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Pharmacodynamic Effect of Methanolic Extract of *Piper guineense* Leaf on Uterine Physiology

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Abstract: Background: The aim of the study was to investigate the pharmacodynamic effect of the methanol extract of the leaf of *Piper guineense* (MeOH-PGL) on rat uterine musculature. Adult female Wistar rats (130-150) gram body weight divided into three groups (n = 10) were orally treated with MeOH-PGL (3.5 mg/g/day) for 3, 7 and 30 days, respectively. The control group was treated orally with isotonic saline (0.9% NaCl) for 3, 7 and 30 days simultaneously. The rats were sacrificed; the uterine muscle strips set up in the organ baths containing Tyrode's solution constantly bubbled with air and warmed at 37°C. The effects of various drugs were tested on the preparations. **Results:** The results showed that repeated oral administration of MeOH-PGL for 3 days increased sensitivity of uterine muscle activity to drugs used, whereas, prolonged administration inhibited the activity of the uterine muscle against the drugs tested. MeOH-PGL was found to contain alkaloids in a greater quantity than cardiac glycosides, flavonoids, polyphenol and tannins. **Conclusions:** The results let to the suggestion that ingestion of *P. guineense* leaf could interfere with the uterine muscle activity of female reproductive system and that, the uterotonic action of the extract could be attributed to the alkaloids contents.

Key words: Repeated administration, leaf of *Piper guineense,* uterine muscle

INTRODUCTION

Piper guineense is commonly used in Southern Nigeria as vegetable supplement due to its aromatic and peppery taste (Udoh *et al*., 1996). In Nigerian herbal medicine, the water extract of the leaf of *P. guineense,* is administered to women after childbirth to enhance uterine muscle contraction for expulsion of debris from the uterus. The results of the preliminary studies showed that the leaf extract of *P. guineense* exhibited cholinergic activity in uterine muscle response which justified its traditional medical usage (Udoh *et al*., 1996).

Mbongue *et al.* (2005) reported on the antifertility effects of aqueous extract of *P. guineense* in Wister rat. Recently, Ekanem *et al.* (2010) attributed the antifertility effects of ethanol extract of *P. guineense* on mice to the activities of some alkaloidal amides on the uterine muscles. In another study, it was reported that the leaf of *P. guineense* extract induced uterine weight increase in immature female rats and enhanced uterine muscle contraction in *in vivo* in a manner similar to estrogen (Udoh, 1999).

Phytochemical data on *P. guineense* revealed that the water extract of the leaf contains cardiac glycosides, alkaloids, saponins, tannins, flavonoids, polyhenol and phelobatnins (Udoh, 1999). It was also confirmed by HPLC analysis that *P. guineense* contains piperanine, dihydrowasanine and N-isobutyl-(E,E)-2, 4-decadienamide (Ekanem *et al*., 2004).

The present study aimed to investigate the effects of repeated ingestion of the MeOH-extract of *P. guineense* in rats uterine muscle.

MATERIALS AND METHODS

Preparation of extract: Fresh leaves of *P. guineense* were purchased from a local market in Calabar, Cross River State Nigeria. A herbarium specimen was deposited in Ethnopharmacology Unit of the Department of Pharmacology, University of Calabar, Calabar, Nigeria. They were washed repeatedly in water to remove unwanted parts, drained to remove excess water and oven dried in a hot air oven at 60°C for 24 h. Dried leaves were crushed to powder with a manual blender and extracted by Soxhlet method with methanol (MeOH) as a solvent at 60°C for 72 h.

One hundred gram of the powder was wrapped in a thimble and inserted into a Soxhlet extractor adopted to a

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pyrex round bottom flask containing 500 mL of methanol (MeOH). The extracting solvent was exhausted from the extract with the help of a rotary evaporator at 40°C. The dry powder of the extract was 9% yield of the powder sample. A stock solution of 100 mg mL^{-1} was prepared from the dry powder of the MeOH-extract for the experiment.

Phytochemistry: The MeOH-extract of the leaf of *P. guineense* was chemically analyzed for its constituents. The presence of chemical constituents of the MeOH-leaf-extract of *P. guineense* were separately identified using the methods described by Sofowora (1984), Odebiyi and Sofowora (1978) and Fadeyi *et al.* (1989).

Treatment: Leaf extract of *P. guineense* (3.5 mg g^{-1}) was administered to three groups of adult female Wister rats weighing between 130 and 150 g (n = 10) daily for 3, 7 and 30 days, respectively. Group four (control) received isotonic saline (0.9% NaCl) for the same number of days instead of the plant extract under the same experimental conditions. The rats were sacrificed on day 4, 8, 15 and 31 after treatments; and the uterine muscle strips (approx. 2.5 cm) were prepared in Tyrode solution (NaCl, 8 g; $CaCl_2$, 0.2 g; KCl, 0.2 g; $MgCl_2$, 0.1 g; NaH_2PO_4, 0.05 g; $NaHCO_3$, 1 g and glucose, 1 g). The preparation was set up in an organ bath capacity of 10 mL containing physiological salt solution constantly bubbled with air and warmed at 37°C. The suspended strip was connected to isotonic transducers (Ugo Basil, UK) via a piece of thread and connected to the microdynometer recorder (Ugo Basil, UK) under a basal tension of 1 g. The experimental set-up was allowed to run for 30 min before commencement of the actual test for data collection.

Concentration response relationships of acetylcholine (SIGMA, USA), Oxytocin (SIGMA, USA), Ergometrin (SIGMA, USA) and Potassium Chloride (M and B, UK) were obtained by addition of graded concentrations of each drug to the bath. During treatments, the rats were allowed free access to water and rate pellets (Afro Feed Mills Limited, Calabar, Nigeria) and the ambient temperature was 28±1°C.

Statistical analysis: Results were expressed as Mean±SEM, based on at least five experiments. Significance was determined by using student's t-test for paired data (Okwuasaba *et al.*, 1990; Bowman and Rand, 1980).

RESULTS

Phytochemical: The MeOH-leaf-extract of *Piper guineense* was found to contain the following chemicals; much more alkaloids than cardiac glycosides, flavonoids, polyphenol and tannins (Table 1).

Table 1: Results of Phytochemical Screening of *P. guineense*

Constituents	Quantity
Alkaloids	+++
Cardiac glycosides	++
Flavonoids	++
Tannins	+

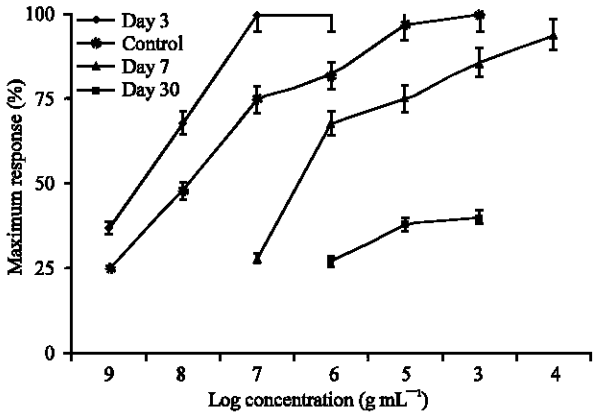

Fig. 1: Dose response curves to the graded concentrations of Acetylcholine on uterine muscle strip

Acetylcholine (Ach)-Ach (1×10^{-5}g mL^{-1}) enhanced uterine muscle contraction of the control rats in a dose related pattern (n = 5). The induced enhancement was potentiated by acute treatment with the extract of *P. guineense* for three days and shifted the dose response curve to the left of the graph (Fig. 1) with $p<0.001$.

Contrarily, sub-acute (7 days) or chronic (30 days) treatment with the same dose of the extract of *P. guineense* antagonized the acetylcholine induced contractions in a time dependent manner and shifted the dose response curves to the right side of the graph (Fig. 1) with $p<0.05$. Competitive antagonism was exhibited by sub-acute treatment, while sub-chronic treatment showed non-competitive antagonism.

Oxytocin (Oxy)-Oxy (2.0×100^{-5}-1×10^{-2} iμ mL^{-1}) induced uterine muscle contractions of normal rats in a dose dependent manner (n = 5). Repeated administration of the leaf extract of *P. guineense* daily for three days significantly ($p<0.01$) potentiated oxytocin induced contractions and shifted the response curve to the left side of the graph (Fig. 2). Repeated treatment with the leaf extract of *P. guineense* daily for seven and thirty days antagonized oxytocin induced concentrations in a time dependent fashion and shifted the dose response curves to the right side of the graph (Fig. 2), n = 5.

Ergometrine (Erg)-Ery (5-100 g mL^{-1}) induced uterine muscle contractions of rats treated with normal saline in the control. Acute treatments with the leaf extract of

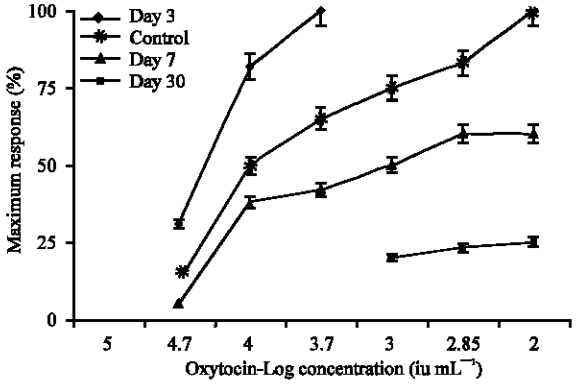

Fig. 2: Dose-response curves to oxytocin on uterine muscle strip

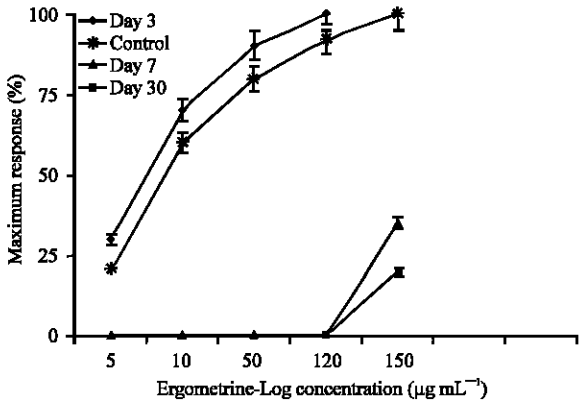

Fig. 3: Dose-response curves to Ergometrine on uterine muscle strip

P. guineense enhanced uterine muscle contractions elicited by ergometrine and shifted the response curve to the left side of the graph. However, the enhanced response was not statistically significant ($p>0.001$). Whereas, sub-acute treatment for 7 days and chronic treatment for 30 days with the extract of *P. guineense* significantly blocked ergometrine-induced uterine contraction ($p>0.001$) as shown in Fig. 3.

The blocking effect was reversed by increased concentration of ergometrine.

Potassium chloride (KCl) (1-50 mm) produced contractions in uterine muscle preparation of control rats in a dose related pattern. Repeated treatment with the leaf extract of *P. guineense* potentiated the induced contractile response in uterine muscle strips and significantly ($p<0.01$) shifted the response curve to the left as shown in Fig. 4. Repeated treatment with the leaf extract of *P. guineense* daily for 7 and 30 days markedly blocked KCl-induced uterine muscle contractions ($n = 5$) and significantly ($p<0.005$) shifted dose response curves to the right side of the graph (Fig. 4). Increased

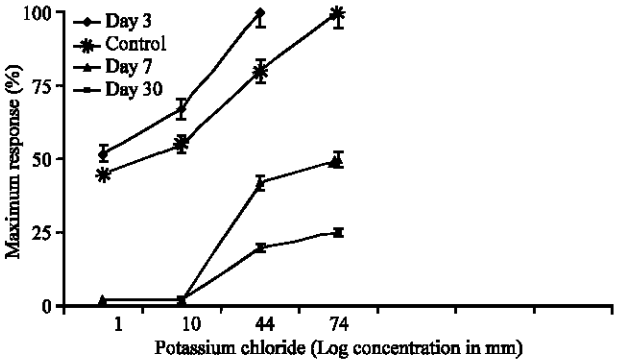

Fig. 4: Concentration-response curves to Potassium chloride (KCl) on uterine muscle strip

concentration of potassium chloride could not overcome the inhibitory effect following sub-acute or chronic extract treatment.

DISCUSSION

The results show that repeated administration of the leaf extract of *Piper guineense* for three days potentiated the enhanced uterine contraction induced by acetylcholine, oxytocin, ergometrine and potassium chloride. Potentiation of drug-enhanced uterine contractions allows assumption that the leaf of *Piper guineense* might sensitise the receptors (such as muscarinic, alpha and antitoxic receptors or entire uterine muscle to drugs. These may account for the report that the leaf extract of *Piper guineense* possesses estrogen-like activity (Udoh, 1999). It is known that when estrogen is secreted into circulation, it increases the growth of the ovarian follicles, increases uterine muscle contractions, excitability and sensitivity of the muscle to drugs (Udoh *et al*., 1999). This observation supports the previous report that the leaf extract of this plant is taken by women giving birth for expulsion of clotted blood and other debris from the uterus (Udoh *et al*., 1996; Braide *et al*., 2003; Udoh, 2007).

The present study also shows that the MeOH-extract of the leaf of *P. guineense* contained more of alkaloids than other chemicals detected. Therefore, the uterotonic action of the extract could be attributed to the alkaloids content of the extract. This is in line with the report of Ekanem *et al.* (2004) who reported a significant amount of alkaloid in the extract of *P. guineense*. However, chronic treatment with estrogen may cause hypertrophy of the uterine muscle and result in the loss of sensitivity to drugs. This presumption supports the results obtained for sub-acute and sub-chronic administration of the extract of the leaf of *P. guineense* (Udoh *et al*., 1999). The

inhibition of the drug induced contractions following repeated administration of the leaf extract of *P. guineense* for 7 or 30 days allows the suggestion that chronic administration of the leaf extract might cause hypertrophy of the uterine and results in the loss of sensitivity to agonist drugs. Such an action is believed to be due largely to persistent increase in the weight of uterine muscle cells induced by the extract. Contractile proteins which might result in the interference with translocation of extracellular Ca^{2+} is necessary for the formation of Ca^{2+} calmodulin complex. The muscle cells which activate myosin light chain kinase and stabilization of sarcoplasmic reticulum membrane with subsequent reduction in the amount of sarcoplasmic reticulum calcium required for activation of the contractile elements resulting in the desensitization of the muscle cells to agonist drug effects.

This finding further suggests that chronic consumption of the leaf of *P. guineense* as vegetable could have great implications in animal reproduction and great caution should therefore be taken consumption of the vegetable especially during pregnancy.

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