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A STUDY OF ULCER INDUCED STOMACH OF ALBINO RATS TREATED WITH IDOGU MIXTURE, A POTENT ANTI-ULCER HERBAL DRUG

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ABSTRACT

Aim: The aim of this study was to assess the potency of the anti-ulcer drug, Idogun on the stomach of ulcer induced albino rats.

Method: Six groups of locally bred albino rats were used. Each group containing five rats of either sex were orally treated respectively with distilled water (ulcer control group), omeprazole 20mg/kg (reference group) and 100, 200, 400 and 800mg/kg extract of Idogun anti –ulcer mixture (experimental group). Rats were sacrificed one hour after oral administration of 1ml absolute ethanol to induce injury in the gastric mucosa.

Results: Tissue sections from ulcer induced rats contained haemorrhage, dense infiltration by mixed inflammatory cells and extensive ulceration. These features were absent in the ulcer induced rats that were subsequently treated with the Idogun mixture. Mild tissue edema and presence of inflammatory cells were observed in the omeprazole treated section.

Conclusion: Idogun anti-ulcer herbal mixture is a potent anti-ulcer drug.

Key words: Idogun mixture, Stomach ulcer, Herbal

INTRODUCTION

The stomach is a very important digestive organ with unique physiology, biochemistry, immunology and histology. All ingested materials, including our nutrition, first have to negotiate this organ hence it is the most important segment within the gastro intestinal tract. This saccular organ has with two surfaces, two curvatures and two orifices and may be divided into seven different sections namely; Cardia, Fundus, Antrum, Pylorus, Lesser curve, Greater curve and the Angularis (Harold and Vishy, 2006; Hunt *et al*., 2015). Stomach ulceration or lesion is a breach in the protective lining (mucosa) of the digestive tract caused by digestion of abnormally high concentration of pepsin and acid or other mechanism which reduces normal protective mechanism of the mucosa. It can also been seen as a break (open wound) in the wall or epithelial lining of the stomach, the duodenum or occasionally the lower oesophagus resulting from digestive action of the gastric juice and or possibly bacterial and mechanical action (Elizabeth, 2010). Without adequate treatment, lesions can result in serious complications such as bleeding, perforation, vomiting of blood (hematemesis), swelling and scarring which cause narrowing and obstruction (Hunt *et al*., 2015). The symptoms of peptic ulcer are abdominal pain (stomach ulcer- pain at meal times and duodenal ulcers pain at three hours after meal), bloating and abdominal fullness, nausea and copious vomiting, loss of appetite and weight loss, foul smelling faeces (melena) and acute peritonitis (Bhat and Sriram, 2013). Stomach ulcers can be diagnosed by Barium contrast x-rays, Esophagogastro-duodenalscopy (EGD)/ gastroscopy, urea breath test for *H. pylori*, direct culture from an EGD biopsy specimen, rapid urease test, measurement of antibody levels in the blood, stool antigen test and histology of

biopsy and treated by reduction of hostile factors, augmentation of protective factors – Antacids, H2 receptor antagonists, inhibition (Omeprazole, Lansoprazole), Surgery and avoidance of chronic use of NSAIDS (Guyton, 2000; Falcao *et al.,* 2008). However, all these only promote healing of ulcers but do not cure ulcer diseases and are also known to have various adverse effects on patients like arrhythmias, impotence, gynaecomastia, arthralgia, hypergastronemia and haemopoetic changes (Akther *et al*., 1992). The use of herbal therapy has been known to have little or no adverse effect as plants have been selected and used as drugs over centuries (Salkat *et al.,* 2009). Plants have great potential to treat both human and livestock diseases (Alawa *et al.,* 2003). Over the past two decades, there has been a tremendous increase in the use of herbal medicine. The pharmacological treatment of diseases began long ago with the use of herbs (Schulz *et al.,* 2001). In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs (Shaw, 1998). In this study, an anti-ulcer herbal concoction used for the cure of stomach ulcers in Southwest Nigeria, Idogun mixture is under focus and as prepared by the herbal practitioner, it contains; whole plant of *Ageratum conyzoides*, roots of *Vernonia amygdalina*, roots *Citrus aurantifolia*, palm oil, palm kernel oil, shea butter, 'otubuyo' (lime juice and ash) and goat meat

MATERIALS AND METHODS

Collection and Extraction of Idogun Mixture

Idogun anti-ulcer drug as prepared by the herbal practitioner contains; whole plant of *Ageratum conyzoides*, roots of *Vernonia amygdalina and Citrus aurantifolia*, palm oil, palm kernel oil, shea butter, lime juice, ash and protein from goat meat. These were collected from farms in Idogun and Owo, Ondo State, Nigeria. About 2000g of the Idogun mixture was dissolved in 15 litres of 70% alcohol, left at room temperature for 3 days and filtered using Whatman filter paper (No 1). The filtrate was concentrated at 40^0C in a water bath for 2 weeks. The extract was packed in brown bottles and stored in the refrigerator. The extract was reconstituted in distilled water for the experiment

Preparation of Test Animals

The animals (male and female rats) used were bred locally in the animal house of the Department of Anatomy, University of Benin, Benin City. They were also acclimatized for two weeks in the animal house. Animals were housed in standard plastic cages, fed with standard pellet diet (Bendel Feeds and Flour Mill, Limited, Ewu, Nigeria) and allowed free access to water. All animals received humane care in accordance with international guidelines (National Research Council of the National Academics, 2011).

Study Design

The study was carried out to confirm the potency of Idogun anti- ulcer mixture on ethanol-induced gastric mucosal ulcers in rats. 6 groups of locally bred albino rats were used. Each was orally treated respectively with distilled water (ulcer control group), omeprazole 20mg/kg (reference group) and 100, 200, 400 and 800mg/kg extract from idogun anti–ulcer concoction (experimental group). Rats were sacrificed one hour after oral administration of 1ml absolute ethanol to induce injury in the gastric mucosa.

Acute toxicity Assessment

The oral median lethal dose (LD_{50}) was carried out with six groups of six mice. Mice of both sexes were randomly distributed into each of the groups and were deprived of food overnight. The control group was given 2ml of distilled water while others received doses as follows: 1g/kg, 2g/kg, 4g/kg, 6g/kg and 8g/kg. After the administration of the extracts, the mice were observed for death, and symptoms of toxicity within three days initially and then for 30minutes each day for another seven days. Signs and symptoms observed include; fast breathing and reduced locomotion. The LD_{50} result for both extracts was then calculated using arithematic formula specified by Angalabiri-Owei and Isirima (2014).

Histopathological Investigation

The tissue specimens were fixed in neutral buffered formalin and processed in Thermo Scientific Spin Tissue Processors STP 120. The tissues were treated for half hour each in three baths of 50% alcohol and were transferred into three baths of 80/20 Ethanol/IPA for half hour each and later treated in three baths of neat Isopropyl alcohol (IPA) for 1 hour each.The samples were allowed to drain for 2 hours before they were immersed in two baths of cell path wax at 56°C for 1½ hours each (Wallington and Drury, 1979).

RESULTS

Results for the acute toxicity testing of Idogun concoction showed death and signs of toxicity at doses 6g/kg and 8g/kg respectively and LD_{50} was calculated as 7g/Kg. Gross appearance of stomach of rats treated with Idogun anti-ulcer concoction as presented in Fig 1 showed perforation for control rats, red coloration for rats dosed with 100mg/kg of the herbal concoction and normal coloration for higher doses of the mixture. Fig 2 is a chart showing the ulcer count for Idogun anti-ulcer concoction.

Histopathological photomicrographs of the stomach of ulcer induced albino rats of this study showed hemorrhage, dense infiltration by mixed inflammatory cells, extensive ulceration (Plate 1) for control rats. Ulceration was seen (Plate 2) in rats treated with 100mg/kg of the herbal concoction and sections treated with higher doses were relatively normal with no ulceration seen but showing mild inflammation at doses 400mg/kg and 800mg/kg (Plates 3-5). Mild tissue edema and presence of inflammatory cells were detected in the omeprazole treated section (Plate 6).

Table 1: Acute toxicity (LD_{50}) test of Idogun drug

GROUP	DOSE	DOSE DIFF	MICE PER GROUP	NO DEAD	MEAN DEAD	SIGNS OF TOXICITY	DOSE DIFF X MEAN DEAD
1	CONT	-------	6	0	0	NONE	0
2	1	1	6	0	0	NONE	0
3	2	1	6	0	0	NONE	0
4	4	2	6	0	0	NONE	0
5	6	2	6	2	1	Calm, fast breathing, reduced locomotion	2
6	8	2	6	2	2	Calm, fast breathing, reduced locomotion, death	4
						SUM	6

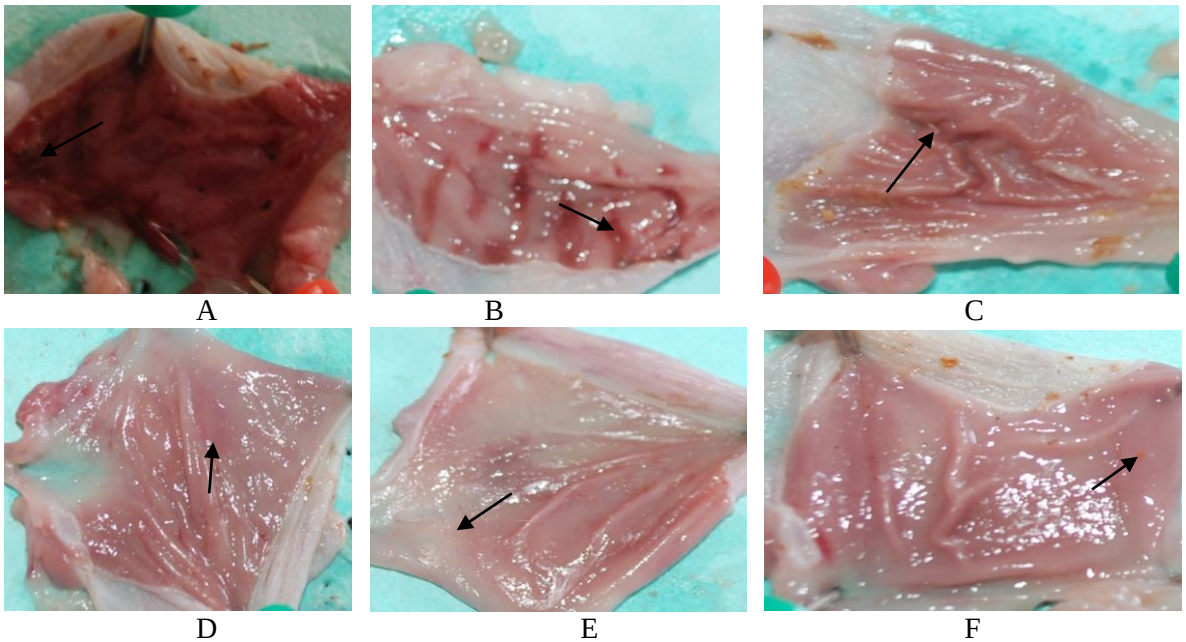


Fig 1. Gross appearance of stomach of rats treated with Idogun anti-ulcer mixture and Control. Arrow; (A, Control) perforation, (B, 100mg/kg) red coloration (C-F, 200-800mg/kg) normal coloration. A-F (Control, 100, 200, 400, 800mg/kg Idogun anti-ulcer mixture and 20mg/kg omeprazole groups respectively)

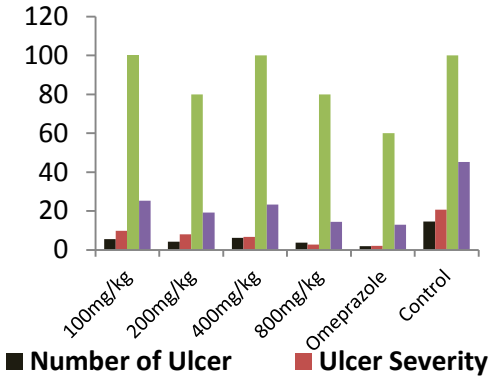
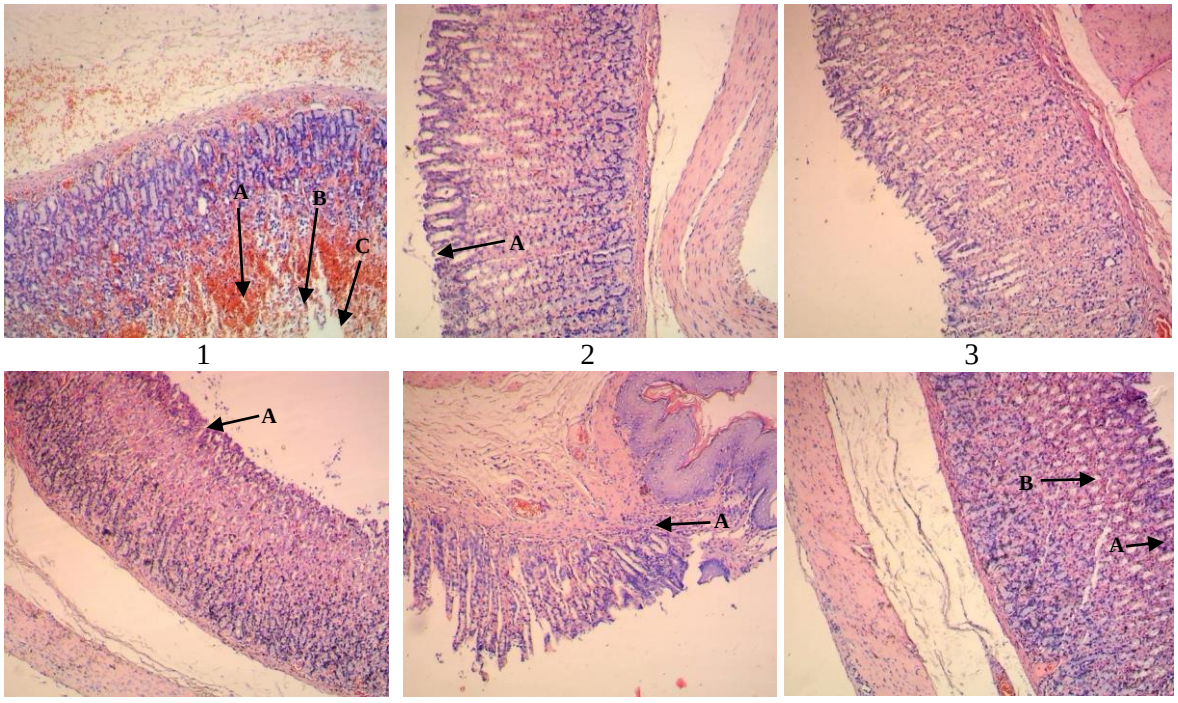


Fig 2.0: Ulcer count –Idogun anti-ulcer concoction



PLATES 1-6: GASTRIC ANTRUM (1) A, Hemorrhage B, Dense infiltration by mixed inflammatory cells C, Extensive ulceration (2) A, Ulceration (3) Normal (4) A, Inflammation - Eosinophil and Neutrophil (5) A, Inflammation (6) A, Mild tissue edema B, Inflammatory cells 1-6 (Control, 100, 200, 400, 800mg/kg Idogun concoction and 20mg/kg Omeprazole groups respectively). H&E x400

DISCUSSION

Peptic ulcers are caused when the natural balances between the aggressive factors of acid, pepsin, defensive mechanism of mucus, bicarbonate, mucosal turnover and blood supply are disturbed (Piper and Stiel, 1986). A number of synthetic drugs are available to treat ulcer. However, they are expensive and capable of producing some side effects like arrhythmias, impotence, gynaecomastia, arthralgia, hypergastronemia and haemopoetic changes (Akther *et al*., 1992). With the present situation of economic decline, traditional medicine is enjoying an enviable patronage. It has maintained greater popularity all over developing countries and the use is rapidly on the increase. In this study, an anti-ulcer herbal conconction used for the cure of stomach ulcers in southwest Nigeria Idogun mixture is under focus. The acute toxicity of the herbal mixture was determined by the calculating the LD_{50} i.e. the dose that will kill 50% of animals of a particular species. LD_{50} has remained a useful tool in safety assessment of substances

(Shivaranda *et al.,* 2009). There were no changes in animals administered with dose of between 1g/kg and 4g/kg of the drugs. The animals that were given 6g/kg showed signs of toxicity they became calm, breathing became very fast and their locomotion was reduced. At dose 8g/kg two out of six animals died. Absence of up to 50% of animal death at oral dose of 8g/kg body weight suggests that Idogun herbal mixture may be largely safe by oral route. Idogun herbal mixture traditional doctors give 30ml of the drug to adult patients in a day i.e. around 1.5g of the local drug in a day. The gross evaluation of gastric lesions showed that rats pre-treated with Idogun concoction prior to administration of ethanol had significant gastric mucosal protection from deleterious effect of the absolute alcohol. The proposed mechanism of action might be; inhibition of apoptosis and necrosis, prevention of oxidative stress which may occur during inflammatory process, amelioration of mucosal architecture due to significant reduction of myeloperoxidase activity, production of tumour necrosis factor alpha, up-regulation of intercellular adhesion molecules and formation of impervious layer over mucosal lining (Di Carlo *et al*., 1999; Bashir *et al*., 2014). Ulcer severity and ulcer count was greatly reduced in Idogun anti-ulcer concoction treated rats as the mean ulcer severity in the herbal mixture was comparable with what was obtained with omeprazole even at 100mg/kg. At 400g/Kg of idogun herbal concoction there was the flattening of the mucosa lobes of the rats treated with them suggesting protection of the site from the effect of absolute alcohol and suppression of formation of ulcers. This is similar to the work of Abidemi *et al*. (2012) who observed that DAS-77 (dried bark of *Mengifera indica* and root of *Carica papaya*) at the peak value of 400mg/kg produced ulcer inhibition values of 98.57% in ethanol-induced rats compared to 100% for Misoprostol. Histological sections of gastric mucosa of rats treated with Idogun anti-ulcer herbal concoction at doses 200mg/kg, 400mg/kg and 800mg/kg showed no ulceration when compared to rats in control group which showed extensive ulceration of the gastric mucosa while rats in omeprazole group had their gastric mucosa protected from ulceration. Similarly, the gross examination of the gastric walls of rats in control group in Abdulla *et al*. (2010) exhibited severe mucosal injury whereas pre-treatment with Cantella asciatica showed protection of gastric mucosa.

CONCLUSION

The study supports the claim of the potency of Idogun mixturen in the management of peptic ulcer.

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