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Original Research Article

Evaluation of Gastroprotective Activities of Fraction Extracts of *Piper guineense* Leaf on Ethanol-Induced Ulcer in Wistar Rats

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Abstract

One of the approaches towards alleviation of peptic ulcer disease is by use of antiulcer agents. Cost factor has an important role to play in the choice of antiulcer agents in ulcer therapy. *Piper guineense*, a flora to tropical Africa, is claimed by traditional medicine as a remedy for peptic ulcer. Despite its wide usage in crude form, purified form of *Piper guineense* are yet to be explored. The aim of the study was to evaluate the gastroprotective activities of fraction extracts of *Piper guineense* on ethanol-induced ulcer in wistar rats. This was done by administering orally, 400mg/kg of various fraction extracts to six test groups, 100mg/kg cimetidine to a positive control group and 5ml/kg Tween 80 (3% v/v) to a negative control group. Histological study and effect of the extracts on stomach weight were conducted. Findings revealed that extracts significantly ($p<0.05$) inhibited gastric ulceration with PF-4 producing 49.02% ulcer inhibition while cimetidine produced 81.93%. Histological study revealed gastroprotection with minor epithelial loss in extract-treated group. The extracts (PF-2, PF-4. EE) respectively produced 23.47%, 27.23% and 33.33% significant ($p<0.05$) increase in stomach weight of rats. In conclusion, the fraction extracts of *Piper guineense* possess gastroprotective activities.

Keywords: *Piper guineense*, Fraction extracts, Gastroprotective, Stomach weight, Ulcer.

INTRODUCTION

Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, being major burden for health care organization (Tanih *et al.*, 2010). Ulcer is basically an inflamed rupturing deterioration of mucus membrane layer of digestive tract (Pradhan *et al.*, 2013; Esen *et al.*, 2018). Factors involved in the development of peptic ulcer are chronic alcohol intake, smoking, excessive stress, chronic use of non-steroidal anti-inflammatory drugs and *Helicobacter pylori* infection and tumors. Ulcers can develop when there is an imbalance between gastroprotectives (mucus, bicarbonate and prostaglandins) and aggressives (acid, pepsin, bile salt and *H. pylori* (Arumugam *et al.*, 2011). One of the recent approaches to peptic ulcer management is by promotion of gastroprotection that enhances mucosal integrity (Saroj *et al.*, 2010). Several orthodox pharmaceutical drugs such as antacids, anticholinergic drugs, histamine H_2-receptor antagonists, and more recently, proton-pump inhibitors have been employed in the management of peptic ulcers but they provoke many adverse effects or drug interactions and may even alter biochemical mechanisms of the body upon chronic usage. In recent years, there has been growing interest in alternative therapies especially from plant source due to their perceived lower side effects, ease of accessibility and affordability (Rates, 2001). Plants are some of the most attractive source of new drugs and some have been shown to have promising result for the treatments of peptic ulcer with minimum side effects (Alkofahi and Atta, 1999; Schmeda-Hirschmann and Yesilada, 2005).Plants with tradomedicinal use in peptic ulcer management therefore need to be screened for potential antiulcer activity and encourage their use in purified forms to reduce or avert possible adverse effects.

Piper guineense is a flora tropical Africa, claimed by traditional medicine as remedy for peptic ulcer. It is also referred to as African black pepper or Ashanti pepper, belongs to the family piperaceae, is a herbacious climber plant 4-10m in length and commonly found in Nigeria with local names "uziza" in Igbo, "iyeree" in Yoruba and "monsoro" in Hausa. The fruits and leaves are commonly sold in Nigeria markets as condiment for food flavoring (Chibuzor and Assumpta, 2014) Ethnomedicinal uses of *Piper guineense* includes: as carminatives and eupeptic (Echo *et al*., 2012), treatment of respiratory infection and syphilis (Okigbo and Igwe, 2007), aphrodisiacs (Noumi *et al*., 1988). Despite its extensive use in crude form in traditional medicine, particularly as a remedy for ulcer, the purified forms of this plant have not been explored

The present work evaluated the gastroprotective activities of fraction extracts of *Piper guineense* leaf on ethanol-induced ulcer in Wister rats.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh leaves of the plant, *Piper guineense*, obtained from a farm land in Naze, Owerri North Local Government Area of Imo State, were identified in the Department of Pharmacognosy, Madonna University, Nigeria, authenticated by a taxonomist, in the herbarium of Department of Plant Science and Biotechnology, University of Port Harcourt, and deposited as voucher specimen with Herbarium Number designated as UPH/P/251

Animal ethics approval

The study was conducted after obtaining full animal ethics approval (Reference number: MAU/SREC/A/17) from University Senate Research and Ethics Committee of Madonna University, Nigeria.

Experimental Animals

The experimental animals used in the study included:

- Healthy adult wistar rats weighing between 170-190g of 12-15 weeks old
- Healthy adult wistar mice weighing between 20-22g of 12-15 weeks old.

These animals were bred in Animal Facility Centre of Madonna University, Nigeria at room temperature. The animals were supplied with clean drinking tap water and fed *ad-libitum* with commercial poultry growers feed (Top feeds ^{R}, Nigeria). The animals were handled in line with the highest standard for the humane and compassionate use of animals in biomedical research as outlined in the Guide for Care and the Use of Laboratory Animals prepared by National Academy of Science and published by the National Institute of Health (NIH) (1986).

Drugs and Chemicals

The following drugs and chemicals were used in the study: Cimetidine (Cadila Pharmaceuticals Pvt Ltd, India), Sucralfate (Moraceae Pharmaceutical Pvt Ltd, Indian), Sodium tetraoxocarbonate IV (Sigma Aldrich Chemie, Germany) Xylene (Chemech, England), 96% Ethanol (Gungsdong Guandgua Chemical Factory ,China), Chloroform (Super Tek Chemicals, India), Glacial acetic acid (Sigma Aldrich Chemie, Germany), Ferric Chloride (Super Tek Chemical, Germany), Tetraoxosulphate VI acid (Hi Media Laboratories Pvt Ltd, India) Hydrochloric acid (Nice Laboratories Reagent, Kevala, India), n-Hexane (Sigma Aldrich Chemie, Germany), Ethylacetate (Rankem, Mumbai, India), Dragendoff's reagent (Super Tek Chemicals, Germany), Tween 80 (3%v/v) (Super Tek Chemicals Germany),

Preparation and extraction of plant material

According to procedure specified by Girma *et al*., (2015), fresh matured leaves of *Piper guineense* were thoroughly washed, air-dried at room temperature for two weeks and coarsely powdered. About 500g of coarsely powdered leaves of *Piper guineense* was macerated in two litres of ethanol (80%) at room temperature with occasional shaking every six hours for 72 hours, after which the filtrate was separated from the marc using filter paper (Whatman No. 1). The marc was re-macerated twice. The obtained filtrates were combined and evaporated in an oven at 40^{o}C.

Calculation of percent yield of plant extract of *Piper guineense*

This was carried out according to the formula demonstrated by Okoli *et al*., (2010) as:

$$\% \text{ Yield} = \frac{\text{Weight(g) of extracted dry residue}}{\text{Weight(g) of macerated powered material}} \times 100$$

Fractionation of ethanol-extract residue

Two chromatographic techniques (thin-layer and column chromatograph) were employed. The thin-layer chromatography was first used to determine the solvent system that gave best resolution, while column chromatography was used to fractionate the crude extract.

Preliminary TLC was carried was conducted on silica gel (F_{254}) plates according to procedure proposed by Stahl (1969) to determine the solvent system that gave best resolution. The following solvent systems were prepared and in the ratio stated below.

Chloroform/Ethylacetate (9:1)
Chloroform/Ethylacetate (8:2)
Chloroform/Ethylacetate/n-Hexane (7:2:1)
Chloroform/Ethanol (1:1)
Chloroform/Ethylacetate/Ethanol (7:2:1)
Chloroform/n-Hexane (1:1)
Ethylacetate/n-Hexane (1:1)
Ethylacetate/Ethanol (1:1)

Fractionation of the extracted-residue was conducted with column chromatography using a glass column of internal diameter of 20mm and length 19cm (Quick-fit England). The column initially packed with sufficient quantity of wet silica gel (F_{254}) was allowed for 24 hours to stabilize. Then a 10g amount of crude extract was dissolved in ethanol, placed on the column and was continuously eluted with the solvent system (Chloroform /Ethylacetate Ethanol; 7:2:1) that gave best resolution in the preliminary thin-layer chromatography.Seventeen-10ml fractions were collected and their TLC mobility (R_f) mobility (R_f) was calculated using the following formula.

$$R_f = \frac{\text{Distance(cm) travelled by the spot from starting point in TLC}}{\text{Distance(cm) travelled by the solvent front in TLC}}$$

Pooling, Labeling and storage of the plant extracts

Fractions that showed similar R_f value were pooled together, evaporated to dryness, labeled appropriately and stored in well-sealed containers as:

EE = Ethanol Extract
PF-1 = Pooled Fraction-1
PF-2 = Pooled Fraction-2
PF-3 = Pooled Fraction-3
PF-4 = Pooled Fraction-4
PF-5 = Pooled Fraction-5

The labeled containers were stored in the refrigerator until when needed.

Phytochemical analysis of ethanol and fraction extracts.

This was conducted using standard procedures specified by Harbone, (1998) to test for presence or absence of various phytochemicals such as flavonoids, saponins, tannins, glycosides, alkaloids, terpenoids and phenolics.

Acute toxicity (LD_{50}) determination

This was conducted with both the ethanol and fraction extracts to establish safe doses of the extracts to be used in subsequent whole animal experiment. A method proposed by Lorke (1983) was employed.

Experimental design

Fifty-six (56) adult wistar rats (170-190g) randomized into eight groups (labeled A – H), consisted of seven animals per group, were fasted of food for 24 hours but allowed free access to water until two hours prior to experiment. Drug and extracts were administered orally via intragastric tube. The doses of extracts administered were safe, as determined in acute toxicity study. Each group of animals was treated as follows:

Group A received 400mg/kg PF-1 orally x stat
Group B received 400mg/kg PF-2 orally x stat
Group C received 400mg/kg PF-3 orally x stat
Group D received 400mg/kg PF-4 orally x stat
Group E received 400mg/kg PF-5 orally x stat
Group F received 400mg/kg EE orally x stat
Group G (positive control) received 100mg/kg cimetidine orally x stat
Group H (negative control) 5ml/kg 3% v/v Tween 80 orally x stat

After 30 minutes following respective treatment as outlined in the experimental design, ulcer was induced by intragastric administration of 5.0ml/kg of 80% ethanol (Dashputre and Naikwade, 2011). Six hours later, the animals were sacrificed under anesthesia and their stomachs cut open along greater curvature.

Macroscopic assessment of stomach

The dissected stomachs as described above were rinsed under tap water, and pinned flat on a flat board. The stomachs were examined with hand lens (x10) to assess ulcer formation. The number of ulcers were counted and scoring made as described by Dashputre and Naikwade (2011) using the following:

Normal colored stomach	0
Red coloration	0.5
Spot ulcer	1
Hemorrhagic streak	1.5
Deep ulcer	2
Perforation	3

Ulcer index and percent inhibition were calculated using equation proposed by Adinortey *et al*., (2013).

$$\text{Ulcer Index} = \frac{\text{total ulcer score}}{\text{number of animals ulcerated}}$$

$$\% \text{ Inhibition} = \frac{\text{ulcer index}_{\text{(control negative)}} - \text{ulcer index}_{\text{(test group)}}}{\text{ulcer index}_{\text{control negative group}}} \times 100$$

Determination of effect of the plant extracts (ethanol and fractions) and standard drug (sulcralfate) on the weight of stomach of the animals after subacute exposure

In this study, fifty-six (56) adult wistar rats (12-15 weeks old) that weighed between 170-190g were randomized into eight groups of seven animals in each group and labeled A-H. The animals in each group were treated as follows daily for four weeks.

Group A received 400mg/kg PF-1 x daily x 4 weeks orally
Group B received 400mg/kg PF-2 x daily x4 weeks orally
Group C received 400mg/kg PF-3 x daily x 4 weeks orally
Group D received 400mg/kg PF-4 x daily x 4 weeks orally
Group E received 400mg/kg PF-5 x daily x 4 weeks orally
Group F received 400mg/kg EE x daily x 4 weeks orally
Group G (positive control) received 250mg/kg Sulcralfate x daily x 4 weeks orally
Group H (negative control) received 5ml/kg 3% v/v Tween 80 x dailyx 4 weeks orally

At the end of four weeks, the animals were sacrificed under anaesthesia and the weight of the stomach determined and recorded.

Histological study

Histological assessment was done by subjecting the isolated stomachs to tissue processing and staining with hematoxylin and eosin (H and E) for histological examination which were observed and recorded with magnification (x60) lenses

RESULTS

Yield of plant extract and fractions

The quantitative yield of the ethanol extract was relatively low (21.08g) when compared to amount (500g) of plant material macerated. Column chromatographic separation yielded a total of seventeen (17) fractions which were pooled into five fractions as earlier explained.

Phytochemical analysis of plant extracts (ethanol and fractions)

The ethanol extract (EE) and fraction extracts (PF-1, PF-2, PF-3, PF-4 and PF-5) contained flavonoids, while glycosides were absent in pooled fraction-2 and pooled fraction-4. There were absence saponins in pooled fraction-1 (PF-1), pooled fraction-2 (PF-2) and pooled fractions-5 (PF-5), while tannins and phenols were absent in pooled fraction – 3 (PF-3) as shown in table 1 below.

Acute toxicity test (LD_{50} determination)

Acute toxicity (LD_{50}) test substantiated that the extracts of *Piper guineense* at the limit test dose of 5000mg/kg body weight of mice did not show any signs of toxicity nor death within 48 hours of observation.

Effect of Piper guineense leaf extracts (ethanol and fractions) and standard drug (cimetidine) on ethanol-induced ulcer in rats

The effect of the plant extracts (ethanol and fractions) and cimetidine on ethanol-induced ulcer in rats is shown in table 2. From the table of result, at oral dose of 400mg/kg body weight, the ethanol extract and fraction extracts produced significant ($P < 0.05$) ulcer inhibition when compared to inhibition obtained with negative control (3% v/v) Tween 80) on ulcer induced at 5ml/kg body weight of 80% ethanol. Among the fraction extracts, the group that received pooled fraction-4 (PF-4) exhibited the highest percentage of ulcer inhibition.

Effect of Piper guineense leaf extracts (ethanol and fractions) and standard drug (sucralfate) on the weight of stomach of animal after sub-acute exposure

The effect of the extracts (ethanol and fraction) on weight of the stomach of the animals were studied by comparing percent increase in weight of the stomach produced by groups that received 400mg/kg of extracts (ethanol and fraction) with that obtained with the negative control group that received 5ml/kg of Tween 80 (3%v/v). From the result as shown in table 3 below, the ethanol extract (EE), pooled fraction-2 (PF-2) pooled fraction-4 (PF-4) produced significant ($P < 0.05$) increase in the weight of stomach of the animal when compared with data obtained with negative control.

Histological examination

Among the pooled fraction extracts, pooled fraction–4 (PF – 4) produced highest ulcer inhibition (49.02%) and increase in stomach weight (27.23%) and therefore was used for histological study. The impact of the pooled-fraction extract (PF-4) and standard drug (cimetidine) on the histoarchitecture of the stomach was studied. Figure 1 shows a photomicrograph of a section of stomach (magnification: x60) from negative control group with arrows indicating erosion (ulceration) of the mucous coat. Figure 2 shows photomicrograph of a section of stomach (magnification: x60) of extract-treated group with arrows showing minor epithelial loss. Figure 3 shows a section of stomach (magnification: x60) of cimetidine-treated group with intact mucosa

Table 1: Phytochemical analysis of *Piper guineense* leaf extracts (ethanol and fraction)

Test	EE	PF-1	PF-2	PF-3	PF-4	PF-5
Carotenoids	+	-	-	+	-	-
Phenols	+	+	+	-	+	+
Saponins	+	-	-	+	+	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	-	+	+
Glycosides	+	+	-	+	-	+
Alkaloids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+

+ = Present

- = Absent

Table 2: Effect of *Piper guineense* leaf extracts (ethanol and fractions) and standard drug (cimetidine) on ethanol-induced ulcer in rats

Treatment group	Dose (oral)	Ulcer index	% Inhibition
A (PF-1)	400mg/kg	5.57 ± 0.43	21.99*
B (PF-2)	400mg/kg	5.79 ± 0.29	18.91*
C (PF-3)	400mg/kg	4.71 ± 0.34	34.03*
D (PF-4)	400mg/kg	3.64 ± 0.21	49.02*
E (PF-5)	400mg/kg	5.29 ± 0.21	25.91*
F (EE)	400mg/kg	4.36 ± 0.30	38.94*
G (Positive control)	100mg/kg	1.29 ± 0.42	81.93*
H (Negative control)	5ml/kg	7.14 ± 0.32	—

Values represent mean ± SEM of seven animals in each group
**Significant relative to negative control (3% v/v Tween 8)*

Table 3: Effect of *Piper guineense* leaf extracts (ethanol and fractions) and standard drug (sucralfate) on the weight of stomach of animals after sub-acute exposure

Treatment Group	Dose (Oral)	Weight of Stomach(g)	% Increase
A (PF-1)	400mg/kg	2.17 ± 0.06	1.88
B (PF-2)	400mg/kg	2.63 ±0.13	23.47*
C (PF-3)	400mg/kg	2.17 ± 0.08	1.88
D (PF-4)	400mg/kg	2.71 ± 0.14	27.23*
E (PF-5)	400mg/kg	2.17 ± 0.06	1.88
F (EE)	400mg/kg	2.84 ± 0.06	33.33*
G (Positive control)	250mg/kg	3.40 ± 0.08	59.62*
H (Negative control)	5ml/kg	2.13 ± 0.06	—

Values represent mean ± SEM of seven animals in each group
**Significant relative to negative control (3% v/v Tween 8*

Table 4: Mean (±SEM) ulcer index of test and control groups of adult Wistar rats after oral administration of 400mg/kg *Piper guineense* leaf extracts and 100mg/kg cimetidine on ethanol-induced ulcer

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
PF-1	7	5.5714	1.13389	.42857	4.5228	6.6201	4.00	6.50
PF-2	7	5.7857	.75593	.28571	5.0866	6.4848	5.00	7.00
PF-3	7	4.7143	.90633	.34256	3.8761	5.5525	3.50	6.00
PF-4	7	3.6429	.55635	.21028	3.1283	4.1574	3.00	4.50
PF-5	7	5.2857	.56695	.21429	4.7614	5.8101	4.50	6.00
EE	7	4.3571	.80178	.30305	3.6156	5.0987	3.00	5.50
NControl	7	7.1429	.85217	.32209	6.3547	7.9310	6.00	8.00
Total	49	5.2143	1.30304	.18615	4.8400	5.5886	3.00	8.00

Table 5: Result of statistical test of significance (ANOVA) of ulcer index values obtained in adult Wistar rats after oral administration of 400mg/kg *Piper guineense* leaf extracts and 100mg cimetidine to test and control groups on ethanol-induced ulcer

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	53.429	6	8.905	13.323	.000
Within Groups	28.071	42	.668		
Total	81.500	48			

Table 6: Multiple comparisons of ulcer index values obtained in adult Wistar rats after oral administration of 400mg/kg *Piper guineense* leaf extracts and 100mg/kg cimetidine to test and control groups on ethanol-induced ulcer

Dependent Variable: Result							
	(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	PF-1	PF-2	-.21429	.43699	.626	-1.0962	.6676
		PF-3	.85714	.43699	.056	-.0247	1.7390
		PF-4	1.92857*	.43699	.000	1.0467	2.8105
		PF-5	.28571	.43699	.517	-.5962	1.1676
		EE	1.21429*	.43699	.008	.3324	2.0962
		NControl	-1.57143*	.43699	.001	-2.4533	-.6895
	PF-2	PF-1	.21429	.43699	.626	-.6676	1.0962
		PF-3	1.07143*	.43699	.018	.1895	1.9533
		PF-4	2.14286*	.43699	.000	1.2610	3.0247
		PF-5	.50000	.43699	.259	-.3819	1.3819
		EE	1.42857*	.43699	.002	.5467	2.3105
		NControl	-1.35714*	.43699	.003	-2.2390	-.4753
	PF-3	PF-1	-.85714	.43699	.056	-1.7390	.0247
		PF-2	-1.07143*	.43699	.018	-1.9533	-.1895
		PF-4	1.07143*	.43699	.018	.1895	1.9533
		PF-5	-.57143	.43699	.198	-1.4533	.3105
		EE	.35714	.43699	.418	-.5247	1.2390
		NControl	-2.42857*	.43699	.000	-3.3105	-1.5467
	PF-4	PF-1	-1.92857*	.43699	.000	-2.8105	-1.0467
		PF-2	-2.14286*	.43699	.000	-3.0247	-1.2610
		PF-3	-1.07143*	.43699	.018	-1.9533	-.1895
		PF-5	-1.64286*	.43699	.001	-2.5247	-.7610
		EE	-.71429	.43699	.110	-1.5962	.1676
		NControl	-3.50000*	.43699	.000	-4.3819	-2.6181
	PF-5	PF-1	-.28571	.43699	.517	-1.1676	.5962
		PF-2	-.50000	.43699	.259	-1.3819	.3819
		PF-3	.57143	.43699	.198	-.3105	1.4533
		PF-4	1.64286*	.43699	.001	.7610	2.5247
		EE	.92857*	.43699	.040	.0467	1.8105
		NControl	-1.85714*	.43699	.000	-2.7390	-.9753
	EE	PF-1	-1.21429*	.43699	.008	-2.0962	-.3324
		PF-2	-1.42857*	.43699	.002	-2.3105	-.5467
		PF-3	-.35714	.43699	.418	-1.2390	.5247
		PF-4	.71429	.43699	.110	-.1676	1.5962
		PF-5	-.92857*	.43699	.040	-1.8105	-.0467
		NControl	-2.78571*	.43699	.000	-3.6676	-1.9038
	NControl	PF-1	1.57143*	.43699	.001	.6895	2.4533
		PF-2	1.35714*	.43699	.003	.4753	2.2390
		PF-3	2.42857*	.43699	.000	1.5467	3.3105
		PF-4	3.50000*	.43699	.000	2.6181	4.3819
		PF-5	1.85714*	.43699	.000	.9753	2.7390
		EE	2.78571*	.43699	.000	1.9038	3.6676

*. The mean difference is significant at the 0.05 level

Table 7: Mean (±SEM) stomach weight of test and control groups of adult Wistar rats after oral administration of 400mg/kg *Piper guineense* leaf extracts and 100mg/kg cimetidine on ethanol-induced ulcer

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
PF-1	7	2.1714	.16036	.06061	2.0231	2.3197	1.90	2.30
PF-2	7	2.6286	.34983	.13222	2.3050	2.9521	2.20	3.00
PF-3	7	2.1714	.20587	.07781	1.9810	2.3618	1.80	2.40
PF-4	7	2.7143	.37607	.14214	2.3665	3.0621	2.30	3.20
PF-5	7	2.1714	.17043	.06442	2.0138	2.3291	1.90	2.40
EE	7	2.8429	.15119	.05714	2.7030	2.9827	2.70	3.10
Control	7	2.1286	.16036	.06061	1.9803	2.2769	1.90	2.30
Total	49	2.4041	.36797	.05257	2.2984	2.5098	1.80	3.20

Table 8: Result of statistical test of significance (ANOVA) of stomach weight values obtained after oral administration of 400mg/kg *Piper guineense* leaf extracts and 100mg/kg cimetidine to test and control groups on ethanol-induced ulcer

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.042	6	.674	11.515	.000
Within Groups	2.457	42	.059		
Total	6.499	48			

Table 9: Multiple comparisons of stomach weight values obtained in adult Wistar rats after oral administration of 400mg/kg *Piper guineense* leaf extracts and cimetidine to test and control groups on ethanol-induced ulcer

Dependent Variable: Result							
	(I) Factor	(J) Factor	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	PF-1	PF-2	-.45714*	.12929	.001	-.7181	-.1962
		PF-3	.00000	.12929	1.000	-.2609	.2609
		PF-4	-.54286*	.12929	.000	-.8038	-.2819
		PF-5	.00000	.12929	1.000	-.2609	.2609
		EE	-.67143*	.12929	.000	-.9323	-.4105
		Control	.04286	.12929	.742	-.2181	.3038
	PF-2	PF-1	.45714*	.12929	.001	.1962	.7181
		PF-3	.45714*	.12929	.001	.1962	.7181
		PF-4	-.08571	.12929	.511	-.3466	.1752
		PF-5	.45714*	.12929	.001	.1962	.7181
		EE	-.21429	.12929	.105	-.4752	.0466
		Control	.50000*	.12929	.000	.2391	.7609
	PF-3	PF-1	.00000	.12929	1.000	-.2609	.2609
		PF-2	-.45714*	.12929	.001	-.7181	-.1962
		PF-4	-.54286*	.12929	.000	-.8038	-.2819
		PF-5	.00000	.12929	1.000	-.2609	.2609
		EE	-.67143*	.12929	.000	-.9323	-.4105
		Control	.04286	.12929	.742	-.2181	.3038
	PF-4	PF-1	.54286*	.12929	.000	.2819	.8038
		PF-2	.08571	.12929	.511	-.1752	.3466
		PF-3	.54286*	.12929	.000	.2819	.8038
		PF-5	.54286*	.12929	.000	.2819	.8038
		EE	-.12857	.12929	.326	-.3895	.1323
		Control	.58571*	.12929	.000	.3248	.8466
	PF-5	PF-1	.00000	.12929	1.000	-.2609	.2609
		PF-2	-.45714*	.12929	.001	-.7181	-.1962
		PF-3	.00000	.12929	1.000	-.2609	.2609
		PF-4	-.54286*	.12929	.000	-.8038	-.2819
		EE	-.67143*	.12929	.000	-.9323	-.4105
		Control	.04286	.12929	.742	-.2181	.3038
	EE	PF-1	.67143*	.12929	.000	.4105	.9323

		PF-2	.21429	.12929	.105	-.0466	.4752
		PF-3	.67143*	.12929	.000	.4105	.9323
		PF-4	.12857	.12929	.326	-.1323	.3895
		PF-5	.67143*	.12929	.000	.4105	.9323
		Control	.71429*	.12929	.000	.4534	.9752
	Control	PF-1	-.04286	.12929	.742	-.3038	.2181
		PF-2	-.50000*	.12929	.000	-.7609	-.2391
		PF-3	-.04286	.12929	.742	-.3038	.2181
		PF-4	-.58571*	.12929	.000	-.8466	-.3248
		PF-5	-.04286	.12929	.742	-.3038	.2181
		EE	-.71429*	.12929	.000	-.9752	-.4534

*. The mean difference is significant at the 0.05 level.

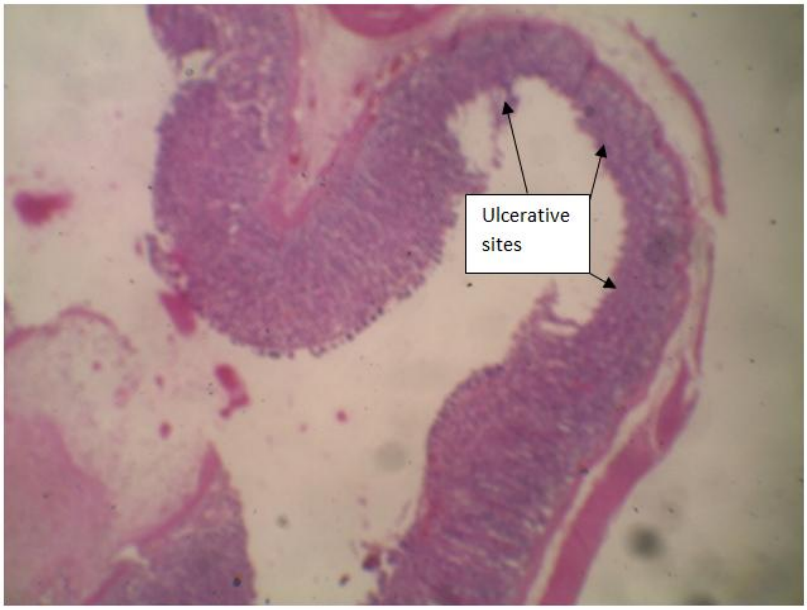

Figure 1: Photomicrograph of stomach (magnification X60) of negative control group showing ulcerative sites

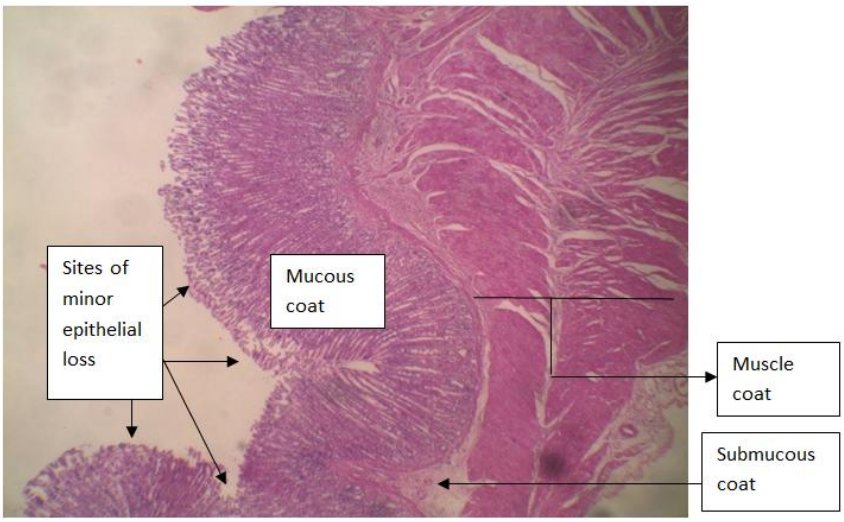

Figure 2: Photomicrograph of stomach (magnification X60) of extract-treated group showing sites of minor epithelial loss

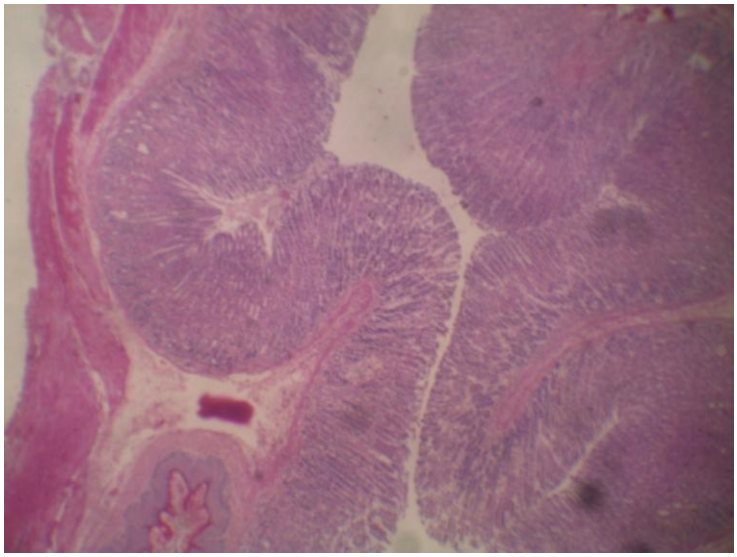

Figure 3: Photomicrograph of stomach (magnification X60) of positive control (cimetidine-treated) group showing intact mucous coat, no ulceration

DISCUSSION

Yield

Judging by the amount of pulverized plant material (500g) in this study, the yield of the crude extract was low (21.08g). This is in correlation with report of Tushar (2017) that biologically active compounds usually occur in plants in low concentration.

Phytochemical Analysis

In this study, phytochemical screening of crude and fraction extracts of *Piper guineense* showed the presence flavonoids, saponins, tannins, glycosides, alkaloids terpenoids and phenolics. Numerous plants phytochemicals have been reported to display antiulcer effect on different models of ulcer through different mechanisms which include antioxidant, antibacterial, antisecretory, anticholinergic and cytoprotective activities (Harsha *et al.,* 2017). Flavonoids have wide range of activities including antiulcer, antioxidant, antidiarrheal and antimicrobial (Rancy and Krishnakumaris, 2015). Nwafor and Akah (2003) reported that flavonoids have the ability to interfere with the release of histamine in gastric mucosa. Tannins which are widely distributed in plants (Finar, 1975), have the ability to augment cell proliferation and enhance mucus secretion (Sharifi-Rad *et al.*, 2018). Tannins being astringent in nature, may precipitate microproteins at the ulcer sites thereby forming an impervious pellicle over the stomach living that renders it less permeable to toxic substances and more resistant to attack by proteolytic enzymes (Nwafor *et al.*, 1996). Terpenoids have been shown to decrease gastric and duodenal lesions and increase production of mucus in experimental ulcers induced by NSAIDs and ethanol, NSAIDs, cysteamine and stress (Memariani *et al.*, 2017).

Acute Toxicity

In this study, of the extracts of *Piper guineense* did not show any toxicity. This was substantiated in acute toxicity (LD_{50}) determination, which indicates that even at LD_{50} of 5000mg/kg body weight of mice, the extracts did not show any obvious signs of toxicity or death within 48 hours of observation. This finding is supported by report of Lorke *et al*, (1983) that compounds with LD_{50} up to 5000mg/kg body weight are considered to have low toxicity potential

Assessment of Antiulcer Properties

From the results in tables 3, PF-4 produced highest reduction of gastric volume (49.02%), which revealed that *Piper-guineense* pre-treatment in ethanol-ulcerated wistar rats significantly ($p<0.05$) inhibited gastric lesion: an effect that support gastroprotective effect of the plant. These findings is in correlation with the report of Saroj *et al.,* (2016) on leaf extract of *Salvadora indica* that produced gastroprotective action against ethanol-induced gastric ulcer in albino rats. Best *et al.,* (1984) reported that gastroprotective agents increase the weight of stomach by increasing the synthesis of sialic acid and hexosamine in the mucosa.. Therefore in this study, the ability of the extracts (EE, PF-2 and PF-4) of *Piper guineense* at 400mg/kg, to significantly ($p<0.05$) increase the weight of stomach is a proof of its gastroprotective action. This action is similar to antiulcer activity of unripe plantain on NSAID-induced ulcer (Ahmad *et al.*, 2016), gastroprotective effect of *Caesalpinia sappan* on gastric lesion induced by necrotizing agent like hydro-alcohol (Challappan *et al*., 2017) and replenishment of levels of non-protein sulfhydrl compound which plays an important role in mucosal protection against ethanol-induced ulcer (Zhang *et al*., 2014; Zakaria *et al*., 2016)

Histology

Treatment with ethanol was associated with changes in the histoarchitecture of the stomach evidenced by disruption and erosion of the mucosal layer with necrotic debris observed in the lumen of ulcer. Shredding of epithelial cells and infiltration of lymphocytes were observed (Figure-1). Pre-treatment with extract of *Piper guineense* (400mg/kg) considerably reduced these changes in the histoarchiteture of the stomach and provided protection against ethanol induced gastric lesions (Figure-2). Treatment with cimetidine (100mg/kg) showed no ulceration (Figure 3)

CONCLUSION

The results of this study conclude that *Piper guneense* possesses gasroprotective activities, hence can be novel remedy in the treatment of gastric ulcer.

REFERENCES

- Tanih, N. F., Ndip, L. M., Clark, A. M., & Ndip, R. N. (2010). An overview of pathogenesis and epidemiology of *Helicobacter pylori* infection, *African Journal of Microbiology Research*, *4*(6), 426-436.
- Pradhan, D., Biswasroy, P., Singh, A., & Suri, K. (2013). Antiulcerogenic activity of ethanol extract of *Curcuma sativus* against NSAID (Aspirin) induced gastric ulcer in Wister albino rats. *International Journal of Herbal Medicine*, *1*, 115-119.
- Esen, S. K., Abdulmecil, A., Zerrin, K., & Yasin, B. (2018). Gastroprotective and antioxidant effects of *Eremurus spetabilis* methanol extract and its isolated components isoorientin on indomethacin induced gastric ulcer in rats, *Acta Cirurgica Brasiteira*, *33*(7), 54-63.
- Arumugam, S., Selvaraj, S. V., Velayutham, S., Natesan, S. K., & Palaniswarmy, K. (2011). Evaluation of antiulcer activity of *Samanea saman* bark on ethanol and stress induced gastric lesions in albino rats. *Indian Journal of Pharmacology*, *43*, 583-590.
- Saroj, K. S., Himanshu, B. S., Priyadarshini, D., Soundarya, G., Kumar, C. K., & Rani, K. U. (2016). Antiulcer activity of ethanolic extract of *Salvadora indica* leaves on albino rats, *Journal of Clinical and Diagnostic Research*, *10*(9), 7-1100(1-2).
- Rates, S. M. K. (2001) Plants as source of drugs. *Toxicology*, *39*(5), 603-611.
- Alkofahi, A., & Atta, A. H. (1999). Pharmacological Screening of antiulcerogenic effects of some Jordanian medicinal plants in rats, *Journal of Ethnophamacology*, *67*(3), 341-345.
- Schmeda-Hirschmann, G., & Yesilada, F. (2005). Traditional medicine and gastroprotective crude drugs. *Journal of Ethnophamacology*, 61-66.
- Chibuzor, O., & Assumpta, O. (2014). Nutritional evaluation of some selected spices commonly used in South-Eastern Nigeria. *Journal of Biology and Agriculture*, *4*(5), 56-60.
- Okigbo, K. N., & Igwe, D. (2007). Antimicrobial effects of *Piper guineense* and *Phylantrus amarus* on *Candida albican* and *Streptococcus feacalis*. *Acta Microbiological Hungarican*, *54*(4), 353-366.
- Noumi, E., Amvan, Z. P. H., & Lontis, D. (1998). Aphrodisiac plant used in Cameroon. *Fitoter*, *69*,5-34.
- National Institute of Health. (1986). *Public Health Services Policy on Humane Care and use of Laboratory Animals.* US Department of Health and Human Services, PP. 99-156.
- Girma, S., Gidan, M., Erko, B., & Mamoh, H. (2015). Effect of crude leaf extract of *Osyris, Quadripartite* on *Plasmodium berghei* in Swiss albino mice, *British Medical Journal of Complementary and Alternative Medicine*, *15*, 184.
- Okoli, A. S., Okeke, M. I., Iroegbu, C. U., & Ebo, P. U. (2010). Extraction and Evaluation of antibacterial principles of *Harungana madagascariensis* leaf, *Phytotherapy Research*, *16*, 183-186.
- Stahl, E. (1969). *Thin-layer chromatography. a laboratory handbook*. 1st edn. Berlin: Springer, pp. 52-55.
- Harbone, J. B. (1998). *Phytochemical methods: a guide to modern techniques of plant analysis*. 5th edn, London, UK: Chapman & Hall, pp 146.
- Lorke, D. (1983). A new approach to practical acute toxicity, *Archieves of Toxicology*, *24*, 275-289.
- Dashputre, N. L., & Naikwade, N. S. (2011). Evaluation of antiulcer activity of methanol extract of *Abutilon indicum* Linn leaves in experimental rats *International Journal of Pharmaceutical Sciences and Drug Research*, *3*(2), 91-100.
- Adinortey, M. B., Ansah, C., Galyuon, I., & Nyarko, A. (2013). Invivo models used for evaluation A of potential antigastroduodenal ulcer agents, *Ulcers*, *20*, 79-85.
- Tushar, F. G. (2017). Effect of extraction method on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*, *Arabian Journal of Chemistry*, *10*(51), 51200-51203.
- Harsha, C., Banik, K., Bordoloi, D., & Kunnumakkara, A. B. (2017). Antiulcer properties of fruits and vegetables: a mechanism based perspective, *Food Chemistry and Toxicology*, *108*, 104-109.
- Rancy, A. T., & Krishnakumari, S. (2015). Phytochemical profiling of *Myristica fragans* seed

extract with different organic solvents, *Asian Journal of Pharmacy and Clincal Research*, *8*, 303-302.

- Nwafor, S. V., & Akah, P. A. (2003). Effect of methanolic extract of *Cissampelos mucronata*: a drug against indomethacin-induced ulcer in rats, *Indian Journal of Experimental Biology*, *41*, 151-183.
- Finar, I. L. (1975). *Organic Chemistry: Sterochemistry and organic chemistry of natural products*. 5th edn (vol 2), London: Longman Group Essex, pp. 771-793.
- Sharifi-Rad, M., Fokou, P. V. T., Sharopov, F., Martorell, M., & Ademiluyi, A. O. (2018). Antiulcer agents: from plant extracts to phytochemicals in healing promotion, *Molecules*, *23*(7), 1751.
- Nwafor, P. A., Okwuasaba, F. K., & Binda L. C. (1996). Gastroprotective effects of aqueous extract of *Khaya senegalensis* bark on indomethacin-induced ulceration in rats, *West African Journal of Pharmacology and Drug Research*, 12, 46-50.
- Memariani, Z. Sharifzadeh, M., Bozorgi, M., Hajimahmoodi, M., Farzaei, M. H., & Gholami, M. (2017). Protective effect of essential oil of *Pistacia atlantica* on peptic ulcer: role of r-pinene, *Journal of Traditional Chinese Medicine*, 37, 57-63.
- Best, R., Lewis, D. A., & Nasser, N. (1984). Antiulcerogenic activity of unripe Banana and Plantain, *British Journal of Pharmacology*, *82*, 107-116.
- Ahmad, M. M., Rahma, M., Rumi, A. K., Islam, S., Huq, F., & Chowdhury, M. F. (1996) Prevalence of *H. pylori* in asymptomatic population - a pilot serological study in Bangledesh. *Journal of Epidemiology*, *7*, 251-254.
- Chellappan, D. R., Purushothaman, A. K., & Brindha, P. (2017). Gastroprotective potential of hydro-alcoholic extract of pattanga (*Caesalpinia Sappan*), *Journal of Ethnopharmacology*, *197*, 294-305.
- Zhang, Y. F., Xie, J. H., Xu, Y. F., Liang, Y. Z., Mo, Z. Z. & Jiang, W. W. (2014). Gastroprotective effect and mechanism of patchouli alcohol against ethanol, indomethacin and stress-induced ulcer in rats, *Chemistry and Biology Interaction*, *222*, 27-36.
- Zakaria, Z. A., Balen, T., & Azemi, A. K. (2016). Mechanism(s) of action underlying gastroprotective effect of ethyl acetate fraction obtained from crude methanolic leaves extract of *Mutingia calabura*, *BMC Complementary and Alternative Medicine*, *16*(78), 209-221.