

The Contractile Effect of Ethanolic Extract of West African Black Pepper (*Piper guineense*) on Isolated Guinea Pig Ileum

Saba, Adebowale Bernard and Tomori, Olayinka Ayotunde
Department of Veterinary Physiology, Pharmacology and Biochemistry,
University of Ibadan, Ibadan, Nigeria

Abstract: The effect of ethanolic extract of West African black pepper (*Piper guineense*) on contractility of gastrointestinal smooth muscle was studied by muscle-bath contractility method using isolated Guinea pig ileum. The effective dose range of the extract on the ileum was determined by the concentration of the extract at the threshold and maximal contractile responses. The dose-response curve for the effect of the extract alone on the strips of isolated ileum and for the extract in the presence of 1×10^{-5} M atropine, 1×10^{-5} M pyrilamine and 1×10^{-5} M cimetidine were obtained. Evaluation of the potency (EC_{50}), affinity (pA_2) of the extract and the maximal response (E_{max}) to the extract were determined in absence (control) and presence of each antagonist (test). Statistical test of the significance of differences of the means between control and test groups was established using the Student's t-test at $p < 0.05$. *P. guineense* contracted the smooth muscle of ileum of guinea pig in a dose-dependent manner. The threshold value for the contraction was 0.9 mg mL^{-1} while the maximal response was obtained at a concentration of 3.6 mg mL^{-1} of extract solution. *P. guineense*-induced contraction was modified by atropine (cholinergic antagonist), pyrilamine (H_1 -receptor antagonist) and cimetidine (H_2 -receptor antagonist). The three antagonists caused a right-ward shift on the dose-response curve and the maximal contractile response of the ileal strips to the extract was attainable in the presence of the antagonists suggesting that atropine, pyrilamine and cimetidine inhibited the effect of the extract competitively. The potency and affinity of the extract were significantly reduced by 1×10^{-5} M atropine, 1×10^{-5} M pyrilamine and 1×10^{-5} M cimetidine. While atropine and pyrilamine did not significantly modify the maximal response, cimetidine significantly depressed the maximal contractile response of ileum to the ethanolic extract of *P. guineense*. The findings in this study strongly suggest that M cholinergic, H_1 and H_2 receptors are involved in the pharmacodynamics of *P. guineense*-induced contraction of gastrointestinal smooth muscle.

Key words: Contractile effect, piper guineense, Ileum, guinea pig

Introduction

Piper guineense, otherwise referred to as Climbing black pepper, Benin pepper, West African or bush pepper; belongs to the plant family called piperaceae (Rehm and Espig, 1991; Amusan and Okorie, 2002; Asawalam, 2006). At least five species of the genus piper are cultivated to produce what is generically called "pepper". The most common is *Piper nigrum* (black pepper); others include *P. longum* (long pepper), *P. cubeba* (cubeb pepper) and *P. guineense* (West African or bush pepper). The pepper plants are either vines or climbing shrubs which can grow up to fifty feet in length. The leaves are glossy; about six inches long (Dalziel, 1937; Irvine, 1961) Black peppercorns are made by picking the pepper berries when they are half ripe and just about to turn red, they are then left to dry which causes them to shrivel and become dark color. Alternatively, green peppercorns are picked while still unripe and green in color, while white peppercorns are picked when very ripe and subsequently soaked in brine to remove their dark outer shell leaving just the white pepper seed (Isawumi, 1984; Jirovetz *et al.*, 2002; Omafuvbe and Kolawole, 2004). The Black pepper is the most pungent and flavourful of

all types of peppers and is available as whole or cracked peppercorns or ground into powder. The reason that pepper was so cherished is that it served important culinary purposes, not only could its pungency spice up otherwise bland foods, but it could disguise a food's lack of freshness, the latter being an especially important quality in the times before efficient means of preservation. (The George Mateljan Foundation, 2006). Studies have shown that apart from the use of these plants as spices and condiments, they have several other wide applications in the local treatment and management of many diseases. Indigenous people value the plants for their ethnomedicinal uses as much as for spicing foods (Stethberger *et al.*, 1996). *P. guineense* is used as anticonvulsant (Pei, 1983; Abila *et al.*, 1993). The fruits and leaves are used as spice for preparing soup for post-partum women. Powder from the dried fruits mixed with honey acts as carminative and relieves stomach aches (DeWitt, 2006). Extract of black pepper has been reported to stimulate digestion of foods by stimulating secretion of digestive enzymes, pancreatic amylases, trypsin and chymotrypsin (Platel and Srinivasan, 2000) and is therefore used for treatment of digestive disorders. The effect of *P.*

guineense on the contractility of the gastrointestinal smooth muscle however needed to be well established in order to have a full understanding of the pharmacological basis for its ethnomedicinal application on the digestive system.

The aim of this study is to therefore investigate the effect of the extract of peppercorns of *P. guineense* on the contractility of the gastrointestinal smooth using isolated ileum of guinea pig.

Materials and Methods

Preparation of plant extract: Fresh samples of half-ripe berries of *P. guineense* were obtained from local market and taken to Department of Botany, University of Ibadan, for identification. The samples were dried on the bench before extraction. The extraction procedure is as described by Harborne (1984). The dried berries were crushed into a coarse powder form and weighed. The powdered peppercorns were continually extracted using absolute ethanol in a Soxhlet extractor. The extract obtained was clarified by filtration through celite on water pump and was then concentrated *in vacuo* using a rotation evaporator at low temperatures. The ethanol remaining in the extract was finally removed by placing small volumes in porcelain dishes in the oven set at low temperature of 40°C. This provided the semisolid materials from which a dose of 100gm of extract 100mL⁻¹ distilled water was made. This formed the stock extract solution from which serial dilutions were made as required in the course of the experiment.

Twelve adult guinea pigs of both sexes were used in this study. Clean ileal segments of 2-3 cm long were prepared from the guinea pigs. One end was fixed to a pin attached to the glass aerating tube and the other to a writing lever which was adjusted for tension (0.5g) and for recording (six times magnification) on Kymograph drum (Biosciences, Kent, England). The whole preparation was set up in 20 mL organ bath containing aerated Tyrode solution (NaCl: 1.0; CaCl₂:0.2; KCl: 0.2; MgCl₂.6H₂O: 0.2; NaHPO₄: 0.05; NaHCO₃: 1 and glucose 2.0g/dl) and maintained at a temperature of 37°C.

Experiment 1: After equilibration for 30 min the aqueous extract were added subsequently in graded doses to the bath, from the lowest dose to determine the sensitivity of the ileal smooth muscle. The threshold value (T.R) is the concentration (mg mL⁻¹) at which the strip of ileum recorded the first contraction, whereas the maximal response (M.R) is the concentration of the extract at which further increases in dose does not produce higher height of contractions.

Experiment 2: Using the dose range between the threshold value and the maximal response, contractile responses of the ileum to the extract was recorded by kymograph. After a dose response curve for the extract had been established, the same preparation was in turn exposed to predetermined doses of 1x10⁻⁵ M atropine sulphate (Nutritional Biochemical Corporation,

Table 1: A measure of sensitivity of isolated guinea pig ileum to ethanolic extract of *Piper guineense*

Dose of extract (mg mL ⁻¹)	Response of Isolated Ileum	Average Height of Contraction (cm)
<0.80	No Response	0.00 (5)
0.90*	Contraction	0.20 (5)
1.20	Contraction	0.45 (5)
1.80	Contraction	0.67 (5)
2.40	Contraction	0.90 (5)
3.30	Contraction	0.92 (5)
3.60**	Contraction	0.96 (5)
3.90	Contraction	0.78 (5)

* = Threshold value (T.R). ** = Maximal Response (M.R). () = Number of observations

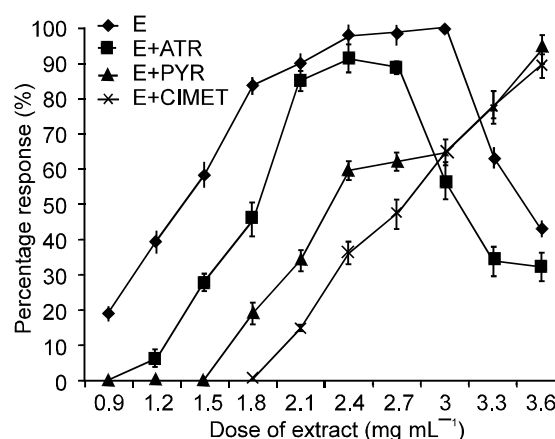


Fig. 1: The dose-response curve for extract alone (E) and in the presence 1x10⁻⁵ M atropine (E+ATR), 1x10⁻⁵ M pyrilamine (E+PYR) or 1x10⁻⁵ M cimetidine (E+CIMET) on isolated guinea pig ileum, Numbers of observation = 5. Standard errors of means are indicated as vertical bars

Cleveland, Ohio, U.S.A), 1x10⁻⁵ M pyrilamine maleate (Poulenc Ltd, Montreal, Quebec) or 1x10⁻⁵ M cimetidine (Sigma Chemical Company, St. Louis, Missouri, U.S.A) as the case may be, for a period of 10 min so as to re-establish another dose-response curve for the extract in the presence of a particular antagonist. The procedure was repeated five times for each agonist and agonist-antagonist interactions using new strip of tissue from different guinea pigs.

Determination of parameters: The heights of the contractions were determined and dose-response curves were plotted for all the recordings. The potency (EC₅₀), affinity (pA₂ = 1/EC₅₀) and efficacy (Emax) of the extract alone and in the presence of antagonists were extrapolated from the graphs (Connaughton and Doherty, 1990; Ortiz De Urbina *et al.*, 1990). The values were recorded as Mean±S.E.M. The test of significance of the difference of the means was determined by Student's t-test (Bailey, 1992).

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Table 2: Pharmacodynamic indices for the effect of extract of *Piper guineense* alone and in the presence of cholinergic and histaminic antagonists

Parameters	Extract Only	Extract+ATR	Extract+PYR	Extract+CIMET
EC ₅₀ (M)	1.28±0.04 ^{a,b,c} (5)	2.21±0.14 ^a (5)	2.20±0.03 ^b (5)	2.28±0.05 ^c (5)
pA ₂ (M)	0.72±0.05 ^{ad,e} (5)	0.34±0.07 ^a (5)	0.29±0.02 ^a (5)	0.36±0.12 ^e (5)
E _{max} (%)	96.00±1.12 ^e (5)	90±4.11 (5)	92±6.22 (5)	82.2±5.09 ^e (5)

Mean with same superscripts on the same row are significant at; p<0.001(a-a, b-b or c-c), p<0.01 (d-d) or p<0.05 (e-e), ATR = Atropine, PYR = Pyrilamine, CIMET = Cimetidine () = Number of observations

Results

The Response of Isolated ileum to extract of *P. guineense*: The extract elicited contractile responses from the ileum. The threshold value for the contractile effect of ethanolic extract on the ileum was obtained at a concentration of 0.9mg mL⁻¹ while the maximal response was attained at 3.60mg mL⁻¹ of the extract solution (Table 1). The dose-response curve obtained for the contractile effect of the extract alone shifted to the right in the presence of 1x10⁻⁵M atropine, 1x10⁻⁵M pyrilamine or 1x10⁻⁵M cimetidine (Fig. 1).

The potency of extract of *P. guineense*: The potency of the *P. guineense*-induced contraction of isolated guinea pig ileum decreased when pretreated with the antagonists. This was exhibited by significant increase in the mean EC₅₀ values of 1.28±0.04 M for extract alone to 2.21±0.14 M in the presence of 1x10⁻⁵M atropine (p<0.001); 2.20±0.03M in the presence of 1x10⁻⁵M pyrilamine (p<0.001) and 2.28±0.05 M in the presence of 1x10⁻⁵M cimetidine (p<0.001) respectively (Table 2).

Affinity of extract of *P. guineense*: There was a decrease from the mean pA₂ values of 0.72±0.05 M obtained for the extract of *P. guineense* alone to 0.34±0.07 M in the presence of 1x10⁻⁵M atropine, the difference of the means is statistically significant (p<0.01). The affinity of the extract was also significantly reduced by pyrilamine (p<0.001) and cimetidine (p<0.05) to mean pA₂ values of 0.34±0.02 M and 0.36±0.12 M (Table 2).

The maximal response of ileum to extract of *P. guineense*: The mean E_{max} value of the extract of *P. guineense* was 96.00±1.12%. The maximal contractile response of the Guinea pig ileum to the plant was slightly reduced by atropine (p>0.05) and pyrilamine (p>0.05) to 90±4.11% and 92±6.22% respectively. Whereas the maximal contractile response of ileal smooth muscle to extract of *P. guineense* was reduced significantly (p<0.05) in the presence of cimetidine to 82.2±5.09% (Table 2).

Discussion

The findings obtained from this study shows that the extract of *P. guineense* produced dose dependent contraction of the smooth muscle of guinea pig ileum.

The antagonists; atropine, pyrilamine or cimetidine individually produced a shift to the right on the dose-response curve. There were also significant decreases in the potency, affinity of the extract and the maximal contractile response of the smooth muscle to the extract of *P. guineense*, in the presence of atropine, pyrilamine and cimetidine. These show that the extract-induced contractions are atropine-, pyrilamine- and cimetidine-sensitive, thereby suggesting that the contractions are mediated by M cholinergic, H₁ and H₂ histaminergic receptors respectively. Stimulation of the cholinergic receptors of the gastrointestinal tract typically elicits contraction, increased secretory activity and relaxation of the sphincter thus paving way for quick transit of luminal content (Brown and Taylor, 2001; Saba *et al.*, 2006). This is also similar for histaminic receptors, where stimulation of H₁- or H₂- receptors produces contraction of the gastrointestinal tract and H₂ receptor is also primarily responsible for evoking gastrin and acid production (Brown and Roberts, 2001). While reports of the direct effect of peppercorns of *P. guineense* on gastrointestinal motility is not readily available, the commercially prepared piperine, the pungent principle in *P. nigrum* and *P. guineense* was only reported to increase HCl and pancreatic enzymes through H₂-receptor mediated pathway but its effect on motility has either been stimulatory (McNamara *et al.*, 2005) or inhibitory (Izzo *et al.*, 2001; Bajad *et al.*, 2001). Piperine has however been reported to cause definite contraction of isolated tracheal strip by stimulation of tetradotoxin-resistant terminal portion of non-cholinergic nerves in guinea pigs (Szolcsanyi, 1983). Contractile response to piperine was dose-dependent in the rat bladder and was partially tetradotoxin- and ruthenium red-sensitive suggesting that contraction of sensory terminals by this agent takes place indirectly as well as by a direct action on sensory receptors. (Patacchini *et al.*, 1990). Piperine caused positive chronotropic and inotropic responses by releasing Calcitonin-Gen Related Peptide (CGRP) from nonadrenergic noncholinergic nerves in the rat (Miyachi *et al.*, 1989). Findings from this study have further shown that mural cholinergic and histaminic receptors are involved in the mediation of *P. guineense*-induced contractions of the gastrointestinal tract.

It can therefore be concluded from this study that peppercorns of *P. guineense* as pungent substances contract gastrointestinal smooth muscle of guinea pig

also through the cholinergic and histaminergic receptors apart from possible mediation through the sensory nerves, vanilloid receptors, or release of CGRP from nerves as previously reported for piperine and other pungent substances on other systems of the body.

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