Research Article

Histological and Histomorphometric Studies of the Effects of Aqueous Leaf Extracts of Ficus Exasperata Vahl (Linn) In Indomethacin-Induced Gastroduodenal Ulcer in Adult Wistar Rats

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1. Abstract

1.1. Objectives

This study was designed to examine the histological and histomorphometric changes in the stomach and duodenum of Wistar rats following indomethacin-induced gastroduodenal lesion and treatment with aqueous extract of F.exasperata leaves.

1.2. Methods

Forty-eight adult male Wistar rats weighing between 150-180 g were randomly assigned into six groups of eight rats each. Group A served as control. A single dose of 20 mg/kg of indomethacin was given to rats in the other groups. Following this, Group B received placebo treatment while Groups C, D, and E were administered the plant extract 12 hourly at doses of 25 mg/kg, 50 mg/kg and 100 mg/kg respectively, and Group F were administered triple regimen- omeprazole (10 mg/kg), Amoxycillin (25 mg/kg), and Clarithromycin (10 mg/kg) 12 hourly. All drug administration's were given via the oral route. The rats were sacrificed under ketamine anesthesia after 14 days of treatment, and the stomach with the proximal 5 mm of duodenum were excised, examined for ulceration, and used for histological and histomorphometric analyses.

1.3. Results

The result showed significant increase (P < 0.05) in the number of gastric and duodenal ulcers and mean total ulcerative areas in group B compared with the F. exasperata and triple regimen treated groups. Histologic examinations revealed markedly increased gastric and duodenal ulceration, inflammation and mucosal architectural distortion in Group B compared with the treated groups. A significant reduction (P < 0.05) in the percentage gastric glandular mucosal thickness and duodenal Villi/Crypt depth ratio for group B compared with the control and the treated groups (which showed no significant differences in the values of these measured parameters when compared among one another and with the control) was observed.

1.4. Conclusion

This study showed that aqueous extract of F. exasperata leaves has ameliorative effects on indomethacin-induced gastroduodenal ulcer in rat model.

2. Keywords: Duodenum; Ficus exasperate; Indomethacin; Stomach; Ulcer

3. Introduction

Gastric and duodenal ulcers are among the commonest gastro-intestinal diseases worldwide. They constitute enormous health burden, and affected people experience severe abdominal pain which negatively impact on their qualities of lives. An estimated 15,000 deaths was projected to occur each year as a consequence of peptic ulcer disease [1]. Gastroduodenal ulcers constitute a devastating Medical condition with tremendous effects on morbidity and mortality. Approximately 500,000 persons develop peptic ulcer disease in the United States of America each year [2]. Seventy percent of the patients are between the ages of 25 and 64 years [3]. The exact prevalence of peptic ulcer disease in Nigeria is not well known, however, Nigeria has been listed some few decades ago as an area of high incidence of PUD [4].

Table 1: Macroscopic measurement of Gastric Lesions

	Average Lesion Number	Mean Mucosal Area (mm²)	Mean Total Ulcer Area (mm²)	Gastric Ulcer Index	Protective Ratio (%)
Group A n = 8	–	660.3 ± 16.0	–	–	–
Group B n = 6	12.3 ± 1.8	690.3 ± 40.6	58.3 ± 19.5	0.80 ± 0.22	–
Group C n = 6	10.2 ± 0.8	725.0 ± 28.8	30.3 ± 6.0	0.42 ± 0.08*	47.5
Group D n = 7	9.3 ± 0.4	712.0 ± 30.5	25.7 ± 5.7	0.36 ± 0.07*	55
Group E n = 7	8.0 ± 0.8	658.9 ± 29.6	23.1 ± 4.1	0.35 ± 0.06*	56.3
Group F n = 7	8.4 ± 0.8	689.9 ± 27.8	22.2 ± 3.5	0.34 ± 0.07*	57.5
Values are mean ± SEM. * - Significantly different when compared with Indo only at $p < 0.05$					

In Nigeria, high rates of poverty and ignorance have contributed to high incidence of acute and chronic gastroduodenal ulcers [5]. There is also an unfortunate trend of increasing use of alcohol in this country [5]. Patronage of local joints that specialize in making concoctions (mixture of herbal products) with very high levels of alcohol and other unhygienic, and probably ulcer provoking substances has become rife among the youths. Such concoctions are advertised to cure sexual dysfunctions, infertility, increase libido and aid general wellbeing. The unfavorable economic situations and hardship encountered by the populace on a daily basis also results in enormous physical, mental stress and depression. This makes a

Table 2: Macroscopic measurement of Duodenal Lesions

	Average Lesion Number	Mean Mucosal Area (mm²)	Mean Total Ulcer Area (mm²)	Duodenal Ulcer Index	Protective Ratio (%)
Group A n = 8	–	86.4 ± 1.5	–	–	–
Group B n = 6	6.8 ± 1.0	92.5 ± 5.3	6.7 ± 1.1	0.73 ± 0.14	–
Group C n = 6	6.2 ± 1.5	97.2 ± 3.5	2.5 ± 0.5	0.25 ± 0.05*	66
Group D n = 7	4.6 ± 0.4	95.3 ± 3.9	1.9 ± 0.3	0.21 ± 0.04*	71.2
Group E n = 7	5.7 ± 1.3	89.6 ± 3.5	1.9 ± 0.7	0.21 ± 0.07*	71.2
Group F n = 7	4.1 ± 0.4	92.9 ± 3.4	1.6 ± 0.6	0.18 ± 0.06*	75.3
Values are mean ± SEM. * - Significantly different when compared with Indo only at $p < 0.05$					

Table 3: Histomorphometric measurements on corpus of stomach

	Mucosal layer (μm)	Subglandular Mucosa layer (μm)	Glandular Mucosa layer (μm)	Percentage glandular mucosa layer (%)
Group A n = 8	890.3 ± 20.0	72.4 ± 3.1	817.9 ± 19.7	91.9 ± 0.4
Group B n = 6	412.2 ± 25.2	74.5 ± 3.7	337.7 ± 25.2	81.6 ± 1.4*
Group C n = 6	759.5 ± 35.0	76.5 ± 4.5	683.0 ± 35.9	89.8 ± 0.9#
Group D n = 7	786.0 ± 30.8	70.9 ± 3.5	715.1 ± 31.6	90.9 ± 0.6#
Group E n = 7	768.4 ± 34.5	74.9 ± 4.2	693.6 ± 33.4	90.2 ± 0.6#
Group F n = 7	778.9 ± 29.2	77.1 ± 3.5	701.7 ± 27.9	90.1 ± 0.4#
Values are mean ± SEM of data obtained. * - Significantly different when compared with Control at $p < 0.05$. # - Significantly different when compared with Indo only at $p < 0.05$				

lot of people result to use of alcohol. Furthermore, most of the drugs in this country are available over the counter and are usually procured without prescription, which are also improperly used. Many of these drugs are ulcerogenic, and it was found that the most

Table 4: Histomorphometric measurements on Pylorus of stomach and duodemum

|	Pylorus				Duodenum		
	Mucosa layer (µm)	Subglandular mucosa layer (µm)	Glandular mucosa layer (µm)	Percentage Glandular mucosa layer (%)	Villi height (µm)	Crypt depth (µm)	Villi height/Crypt depth ratio
Group A n = 8	696.0 ± 10.1	78.5 ± 3.3	617.5 ± 9.4	88.8 ± 0.4	620.5 ± 18.6	290.8 ± 12.8	2.18 ± 0.13
Group B n = 6	342.0 ± 16.1	75.0 ± 5.6	267.0 ± 14.1	78.0 ± 1.4*	335.2 ± 38.3	301.3 ± 16.7	1.15 ± 0.13*
Group C n = 6	603.5 ± 12.0	78.5 ± 4.2	525.0 ± 9.7	87.0 ± 0.5#	459.7 ± 19.9	272.5 ± 19.9	1.73 ± 0.11#
Group D n = 7	613.9 ± 10.3	72.4 ± 3.6	541.4 ± 10.5	88.1 ± 0.6#	441.7 ± 28.9	277.3 ± 15.6	1.64 ± 0.15#
Group E n = 7	621.4 ± 6.4	75.7 ± 4.5	545.7 ± 7.2	87.8 ± 0.7#	442.1 ± 33.2	260.7 ± 19.4	1.74 ± 0.18#
Group F n = 7	610.0 ± 7.6	74.7 ± 3.9	535.3 ± 9.1	87.7 ± 0.7#	449.0 ± 26.3	277.6 ± 18.9	1.69 ± 0.18#
Values are mean ± SEM of data obtained							
* - Significantly different when compared with Control at p< 0.05							
# - Significantly different when compared with Indo only at p< 0.05							

commonly abused drugs in Nigeria are NSAIDs [6]. These factors are responsible for the high rates of PUD in Nigeria [7] found out

that PUD was among the leading causes of morbidity and mortality in Nigeria.

There is an interplay of mucosal aggressors and protectors involved in the aetiogenesis of gastroduodenal ulcers. Mucosal aggressors include: secreted acid, pepsin, bile salts, Helicobacter pylori infection, non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, cigarette smoke, and various food substances [8, 9]. The mucosal protective factors are the normal gastric mucosal cells and secretion of mucus, bicarbonates, prostaglandins, nitric oxide and adequate blood supply to the stomach and duodenum [8]. These factors work together to prevent the development of gastric and duodenal ulceration. Gastric and duodenal ulcerations usually result when there is imbalance in the aggressive (ulcer-provoking) and protective factors, such that the aggressors overwhelm the protective mechanisms [8, 10-11].

Ulcer of the stomach and the duodenum can occur as acute ulcers, or as chronic lesions as seen in peptic ulcer disease. Exposure to irritants or corrosive agents, stress and chronic debilitating diseases cause acute gastroduodenal ulceration. Majority of the chronic gastroduodenal ulceration (peptic ulcer disease) In contrast with acute gastroduodenal ulcerations, are associated with infection by a bacterium known as Helicobacter pylori [12]. The other previously outlined aggressors may assist H. pylori in causing the peptic ulcer disease [12]. Both acute gastric and duodenal ulcers can be caused by distruption of blood supply to these organs as in portal hypertension [9].

Non-steroidal anti-inflammatory drugs (NSAIDs) are drugs with anti-inflammatory properties that are used to treat acute and chronic debilitating inflammatory conditions like rheumatoid arthritis and gout [13]. Their mechanism of action is via the inhibition of the cyclo-oxygenase enzyme (COX) in the body, thereby causing reduction in the generation of prostaglandins. Two isoforms of cyclo-oxygenase enzyme (COX) have been found: COX-1 and COX-2. COX-1 is produced constitutively in the tissues while COX-2 is the inducible form, which is produced at sites of inflammation [14]. Inhibition of COX enzymes by NSAIDs distrupts the production of protective prostaglandins and cause gastric and duodenal mucosal erosion and ulceration [2]. Other protective COX-2 mediated effects like enhancement of gastric mucosal protection and stimulation of mucus and bicarbonate secretion, epithelial cell proliferation and augmentation of mucosal blood flow are also lost in NSAID use [2]. Coexisting H. pylori infection increases the likelihood
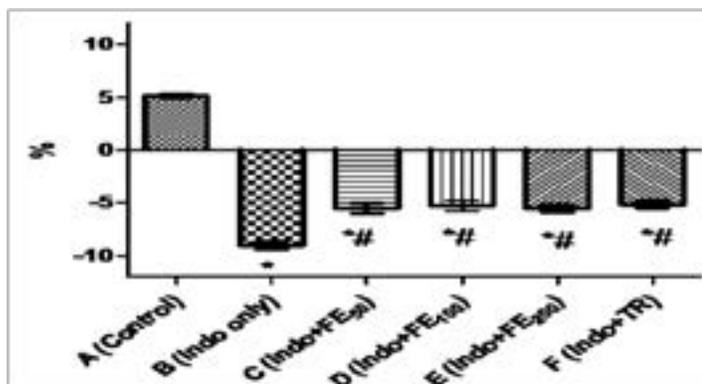
Figure 1: The percentage body weight gain/loss of control and treated rats. * - Significantly different when compared with Control at p< 0.05 # - Significantly different when compared with Indo only at p< 0.05
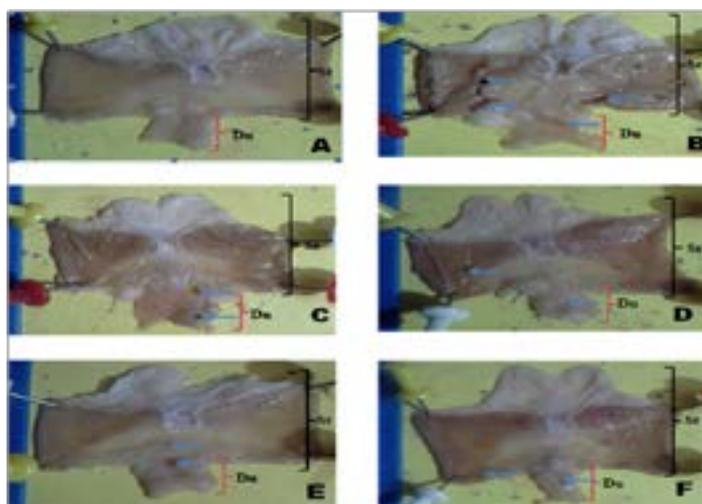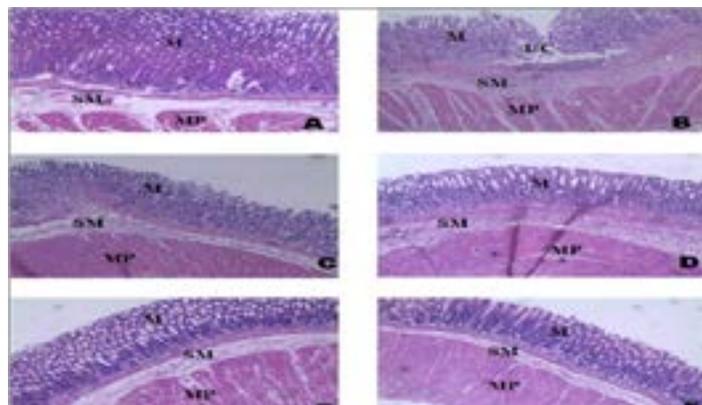

Figure 2: Macroscopic pictures of the stomach and duodenum. A (Control), B (Indo only), C (Indo+FE$_{50}$), D (Indo+FE$_{100}$), E (Indo+FE$_{200}$), F (Indo+TR). St- Stomach, Du- Duodenum, Blue arrows- Ulcers.
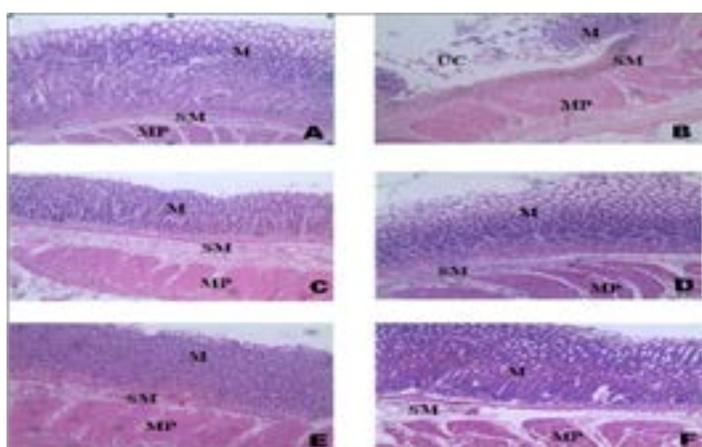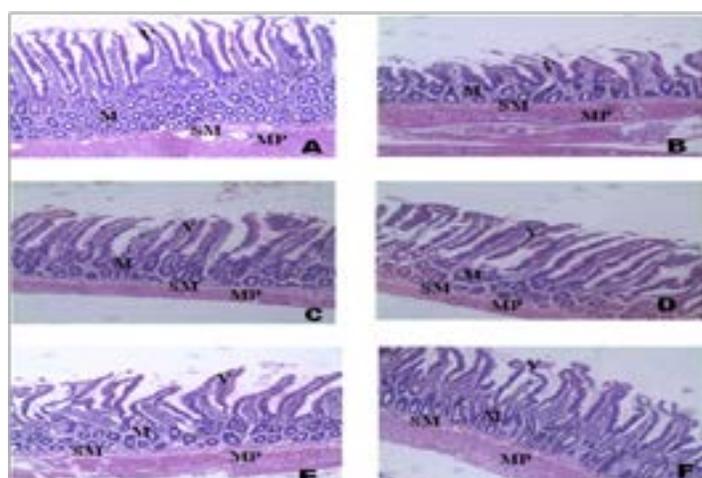

Figure 3: Photomicrographs of Corpus of the Stomach. A (Control), B (Indo only), C (Indo+FE$_{50}$), D (Indo+FE$_{100}$), E (Indo+FE$_{200}$), F (Indo+TR). H&E X 100. M- Mucosa, SM- Submucosa, MP- Muscularis propria, UC- Ulcer crater.

Figure 4: Photomicrographs of Pylorus of the Stomach. A (Control), B (Indo only), C (Indo+FE$_{50}$), D (Indo+FE$_{100}$), E (Indo+FE$_{200}$), F (Indo+TR). H&E X 100. M- Mucosa, SM- Submucosa, MP- Muscularis propria, UC- Ulcer crater

Figure 5: Photomicrographs of the Duodenum. A (Control), B (Indo only), C (Indo+FE$_{50}$), D (Indo+FE$_{100}$), E (Indo+FE$_{200}$), F (Indo+TR). H&E X 100. V- Villus, M- Mucosa, SM- Submucosa, MP- Muscularis propria.

Figure 6: Photomicrographs of the Corpus of the Stomach. A (Control), B (Indo only), C (Indo+FE$_{50}$), D (Indo+FE$_{100}$), E (Indo+FE$_{200}$), F (Indo+TR). VVG X 100. M- Mucosa, SM- Submucosa, MP- Muscularis propria, Red staining- Collagen fibres, Brown-black staining- Elastic fibres.
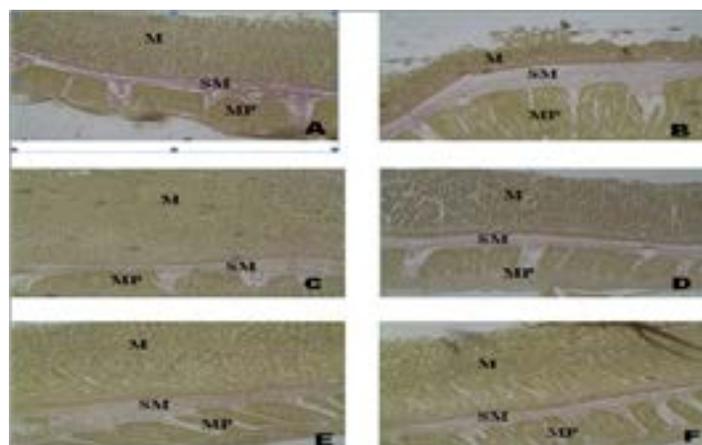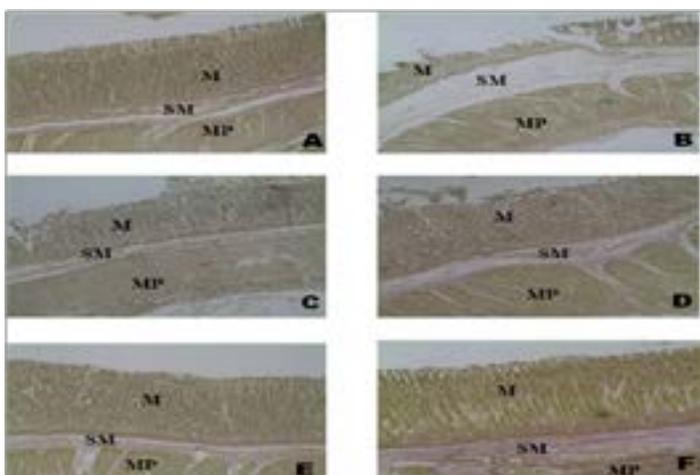
and intensity of NSAIDs-induced damage [2]. NSAIDs use is responsible for approximately one-half of perforated ulcers, which occur most commonly in older patients who are taking NSAIDs for cardiovascular disease or arthropathy [15].
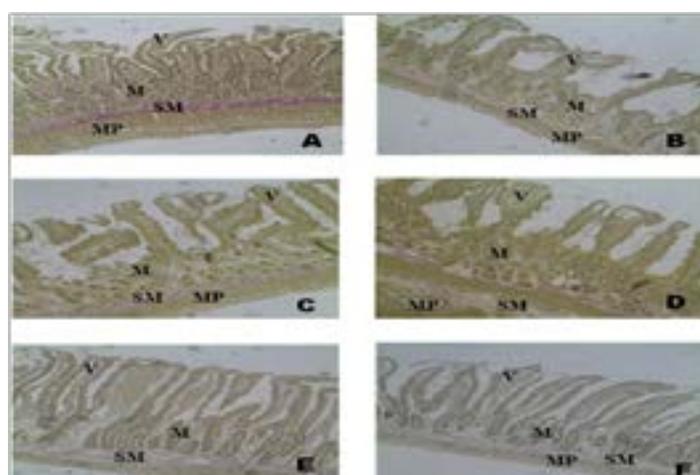
Figure 7: Photomicrographs of the Pylorus of the Stomach. A (Control), B (Indo only), C (Indo+FE$_{50}$), D (Indo+FE$_{100}$), E (Indo+FE$_{200}$), F (Indo+TR). VVG X 100. M- Mucosa, SM- Submucosa, MP- Muscularis propria, Red staining- Collagen fibres, Brown-black staining- Elastic fibres.

Plants of the genus Ficus are well known all over the World as fig plants [16]. They belong to the Mulberry family (Moraceae), which comprises about 800 species that naturally occur in the tropical and warm temperate regions of the World [17]. F. exasperata is an afro-tropical tree with scabrous, oval-shaped leaves. The tree can grow up to about 20m tall and prefers forest habitats [18]. The tree is popularly referred to as 'Sandpaper leaf tree' because of the rough surface of the leaves. The leaves are called 'Ewe ipin' by the Yoruba people of the Southwestern part of Nigeria. This plant is widely spread in West Africa in all kinds of vegetation [18].

The plant is well regarded among the practitioners of traditional medicine and healers in Africa as a highly medicinal plant, as different parts of it are used to treat various medical conditions. The leaves and other parts of the plant are considered to be effective in treatment of diabetes, skin diseases, ulcers, dysentery, diarrhea, stomach ache, haemorrhoids and as carminative, astringent, anti-inflammatory, anti-oxidant and anti-cancer agents [19-21]. They are also locally used to treat hypertension, hypoglycemia, obesity, leprosy, tuberculosis, respiratory tract infections, asthma, ophthalmic and oral infections, nasopharyngeal irritation, arthritis, rheumatism, gout, oedema, kidney disorders and venereal diseases [20, 22]. Various parts of the tree are used for many purposes in the household and for industrial use. The leaves are used as feeds for animals like sheep, goats and chimpanzees [23]. The leaves are also employed in the stabilization of palm oil to enhance their keeping qualities [24].

The leaves as well as the bark and roots of FE have been reported to contain steroids, flavinoids, phlobatannins, tannins and saponins

Figure 8: Photomicrographs of the Duodenum. A (Control), B (Indo only), C (Indo+FE$_{50}$), D (Indo+FE$_{100}$), E (Indo+FE$_{200}$), F (Indo+TR). VVG X 100. V- Villus, M- Mucosa, SM- Submucosa, MP- Muscularis propria, Red staining- Collagen fibres, Brown-black staining- Elastic fibres.

[19, 21]. In addition to these biochemical entities, OGUNLEYE [25] also reported the presence of cardiac glycosides in the aqueous leaf extract. The diverse medicinal usefulness of this plant has been attributed to these phytochemical constituents.

However, despite the beneficial roles and medicinal values of F. exasperata, there is paucity of information on its micro anatomical effects on the stomach and duodenum, and on its role in the management of gastroduodenal ulcer. This study is therefore aimed at evaluating the ameliorative properties of Ficus exasperata, with a view to elucidating its effects on the microanatomy of the stomach in indomethacin- induced gastro-duodenal ulcer in rat models.

4. Materials and Methods

4.1. Animal Care and Management

Forty-eight adult male wistar rats (Rattus norvegicus) weighing between 150 to 180 g were used for the study. The rats were housed in plastic cages in the Animal Holding of the Department of Anatomy and Cell Biology of Obafemi Awolowo University (OAU), Ile-Ife under standard laboratory conditions of light, temperature humidity. They were fed with standard laboratory rat pellets obtained from veterinary care centre and allowed access to clean water ad libitum. The rat cages were cleaned with the wood filings used as rat beddings changed every 48 hours.

4.2. Plant Material and Extract Preparation

Fresh leaves of Ficus exasperata were plucked from the plant shrubs and trees within Obafemi Awolowo University (OAU) campus in Ile-Ife, Nigeria. These leaves were authenticated by a Taxonomist at the

Department of Botany OAU. A voucher specimen was deposited at the herbarium for future reference (voucher number IFE17400). The leaves were cleaned and air-dried at room temperature for four weeks. The dried leaves were pulverized using an electric blender. The extraction was done using percolation method with Soxhlet extractor. The dissolved extract was filtered through Whatmann No. 1 filter paper and the filtrate was concentrated in a vacuum rotary evaporator at $60\,^{0}$C and freeze-dried.

4.3. Experimental Design

- The animals were randomly assorted into 6 groups (groups A, B, C, D, E, and F) of eight rats each. The rats were commenced on their respective treatment after overnight fasting. Administration of all drugs was through the oral route.

- Group A was the normal control group served with distilled water at the dose of 3 ml/kg 12 hourly

- Group B was served20 mg/kg indomethacin dissolved in distilled water, followed 24 hours later by administration of distilled water at 3 ml/kg12 hourly

- Groups C was served 20 mg/kg indomethacin dissolved in distilled water, followed24 hours later by administration of aqueous extract of Ficus exasperata dissolved in distilled water at dosage of 25 mg/kg 12 hourly(50 mg/kg/day)

- Group D was served 20 mg/kg indomethacin dissolved in distilled water, followed24 hours later by administration of aqueous extract of Ficus exasperata dissolved in distilled water at dosage of 50 mg/kg 12 hourly (100 mg/kg/day)

- Group E was served 20 mg/kg indomethacin dissolved in distilled water, followed24 hours later by administration of aqueous extract of Ficus exasperata dissolved in distilled water at dosage of 100 mg/kg 12 hourly (200 mg/kg/day)

- Group F was served 20 mg/kg indomethacin dissolved in distilled water, followed24 hours later by administration of Omeprazole 10 mg/kg 12 hourly + Amoxycillin 25 mg/kg 12 hourly and Clarithromycin 10 mg/kg 12 hourly, all dissolved in distilled water,

- All treatments were given for a period of 14 days.

4.4. Animal Sacrifice and Macroscopic Examination of Stomach and Duodenum

The rats were sacrificed under ketamine anesthesia. A midline incision was made along the anterior abdominal wall. The stomach and the proximal 5 mm of the duodenum were excised. The stomach was opened along the greater curvature, and this incision was extended to the duodenum to open it. The stomach with the duodenum were washed in normal saline, and pinned to a dissecting board for macroscopic examination and permanent photography using Sony Cyber shot DSC-S2000 Digital Camera. A centimeter rule graduated in 1 mm was photographed together with the tissue. The images captured were imported into Image J software for proper measurement and determination of macroscopic ulcer index. Image Processing and Analysis in JAVA (Image J) Software by the National Institute of Health (NIH, USA) was used to measure areas of gastric lesions in mm^2. The numbers of gastric lesions were also noted and documented.

Ulcer Index (UI) was calculated by the method of Ganguly [26]

$UI = 10/x$

Where x = Total mucosal surface/Total ulcerated area

In case of petechial haemorrhages, 5 of them were considered to be equivalent to 1 mm^2 of ulcer.

Protective Ratio (PR) was calculated as follows:

$$PR\,(\%) = \frac{(a-b)x}{a}\,100$$

a- the ulcer index of the negative control group.

b- The ulcer index of the experimental group (extract or drug treated).

4.5. Histological Preparation

The gastric and duodenal tissues were fixed in 10 % formol-saline for histological procedure. Haematoxylin and Eosin (H&E) stains were used for demonstration of general micro architecture, and Verhoeff van Gieson stain for collagen and elastic fibres.

4.6. Photomicrography and Histomorphometric Study

All stained sections were examined under Leica DM 750 research microscope connected to a Leica ICC50 camera and photomicrographs of the sections were taken and archived. The photomicrographs taken were imported to OpenOffice.org™ software for histomorphometric measurements on the gastric corpus and pylorus, and duodenum. The following parameters were measured for the gastric corpus and the pylorus;

1. Mucosal layer: from the gastric epithelial lining to the

muscular is mucosa.

2. Sub glandular mucosal layer: from the lowermost glands in the lamina propria to the muscular is mucosa.

3. Glandular mucosa layer: calculated by subtracting the sub glandular mucosal from the mucosal layer.

The percentage of mucosa that is glandular was calculated as:

$$\%\text{Glandular Mucosal layer} = \frac{\text{Glandular Mucosal layer} \times 100\%}{\text{Mucosal layer}}$$

For the duodenum, the following parameters were measured:

1. Villi height (μm)

2. Crypt depth (μm)

3. Villi height/ Crypt depth ratio was calculated

All the measurements were made in micrometer (μm) at X100 magnification.

4.7. Statistical Analysis

Data were recorded as Mean ± Standard error of mean and subsequently analysed using one way Anova with Newman-Keuls Multiple Comparison Test. Graph Pad Prism 5 (Version 5.03, Graph Pad Inc.) was the statistical package used for data analysis. P-values < 0.05 were considered statistically significant.

5. Results

5.1. Body Weights of Animals (g)

There was a weight drop in all the groups that had gastroduodenal ulcer induction with indomethacin which was shown as percentage weight gain/loss in (Figure 1). The percentage body weight loss was significantly higher in group B (-9.0±0.40%) compared with group C (-5.4±0.52%), group D (-5.5±0.49%), group E (-5.7±0.36%) and group F (-5.3±0.35%). There was no significant difference observed between the treated groups when compared with one another. There was however, weight gain in the normal control group which was significant when compared with the other groups (5.1±0.17%).

5.2. Macroscopic Examination of Stomach and Duodenum

Indomethacin induced ulceration and haemorrhagic lesions in the stomach and duodenum of the animals that received it. The lesions in the various animal groups are shown in (Figure 2). The lesions were distributed evenly in the corpus and pyloric regions of the stomach, but not in the non-secretary portion. Gastric and duodenal lesions were most severe in group B, with presence of numerous ulcers

in the mucosae. Treatment with FE and ulcer triple regimen (TR) however resulted in less ulceration. There were no lesions seen in the control group. The gastric lesions were significantly more in the Indo only group (gastric ulcer index= 0.80± 0.22) compared with the treated groups with significantly lesser numbers of ulcers as shown in (Table 1). TheIndo+FE$_{50}$, Indo+FE$_{100}$, Indo+FE$_{200}$ and Indo+TR groups had gastric ulcer indices of 0.42±0.08, 0.36±0.07, 0.35±0.06, and 0.34±0.07 respectively. There were no significant differences in the ulcer indices between the various extract treated groups (p< 0.05).The gastric ulcer protective ratios for the various treated groups were comparable. The ulcer indices in the duodenum follow a similar trend as seen in the stomach and were depicted by (Table 2). Group B had a significantly increased duodenal ulcer index (0.73±0.14) when compared with groups C (0.25±0.05), D (0.21±0.04), E (0.21±0.07), and F (0.18±0.06). The duodenal ulcer protective ratios for the different treatment groups showed no significant difference.

5.3. Histological Observations

5.3.1. Haematoxylin and Eosin (H and E) Staining:

Sections from the corpus (Figure 3) showed normal histology in the control group. The layers of the gastrointestinal tract, viz; the mucosa, submucosa, and muscularis propria are presented. There was preservation of the arrangement of glands and general histoarchitecture in the control. The corpus showed closely packed, well arranged glands in the mucosa, with the gastric pits made of straight tubular glands. There was no ulceration seen in the control group. There was however, extensive ulceration of the mucosal lining in the indo only group. This was accompanied by infiltration by mixed mononuclear and polymorphonuclear cells. There was loss of glands within the lamina propria. The treated groups (groups C, D, E and F) had mild ulceration of the mucosal lining and presence of fewer inflammatory cells within the lamina propria. The pyloric region of the stomach showed presence of branched, coiled tubular glands of the gastric pits (Figure 4).The glands were lined by mucus-secreting cells as well as parietal and chief cells. There was extensive loss of glands and ulceration of the mucosa in group B, the control group showed no ulceration, while the F. exasperata and triple regimen treated groups had mild mucosal ulceration. The mucosa, submucosa, and muscularis propria of the duodenum in the various animal groups can be appreciated in (Figure 5). The lining of the glands contain numerous goblet cells, and the mucosa is thrown into villous projections. The control group showed normal villous structure. There was a great disruption of the villar epithelium with shortening of the villi, and the lamina propria had been infiltrated by

polymorphonuclear and mononuclear inflammatory cells in group B. In the F. exasperata and triple regimen treated rats in groups C, D, E, and F, mild villous distortion, with minimal inflammation of the mucosae was observed (Figure 6).

5.3.2. Verhoeff Van Gieson Staining:

Collagen fibres stain red while elastic fibres stain brown-black as shown in Figure6-8. Staining for collagen fibres could only be demonstrated in the submucosa, while staining for elastic fibres was more intense in the muscularis propria and within the glands of the mucosa, and not in the lamina propia and the submucosa. Normal staining pattern for collagen and elastic fibres was observed for the control group. There was marked reduction in the intensity of staining for collagen fibres in the gastric corpus, pylorus and the duodenum in group B, especially in the regions of ulceration. The F. exasperata and triple regimen treated groups however, showed moderate reduction in the staining intensity for collagen fibres. Moderate attenuation in staining intensity for elastic fibres was observed for group B, while the F. exasperata and triple regimen treated groups showed no obvious difference when compared with the control (Figure 7).

5.3.3. Histomorphometric Observations:

Indomethacin caused a significant reduction ($p < 0.05$) in the thickness of the corporal total and glandular mucosal layers in all the animal groups that received it, compared with control (Table 3). However the effects of the indomethacin on the corporal mucosa was attenuated in the F exasperata and triple regimen treated groups, and there were significant differences ($p < 0.05$) in the measured mucosal and glandular mucosal layers in the F. exasperata and triple regimen treated groups compared with the group B. There were no significant differences among the treated groups when compared with one another. The calculated percentage glandular mucosal layer showed comparable results among the various treatment groups and the control but there are significant differences ($p < 0.05$) between any of these groups and group B (Table 3). Indomethacin caused a significant reduction ($p < 0.05$) in the thickness of the pyloric total and glandular mucosal layers in all the animal groups that received it, compared with control (Table 3 and 4). However the effects of the indomethacin on the pyloric mucosa was attenuated in the F exasperata and triple regimen treated groups, and there were significant differences ($p < 0.05$) in the measured mucosal and glandular mucosal layers in the F. exasperata and triple regimen treated groups compared with the group B (Figure 8). There were no significant differences among the treated groups when compared

with one another. The calculated percentage glandular mucosal layer showed comparable results among the various treatment groups and the control but there are significant differences ($p < 0.05$) between any of these groups and group B. Ulceration caused loss of the superficial layers of the mucosa resulting in significant shortening of the corporal and pyloric glandular mucosal layer thickness in group B ($337.7 \pm 25.2 \mu m$, 267.0 ± 14.1 μm respectively) compared with the other groups (Tables 3 & 4). There was no significant difference in the percentage glandular mucosal layer of the control ($817.9 \pm 19.7 \mu m$, 617.5 ± 9.4 μm) and in groups C (683.0 ± 35.9 μm, 525.0 ± 9.7 μm), D (715.1 ± 31.6 μm, 541.4 ± 10.5 μm), E (693.6 ± 33.4 μm, 545.7 ± 7.2 μm), and F (701.7 ± 27.9 μm, 535.3 ± 9.1 μm). Significant reduction ($p < 0.05$) of the villi height and the villi height/crypt depth ratio was observed in all the indomethacin-treated groups compared with the control. This was shown in (Table 4). However, treatment with F. exasperata and triple regimen caused some preservation of the villi height and the villi height/crypt depth ratio with significant differences ($p < 0.05$) between the treated groups and group B. There was marked reduction in the mean villi height/crypt depth ratio in group B (1.15 ± 0.16), and a significant difference was observed when compared with the control group (2.18 ± 0.13) ($p < 0.05$). There were no significant differences in the mean villi height/crypt depth ratio among groups C (1.73 ± 0.11), D (1.64 ± 0.15), E (1.74 ± 0.18), and F (1.69 ± 0.18) and with the control (2.18 ± 0.13).

6. Discussion

A significant decrease in the body weight of the rats treated with indomethacin was observed in this study. The weight loss was however significantly less in the rats that had treatment with FE and TR. This weight loss may be due to reduction in appetite of the rats following treatment with indomethacin. Previous study by [27] corroborated our finding [27] reported that indomethacin caused reduction in meal size and meal number thereby leading to reduction in spontaneous food intake. This finding was attributed to increased production of appetite-suppressing cytokines such as TNF-alpha and IL-1 alpha [27]. The mechanisms of loss of appetite and weight reduction in NSAID's-induced ulceration in laboratory animals however, need to be further studied [28] reported significant weight loss in their experimental animals following induction of gastric ulcer with aspirin. This was attributed to reduced digestive functions following debilitating injuries to the mucosa. This may partly explain the weight loss in the indomethacin-treated rats in this study. Generation of free radicals and their systemic release has been linked to weight loss [29]. Release of free radicals caused by

treatment with indomethacin in this study may therefore play a role in causing the observed weight loss.

Indomethacin belongs to a family of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs). They are commonly employed in the treatment of painful inflammatory conditions. Indomethacin causes gastric and duodenal ulcerations by diverse mechanisms. It acts via the inhibition of cyclo-oxygenase (COX) enzyme to inhibit the secretion of protective prostalglandins in the mucosae of stomach and duodenum, which in turn inhibits the release of mucus, a protective factor against gastrointestinal damage [30-32]. Besides providing significant buffering capacity for the neutralization of luminal acid, the mucus also offers protection against both endogenous aggressors and exogenous gastrotoxic agents such as indomethacin, thereby enhancing the rate of local healing process [33]. This important action of mucus is lost by the loss of protective mucus secretion in indomethacin use. Indomethacin has also been found to cause generation of free radicals and inhibits the antioxidant mechanisms of the body [34]. Generation of oxygen free radicals and lipid per oxidation play a key role in the development of the gastric mucosal lesions induced by indomethacin [35-37]. In addition to causing gastric ulceration and other lesions, indomethacin has been reported to cause lesions in other parts of the intestine, especially the proximal duodenum [38].

In this study, single dose (20 mg/kg) of indomethacin administration caused gastric and duodenal ulcerations. The ulcerations resulted from the loss of protective mucosal prostaglandins and mucus secretion and production of free radicals that resulted in the necrosis of the lining epithelium and the glands. This is in agreement with previous works by [39, 40]. Where indomethacin was reported to have caused unfavorable alterations in the gastric and duodenal secretary activities.

Gastroduodenal ulcers were demonstrated macroscopically, histologically and supported by histomorphometric measurements in this study. Histological findings of the present study revealed the gastric and duodenal ulcerations of indomethacin treated groups. There were lesser ulcerations and better preservation of mucosal tissue integrity in the FE and TR treated groups compared with group B. A combination of events including release of preformed mucus, wound retraction and re-epithelialization are involved in ulcer-healing process after toxicological injury [41]. Histological sections show better preserved tissue integrity in the lining epithelium and glandular mucosae of the stomach and duodenum in the FE and TR treated rats compared with the group B.

The histological findings were complemented by histomorphometric measurements which showed significantly reduced mucosal and glandular layers of the stomach and significant shortening of the duodenal villi of indomethacin treated rats. The FE and TR treated groups however, have lesser degree of reduction of gastric glandular mucosal layer and the villi height of the duodenum compared to group B. There was an increase, albeit insignificant, in the thickness of the sub glandular mucosal layer in the indo only group because of the greater inflammation in this region of the stomach and duodenum in the rats in group B compared with the other groups.

In this study, VVG demonstration of the collagen and elastic fibres showed staining for collagen to be largely confined to the submucosal part of the walls of the stomach and duodenum, and only minimal staining in other parts of the wall, while the elastic fibres were more densely localized in the muscularis propria and the mucosa. This observation was in agreement with the findings of [42]. The observed reduction in the intensity of staining for collagen and elastic fibres in this study was due to dense inflammation and infiltration of the stomach and duodenal wall by mononuclear and polymorphonuclear inflammatory cells which caused destruction of the glands and extraglandular fibrocollagenous connective tissue. This was attenuated in the FE and TR treated rats.

In this study, the effects of FE extract on gastroduodenal ulcer, assessed by using macroscopic examination and ulcer index determination, histology and histomorphometry study were shown to be comparable with the effects of TR treatment. The gastric and duodenal ulcer protective ratios for the 3 different doses of FE used were not significantly different, and comparable with the protective ratios with the standard TR. Among the 3 different daily dosages of FE used for this study (FE_{50}, FE_{100}, and FE_{200}), the FE_{100} and FE_{200} had greater ameliorative effects on the stomach and duodenum (lesser ulcer indices and higher protective ratios) compared with the FE_{50}, although there were no significant differences in these measured parameters.

7. Conclusion

This study showed that aqueous extract of the leaf of FE has remarkable ameliorative effects on indomethacin-induced gastroduodenal ulcer in rat model, and may be useful in the clinical management of gastroduodenal ulceration, and possibly peptic ulcers. The anti-ulcer properties of this plant may be due to the potent

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anti oxidative agents such as steroids, flavinoids, phlobatannins, tannins and saponins present in the leaf [19, 21]. The actions of these anti-oxidants oppose the actions of free radicals generated by indomethacin and other NSAID's, thereby enhancing healing of gastroduodenal ulcer.

8. Recommendation

We recommend further studies to firmly establish the clinical usefulness of FE in the treatment of gastroduodenal ulcers, and to determine the effective and safe dose for this plant in man.

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