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Original Article

An *in vivo* study of anti-gastrointestinal motility effect of fraction extracts of *Piper guineense* leaf in albino mice

Tharcitus Chilaka Onwudiwe[1], Prince Chiazor Unekwe[2], Athanasius Ugochukwu Aligwekwe[3], Chidmma Nwamaka Nzerem[4]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Elele, Nigeria, [2]Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Science, Nnamdi Azikiwe University, Awka, Nigeria, [3]Department of Anatomy, Faculty of Basic Medical Science, Madonna University, Elele, Nigeria, [4]Department of Computational Biology, Broad Institute of Massachusetts Institute of Technology, Cambridge, Massachusetts, United States

ABSTRACT

Diarrhea is associated with dysfunction of gastrointestinal tract motility and it is the leading cause of morbidity particularly in children worldwide. It is characterized by increased peristalsis of the gut, hence resulting to massive loss of fluid and electrolytes. Available synthetic medications for the management of diarrhea provoke undesirable effects, hence necessitating exploitation of alternative remedies, which plants can offer. This work is an *in vivo* study of anti-gastrointestinal motility effect of fraction extracts of *Piper guineense* leaf in albino mice. Ethanol-extracted residue was fractionated to produce pooled fraction extracts, labeled PF1 - PF5. The extracts were orally administered at 400 mg/kg to different test groups while the positive and negative control groups received orally, 5 mg/kg Atropine Sulfate and 5 ml/kg 3% v/v Tween 80, respectively. Five minutes later 0.5 ml of 5% charcoal suspension in 10% tragacanth aqueous solution was administered orally. The intestinal length from pylorus to ileocecal region of the animals was measured. The distance moved by charcoal in the intestine at 0.5 h, 1 h, 2 h, and 4 h was determined and expressed as percent intestinal transit. Result showed that the extracts significantly ($P < 0.05$) reduced the movement of charcoal when compared to the values of negative control, hence, suggesting anti-motility effect of *P. guineense* leaf. The finding of this study has, therefore, authenticated the tradomedicinal claim on *P. guineense* as a remedy for various forms of stomach disorder such as diarrhea and ulcer.

Keywords: Activated charcoal, diarrhea, fractionation, intestinal length

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INTRODUCTION

Gastrointestinal motility refers to the contraction of the smooth muscles of gastrointestinal tract that create a force that moves food from the mouth through the pharynx, esophagus, stomach, small and large intestines, and out of the body. The physiology of gastrointestinal tract motility can be altered by drugs, pathogens, chronic diseases, malnutrition, and neurohormonal factors, thereby leading to changes in the transit time of materials in the gut and ability of intestinal epithelium to either secrete or absorb fluids.[1]

Diarrhea is one of the gastrointestinal motility-related disorders that affect a significant proportion of the world population. Diarrhea has been reported as the leading cause of morbidity and mortality particularly in the underdeveloped and developing countries.[2-4] A report by the World Health Organization shows an increasing global incidence of diarrhea in children, hence, making it the second leading cause of death among children below 5 years of age, and accounting for 3.6% of global disease burden.[5-7] A major physiologic change primary to diarrhea is increase in smooth muscle contraction and uncontrolled peristalsis of the gastrointestinal tract, which result to massive loss of fluid and electrolytes. Furthermore, increase in gastrointestinal tract contractility reduces the transit time, thereby altering absorption of drugs and nutrients from the gut.

Several synthetic medications are available for management of diarrhea and other gastrointestinal motility-related diseases. Unfortunately, these medications are unavailable, expensive

Address for correspondence: Tharcitus Chilaka Onwudiwe, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Elele, Nigeria. E-mail: onwudiwetc@gmail.com

and provoke numerous undesirable interactions that limit their use to a few situations. To overcome these problems, there is a growing interest to develop alternative medications of plant origin. Plants abound in nature and have been considered as promising source for development of new agents with safe therapeutic window.[8] Furthermore, the World Health Organization,[9] encouraged the use of medicinal plants in the management of diarrhea *Piper guineense*, commonly found in South Eastern Nigeria, is a spice plant from family Piperaceae, and genus piper. It is popularly referred to as "African Black Pepper." In traditional medicine, *P. guineense* is claimed to be a remedy for various forms stomach disorders, including diarrhea, peptic ulcer, and bowel inflammatory disease. Some studies have reported other ethnomedicinal activities of *P. guineense* as an antibacterial agent,[10] a larvicidal agent,[11] and a hypolipidemic agent.[12] Despite its wide range use in traditional medicine, there is lack of comprehensive knowledge of the use of *P. guineense* as an anti-motility and/or anti-diarrheal agent. This study, therefore, was undertaken to scientifically confirm the traditional claim on *P. guineense* as a remedy for diarrhea.

MATERIALS AND METHODS

Plant Material Collection and Confirmation

Matured fresh leaves of the plant having been collected from a farm land in Owerri, Nigeria, was confirmed as *P. guineense* by a taxonomist and deposited as voucher specimen (Herbarium Number: UPH/P/251) in the herbarium of Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

Animal Ethics Permit

Animal ethics permit "MAU/SREC/A/17" was approved by Senate Research and Ethics Committee of Madonna University, Nigeria. Compliances to animal handling as published by National Institute of Health[13] were strictly followed.

Experimental Animals

Healthy adult albino mice that weighed between 20 and 22 g and of 12–15 weeks of age were employed in the study. The mice were reared at Animal Facility Unit of Madonna University, Nigeria, in a well-ventilated room and were kept in secured cages, floored with adequate wood shavings. The mice were allowed unlimited access to clean drinking tap water and adequately fed with commercial poultry growers feed (Top feeds^{R}, Nigeria).

Study Area

Experimental studies were conducted at the Animal Facility Center of Madonna University, Nigeria, Elele Campus.

Reagents and Drugs

The following reagents and drugs were employed: Chloroform (Super Tek Chemicals, India), Tween 80 (3%v/v) (Super Tek Chemicals Germany), Ethylacetate (Rankem, Mumbai, India), Dragendoff's reagent (Super Tek Chemicals, Germany), n-Hexane (Sigma-Aldrich Chemie, Germany), 96% Ethanol (Gungsdong Guandgua Chemical Factory, China), Activated charcoal (Rambaxy, India), and Atropine Sulfate (British Drug House, London).

Plant Material Extraction

Employing the procedure proposed by Girma *et al*.,[14] as reported earlier by Onwudiwe *et al*.,[15] about 3 kg of matured fresh leaves of *P. guineense* were washed thoroughly with clean tap water and kept under shade for about 14 days to air-dry. The dried leaves were ground into coarse powder. At ambient temperature, about 500 g was soaked in 2 L of ethanol (80%) for 3 days with occasional agitation every 6 h. The resulting mixture was filtered through filter papers (Whatman, No. 1) and filtrate separated from the marc. After filtration, all the obtained filtrates were put 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 residue obtained.

Chromatographic Fractionation of Ethanol-extracted Residue

Thin-layer chromatography (TLC) as proposed by Stahl[16] and column chromatography as proposed by Harbone[17] was employed to produce fraction extracts from ethanol-extracted residue. Eight solvent systems constituted in different ratios, were subjected to preliminary TLC and the one that gave the best resolution was obtained.[15] The Chloroform/Ethylacetate/Ethanol (7:2:1) which gave the best resolution, was subsequently used as the eluting solvent system in column chromatography and in determining TLC mobility (R_f values) of fractions. A 10 g amount of ethanol-extracted residue was used in column chromatography to obtain seventeen-10 ml fractions, labeled FE-1 to FE-17. TLC mobility of the obtained fractions was conducted and R_f value calculated as follows: [15]

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

Pooling, labeling, and storage of the plant extracts

Fractions that showed very close R_f value and similar color reaction were considered to be the same, and therefore, pooled together in a previously weighed clean beaker. The pooled fractions were 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). The beakers and their content were placed in an oven at 40°C until dried solid mass was obtained. After drying, the labeled containers were stored in the refrigerator until when needed.

Calculation of percent yield of extracts

The beakers and their dried content were re-weighed and the initial weight of empty beakers was subtracted. The difference was taken as the actual weight of extracted residue. Using the

formula proposed by Okoli *et al*.,[18] percent yield was calculated as the ratio of weight (g) of the extracted residue to weight (500 g) of the soaked powdered material multiply by hundred.

Acute Toxicity Study

Employing the method proposed by Lorke,[19] both the ethanol and fraction extracts were tested for acute toxicity, to ascertain doses that could be safe in subsequent whole animal experiment.

Study Protocol

Ninety-six healthy adult albino mice (aged: 12–15 weeks and weighed: 20–22 g) were randomly placed into eight experimental groups that made up of six test groups (labeled A–F), one positive control group (labeled G), and one negative control group (labeled H). Each of the test and control groups consisted of 12 mice per group (i.e., $n = 12$). The mice were deprived of food for 24 h before the study but were given access to drinking water until 2 h to the experiment. Drug and extracts were orally administered through intragastric tube and the dose of extracts administered was safe, as ascertained in acute toxicity study. Mice in each group were treated as follows:

- A dose of 400 mg/kg PF-1 was given orally to each mouse in Group A
- A dose 400 mg/kg PF-2, was given orally to each mouse in Group B
- A dose of 400 mg/kg PF-3, was given orally to each mouse in Group C
- A dose of 400 mg/kg PF-4, was given orally to each mouse in Group D
- A dose of 400 mg/kg PF-5, was given orally to each mouse in Group E
- A dose of 400 mg/kg EE, was given orally to each mouse in Group F
- A dose of 5 mg/kg atropine sulfate, was given orally to each mouse in Group G (positive control)
- A dose of 5 ml/kg 3% v/v Tween 80, was given orally to each mouse in Group H (negative control).

Five minutes after various drug treatment, 0.5 ml of 5% charcoal (25 mg) suspension in 10% aqueous solution of Tragacanth powder[20] was administered to each mouse orally. After ½ h, 1 h, 2 h, and 4 h, three animals in each group were sacrificed under anesthesia. The small intestine and large intestine were isolated. The intestinal distance (centimeters) moved by the leading edge of charcoal meal from the pylorus, and total length (centimeter) of intestine from pylorus to ileocecal region were measured using meter rule. Using the formula proposed by Miller *et al*.,[21] percent small intestinal transit (% SIT) was calculated as follows:

$$\%SIT = \frac{Distance\,(cm)\,travelled\ by\ charcoal}{Total\ length\,(cm)\,of\ \ small\ intestine} \times 100$$

Statistical Data Analysis

The obtained data expressed as mean ± standard error of mean were analyzed statistically using one-way analysis of variance. Significance was accepted at $P < 0.05$.

RESULTS

TLC Mobility (R_f values) and Pooling of Fractions

As shown in Table 1, the 17 fractions obtained by column chromatographic separation were pooled based on their R_f values. PF-1 consisted of FE-1, FE-2, and FE-3; PF-2 consisted of FE-4 and FE-5; PF-3 consisted of FE-6, FE-7, and FE-8; PF-4 consisted of FE-9, FE-10, FE-11, FE-12, and FE-13; and PF-5 consisted of FE-14, FE-15, FE-16, and FE-17.

Yield of Ethanol and Pooled Fraction Extracts

The quantitative yield of the ethanol extracted residue was 4.22% of soaked powdered material (500 g). The PF-4 gave the highest yield of 38.1% when compared to the quantity of fractionated residue (10 g).

Toxicity (Acute) Study

At 5000 mg/kg extracts administered in mice, no signs of toxicity or mortality were observed within 48 h in the treated groups of mice. The LD_{50} of the extracts of *P. guineense* leaf, therefore, might be >5000 mg/kg.

Charcoal Meal Test

The result of the charcoal meal test in Tables 2 and 3 revealed that both the ethanol and fraction extracts at oral dose of 400 mg/kg significantly ($P < 0.05$) reduced the distance

Table 1: TLC mobility (RF value) and pooling of fractions

Fraction	Aliquot (ml)	RF value	
FE-1	10	0.801	Pooled fraction-1 (Pf-1)
FE-2	10	0.798	
FE-3	10	0.80	
FE-4	10	0.757	Pooled fraction-2 (Pf-2)
FE-5	10	0.753	
FE-6	10	0.701	Pooled fraction-3 (PF-3)
FE-7	10	0.703	
FE-8	10	0.698	
FE-9	10	0.634	Pooled fraction-4 (PF-4)
FE-10	10	0.638	
FE-11	10	0.635	
FE-12	10	0.643	
FE-13	10	0.641	
FE-14	10	0.616	Pooled fraction-5 (PF-5)
FE-15	10	0.595	
FE-16	10	0.618	
FE-17	10	0.607	

Table 2: Mean small intestinal length (cm) and mean distance (cm) travelled by charcoal meal in mice

Treatment Group	Mean Intestinal Length (cm)	0.5 h	1 h	2 h	4 h
A (PF-1)	39.64±0.24	7.5±0.16	12.3±0.16	19.2±2.83	32.4±0.25
B (PF-2)	39.69±0.24	8.2±0.13	10.3±0.27	21.6±0.26	33.7±0.27
C (PF-3)	39.96±0.21	6.7±0.15	13.6±0.20	20.2±0.20	32.2±0.34
D (PF-4)	39.70±0.32	7.4±0.10	13.3±0.15	22.5±0.18	32.8±0.21
E (PF-5)	39.60±0.22	8.4±0.12	14.2±0.17	20.8±0.08	30.3±0.41
F (EE)	38.81±0.32	6.6±0.08	11.4±0.17	26.4±0.19	31.6±0.33
G (Positive control)	39.24±0.85	2.3±0.11	3.8±0.09	9.6±0.22	11.4±0.40
H (Negative control)	39.77±0.21	8.8±007	16.5±0.15	29.8±0.17	37.3±0.17

Values represent mean±SEM. Significant relative to negative control at $P<0.05$

Table 3: Mean percent small intestinal transit of charcoal meal in mice

Treatment Group	0.5 h	1 h	2 h	4 h
A (PF-1)	19.14±0.43	31.14±0.30	56.02±0.64	81.70±1.08
B (PF-2)	20.74±0.35	25.91±0.60	54.34±0.67	82.80±1.04
C (PF-3)	16.77±0.40	34.03±0.89	50.47±0.39	80.58±0.62
D (PF-4)	18.64±0.33	33.43±0.41	56.69±0.45	82.63±0.33
E (PF-5)	21.22±0.35	35.51±0.34	52.55±0.35	76.54±1.21
F (EE)	17.00±0.24	29.20±0.28	68.10±1.04	81.41±1.01
G (Positive control)	5.86±0.26	9.69±0.33	24.46±1.53	29.08±1.95
H (Negative control)	22.13±0.24	41.42±0.58	74.93±0.46	83.07±0.79

Values represent mean±SEM. Significant relative to negative control at $P<0.05$

travelled by charcoal meal when compared to negative control (5 ml/kg 3% v/v Tween 80).

DISCUSSION

Yield of Extract

As reported in earlier study by Onwudiwe *et al*.,[15] the quantitative yield of the ethanol-extracted residue was low (4.2%), and this finding is in agreement with an existing report that biologically active substances occur in plants in low amounts.[22]

Acute Toxicity

At LD_{50} of up to 5000 mg/kg of *P. guineense* leaf extracts, no signs of toxicity nor lethality was observed. Results of similar studies have reported that absence of obvious signs of mortality up to 5000 mg/kg of compounds is an indication safety.[6,23] Some reports have indicated that plants have advantages of toxicity consideration based on their long-term use,[24-26] therefore the result (i.e., LD_{50} > 5000 mg/kg) obtained from this study may explain why *P. guineense* leaves have been used over the years in tradomedicine as a remedy for various stomach disorder without any record of toxicity.

Charcoal Meal Test

Peddreddy[27] reported that motility function of the gastrointestinal tract is associated with digestion and nutrient absorption process as well as generation of force that propels waste matters from the gut. Increase gastrointestinal tract motility interferes with digestion and absorption process thereby leading to diarrhea and diminished absorption of nutrient.[28,29] From the result of this study, the ability of the extracts of *P. guineense* leaves to significantly ($P < 0.05$) inhibit intestinal movement of charcoal meal, may suggest that the plant possesses anti-motility property which can be exploited in the management of diarrhea. This finding is in correlation with the report that mixture of *Matricaria chamomilla* (Chamomile) and *Illicium verum* (Star anise) effectively reduces gastrointestinal tract motility that modulates diarrhea when compared to control group.[2]

Gastrointestinal tract motility describes the contraction of muscles that propels the gastric content.[4] Gastric ulcer, like other irritants increases gastrointestinal tract motility and most anti-ulcer drugs decrease gastrointestinal tract motility.[30] Studies have shown that anti-spasmodic activity and decreased gastrointestinal tract motility leading to flattening of mucosal fold is associated with gastroprotection.[31,32] Therefore, in this study, the ability of the extracts of *P. guineense* leaves to significantly ($P < 0.05$) decrease gastrointestinal tract motility when compared to negative control, may indicate anti-ulcer property of the plant. This finding conforms with the report that aqueous root extracts of *Guiera senegalensis* exhibits anti-diarrheal and anti-ulcer effects in rodents through inhibition gastrointestinal tract motility.[33] Reduction of propulsive movement of the gut (i.e., anti-GIT motility) is considered in the treatment of ulcer because decreased gastrointestinal tract motility will prevent speedy evacuation of anti-ulcer agent, thereby promoting healing.[34,35] One notable side effect of conventional anti-ulcer drugs is diarrhea.[36,37] Therefore,

decreased gastrointestinal tract motility exhibited by extracts of *P. guineense* leaf is an essential feature of anti-diarrheal activity which is not only desirable but also an advantage over the conventional anti-ulcer drugs.

CONLUSION

P. guineense leaf extracts decreased gastrointestinal motility, which accounts for anti-diarrheal and anti-ulcer activities of the plant, hence, confirming the tradomedicinal use in management of various forms of stomach disorder.

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