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Evaluation of the Proposed Inhibitory Effect of the Aqueous Stem-Bark Extract of *Ficus exasperata* on Uterine Preparations *in vitro*

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Abstract: The effect of the aqueous stem-bark extract of *Ficus exasperata* (ASE) was studied on oxytocin- and acetylcholine-induced uterine contractions in uterine preparations isolated from non-pregnant Sprague-Dawley rats in oestrus. Preliminary phytochemical analysis was also performed. There were no statistically significant increases in the concentrations of oxytocin and acetylcholine required to elicit 30 and 50% of maximum response (EC_{30} and EC_{50}, respectively) in the presence of the extract. Salbutamol and atropine, however, significantly inhibited the effects of oxytocin and acetylcholine, respectively. Phytochemical analysis revealed the presence of alkaloids, tannins and saponin glycosides. These results indicate that ASE possesses no inhibitory effect on the non-pregnant rat uterus as claimed by traditional healers.

Key words: *Ficus exasperata*, uterine segments, pre-term contraction, oxytocin-induced contraction, uterine inhibition

INTRODUCTION

In recent times herbal remedies are becoming indispensable and constitute an integral part of primary health care systems in some nations.

The plant, *Ficus exasperata* Vahl. (Moraceae) is a small tree or shrub up to 20 m tall with scabrous, ovate leaves. It bears figs, which usually appear in pairs in the leaf axils. The bark is smooth, grayish cream with brown streaks and it exudes gummy sap. This plant is popularly referred to as sand paper tree in Nigeria. It is also known by local names such as; Kawusa (Nupe), Ameme (Edo), Erepin (Yoruba), Anwerenwa (Igbo).

Different parts of this plant are traditionally used for treatment of various ailments or disorders in Nigeria and across the African continent. We were informed by some traditional healers that the leaves and bark are used as an anthelminthic and the root bark is used for treatment of asthma. It has also been reported that the leaves are potent inhibitors of intestinal motility and have significant anti-ulcer activity. Their claims have been supported by the studies of Keita *et al.* (1999), Ake (1990) and Akah *et al.* (1997, 1998). Ijeh and Ukweni (2007) reported the use of the aqueous extract of the bark in hastening the expulsion of placenta in cows, after calf delivery. The stem bark and the leaves are also used by traditional healers in parts of Southern Nigeria and they claim that it is useful in dysmenorrhoea. The stem-bark is scraped, cleaned, dried under a shade and boiled in water. The resulting decoction is then administered. We found no relevant literature for this use.

There is as yet, no report on the pharmacological effect of the stem-bark extract on the uterus. This study was therefore undertaken to examine the inhibitory effect of the aqueous stem-bark on the uterus of non-pregnant rats prompted by the need to search for safe tocolytic agents necessary for the treatment of preterm labour, which accounts for 40-50% of preterm deliveries (Goldenberg, 2002).

MATERIALS AND METHODS

Plant material: The fresh stem barks of *Ficus exasperata* were collected in Benin, Edo state, Nigeria between the months of March and April, 2006. The plant was identified at the Taxonomy Department, Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria and has a herbarium No. F.H.I.107312.

Extraction: The stem barks were cleaned, air-dried for 8 days and ground into powder. The powder was macerated in distilled water for 48 h. The extract was decanted, filtered and concentrated in a vacuum evaporator (Buchi R110, Germany) at 70°C and dried in an oven set at 40°C. The sample gave a yield of 1.7% w/w.

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Animals: Female Sprague-Dawley rats, weighing between 160-180 g were used. They were bred locally at the Laboratory Animal unit of the Department of Pharmacology and Toxicology, University of Benin, Nigeria. The animals were maintained under standard conditions and had free access to standard diet (Ladokun Feeds limited, Ibadan, Nigeria) and water. They were handled according to standard guidelines for use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory animals, 2002).

Experiment on the isolated rat uterus: The animals were pre-treated with diethylstilboestrol (0.1 mg kg^{-1} i.p.) 24 h prior to the commencement of the experiment (Amos *et al*., 1998). Oestrus was confirmed by observation of vaginal smears. The rats were sacrificed under anaesthesia by injecting urethane 1.75 g kg^{-1} i.p. Uterine segments, 1.5 cm in length were rapidly dissected out and freed of adhering tissues. These were mounted in 35 mL organ baths containing physiological salt solution of the following composition in g/5 L: NaCl 45.0, $NaHCO_3$ 2.5, D-Glucose 2.5, KCl 2.1 and $CaCl_2.2H_2O$ 1.32. The lower end of the tissue was attached to a tissue holder by means of silk suture and the upper end to a Ugo Basile isometric force-displacement transducer (model 82145) connected to a Ugo Basile unirecorder (Model 7050). The solution was maintained at 37°C and continuously aerated. The preparations were equilibrated for 45 min at resting tension of 0.75 g before the start of the experiment. Concentration-response relationships of oxytocin and acetylcholine were determined in the absence and presence of two concentrations (0.04 and 0.32 mg mL^{-1}) of ASE, salbutamol (10^{-6} mg mL^{-1}) and atropine (10^{-7} mg mL^{-1}). The concentrations were fixed from results of preliminary experiments.

Phytochemical analysis: The aqueous stem-bark extract of *F. exasperata* was screened for glycosides, tannins, saponins, alkaloids, triterpenes and anthracenes by using the methods of Evans (2002).

Drugs: Diethylstilboestrol and acetylcholine were obtained from Sigma (UK), salbutamol from GlaxoSmithKline (England) and oxytocin and atropine from Laborate Pharmaceuticals (India). These were prepared fresh by dissolving in physiological salt solution (composition stated above) with the exception of diethylstilboestrol, which was constituted in ethanol from Sigma (UK). Other chemicals were of analytical grade and were obtained from Sigma, May and Baker (UK) and BDH chemicals (UK).

Statistical analysis All values are expressed as Mean±SEM (standard error of mean) and n represents the number of rats from which uterine segments were obtained. The EC_{30} and EC_{50} (concentration which produced 30 and 50% of maximum oxytocin and acetylcholine induced contractions) were computed for each concentration-response experiment. Comparisons were made using one-way ANOVA with Dunnett post hoc test. Statistical significance of $p<0.05$ was used in all cases.

RESULTS

Effect of extract on oxytocin- and acetylcholine-induced uterine contractility: The extract did not inhibit the contractions induced by oxytocin. Though the concentration-response curve of oxytocin was shifted slightly to the right in the presence of the extract, there was no statistically significant increase in the EC_{30} and EC_{50} values of oxytocin as shown in Table 1. Salbutamol (10^{-6} mg mL^{-1}) on the other hand significantly ($p<0.01$) inhibited the EC_{30} and EC_{50} of oxytocin (Table 1) while also significantly ($p<0.01$) depressing the E_{max} (Fig. 1).

The extract did not also inhibit acetylcholine-induced contractions, computation of the EC_{30} and EC_{50} values were not significant at both concentrations of extract used (Table 2). There was also no significant difference in the E_{max} as shown in Fig. 2. However, atropine (10^{-7} mg mL^{-1}) significantly inhibited the EC_{30} and EC_{50} values of extract ($p<0.01$, Table 2) with no significant difference in E_{max} (Fig. 2).

Phytochemical constituents of extract: Results of the preliminary phytochemical analysis of the aqueous stem-bark extract of *F. exasperata* revealed the presence of alkaloids, tannins, saponins and cardiac glycosides (Table 3).

Table 1: Concentrations of oxytocin producing effects at 30 (EC_{30}) and 50% (EC_{50}) of maximal response

Drugs	EC_{30} (IU mL^{-1})±SEM	EC_{50} (IU mL^{-1})±SEM
Oxytocin ($\times10^{-4}$IU mL^{-1})	1.26±2.83	1.83±5.12
Oxytocin + extract (0.04 mg mL^{-1})	1.67±4.81	1.81±3.22
Oxytocin + extract (0.32 mg mL^{-1})	1.71±2.43	1.78±3.87
Oxytocin + salbutamol (10^{-6} mg mL^{-1})	18.31±6.53**	0 **

The EC_{30} and EC_{50} values of oxytocin in the absence of extract and antagonists are controls. These values were compared to those in the presence of extract and salbutamol. Results indicate that the extracts did not significantly increase the EC_{30} and EC_{50} values of oxytocin. A greater and significant increase was observed in the presence of salbutamol which increased both EC_{30} and EC_{50} values. **$p<0.01$ compared to oxytocin alone, n = 5 rats

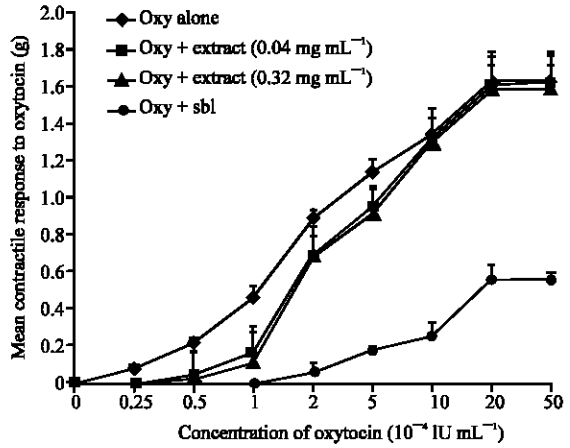

Fig. 1: Concentration - response relationships of oxytocin (10^{-4} IU mL^{-1}) in the absence and presence of extract (0.04 and 0.32 mg mL^{-1}) and salbutamol (10^{-6} mg mL^{-1}). The extract pushed the curve slightly to the right however maximum responses were completely restored without a depression or a shift. Similarly salbutamol inhibited oxytocin but with a greater degree of shift and a depression in maximum responses, n = 5 rats

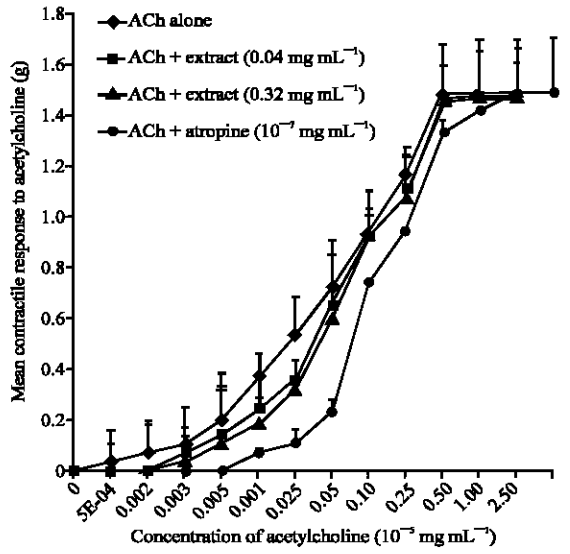

Fig. 2: Concentration-response relationships of acetylcholine (10^{-5} mg mL^{-1}) in the absence and presence of extract (0.04 and 0.32 mg mL^{-1}) and atropine (10^{-7} mg mL^{-1}). In the presence of the extract, the curve for acetylcholine was shifted minimally to the right and there were no significant differences in the maximum responses obtained. There were no significant differences among the concentrations of extract in their efficacy in inhibiting acetylcholine induced contractions, n = 5 rats

Table 2: Concentrations of acetylcholine (ACh), producing effects at 30 (EC_{30}) and 50% (EC_{50}) of maximal response

Drugs	EC_{30} ($\times 10^{-6}$ mg mL^{-1})±SEM	EC_{50}($\times 10^{-6}$ mg mL^{-1})±SEM
ACh($\times 10^{-6}$ mg mL^{-1})	0.04±0.13	0.02±0.22
ACh + extract (0.04 mg mL^{-1})	0.04±0.11	0.03±0.18
ACh + extract (0.32 mg mL^{-1})	0.04±0.25	0.03±0.09
ACh + atropine (10^{-7} mg mL^{-1})	0.09±0.18**	0.06±0.12**

The EC_{30} and EC_{50} values of acetylcholine in the absence of extract and antagonists are controls. These values were compared to those in the presence of extract and atropine. Results indicate that the extracts did not significantly affect the EC_{30} or the EC_{50} of acetylcholine. However, in the presence of atropine both EC_{30} and EC_{50} values were significantly increased. **: $p < 0.01$ compared to ACh alone. n = 5 rats

Table 3: Preliminary phytochemical analysis of the aqueous extract of *F. exasperata* stem-bark

Constituents	Observation
Carbohydrates	Present
Reducing sugars	Present
Glycosides	Present
Cardiac glycosides	Present
Cyanogenetic glycosides	Absent
Saponins	Present
Tannins	Present
Alkaloids	Present
Anthracene derivatives	Absent

The stem-bark of *F. exasperata* has been shown to be devoid of cyanogenetic glycosides and derivatives of anthracene while containing glycosides with reducing sugars amongst which are cardiac glycoside, saponins and tannins

DISCUSSION

One of the mechanisms proposed for the pathogenesis of preterm labour is the early idiopathic activation of the normal labour process (Goldenberg, 2002) amongst which include: oxytocin initiation, which necessitated the employment of oxytocin in this study. Oxytocin, a cyclic nanopeptide, is a known endogenous stimulator of uterine contraction via stimulation of increased intracellular calcium ions concentration. Thus oxytocin inhibitors or antagonists might prove useful in alleviating or arresting preterm contractions (Pak, 1994).

Acetylcholine was also employed in this study to assess the effect of the extract on cholinergic stimulation. Cholinergic innervation to the uterus is well documented (Higby *et al*., 1993) and may possibly play a role in preterm contractions. Acetylcholine is an endogenous muscarinic receptor stimulator that produces uterine smooth muscle contraction via activation of M_2 and M_3 receptors located within the myometrium (Pennefather *et al*., 1994). Again, inhibitors of acetylcholine might also prove useful in counteracting preterm contractions.

The aqueous stem-bark extract of *F. exasperata* has been shown in this study to have no inhibitory effect on oxytocin-or acetylcholine-induced uterine contractions. There are a number of agents that inhibit uterine smooth

muscle contraction. They include: β-sympathomimetic agents, magnesium sulphate, prostaglandin synthetase inhibitors, calcium channel blockers, oxytocin antagonists, nitric oxide donors etc. They cause uterine relaxation by various mechanisms. Their relevance in uterine relaxation arises from the need to decrease the incidence of preterm labour, which is a leading cause of preterm delivery, neonatal mortality and morbidity, accounting for 60-80% of deaths of infants without congenital anomalies (Goldenberg, 2002).

Salbutamol is a β_2-selective sympathomimetic agent and is one of the primary drugs utilized for the treatment of preterm labor (Goldenberg, 2002). It physiologically counteracts uterine contractions by activation of adenylate cyclase resulting in an increase in cAMP-dependent protein kinase (Engstrom *et al*., 1999), which phosphorylates myosin light chain kinase and prevents the interaction of myosin and actin necessary for uterine smooth muscle contraction. It is however encompassed by numerous maternal side effects, which accompanies its administration e.g., maternal cardiopulmonary effects and even maternal death. Foetal and neonatal deaths, with histological evidence of myocardial ischemia, have also been reported. Thus the search for safer alternatives with minimal side effects is on the increase (Higby *et al*., 1993). Salbutamol physiologically antagonized the effect of oxytocin in this study observed by the depression of the maximum response compared to the extract. Thus the purported belief by traditional healers that the stem-bark of *F. exasperata* inhibits uterine contractions is unfounded as this study has shown. However, further studies on the pure fractions may reveal other properties of the extract on the uterus. Atropine is a direct muscarinic receptor inhibitor and competes with acetylcholine and other muscarinic agonists for a common binding site on the muscarinic receptor. This binding site is in a cleft predicted to be formed by several of the receptor's seven transmembrane helices (Palczewski *et al*., 2000). Antagonism by atropine is competitive and can thus be overcome if the concentration of the muscarinic agonists at receptor sites of the effector organ is increased sufficiently. Atropine significantly inhibited the effect of acetylcholine whilst the extract did not.

In concordance with the foregoing, this study has disproved traditional beliefs that the aqueous stem-bark extract of *F. exasperata* relaxes uterine smooth muscles and thus cannot be useful in counteracting conditions of hyperstimulation of the uterus such as dysmenorrhoea and preterm labour.

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