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RESEARCH ARTICLE

Assessment of Gastric Acid Anti-Secretory Effects of Fraction Extracts of *Piper guineense* Leaf on Histamine-Induced Gastric Ulcer in Wistar Rats

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ABSTRACT

Despite many etiological factors, gastric acid secretion is assumed to play a central role in the development of gastric ulcer. Blockade of gastric acid secretion therefore, seems to be the key therapeutic objective of gastric ulcer disease. Available acid-blocking/anti-secretory drugs used in the management of gastric ulcer are associated with high cost, unwanted side effects and treatment relapse. Extracts from plants can be useful and may serve as an alternative to conventional drugs in gastric ulcer management. This work was aimed at assessing the gastric acid anti-secretory effects of fractionated ethanol extract of *Piper guineense* leaf on histamine-induced ulcer in Wistar rats. This was conducted by administering orally, 400mg/kg of fraction extracts obtained by fractionation of ethanol-extracted residue, to fifty six adult Wistar rats randomized into eight groups (n=7) of animals followed by induction of ulcer with 40mg/kg histamine, orally. The effects of the plant extracts on histamine-induced ulcer, gastric pH and gastric volume were ascertained. From the results, all the extracts significantly ($p<0.05$) produced ulcer inhibition, increased gastric pH and reduced gastric volume. This study concludes that extracts of *Piper guineense* leaf possess antiulcer effects, which may occur via histamine-2-receptor blockade and/or anti-secretory mediated activities.

Keywords: Gastric acid; Fraction extracts; Histamine; Antisecretory; Piper guineense

INTRODUCTION

Gastric ulcer being one of the major health disorders that affects significant proportion of global population, continues to increase both in occurrence and prevalence.[1,2] Gastric ulcer has been described[2,3] as the new "plague" of the 21st century, with increasing morbidity. Pathogenesis of gastric ulcer is linked to an imbalance between the gastroprotective and destructive factors of the stomach.[3] Gastric ulcer develops when the destructive factors are in excess of the gastroprotective factors, or as a result of diminished mucosal protective mechanisms.[4–6] The destructive factors include gastric acid, pepsin, non-steroidal anti-inflammatory drugs (NSAIDs), excessive consumption of alcohol, free radicals, poor diet, stress and infection by *Helicobacter pylori*.[7,8] Recently, prevention and treatment of gastric ulcer have posed some challenges to medicine, thus, have stimulated a lot of research interest. Recent approaches in the management of gastric ulcer involve blocking secretion of gastric acid, using conventional histamine$_2$-receptor antagonists and proton pump inhibitors. Although conventional drugs have reasonably reduced the morbidity and mortality rates, they have some short-comings. High treatment failure and relapse rates, drug-drug interaction, high cost and unwanted side effects[3] pose complexity in routine management of this ailment. On this note and on the basis of preponderance of evidence demonstrating the effectiveness of medicinal plants,[9–11] attention of researchers has been drawn to medicinal plants as an alternative in the pharmacotherapy of ulcer diseases. Plant can serve as a novel source of therapeutic agents for management of ulcer, whereby extracts with anti-secretory ability, in isolation and/or in combination with other agents, can provide gastroprotective actions. This formed the justification for

study of antiulcer activity of *Piper guineense* leaf, claimed by traditional medicine as remedy for various forms of stomach disorders in the Eastern part of Nigeria.[12]

Piper guineense, a native of Tropical regions of Central and West Africa, is a perennial plant cultivated in Nigeria where it is locally known as "iyere" among the Yorubas, "uziza" among the Igbos and "monsoro" among the Hausas. *Piper guineense* is commonly used in Nigeria as food spices and appetizer.[13] Studies have reported various ethnomedicinal uses of *Piper guineense* which include: treatment of female infertility and low sperm count in men,[14] weight control,[15] fish preservation,[16] carminative and eupeptic[17] and insecticidal activity.[18] Previous report found that alkaloids, flavonoids, saponins, tannins, resins and essential oil are present in *Piper guineense* leaves.[19]

We had earlier reported that the fraction extracts of *Piper guineense* leaf exhibited significant antiulcer activities against indomethacin-induced ulcer in Wistar rats.[20] Nevertheless; its effect on gastric acid secretion was neither examined nor explored in our previous study. The present study, therefore, assessed the gastric acid anti-secretory effects of fraction extracts of *Piper guineense* leaf on histamine-induced ulcer in Wistar rats, with a view to establish the possible mechanism(s) of antiulcer activity, and to scientifically justify the tradomedical claim as ulcer remedy.

MATERIALS AND METHODS

Plant collection, identification and authentication

Piper guineense fresh leaves, were harvested from a farm land in Naze, Owerri, Imo State, Nigeria, during rainy season in the month of August, 2018. The plant material was identified, authenticated and deposited as voucher specimen (Herbarium Number: UPH/P/251) for further reference in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigreia.

Experimental animals/Animal ethics approval

Healthy adult Wistar rats of 12-15 weeks old that weighed between 170 and 190g and healthy adult albino mice of 12-15 weeks old that weighed between 20 and 22g were employed in the study. The animals were reared in Animal Facility Unit of Madonna University, Nigeria, under well-ventilated room temperature. They were housed in secured cages, floored with adequate wood shavings/saw-dusts, fed freely with commercial poultry growers feed (Top feeds^{R}, Nigeria) and supplied with clean drinking tap water. Animal ethics approval (Reference number: MAU/SREC/A/17) was granted by University Senate Research and Ethics Committee of Madonna University, Nigeria

Animal handling compliances as published earlier[21] were strictly followed.

Study area

All studies that involved the use of animals were conducted at the Animal Facility Unit, Madonna University, Nigeria, Elele Campus.

Drugs and reagents

Drugs and reagents employed in this work included the following: Histamine (Fine Chemicals, Mumbai, India), Phenolphthalein (Fine Chemicals, Mumbai, India) Cimetidine (Cadila Pharmaceuticals Pvt Ltd, India), Chloroform (Super Tek Chemicals, India), Sodium Hydroxide (Rankem Mumbai, India), Tween 80 (3%v/v) (Super Tek Chemicals, Germany), Ethylacetate (Rankem, Mumbai, India), Dragendorff's reagent (Super Tek Chemicals, Germany), n-Hexane (Sigma Aldrich Chemie, Germany) Tween 80 (3%v/v) (Super Tek Chemicals, Germany), 96% Ethanol (Gungsdong Guandgua Chemical Factory, China), Topfer's reagent (Fine Chemicals, Mumbai, India)

Extraction of crude (ethanol) extract.

As indicated in our earlier study,[22] about three kilograms of matured fresh leaves of *Piper guineense* were properly washed in water to remove foreign matters and air-dried under shade for two weeks to obtain a dry mass for powdering. Using a roller grinder, the dried leaves were ground into coarse powder, and about 500g was soaked in two liters of ethanol (80%) at ambient temperature. The soaked material was occasionally stirred four times a day (every six hours) for three days. The resultant solution was filtered through filter papers (Whatmann, No 1) to separate the filtrate from the marc. The marc was re-soaked and re-filtered. After filtration, all the obtained filtrates were gathered together in a previously weighed clean empty beaker. The beaker and its content were placed in an oven at 40°C until ethanol was evaporated and dried solid residue obtained.

Preparation of fraction extracts

Thin-layer chromatography (TLC)[23] and column chromatography[24] as reported earlier[22] were employed in the preparation of fraction extracts from ethanol-extracted residue. Preliminary thin-layer chromatography (TLC) was performed on eight solvent systems constituted in different ratios, to obtain the one that would give the best resolution.[22] The solvent system (Chloroform /Ethylacetate/Ethanol; 7:2:1) that gave the best resolution was subsequently used in column chromatography to obtain the fraction extracts and to determine their TLC mobility (R_f values). In column chromatographic procedure, 10g of ethanol-extracted residue was dissolved in 10ml ethanol, placed on the column and then continuously eluted with Chloroform /Ethylacetate/Ethanol (7:2:1) solvent system. Seventeen-10ml fractions were collected (labeled FE-1 to FE-17) and

their TLC mobility (R_f values) was calculated as follows[22]

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

Pooling, labeling and storage of the plant extracts

Fractions with similar color reaction and R_f value were appropriately gathered together in a previously weighed clean beaker, then labeled as: pooled fraction-1 (PF-1); pooled fraction-2 (PF-2); pooled fraction-3 (PF-3); pooled fraction-4 (PF-4) and pooled fraction-5 (PF-5). Each beaker and its content were placed in an oven at 40°C to expel the eluting solvent system to obtain a dried solid mass. After drying, the labeled containers were stored in the refrigerator until when needed.

Calculation of percent yield of extracts

Various beakers and their dried content were re-weighed, compared with the initial weight of empty beakers, and difference in weight was noted. Using the formula proposed by,[25] percent yield was calculated as the ratio of weight (g) of the extracted residue to weight (500g) of the soaked powdered material multiplied by hundred

Toxicity (acute) study

Both the ethanol extract and fraction extracts were tested for acute toxicity, to ascertain doses that could be safe in subsequent whole animal experiment. Healthy adult albino mice (20-22g) of 12-15 weeks old that fasted over night were used. A method involving two phases as demonstrated by[26] that used a minimal number of thirteen (13) animals was employed. In this method, defined doses of 10mg/kg, 100mg/kg and 1000mg/kg of the extracts were respectively administered orally to three groups of animals (n=3) in the phase one of the experiment, after which, the animals were observed for 24 hours for any sign of toxicity and death. Based on the result obtained in phase one, the phase two of the experiment was conducted, in which defined doses of 1000mg/kg, 1600mg/kg, 2900mg/kg and 5000mg/kg of the extracts were respectively administered to four groups of animals (n=1) and then observed for sings of toxicity or death for another 24 hours

Study design

Fifty six healthy adult Wistar rats of 12-15 weeks old that weighed between 170 and 190g were randomly placed into eight experimental groups comprising of six test groups (labeled A to F) and two control (positive and negative) groups (labeled G and H respectively). Each test and control groups consisted of seven animals per group (n=7). Each group was fasted for 24 hours prior to the study but was allowed liberty to drink water until two hours to the experiment. Drug and extracts were orally administered through intra-gastric tube and the dose of extracts administered was safe, as ascertained in acute toxicity study. Various groups of animals were treated in following way:

Each rat in Group A received at once, 400mg/kg PF-1, orally

Each rat in Group B received at once, 400mg/kg PF-2, orally

Each rat in Group C received at once, 400mg/kg PF-3, orally

Each rat in Group D received at once, 400mg/kg PF-4, orally

Each rat in Group E received at once, 400mg/kg PF-5, orally

Each rat in Group F received at once, 400mg/kg EE, orally

Each rat in Group G (positive control) received at once, 100mg/kg cimetidine, orally

Each rat in Group H (negative control) received at once, 5ml/kg 3% v/v Tween 80, orally

Following the respective treatment above (i.e. 30 minutes later), ulcer was induced in each animal via intragastric administration of a dose of 40mg/kg histamine.[27]

Isolation of stomach and collection of gastric content

Six hours after the induction of ulcer, the animals were humanely sacrificed under anesthesia and their stomachs dissected. Gastric content of each animal was drained into a separate graduated tube while the stomach was washed under tap water.

Macroscopic assessment of gastric mucosal lesion

Using hand lens (magnification: x10), the washed stomachs were pinned flat on a board and examined for ulcer/lesion formation. The number of ulcers was counted and scoring made as proposed by[28] as follows:

Normal colored stomach = 0;

Red coloration = 0.5;

Spot ulcer = 1;

Hemorrhagic streak = 1.5;

Deep ulcer (ulcer > 3 but $\leq$ 5mm) = 2;

Perforation(> 5mm) = 3

Equations proposed in,[29] were used to calculate the ulcer index and percent ulcer inhibition as follows:

$$Ulcer\ Index = \frac{Total\ ulcer\ score}{Number\ of\ animals\ ulcerated}$$

$$\%\ Ulcer\ Inhibition = \frac{Ulcer\ index\ control\ negative - Ulcer\ index\ test\ group}{Ulcer\ index\ control\ negative\ group}\ x\ 100$$

Determination of gastric pH

Gastric pH was determined according to procedure specified by[27]. In this procedure, an 1ml aliquot of gastric juice was diluted with 1ml of demineralized water, and pH of the resulting solution was measured using pH meter (ADWA AD 8000).

Measurement of volume, free acidity and total acidity of gastric content

The gastric content of each animal was centrifuged at 3000 rpm for ten minutes, after which, the supernatant portion was decanted and measured using measuring cylinder to obtain the gastric volume.

Determination of level of free acidity and total acidity of the gastric content were done according to Topfer's method of gastric analysis as described by[30]. In this method, 1ml aliquot was pipette out into 100ml conical flask, two drops of Topfer's reagent (Dimethyl-amino-azo benzene) were added and titrated with 0.01N Sodium hydroxide until all traces of pink color disappeared and the color of the resulting solution turned into yellowish orange. The quantity of Sodium hydroxide added was recorded and this was taken to correspond to free acidity. Titration was continued until pink color of the obtained solution reappeared. Again the total quantity of Sodium hydroxide added was recorded and this volume was taken to correspond to total acidity. Using the equation proposed by[31], acidity was calculated as follow:

$$Acidity = \frac{V\ x\ N\ x\ 100mEq}{0.1}$$

Where V = volume of Sodium hydroxide

N = Normality of Sodium hydroxide

The calculated values of free and total acidity are expressed as mEq/L[31]

STATISTICAL ANALYSIS OF DATA

Data are expressed as mean ± standard error of mean (SEM) of seven animals. Results were analyzed statistically using one-way analysis of variance (ANOVA). Post-Hoc (Duncan's) multiple comparisons were used to compare the values of the test and the negative control groups using standard statistical Software Package of Social Science (SPSS) version 24. P<0.05 was considered as significant.

RESULTS

R_f values and pooling of fractions

Column chromatographic separation produced a total of seventeen-10ml fractions. Based on the R_f values, FE-1, FE-2 and FE-3 were pooled together as pooled fraction-1(PF-1); FE-4 and FE-5 were pooled together as pooled fraction-2 (PF-2); FE-6, FE-7, and FE-8 were pooled together as pooled fraction-3(PF-3); FE-9, FE-10, FE-11, FE-12, and FE-13 were pooled together as pooled fraction-4 (PF-4); FE-14, FE-15, FE-16 and FE-17 were pooled fraction-(PF-5).

Yield of ethanol and pooled fraction extracts

As shown in Table 1, the quantitative yield of ethanol extract was 21.08g. Among the pooled fractions, pooled fraction-4 (PF-4) gave the highest yield of 3.81g.

Table 1: Yield of Ethanol and Pooled Fraction Extracts

Extract	Yield (g)	% Yield
Ethanol	21.08	4.2
Pf-1	1.23	12.3
Pf-2	0.92	9.2
Pf-3	1.84	18.4
Pf-4	3.81	38.1
Pf-5	2.11	21.1

Toxicity (acute) study

The acute toxicity study did not produce any toxicity symptoms at 5000mg/kg in mice. No mortality was observed within 48 hours in the treated groups, hence, the LD_{50} of the extracts of *Piper guineense* leaf could be greater than 5000mg/kg.

Effects of extracts and standard drug (cimetidine) on histamine-induced ulcer in Wistar rats

The effects of the extracts and standard drug (cimetidine) on histamine ulcer model in Wistar rats were studied by comparing the percent inhibition produced by the extract-treated groups (test groups) with that produced by the 3%v/v Tween 80-treated group (negative control). From the result obtained, cimetidine at 100mg/kg and the extracts (ethanol and fractions) at 400mg/kg, significantly ($P < 0.05$) inhibited ulcer induced with histamine (40mg/kg). PF-3 produced the highest ulcer inhibition of 20.85% as shown in Table 2

Table 2: Effect of plant extracts (ethanol and fractions) and standard drug (cimetidine) on histamine-induced ulcer in Wistar rats

Treatment group	Dose (oral)	Ulcer index	% Inhibition
A (PF-1)	400mg/kg	5.78 ± 0.18	15.74*
B (PF-2)	400mg/kg	5.64 ± 0.21	17.78*
C (PF-3)	400mg/kg	5.43 ± 0.17	20.85*
D (PF-4)	400mg/kg	5.50 ± 0.31	19.83*
E (PF-5)	400mg/kg	5.79 ± 0.26	15.60*
F (EE)	400mg/kg	4.93 ± 0.30	28.13*
G (positive control)	100mg/kg	2.07 ± 0.93	69.83*
H (negative control)	5ml/kg	6.86 ± 0.28	—

Values represent mean ±SEM of seven animals in each group
*Significant relative to negative control (3% v/v Tween 80), $P < 0.05$.

Effect of extracts on gastric p H

Table 3 reveals that both the ethanol and fraction extracts significantly ($p< 0.05$) increased gastric acid pH. Pooled fraction-2 (PF-2) produced the highest increase (41.94%) in gastric pH.

Table 3: Effect of plant extracts (ethanol and fractions) and standard drug (cimetidine) on gastric pH in Wistar rats

Treatment group	Dose (oral)	Gastric pH	% increase pH
A (PF-1)	400mg/kg	3.7±0.05 *	19.35*
B (PF-2)	400mg/kg	4.4 ±0.1 *	41.94*
C (PF-3)	400mg/kg	3.6 ±0.04 *	16.13*
D (PF-4)	400mg/kg	4.2 ±0.07*	35.48*
E (PF-5)	400mg/kg	3.4 ±0.03*	9.68*
F (EE)	400mg/kg	3.5 ±0.04*	12.90*
G (Positive control)	100mg/kg	4.8 ±0.05 *	54.84*
H (Negative control)	5ml/kg	3.1 ±0.08	-

Value represent mean ±SEM of seven animals in each group
*Significant relative to negative control (3% v/v Tween 80), (P< 0.05)

Effects of extracts and standard drug (cimetidine) on volume, free acidity and total acidity of gastric content in Wistar rats

Table 4 shows that the ethanol extract (EE), pooled fraction-3 (PF-3), pooled fraction-4 (PF-4) and pooled fraction-5 (PF-5) significantly reduced gastric volume (46.01%, 44.15%, 48.94% and 43.09% respectively); free acidity (54.90%, 47.84%, 33.82% and 35.05% respectively) and total acidity (34.54%, 34.60%, 46.18% and 29.35% respectively) compare to negative control (3% v/v Tween 80). Although gastric content volume and total acidity were significantly (P<0.05) reduced by pooled fraction-1 (PF-1), it did not reduce free acidity.

DISCUSSION

Yield

This study reveals that the quantitative yield of the ethanol-extracted residue is low (21.08g) when judged with the amount (500g) of soaked powdered material. Pooled fraction-4 (PF-4) although comprised of five fractions, gave a low yield (3.81g) in relation to the proportional amount of soaked material. This finding conforms to the previous report[32] that active biological principles usually occur in plants in low concentration.

Toxicity (acute) study

Acute toxicity test was conducted to assess the adverse effects that may occur within a short time after administrating a single dose of a test substance[33]. Although earlier reports[34,35] suggest that medicinal plants have advantages of toxicity considerations because of their long term use by humans, we conducted acute toxicity test of the extracts with the principal aim of ascertaining safe dose that could be used in further pharmacological screening. The result obtained indicated that up to LD_{50} of 5000mg/kg of the leaf extracts, no symptom of toxicity was noticed, and therefore, it was judged to be safe for use in further pharmacological screening. This finding is substantiated by the report that compounds that do not show adverse effects when given in doses of 3000mg to 5000mg per kilogram body weight are essentially non-toxic[36].

Antiulcer activity on histamine-induced ulcer

Earlier report[37] showed that histamine plays an essential role in the pathogenesis of ulcer since it is a potent stimulator of H_2-receptors of parietal cells. Further report[29] indicated that histamine causes gastric ulcers by its potent stimulation of parietal cells that secrete gastric acid, and by its vasodilating capability, which leads to increased vascular permeability. These bio-pharmacological actions enable its use as an ulcer model for evaluating agents with anti-secretory and/or H_2-receptor-blocking potential[38]. In this sutydy, the ability of *Piper guineense* ethanol and fraction extracts to block histamine-induced ulcer may suggest H_2-receptor blockade and/or anti-secretory as the mechanism of action. This finding is similar to earlier report[39] that Cudrania tricuspidata ethanol extract prevents H_2-receptor related diseases like hyperacidity and reflux esophagitis.

Gastric pH, volume, free acidity and total acidity

Earlier report[40] suggested that elevated concentration of the hydrogen ion is a destructive factor that promotes gastric damage via decreasing pH of the gastric juice. The result of this study shows that pre-treatment with *Piper guineense* leaf extract significantly (P<0.05) elevated gastric pH levels with simultaneous decrease in gastric secretion (volume); an effects that support anti-secretory and/or H_2 receptor-blocking activity of the leaves. These findings correlate with the earlier report[41] on Cuphea ignea, which significantly increased gastric pH, and reduced gastric volume.

CONCLUSION

Piper guineense leaf extracts inhibit histamine-induced ulceration, an action mediated by their anti-secretory and/or H_2 receptor blocking mechanism(s), as evidenced by its ability to elevate gastric pH, and simultaneously decrease gastric secretion (volume). This finding has authenticated the tradomedicinal claim on the plant as a remedy for ulcer. However, this claim can further be substantiated by clinical trials.

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Table 4: Effect of plant extracts (ethanol and fractions) and standard drug (cimetidine) on volume, free acidity and total acidity of the gastric content in Wistar rats

Treatment group	Dose (oral)	Gastric volume(ml)	Free Acidity(mEq/L)	Total Acidity (mEq/L)
A (PF-1)	400mg/kg	2.99 ± 0.18(20.48%)*	40.68 ± 0.62 (2.35%)	62.57 ± 0.74(24.16%)*
B (PF-2)	400mg/kg	3.17 ± (15.69%)*	25.24 ± 1.17 (39.41%)*	45.17 ± (45.16%)*
C (PF-3)	400mg/kg	2.10 ± 0.15 (44.15%)*	21.73 ±0.65 (47.84%)*	53.86 ±0.28 (34.60%)*
D (PF-4)	400mg/kg	1.92 ± 0.17 (48.94%)*	27.57 ± 0.41(33.82%)*	44.33 ±1.80 (46.18%)*
E (PF-5)	400mg/kg	2.14 ± 0.17 (43.09%)*	27.06 ± 0.80 (35.05%)*	58.19 ±0.52 (29.35%)*
F (EE)	400mg/kg	2.03 ± 0.13 (46.01%)*	18.79 ±0.58 (54.90%)*	53.91 ±0.26 (34.54%)*
G (positive control)	100mg/kg	1.29 ± 0.20 (65.69%)*	5.64 ± 0.51(86.46%)*	11.83 ±0.53 (85.64%)*
H (negative control)	5ml/kg	3.76 ± 0.09 (0.00%)	41.66 ± 0.63 (0.00)	82.36 ±0.41 (0.00%)

Values represent mean±SEM of seven animals in each group
*Significant relative to negative control (3% v/v Tween 80) data, ($p < 0.05$).

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