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Short Communication

In vitro tocolytic effects of the glycosidic constituents of the fruits of *Piper guineense*

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ABSTRACT: The tocolytic effect of the butanol fraction of the glycoside extract of the fruits of *Piper guineense*, was pharmacologically screened by measuring the uterine contractility. A state of oestrous was induced in three female rats through intramuscular injection of 0.1 *µ*g per 100 g body weight of stilboestrol. The animals were sacrificed; the two horns of the uterus were carefully dissected free of fat and connective tissues and divided into two pieces. Dose response curves were obtained using oxytocin alone, oxytocin plus butanolic fraction of the glycoside extract, and oxytocin plus salbutamol. Pretreatment of the uterine tissues with the glycoside extract or salbutamol resulted in inhibition of uterine contraction at low concentrations of oxytocin. The dose response curve of oxytocin inhibition by the glycoside extract is similar to that of salbutamol. This suggests that the bioactive component of the extract could be a competitive antagonist of oxytocin action.

KEYWORDS: *Piper guineense,* fruits, glycosidic constituents, butanol fraction, tocolytic effect.

INTRODUCTION

Tocolytics (uterine relaxants or uterine sedatives) are substances that relax or decrease uterine contractility or motility while substances that induce uterine contractility are known as oxytocics (uterotonics). Both the tocolytics and oxytocics act on the oxytocin receptors present in the smooth muscles of the myometrium as either antagonists or agonist respectively. The concentration of the oxytocin receptors is lower in non-pregnant state or at early pregnancy. As the pregnancy advances there is marked increase in the number of the oxytocin receptors in the myometrium. Also, the sensitivity of the oxytocin receptors is low during the first and second trimesters. However, there is tremendous increase in the sensitivity of the receptors. Before term, uterine contractility is not desired since it can lead to the expulsion or abortion of the embryo or foetus. Tocolytics become useful in delaying or postponing the pre-term labour, arresting the threatened abortion, reducing the harm to the foetus and allowing the foetus to mature. At term, uterine contraction becomes necessary for the safe delivery of the foetus. Dysfunctional uterine contractility during pregnancy could lead to preterm delivery with consequent prenatal morbidity and mortality. Thus a research into a safe and efficacious tocolytic is vital.

In different part of the world, traditional healers use plants to prevent preterm births. Studies have shown that these plants have tocolytic effect. For example, aqueous extract of the leaves of *Ficus exasperata* (Moraceae) has been shown to inhibit oxytocin-induced uterine contractions (Bafor *et al.*, 2011). Also, chloroform and methanol extracts of the rhizome of *Curcuma aeruginosa* Roxb. (Zingiberaceae) have inhibitory effects on oxytocin induced uterine contraction

(Thaina *et al.*, 2009). Oroxylin A, isolated from the root of *Scutellaria baicalensis* has also been reported to have tocolytic effect (Shih *et al.*, 2009). In Nigeria, *Piper quineense* (Piperaceae) is traditionally added to food due to the claims that it aids conception. Entries in the Jstor Plant Science database show that the fruits of *Piper quineense* is used as an antiabortifacient. The common names of the plant are Benin pepper and Ashanti pepper. In Nigeria the plant is commonly called Masoro (Hausa), Iyere (Yoruba), Uziza (Igbo), Adusa (Efik), Enie (Edo), Oziza (Ika) and Eshasha (Urhobo).

Previously, there was a report on the effects of the aqueous extracts of the fruits of *P. guineense* on the male reproductive functions of Wistar rats (Mbongue *et al.*, 2005). Also the antifertility effects of the ethanol extract of the seeds of *P. guineense* on female mice were attributable to the activities of some alkaloidal amides (Ekanem *et al.*, 2010).

Udoh *et al.* (2012) studied the pharmacodynamic effect of methanolic extract of the leaves of *P.guineense* on uterine physiology and showed that there was an initial potentiation of drug-enhanced uterine contraction. However, the study also showed that repeated administration of the leaf extract inhibited the drug-induced uterine contraction (Udoh *et al.* 2012).

Phytochemical screening of both the fruits and leaves of *P.guineense* showed the presence of alkaloids, glycosides, tarponins and volatile oil (Udoh *et al.*, 2012; Ejele *et al.*, 2012). In the light of these reports, it is essential to unravel how the active components of these extracts exert their purported inhibition of uterine functions. This study is aimed at investigating the effect of glycosidic constituents of the fruits of *Piper guineense* on the isolated uterus of Wistar rats.

METHODOLOGY

Extraction of glycoside constituents of Piper guineense.

The fruits of *piper guineense* collected from Aluku village, Edo state, Nigeria was authenticated in the Department of Pharmacognosy, University of Benin, Benin City, Nigeria. The fruits were kept in open air at room temperature for three days, followed by oven drying for three days at 60 ^{o}C. Two hundred and fifty (250) grammes of the powdered fruits were extracted for 24 hours using Soxhlet apparatus with 1.5 litres of methanol. The extract was filtered and concentrated in a rotary evaporator to remove the methanol in the extract. This concentrated extract was transferred to a conical flask. A 200 ml sample of the sample was made acidic by adding dilute sulphuric acid (10% solution). The acidified extract was mixed with 50 ml chloroform kept for 5 minutes to achieve phase separation. The aqueous layer was separated from the chloroform layer, and a few drops of distilled water was added to the aqueous layer to obtain a precipitate. The procedure was repeated using the original Soxhlet extract, and the aqueous and organic chloroform layers were separated. The aqueous layer was successively extracted with portions of chloroform and added to the first chloroform portion. The final chloroform portion was concentrated using a rotary evaporator. This portion contains the alkaloid while the aqueous phase contains the glycoside.

To the aqueous portion, n-butanol (n-butyl alcohol) was added, shaken together thoroughly in a separating funnel and left for 5 minutes. The butanol portion was then separated from the aqueous portion. This procedure gave rise to two fractions. The butanolic fraction (PG/A) is the butanol layer obtained after saturating the aqueous portion with butanol. The second fraction is the aqueous fraction (PG/B), which is obtained after saturating the aqueous portion with butanol.

Some distilled water was added to the butanol fraction in a separating funnel in order to remove the impurities. Test for reducing sugars presence of glycoside was done for the two fractions (1ml of extract) using Fehling solution after hydrolysis with 10% sulphuric acid, and treatment with 10% sodium hydroxide (NaOH) to make the solution alkaline. Both fractions were found to be positive for glycosides.

A pilot test showed that the butanol fraction has greater pharmacological activity on the oestrous rat uterus and this fraction was subjected to further pharmacological screening. The purified butanol fraction was air-dried, and 1.777 g of the butanolic fraction was suspended in a small volume of 0.5% carboxymethyl cellulose (CMC) solution. The sample was made up to 17.77 ml to give a 100 mg/ml stock solution.

Induction of oestrous state

Oestrous state was induced in three female rats through intramuscular injection of stilboestrol at 0.1 μg per 100 g body weight. This was aimed at improving the sensitivity of the uterus. In order to confirm that the rats were in oestrous, vaginal swabs were taken and examined microscopically for the presence of cornified squamous cells. The animals were sacrificed swiftly, and the two horns of the uterus were carefully dissected free of fat and connective tissues and divided into two pieces. The uterine tissues (n=12) were divided into three groups: oxytocin alone, oxytocin plus butanolic fraction of the glycoside extract, and oxytocin plus salbutamol. The tissues were suspended in 50 ml organ bath containing dejalon solution at 32 ^{o}C and aerated by means of air pump. The responsiveness of uterine tissues was tested by administration of oxytocin. The tissues were allowed to rest for 60 minutes. Dose response curves were obtained using oxytocin alone, oxytocin plus 2 mg of butanol fraction of the glycoside extract, and oxytocin plus 0.05 μg of salbutamol. Uterine contractions were recorded with the aid of a unirecorder and transducer. The pretreatment time was 15 seconds, contact time was 45 seconds while relaxation time (rest) was 2 minutes.

RESULTS AND DISCUSSION

In this study, we sought to investigate the *in vitro* effects of the glycosidic constituents of the fruits of *Piper guineense* on oxytocin-induced uterine contraction. We carried out butanolic extraction of the dried fruits and used Fehling's test to demonstrate the presence of glycosidic constituents in the extracts.

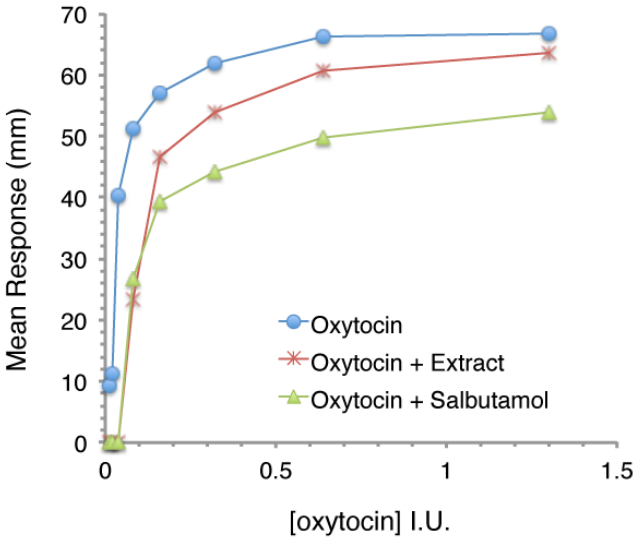

Figure 1: Effect of salbutamol and the butanolic fraction of the glycoside constituents of the fruits of *Piper guineense* on oxytocin-induced contractions of the uterus of rat. Uterine tissues from Wistar rats were pre-treated with salbutamol (0.05 µg) or 2 mg of the extracts prior to induction of uterine contraction with the indicated amounts of oxytocin.

Oxytocin is an oxytocic agent that induces uterine contraction. It is used to induce abortion or induce/augment labour and to minimize blood loss from the placental site. In this study, we used oxytocin to produce uterine contraction and to show the effect of the glycoside in the presence of oxytocin. Salbutamol is a tocolytic drug that relaxes or decreases uterine contractility or motility. It is used to prevent uncomplicated premature labour. In this study, salbutamol was used to inhibit oxytocin-induced uterine contraction, and the effect compared to the effect of the glycoside extract on the uterus. Stilboestrol is a nonsteroidal oestrogen, and was used to induce oestrus. Administration of stilboestrol improves the sensitivity of the uterine tissue to the action of oxytocin.

The glycoside extract at a dose of 2 mg (total dose in a 50 ml organ bath) was found to inhibit the oxytocic effect of oxytocin on the uterus. The dose-response curve of oxytocin in the presence of 2 mg of the extract shifted to the right of the dose-response curve of oxytocin alone (Figure 1). The glycoside tends to make the oxytocin two times less potent as an agonist (Table 1). In addition the glycoside extract exhibited a competitive antagonism since the antagonism was overcome with increase in the dose of the agonist. These trends suggest that the glycoside extract competes with the oxytocin for the oxytocin receptors. Salbutamol at a dose of 0.05 µg (total dose in a 50 ml organ bath) was also found to competitively antagonize the oxytocic effect of oxytocin on the rat uterus. The salbutamol tends to make the oxytocin four times less potent an agonist at that dose (Figure 1). The dose-response curve of the oxytocin in the presence of the salbutamol has shifted to the right of that of oxytocin alone.

At the doses used, salbutamol showed a higher tocolytic effect compared to the glycoside extract. The observed difference may be due to the crude nature of the extract. Further purification and refinement of the crude glycoside extract and isolation of the compound that possesses the tocolytic effect could result in higher degree of antagonism and consequently a better tocolytic effect.

Table 1: Effect of salbutamol and the butanolic fraction of the glycoside constituents of the fruits of *Piper guineense* on oxytocin-induced contractions of the uterus of rat.

	Mean Uterine Response (mm)		
[Oxytocin] I.U.	Oxytocin alone	Oxytocin + Extract	Oxytocin + Salbutamol
0.01	9.3 ± 5.1	0	0
0.02	11.3 ± 5.0	0	0
0.04	40.3 ± 10.7	0	0
0.08	51.3 ± 6.7	23.3 ± 4.9	26.7 ± 5.1
0.16	57.0 ± 6.7	46.7 ± 1.5	39.3 ± 5.7
0.32	62.0 ± 5.6	54.0 ± 2.7	44.3 ± 6.0
0.64	66.3 ± 4.7	60.7 ± 3.2	49.7 ± 4.0
1.3	66.7 ± 4.9	63.7 ±3.2	54.0 ± 3.6

Uterine tissues from Wistar rats were pre-treated with salbutamol (0.05 µg) or 2 mg of the extracts prior to induction of uterine contraction with the indicated amounts of oxytocin. The data shown here are the same shown in graphic format in Figure 1.

The results obtained in this study are not in complete agreement with previous studies on the effects of this plant on uterine physiology. We suggest that the main cause of the difference is the effect of the glycoside constituents of the extract used in this work. According to Ekanem *et al.* (2010), the uterotonic effect of the seed was attributable to the alkaloidal amides. The uterine inhibitory effect observed in repeated administration of the leaf of *Piper guineense* (Udoh *et al.*, 2012) could be due to increased concentration of the glycoside constituents with repeated administration. Further studies that look at the specific effects of active alkaloids and glycosides of the plant on uterine contraction could provide more details on the potential use of *Piper guineense* extracts

and their safety. The clinical implication of the findings reported here are significant. Ingestion of the fruits at term could trigger potential herbal-physiologic state interactions with the consequence of delaying the onset of labour.

Conclusion

The results showed that the glycoside constituents of the fruits of *Piper guineense* possess tocolytic effect. The dose response curve of oxytocin inhibition by the glycoside extract is similar to that of salbutamol. This suggests that the bioactive component of the extract could be a competitive antagonist of oxytocin action. Further studies are required to compare the effects of the alkaloidal and glycosidic constituents of the fruits of *P.guineense* on uterine contraction, and since the uterus has other parasympathetic innervations, such studies could reveal if the alkaloidal and glycosidic constituents are specific for the oxytocin receptors or non-specific.

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