 ± 2.32	74.23 ± 4.73	1.33 ± 0.17	5.87 ± 0.28	4.95 ± 0.13	0.39 ± 0.08	3.42 ± 0.61[a]	2.55 ± 0.72[a]	0.95 ± 0.10[a]	1167± 333.3	1337± 168.4	1987± 415.7
C	Ulcer + CQ	245.50 ± 9.96*	110.70 ± 6.05*	13.40 ± 0.63*	16.03 ± 0.98	7.35 ± 0.42*	0.23 ±0.05 *	5.84 ± 1.49[a]	3.92 ± 1.01[a]	1.41 ± 0.21[a]	1703 ± 296.7	1307 ±296. 7	666.7 ± 88.19

*p< 0.05 9 c.f normal saline treated, ulcerated group (B); [a]p<0.05 c.f normal saline treated, no ulcer group (A). NA= not available

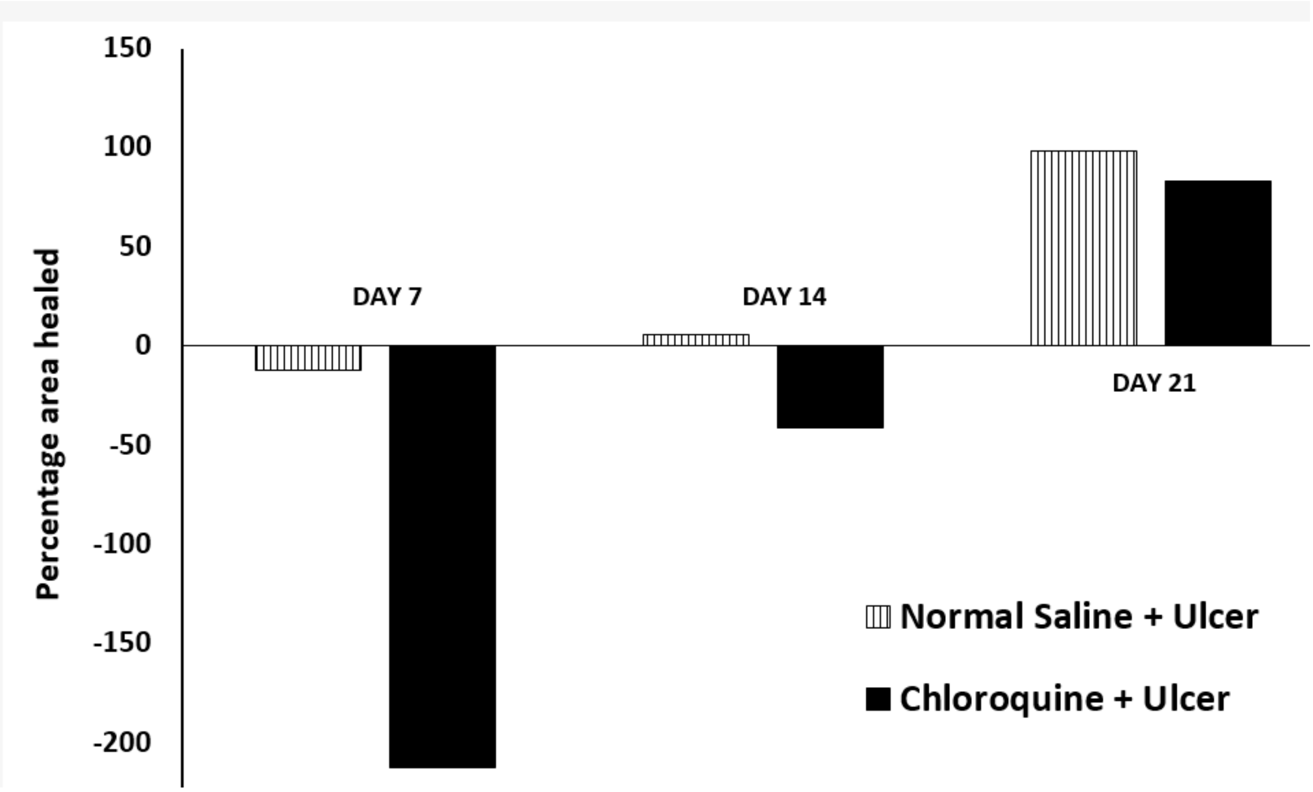

Figure 1
Percentage area of original ulcer healed after 7, 17 and 21 days of induction of acetic acid induced ulcer (calculated based on assumption of an initial circular ulcer with diameter of 10mm for each animal).

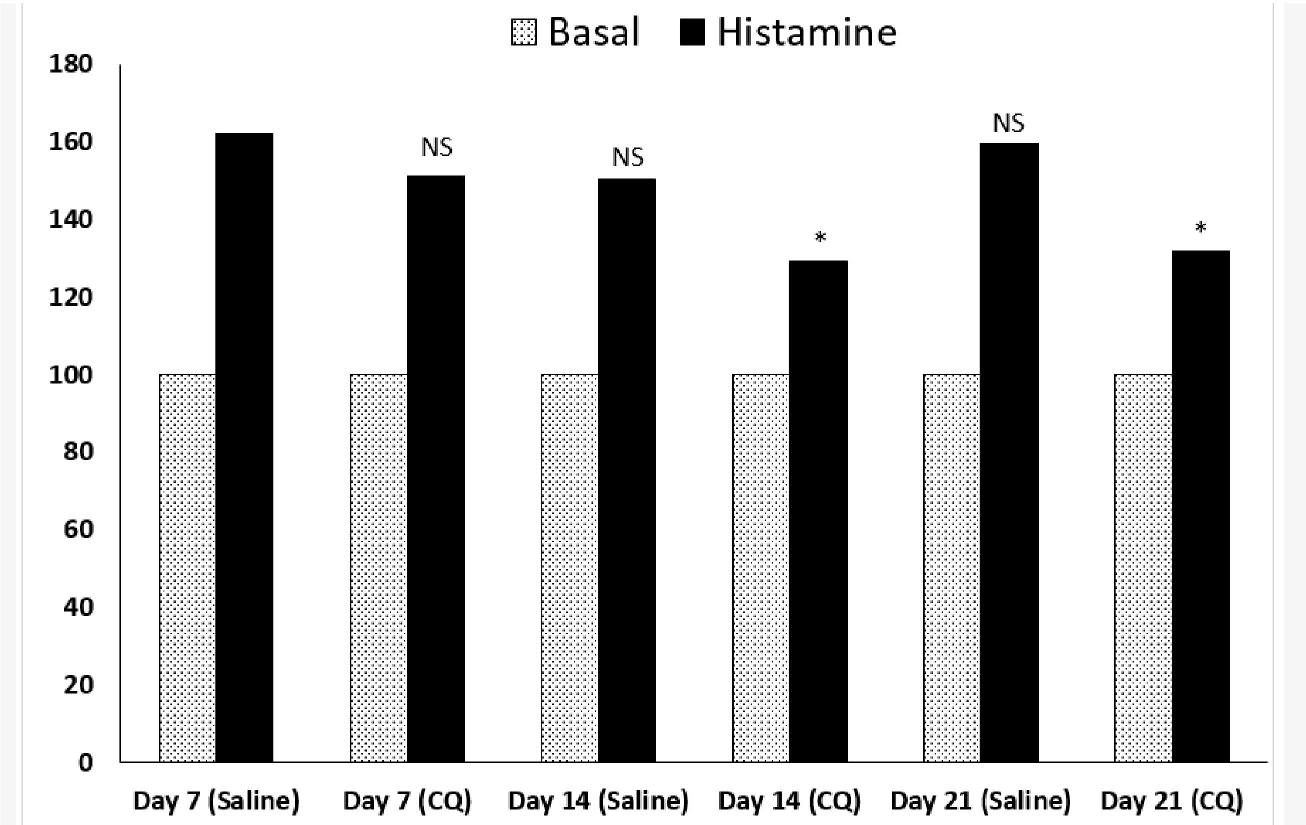

Fig. 2
Basal gastric acid secretion in normal and chloroquine treated rats after acetic acid induced ulceration. Each bar represents mean ± SEM of 5 rats per group

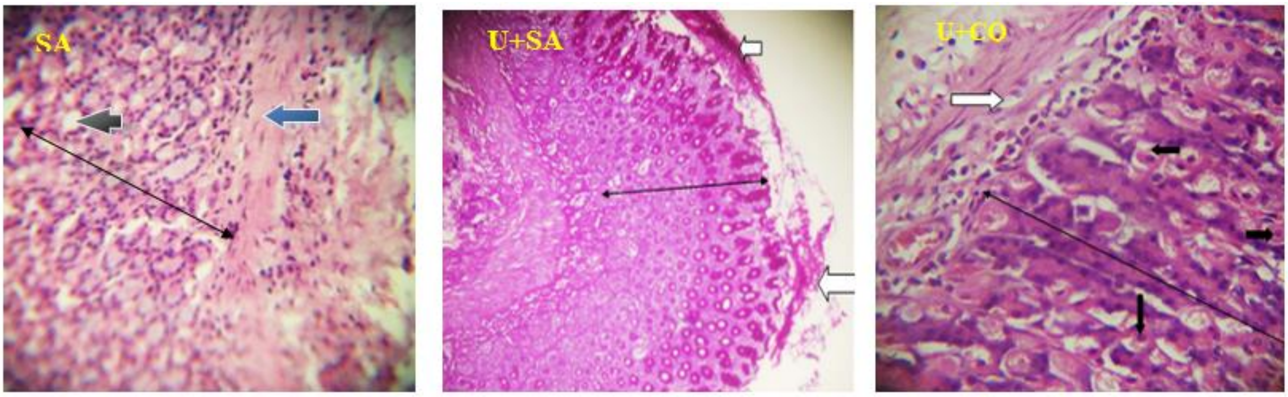

Plate 1:
Photomicrograph of a stomach section (Mag x 400; PAS staining) by day 7 showing C**ontrol (SA)** normal mucosa surface epithelial layer. The mucosa layer (spanned) shows no infiltration of inflammatory cells.the gastric gland and lamina propria appear normal and not infiltrated (black arrow). The submucosa layer (blue arrow) shows no infiltration of inflammatory cells. There is no ulcer, nor haemorrhage seen. The circular muscle appears normal (red arrow). **Ulcer and Saline treated** (**U+SA):** there is minimal suppurative inflammation at the submucosa level. The lamina propria are also infiltrated moderately by inflammatory cells consisting of lymphocyte, plasma cells, and few polymorphs. Parietal cells are present and appear normal. The PAS reaction shows both surface and intracellular mucin production. Parietal cell count = 350 cells/field10 fields counted = 3500 cells/field. **Ulcer and Chloroquine (3 mg/kg b.w) treated** (**U+CQ):** there is abundant surface mucin (white arrow) and intracellular mucin (slender arrow). There is an extensive mucin production in glandular mucus secreting cells (slender arrow). Spanned is the mucosa layer. There is also minimal inflammation in the submucosal layer (white arrow), there is no edema nor haemorrhage.. The parietal cells are present and appear normal (black arrow)

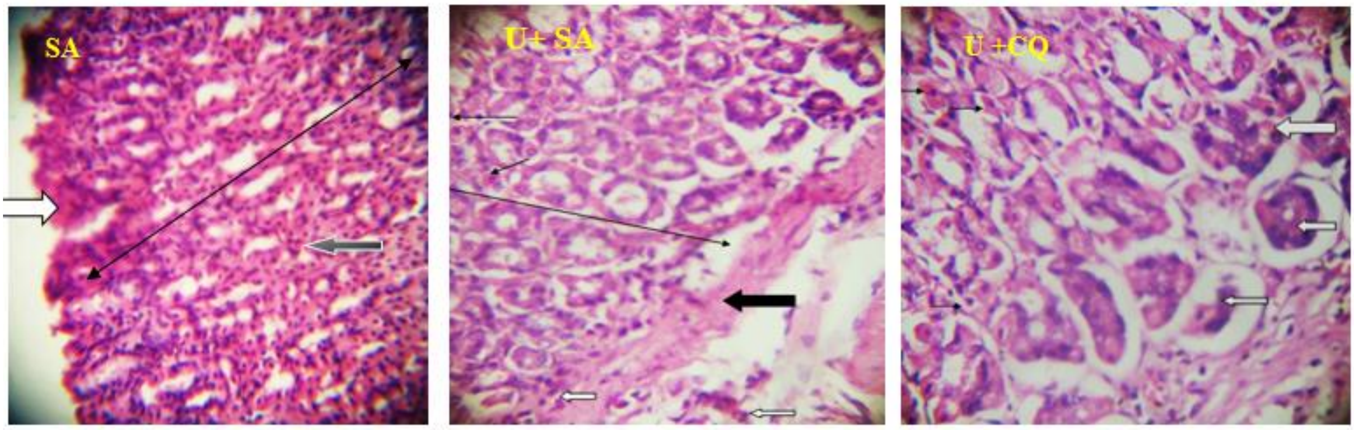

Plate 2:
Photomicrograph of a stomach section (Mag x 400; PAS stain) by day 14 showing **Control (SA**) normal mucosa surface epithelial layer (white arrow). the mucosa layer (spanned) shows no infiltration of inflammatory cells.the gastric gland and lamina propria appear normal and not infiltrated (black arrow). the submucosa layer (blue arrow) shows no infiltration of inflammatory cells. there is mild haemorrhage seen below the submucosa layer**. Ulcer + Saline treated (U+SA):** There is infiltration of the submucosa and mucosa layer by inflammatory cells, including lymphocyte, plasma cells, and polymorphs. There is evidence of oedema at the submucosa level. Parietal cells are present and normal. The PAS reaction shows abundant surface and intracellular mucin production. No ulceration, parietal cells are present and normal. Parietal cell count = 401 cells/field 10 field counted = 4010 cells**. Ulcer and Chloroquine (3mg/kg b.w) treated (U+CQ):** The stomach section shows minimal inflammation mostly seen at sub mucosal region, but sparingly within lamina propria. There is focal regeneration process involving all the glands of the gastric epithelium. The parietal cells are present and are normal. The PAS reaction shows mucin production limited to intracellular. There is no epithelium surface mucin. Parietal cell count = 392 cells/field.10 field counted = 3920 cells

DISCUSSION

Gastric ulcer is a deep defect in the gastric wall penetrating the entire mucosal thickness and the muscularis mucosa (Tarnawski, 2000). The healing of gastric ulcer involves reconstruction of mucosal architecture and the filling of its defects with epithelial and connective tissue cells. Ulcer healing process includes inflammation, tissue formation (granulation tissue formation, angiogenesis, and re-epithelialization), and tissue remodeling (Tarnawski 2000). In this study, it was observed that groups treated with chloroquine had elevated gastric ulcer score implying a delay in the wound closure. It might probably be that chloroquine slowed down certain stages of gastric ulcer healing or exert inhibitory signals on certain mediating gastric ulcer healing cells.

Suppression of gastric acidity has long been known to be a major requirement for healing in an injured stomach. There are conflicting views on the role played by chloroquine as regards gastric acidity and secretions: Etimita et al., (2005) reported that chloroquine is a weak stimulant for gastric acidity as it competes and binds to the histamine H-receptors. However, Ajeigbe et al., (2008 a and b) reported that chloroquine potentiates gastric secretions as well as acidity via the H2 receptors and Muscarinic receptors thus aggravating ulceration as it increases gastric pH and volume during gastric ulceration.

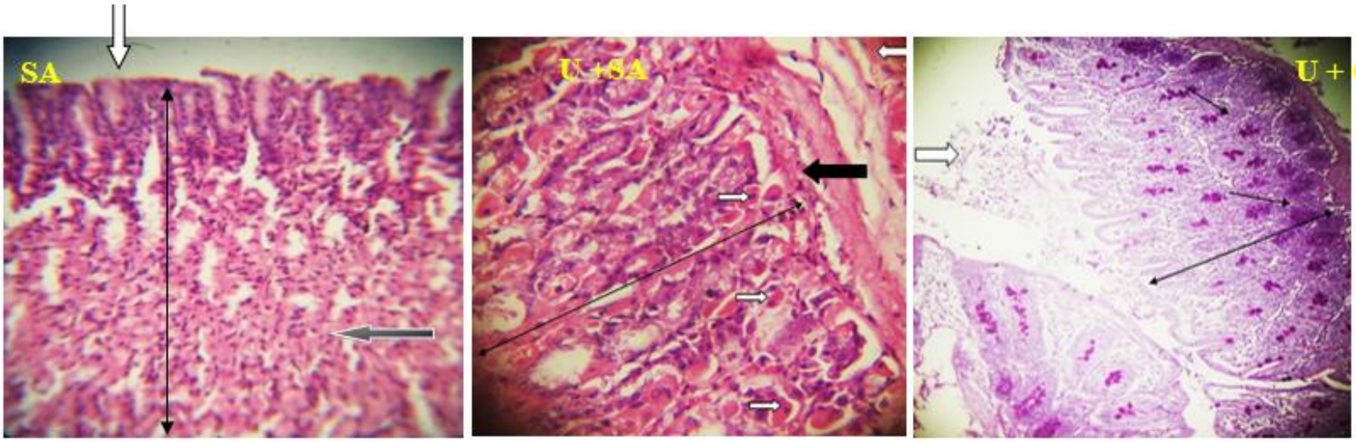

Plate 3:
Photomicrograph of a stomach section (Mag x 400; PAS stain) by day 21 showing **Control (SA)** normal mucosa surface epithelial layer (white arrow). The gastic pit appear normal(slender arrow). The mucosa layer (spanned) shows no infiltration of inflammatory cells.the gastric gland and lamina propria appear normal and not infiltrated (black arrow). The submucosa layer (blue arrow) shows no infiltration of inflammatory cells. There is no ulcer, nor haemorrhage seen. The circular muscle appears normal (red arrow). **Ulcer + saline treated** (**U+SA):** There is moderate to severe infiltration in the submucosa, and lamina propria. The parietal cells are present, and abundant. There is no haemorrhage seen. No ulceration. Parietal cell count 596 cells/field. 10 fields counted = 5960 cells **Ulcer + Chloroquine (3mg/kg b.w) treated (U+CQ):** The stomach section shows lymphoid aggregate with prominent germinal centre. There is moderate infiltration of lamina propria by inflammatory cells consisting of majorly lymphocytes, plasma cells, and few polymorphs. Parietal cells are lacking, however there is prominent regeneration process involving all the glands of the gastric epithelium. The PAS reaction shows focal surface mucin as well as intracellular mucin productions. Parietal cell count is equal to or less than 2/ field

In this study, gastric secretion was enhanced in the chloroquine treated groups by days 7 and 14 but delineated by day 21 of gastric ulcer healing. Observations from this study by days 7 and 14 on basal and histamine stimulated gastric secretions where in line with those of Ajeigbe et al., 2008a and b. However, a reversal of trend in gastric secretion of the chloroquine treated group by day 21 might probably imply that chloroquine has an inhibitory effect on gastric secretion when administered over a long period and is worthy of further investigation.

There were increased parietal cell counts in the chloroquine treated groups both on days 7 and 14 post ulceration. However, this might have been responsible for the observed delay in the closure of gastric ulcer as increased parietal cell count is directly proportional to increase in gastric acid thus resulting in erosion of the gastric mucosa (Goodman and Gilman, 2005, Brunton et al., 2005). However, by day 21 of gastric ulcer healing, it was observed that there was an appreciable decrease in the parietal cell count which probably accounted for the reduced gastric secretion hence increased gastric ulcer healing rate. It may be that prolonged administration of chloroquine have inhibitory activities on gastric mucosa cells (parietal cells) during chronic gastric ulcer healing.

In the course of this study it was observed that chloroquine caused elevated levels of lipid peroxidation probably as a result of increased production of free radicals or the inability of chloroquine to suppress generation of oxidative stress. This elevated level of lipid peroxidation is in support of earlier findings by Ajeigbe et al., (2012). Free radicals have been documented to exert adverse effect on healing of gastric ulcers hence prolonging or hindering its closure (Perry et al., 1986, Ajeigbe et al., 2008a and b). It might very well be that the delay in chronic gastric ulcer healing observed in the chloroquine treated group might have been as a result of a synergistic activity of increased gastric secretion, parietal cell count and lipid peroxidation.

Studies in progress are aimed at explaining the complex activities of chloroquine on the gastric mucosa (and its cells) during chronic gastric ulcer healing. Observations from this preliminary study revealed that prolonged administration of chloroquine might exert inhibitory activities on parietal cells. This study also reveals that chloroquine delays chronic gastric ulcer healing in rats.

REFERENCES

Adeniyi O.S, Emikpe B.O., Olaleye S.B (2014). Gastric Mucosa Re-epithelisation, Oxidative Stress and Apoptosis During Healing of Acetic Acid -Induced Ulceration in Thyroxine Treatment and Thyroidectomy on Rats. J. Afr. Ass. Physiol. Sci. 2 (1): 57-66

Ajeigbe, K.O., Olaleye, S.B., Nwobodo, E.O. (2008a). Effects of Amodiaquine Hydrochloride and Artemisinin on Indomethacin induced Lipid peroxidation in rats. Pak. J. Biol. Sci., 11 (17): 2154-2158.

Ajeigbe, K.O., Nwobodo, E.O., Oyesola, T.O., Ofusori, D.A., Olaleye, S.B. (2008b). Chloroquine phosphate potentiates indomethacin and HCl/Ethanol-induced gastric mucosa injury in rats. Int. J. Pharmacol., 4 (6): 482-486.

Ajeigbe K.O., Emikpe B.O., Olaleye S.B. (2012). Augmentation of gastric acid secretion by
chloroquine and amodiaquine in the rat stomach. Niger J. Physiol,Sci. (27): 089-094.

Al Mofleh, I.A. (2010). Spices, herbal xenobiotics and the stomach: Friends or foes? World J Gastroenterol. 16 (22): 2710-2719.

Barth H, Lorenz W, Troidl H (1975). Effect of amodiaquine on gastric histamine methyltransferase and on histamine-stimulated gastric secretion..Br J Pharmacol; 55(3): 321–327.

Bertleff M.J., Lange J.F. (2010). Perforated peptic ulcer disease: a review of history and treatment. Dig Surg.;27(3):161-9..

Brunton, L.L., Lazo, J.S., Parker, L.K. (2005). Pharmacotherapy of gastric acidity, peptic ulcers and gastroesophageal reflux. In Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th Edition McGraw-Hill Companies. www.accessmedicine.com

Chen N., Wilson D.W, Pasay C et al. (2005). Origin and dissemination of chloroquine-resistant Plasmodium falciparum with mutant pfcrt alleles in the Philippines. Antimicrobial Agents and Chemotherapy 49 , 2102–2105.

Davies, R.E. (1951). The mechanism of Hydrochloric acid production by the stomach. Biol. Rev.26 (1): 87-120.

Dent J (2002). Gut. Management of reflux disease;50(Suppl 4): iv67–iv71.

Douglas W. B., Stephen G. C., Richard H. H (1990). Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression. Gastroenterology: Vol. 99,No.2 Pages 345–351.

Falade C.O (2014). Fighting a moving target: malaria the pains and the gains: an inaugural lecture delivered at the University of Ibadan on Thursday, 27 November, 2014.University of Ibadan Press. ISBN:9789788456650

Farombi, E.O., Olowu, B.I., Emerole, G.O. (2000). Effect of three structurally related antimalarial drugs on liver microsomal components and lipid peroxidation in rats. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 126 (3); 217-224.

Farombi, E.O. (2006). Genotoxicity of chloroquine in rat liver cells: Protective role of free radical scavengers. Cell Biology and Toxicology:22, Issue 3, pp 159–167.

Geibel JP (2005). Role of potassium in acid secretion. World J Gastroenterol;11(34):5259-5265

Ghosh, M.N., Schild, H.O. (1958). Continuous recording of acid gastric secretion in the rat. Brit. J. Pharm. 13: 54-61.

Goodman CA, Coleman PG, Mills AJ. (2000). Economic Analysis of Malaria Control in Sub-Saharan Africa . Geneva: Global Forum for Health Research.

Goodman, C.S., Gilman, B.T. (Eds.), Goodman and Gilman's:The Pharmacological Basis of Therapeutics, 10th ed. McGraw -Hill, New York,2007:.1005 –1019.

Happi CT, Gbotosho GO, Sowunmi A, Falade CO, Akinboye DO, Gerena L, Kyle DE, Milhous W, Wirth DF, Oduola AM (2004). Molecular analysis of Plasmodium falciparum recrudescent malaria infections in children treated with chloroquine in Nigeria. Am J Trop Med Hyg 70: 20-26.

Iskander FA., Ahmed YY., Nassar AM., Tantawy SA., Kamel ZM., Farsi JMA., Abdel-Fattah NA (1990). Experimental studies on the effect of Primaquine (antimalarial drug) on the gastric mucosa of albino rat. Sci. Med. J. Cai. Med. Synd. Vol 2.(2).pg 1-19

Landewe R., Miltenburg A., Verdonk M., Verweij C (1995). Chloroquine inhibits T-cell proliferation by interfering with IL-2 production and responsiveness. Clin. Exp. Immunol, 102:144-151

Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, Takala SL, Taylor TE, Plowe CV (2006). Return of chloroquine antimalarial efficacy in Malawi. N Engl J Med 355: 1959-1966

Magwere, T., Yogeshkumar, S.N., Julia, A.H. (1997). Effects of Chloroquine Treatment on Antioxidant Enzymes in Rat Liver and Kidney. Free Rad Bio.Med. 22(1-2): 321-327

Nneli R. O., Nwafia W. C., Oji J. O. (2007). Diets/dietary habits and certain gastrointestinal disorders in the tropics: a review. Nigerian journal of physiological sciences 22 (1-2): 1-13

Obaseiki-Ebor, E.E., Obasi, E.E. (1986). Aspects of Chloroquine mutagenicity. Mutat Res. 175(2):51-9.

Ogungbamigbe T O, Ojurongbe O, Ogunro P S, Okanlawon B M, Kolawole S O (2008).

Chloroquine resistant Plasmodium falciparum malaria in Osogbo Nigeria: efficacy of amodiaquine + sulfadoxine-pyrimethamine and chloroquine + chlorpheniramine for treatment. Mem Inst Oswaldo Cruz , Rio de Janeiro 103 (1):79-84

Ogunkunle O.O., Fehintola F.A., Ogungbamigbe T.O., Falade C.O. (2011). Comparative cardiac effects of chlorproguanil/dapsone and chloroquine during treatment of acute uncomplicated falciparum malaria infection in Nigerian childrenAfrican Journal of Biomedical research.; 14; 161 -167

Olaleye S.B, Ajeigbe K.O., Emikpe B.E (2012). Effect of Sulfadoxine-Pyrimethamine and Artesunate on Gastric Acid Secretion and Parietal Cell Mass in Rats. Afr. J. Biomed. Res.Vol.15; 23 - 28

Perry M.A., Wadhwa S., Parks D.A., Pickard W., Granger, D.N. (1986). Role of oxygen radicals in ischemia-induced lesions in the cat stomach. Gastroenterology 90: 362-367

Picot, SF., Peyron A., Donadille., Vuillez., Barbe G., Ambroise- Thomas (1993). Chloroquine- induced inhibition of the production of TNF but not IL-6, is affected by disruption of iron metabolism. Immunology, 80:127-133.

Schubert ML . (2005). Gastric secretion. Curr Opin Gastroenterol.;21(6):636-43.

Sylvester C.N., Uchenna I.O., Obienu, Neri P., (2012). Time trends of upper gastrointestinal diseases in Nigeria. Annals of Gastroenterology 25, 1-5

Tarnawski A. (2000). Molecular mechanism of ulcer healing. Drug News & Perspective 13:158-168.

Tarnawski, A., Szabo, I.L., Husain, S.S., Soreghan, B., (2001). Regeneration of gastric mucosa during ulcer healing is triggered by growth factors and signal transduction pathways. J. Physiol. Paris 95, 337– 344.

Toler SM (2004). Oxidative stress plays an important role in the pathogenesis of drug-induced retinopathy. Exp Biol Med (Maywood).;229(7):607-15.

Tulassay Z, Herszényi L. (2010). Gastric mucosal defense and cytoprotection. Best Pract Res Clin Gastroenterol. 24(2):99-108. doi: 10.1016/j.bpg.2010.02.006.

Ursos L.M., Roepe P.D. (2002). Chloroquine resistance in the malarial parasite, Plasmodium falciparum..Med Res Rev.;22(5):465-91.

Wang J.Y, Yamasaki S., Takeuchi K., Okabe S. (1989). Delayed healing of acetic acid induced gastric ulcers in rats by indomethacin. Gastroenterol. 96:393-402.

Wellems, T. E (1992). How Chloroquine works. Nature. 355, 6356: 108-109.

Wellems T.E., Plowe C.V., (2001). Chloroquine-resistant malaria..J Infect Dis.: 15;184(6):770-6.

WMA/APS (2002). Guiding principles for research involving animals and human beings. Am J. Physiol. Regul. Integr. Comp. Physiol., 283: R281-R283.

Yao, X., Forte, J.G. (2003). Cell biology of acid secretion by the parietal cell. Annu Rev Physiol 65:103-131