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Attenuation of Experimental Gastric Ulceration by Sulfadoxine-pyrimethamine in Albino Rats

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The aim of the present study was to investigate the effect of the therapeutic dose of sulfadoxine-pyrimethamine on experimentally induced gastric ulceration in albino rats. Rats were given sulfadoxine-pyrimethamine (22.5 mg kg^{-1}) intramuscularly for 24 h after formation of ulcers induced by indomethacin or by acidified ethanol. Upon sacrifice, colorimetric assays were applied to determine the concentration of protein and mucus, activities of catalase and superoxide dismutase and lipid peroxidation in homogenized gastric mucosal samples. Sulfadoxine-pyrimethamine was observed to alleviate gastric lesions produced either by indomethacin or acidified ethanol. Also, the drug seemed to attenuate the indomethacin or acidified ethanol induced effects on gastric juice volume, pH and acid output. On the other hand, thiobarbituric acid reactants (TBAR) was decreased and superoxide dismutase (SOD) and catalase (CAT) activities increased in the gastric mucosal samples, though, however, protein and mucus concentrations remained statistically unchanged. The use of sulfadoxine-pyrimethamine may be safe on the integrity of the stomach, especially in existing gastric ulcers. It ameliorates oxidative stress in the gastric mucosa caused by indomethacin and acidified ethanol.

Key words: Sulfadoxine-pyrimethamine, thiobarbituric acid reactants, superoxide dismutase, ulcer, oxidative stress

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INTRODUCTION

Sulfadoxine-pyrimethamine combination therapy is one of the most important second-line treatment of chloroquine-resistant *Plasmodium falciparum* infections (Peters, 1987). However, some countries have adopted its use as first-line treatment because of the high prevalence of chloroquine resistance (Bloland *et al*., 1993). Sulfadoxine-pyrimethamine is a synergistic combination of antifolate drugs that act against parasite-specific enzymes, dihydropteroate synthase and dihydrofolate reductase (Plowe *et al*., 1997). It has long been proved to be significantly more effective than chloroquine (Randrianasolo *et al*., 2004; Menard *et al*., 2007). Its high efficacy is established even as a constituent of either Artemisinin Combination Therapy (ACT) or Non-Artemisinin Combination Therapy (NACT) (Menard *et al*., 2007).

Despite challenges faced by the resistance of *Plasmodium falciparum*, sulfadoxine-pyrimethamine is still preferred in many parts of malaria endemic areas. This may be due to its affordable price (Bell and Winstanley, 2004) and less severe side effects (Nguoesse *et al*., 2001; Menard *et al*., 2007).

The prevalence of peptic ulceration has been shown to be high in Africa and Asia (Amure and Elegbe, 1975; Sonnenberg, 1985) which are also malaria endemic regions. Peptic ulcer results from increased formation of Reactive Oxygen Species (ROS) and/or decreased antioxidant reserve in the gastric mucosa, a condition termed as oxidative stress. Hence, its occurrence is associated with oxidative stress increases by pro-ulcerative factors in the gut like *Helicobacter pylori* (Yamaguchi and Kakizoe, 2001) use of non steroidal anti-inflammatory drugs (NSAIDs) (Rostom *et al*., 2000), smoking (Ma *et al*., 2000), psychological stress (Mawdsley and Rampton, 2006) and dietary intake of potential ulcerogens (Ibironke *et al*., 1997). Lipid peroxidation (LPO) a result of the reaction of oxyradicals and polyunsaturated fatty acids has been suggested as an attack factor in the gastric mucosa (Guo *et al*., 2005). Also glutathione, an endogenous sulfhydryl compound (Hung, 2000) and other antioxidative enzymes are important substances in the cellular defense system.

Current literature shows little or no attention drawn to possible role of the use and/or misuse of sulfadoxine-pyrimethamine as an antimalarial on the etiology of inflammatory disorders of the gastrointestinal system. Therefore, we present findings on the attenuation of the experimental gastric ulceration by therapeutic dose of sulfadoxine-pyrimethamine, especially in relation to the enzymatic antioxidant defense system in the stomach of the rat.

MATERIALS AND METHODS

This study was carried out, during the period July and August 2007, in the Department of Physiology, School of Basic Medical Sciences, Igbinedion University, Okada, Edo State, Nigeria.

Drugs: Sulfadoxine-pyrimethamine (FANSIDAR) and indomethacin were obtained from a local pharmacy registered by the Pharmacists' Council of Nigeria. All other reagents were of analytical grade and obtained from the British Drug Houses, Poole, UK.

Animals: Forty male healthy adult albino rats of Wistar strain weighing between 180 and 220 g were used in the study. The animals were housed under standard conditions of temperature (23±2°C), humidity (55±15%) and 12 h light (7.00 am-7.00 pm).

They were kept in wire meshed cages and fed with commercial rat pellets (Ladokun Feeds Ltd., Ibadan, Nigeria) and allowed water *ad libitum*.

Experimental design: The animals were divided into 5 groups of 8 rats each. Group 1 animals were treated with normal saline after 36 h fasting. Group 2 was treated orally with indomethacin dissolved in sodium bicarbonate (40 mg kg^{-1}) after 36 h fasting. Group 3 was treated with 1.0 mL HCl/ethanol mixture (0.15 N HCl in 70% ethanol). Group 4 and 5 received sulfadoxine-pyrimethamine intramuscularly (22.5 mg kg^{-1}) 4 h after indomethacin or acidified ethanol administration, respectively.

Ulcer induction and index determination

Indomethacin induced ulceration: Four hours later (for the treated group) and 24 h later (for the experimental group), the animals were killed with ether anaesthesia. The stomachs were opened along the greater curvature, washed in normal saline to remove debris and pinned on a cork mat for ulcer scoring. This was done by locating the wounds in the glandular regions under a simple microscope. The lengths (mm) of all the elongated black-red lines parallel to the long axis of the stomachs in the mucosa was measured. The ulcer index was calculated by adding the lengths of all the lesions in the glandular region of the stomach (Rifat-uz-Zaman and Khan, 2004; Tanaka *et al*., 1993). The wounds were assessed independently by three observers.

HCl/Ethanol induced ulceration: Thirty six hour fasted rats were given 1.0 mL HCl/ethanol mixture containing 0.15 N HCl in 70% ethanol (Anadan *et al*., 1999). Four hour later (for the treated control) and 24 h later (for the

experimental group), the animals were sacrificed under sodium pentobarbitone anaesthesia (60 mg kg^{-1} i.p.). The stomachs were removed, inflated with 10 mL of 2% formaldehyde for 10 min to fix the tissue walls and opened along the greater curvature. The hemorrhagic lesions were stretched out on a glass plate and their sizes were estimated using an underlying graph paper with a 1 mm^2 grid. Lesions areas were summed up per stomach and expressed as % of the total mucosal area.

Gastric juice volume, pH and acid output: Four hour gastric juice collection via., the pyloric cannula was drained into a graduated test tube and centrifuged at 2000 rpm for 10 min. The supernatant volume and pH were recorded. The total acid content of the gastric juice was also determined by titration to pH 7.0 with 0.05 N NaOH, using phenolphthalein as indicator.

The protein content was also estimated as described by Lowry *et al.* (1951).

Determination of lipid peroxidation: Lipid peroxidation was assayed by measuring the thiobarbituric acid reactants (TBAR) products using the procedure of Walls *et al.* (1976). The glandular portion of the stomach was scrapped and 1 g of the portion was suspended in 4 mL of ice cold physiological saline and homogenized. The homogenate was supplemented with 0.75 g L^{-1} TBA in 0.1 mol L^{-1} HCl. The reactants were then supplemented with 5 mL n-butanol-pyridine mixture, shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm min^{-1}. Absorbance was then read at 532 nm and the results expressed as nmol TBA per 100 mg wet tissue.

Determination of catalase activity: Activity of catalase in gastric mucosa was determined according to the procedure of Sinha (1972). This method is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate so produced is measured calorimetrically at 530 nm.

Determination of superoxide dismutase (SOD) activity: A method originally described by Misra and Fridovich (1972) as reported by Magwere *et al.* (1997) was employed. This method is based on the ability of superoxide dismutase to inhibit the autoxidation of epinephrine caused by O_2 generated by xanthine oxidase reaction.

Determination of gastric mucous: Adherent gastric glandular mucous was measured by the method of Corne *et al.* (1974). The excised stomachs were soaked for 2 h in 0.1% Alcian blue dissolved in buffer solution containing 0.1 M sucrose and 0.05 M sodium acetate (pH adjusted to 5.8 with hydrochloric acid). After washing the stomach twice in 0.25 M sucrose (15 and 45 min), the dye complexed with mucous was eluted by immersion in 10 mL aliquots of 0.5 M $MgCl_2$ for 2 h. The resulting blue solution was shaken with equal volumes of diethyl ether and the optical density of the aqueous phase measured at 605 nm using a spectrophotometer.

Using a standard curve, the absorbance of each solution was then used to calculate the various concentration of the dye and the weight of dye (expressed in mg). The weight of the dye was then expressed over the weight of the stomach.

Statistical analysis: The data obtained were expressed as Mean±SEM (Standard Error of Means of eight observations) and analysed statistically by application of the Statistical Package for Social Sciences (SPSS).

The student's t-test was applied and p-values were determined. Differences were considered significant at $p<0.05$ and highly significant at $p<0.001$.

RESULTS

Development of gastric lesions: Indomethacin and acidified ethanol caused severe damage to the stomachs of the rats. The mean ulcer score of the indomethacin and acidified ethanol treated animals is 8.58±1.44 mm and 7.15±0.55%, respectively. On administration of sulfadoxine-pyrimethamine, the severity of the lesions was decreased (Table 1, $p<0.05$). Also shown in the Table 1 is the result of protein and mucous concentration of the glandular stomach. Although protein and mucous content markedly decrease in response to indomethacin

Table 1: Effects of sulfadoxine-pyrimethamine treatment on indomethacin- and acidified ethanol-induced gastric mucosa injury in the rat

Groups	Treatment	Ulcer score (mm; % of total mucosal area)	Gastric mucus (mg g^{-1})	Protein (mg g^{-1})
1	Saline	0.00	33.50±0.95	10.60±0.52
2	Indomethacin (40 mg kg^{-1})	8.58±1.44**	30.25±0.70*	8.20±0.15*
3	Indomethacin (40 mg kg^{-1})+sulfadoxine-pyrimethamine (22.5 mg kg^{-1})	7.47±0.52*	29.89±0.95	7.95±0.60
4	Acidified ethanol (1 mL of 0.15 N HCl in 70% ethanol)	7.15±0.55**	30.50±0.65*	8.52±0.21*
5	Acidified ethanol (1 mL of 0.15 N HCl in 70% ethanol)+ sulfadoxine-pyrimethamine (22.5 mg kg^{-1})	5.54±0.25*	30.75±0.55	8.25±0.41

Mean±SEM, n = 8, Indomethacin: Highly significant from normal saline treated $p<0.001$**, significant $p<0.05$, Sulfadoxine-pyrimethamine: Significant $p<0.05$*, from indomethacin-and acidified ethanol-treated

Table 2: Gastric juice profile of saline, indomethacin, acidified ethanol and sulfadoxine-pyrimethamine treated rats

Groups	Treatments	Volume (mL)	pH	Acid output (x10^{4}mmol/4 h)
1	Saline	3.50±1.20	2.51±0.51	8.10±0.25
2	Indomethacin (40 mg kg^{-1})	6.20±0.75*	1.50±0.20*	11.05±0.16**
3	Indomethacin (40 mg kg^{-1})+sulfadoxine-pyrimethamine (22.5 mg kg^{-1})	5.00±0.10*	2.26±0.05*	9.12±0.55*
4	Acidified ethanol (1 mL of 0.15 N HCl in 70% ethanol)	8.35±1.05**	2.50±0.40	10.15±0.30*
5	Acidified ethanol (1 mL of 0.15 N HCl in 70% ethanol)+ sulfadoxine-pyrimethamine (22.5 mg kg^{-1})	6.10±0.57*	2.60±0.10	7.95±0.55*

Mean±SEM (n = 8). Indomethacin: Highly significant from normal saline treated p<0.001**, significant p<0.05*. Sulfadoxine-pyrimethamine:Significant p<0.05* from indomethacin- and acidified ethanol-treated

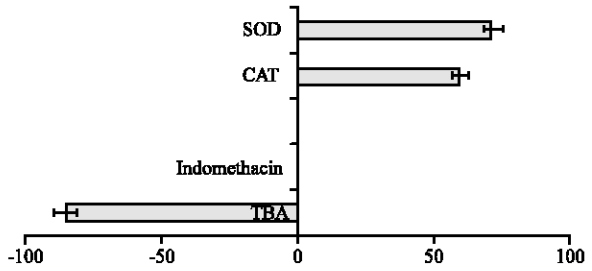

Fig. 1: Thiobarbituric acid (TBA) reactants; 85.0% decreased, superoxide dismutase (SOD); 71.5% increased and catalase (CAT); 60.0% increased activities in ulcerated (indomethacin) gastric mucosa treated with sulfadoxine-pyrimethamine (Mean±SEM, n = 8) (p<0.05)

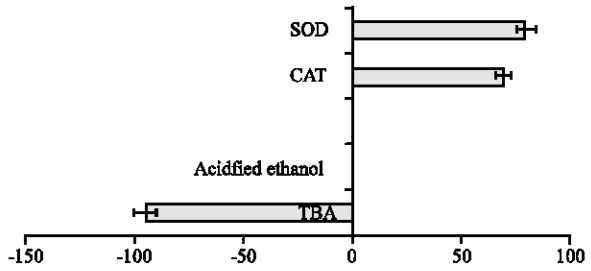

Fig. 2: Thiobarbituric (TBA) reactants; 95.0% decreased, superoxide dismutase (SOD); 80.5% increased and catalase (CAT); 70.0% increased activities in ulcerated (acidified ethanol) gastric mucosa treated with sulfadoxine-pyrimethamine (Mean±SEM, n = 8) (p<0.05)

and acidified ethanol pre-treatment, it remained statistically unchanged after sulfadoxine-pyrimethamine administration.

Gastric juice profile: Gastric secretion volume, pH and acid output were all increased significantly by indomethacin and acidified ethanol. However, these gastric changes were attenuated on administration of sulfadoxine-pyrimethamine as shown in Table 2.

Lipid peroxidation and anti-oxidative enzymes: Figure 1 and 2 show the lipid peroxidation, catalase and superoxide dismutase activities in the ulcerated gastric mucosa after sulfadoxine-pyrimethamine treatment, expressed as percentage of the control value.

Lipid peroxidation is measured as the amount of TBA reactants in the gastric mucosa. In the indomethacin treated control animals, it was 620±71 mmol g^{-1} tissue. sulfadoxine-pyrimethamine significantly decreased gastric TBA reactants level to 85.0±5.3% of the control value. Similarly, in the acidified ethanol treated control animals lipid peroxidation was 640±37 mmol g^{-1} tissue; which was significantly decreased on sulfadoxine-pyrimethamine administration to 95.0±5.5% of the control value.

On the other hand, sulfadoxine-pyrimethamine administration increases gastric mucosal SOD activity, which averaged 320±37 and 351±20 nkat g^{-1}, to 71.5±5.1% of indomethacin-treated control value and 80.5±5.7% of acidified ethanol treated control value, respectively.

Similarly, sulfadoxine-pyrimethamine increases gastric mucosal catalase activity to 60.0±4.5% and 70.0±5.3% of indomethacin and acidified ethanol control values, respectively.

DISCUSSION

The findings of the present study show that sulfadoxine-pyrimethamine, an important drug used in the chemotherapy of malaria in the tropics, was able to ameliorate existing gastric ulcers in albino rats. Earlier studies have implicated other commonly used antimalarials like chloroquine phosphate and amodiaquine hydrochloride in the etiology of gastric ulcers (Ajeigbe *et al*., 2008a, b). Interestingly, however, artemisinin, the most rapidly acting and potent antimalarial (Nosten *et al*., 2000) inhibits experimentally-induced gastric lesions in albino rats (Ajeigbe *et al*., 2008b; Olaleye *et al*., 2008). This depicts somewhat gastroprotective nature of sulfadoxine-pyrimethamine and artemisinin and suggest the safety of the drugs on the integrity of gastric mucosal architecture at therapeutic dose.

Gastrointestinal wall integrity is known to be controlled by two opposing forces: aggressive forces and

the defensive force (Corne *et al*., 1974). The aggressive force encompasses the increase in acid output and subsequent lipid peroxidation, which is a result of the reaction of oxyradicals and polyunsaturated fatty acids. The defensive force are gastro protective and involve anti-oxidative enzymes; SOD which catalyses the dismutation of superoxide radical anion (O_2^-) into less noxious hydrogen peroxide (H_2O)$_2$ and CAT or glutathione peroxidase that inactivate H_2O_2 by the degradation into water (Masuda *et al*., 1995).

Indomethacin and acidified ethanol has been known to cause lipid peroxidation (Kapui *et al*., 1993; Anadan *et al*., 1999) with depletion of endogenous antioxidants. In the present study, lipid peroxidation, as measured by the amount of TBA reactants, was decreased on sulfadoxine-pyrimethamine administration. This implies that sulfadoxine-pyrimethamine inhibits the lipid peroxidation/apoptosis activity of indomethacin and acidified ethanol. Also, acid output was significantly decreased in both the indomethacin and acidified ethanol pre-treated rats. It is well known that gastric acid secretion plays a role in gastric ulcer; which explains the mechanism of action of many anti-ulcer drugs (Schmassmann,1998).

Moreover, depletion of the antioxidant reserve in the gastric mucosa is an important factor in the pathogenesis of peptic ulceration. Hence, increase in superoxide dismutase and catalase in the present study underscore the gastroprotective tendencies of sulfadoxine-pyrimethamine, because they are known as scavengers which mop up free radicals predisposing the stomach to inflammation. But, however, there is no appreciable difference in protein and mucus concentration on administration of the antimalarial. The reason for this is unclear. Further studies are still needed to investigate the role of other anti-oxidative enzymes on the amelioration of gastric ulcers by sulfadoxine-pyrimethamine.

Conclusively, the results of the present study show that there is attenuation of ulceration in the stomach of rats treated with sulfadoxine-pyrimethamine. If these findings are extrapolated to humans, they suggest that use of sulfadoxine-pyrimethamine as an antimalarial may be safe on the integrity of the stomach especially in peptic ulcer prone individuals.

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