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Evaluation of the Uterotonic Activity of the Aqueous Leaf Extract of *Ficus exasperata* Vahl (Moraceae)

Enitome E. Bafor, Eric K. Omogbai and Raymond I. Ozolua
Department of Pharmacology and Toxicology, Faculty of Pharmacy,
University of Benin, 300001, Nigeria

Abstract: The leaves of *Ficus exasperata* Vahl Enum. Pl. vahl (Moraceae) are used by traditional healers in Southern Nigeria to arrest preterm contractions in pregnant women and are also used as abortifacients in some parts of Africa. In this study the purported uterotonic activity of the aqueous leaf extract of *F. exasperata* (AET) was investigated *in vitro*. AET was obtained from the fresh leaves of the plant. The effect of the extract on rhythmic spontaneous uterine contractions was investigated and the extract was also directly tested on uterine tissues. The effect of the extract was compared with those of acetylcholine. The extract, at concentrations ranging from 2.5×10^{-2} to 100×10^{-2} mg mL^{-1}, significantly increased the frequency ($p<0.05$) but not the amplitude of spontaneous contractions and directly stimulated uterine contractions. Acetylcholine likewise, concentration-dependently stimulated uterine contractions and significantly increased the frequency ($p<0.05$) of spontaneous contractions. The aqueous leaf extract of *F. exasperata* at the concentrations used in this study stimulates uterine contractility which may account for its use in easing childbirth in some parts of Africa.

Key words: Uterine contraction, *Ficus exasperata*, uterus, acetylcholine, aqueous extract

INTRODUCTION

The use and awareness of medicinal plants has increased over the years. In some African countries, up to 90% of the population still relies exclusively on plants as a source of medicine (Hostettmann *et al*., 2000).

One plant used by traditional healers in Nigeria is *Ficus exasperata* Vahl Enum. Pl. vahl. The plant belongs to the family Moraceae (Umerie *et al*., 2004) and is locally known as sandpaper tree in Nigeria (Ijeh and Ukweni, 2007) due to the well known rough or scabrous surface of its leaves. Its ethnic names include: Kawusa (Nupe); Ameme (Edo); Erepin (Yoruba); Anwerenwa (Igbo) and Umwemwe (Etsako).

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. In Congo, extracts of the bark are used by traditional birth attendants to ease childbirth (Ijeh and Ukweni, 2007). In Ivory Coast, the leaves are used to counteract dysmenorrhoea (Ake, 1990). Baerts and Lehmann (1991) reported the use of the leaves as an oxytocic to hasten childbirth and as an abortifacient.

From the foregoing, it would appear that the leaves of *F. exasperata* are used traditionally for relaxing the uterus by some herbal medical practitioners (Ake, 1990) and in enhancing contractions of the uterus by others as Baerts and Lehmann (1991). Our unpublished data indicate that the aqueous leaf extract of the plant at 1.0×10^{-2} mg mL^{-1} significantly inhibited oxytocin-induced uterine

Corresponding Author: E.E. Bafor, Department of Pharmacology and Toxicology, University of Benin, Benin City 300001, Nigeria Tel: +234-802-352-4422

contractions. This study is therefore aimed at investigating the effects of higher concentrations of the extract on the isolated uterus, in order to determine if the reported dual uterine actions of the extract are concentration-dependent.

MATERIALS AND METHODS

Preparation of the Plant Material

The leaves of *F. exasperata* were collected in September, 2006 from the premises of the University of Benin, Benin City, Nigeria. The plant was identified by Dr. B. Ayinde of the Department of Pharmacognosy and authenticated by Mr. Felix Usang of the Forest Research Institute of Nigeria, Ibadan, where a herbarium sample with voucher number F.H.I.107312 was prepared and deposited. A specimen voucher was also deposited in the Department of Pharmacognosy, University of Benin.

Extraction

The fresh leaves were manually rendered free of adulterants, cleaned by rinsing in clean water and ground. The aqueous leaf extract was obtained by macerating the ground leaves (2 kg) in distilled water (2 L) for 24 h. The resulting decoction was decanted, filtered and concentrated under pressure in a rotary evaporator (R110 Buchi, Switzerland) at 60°C and dried to a constant weight in an oven set at 40°C. The dried extract gave a yield of 20.14% w/w and was stored in an air-tight container at about 4°C until required.

Animals

Adult female Sprague-Dawley rats (160-200 g) bred in the animal house Department of Pharmacology and Toxicology, University of Benin, Nigeria were used. The animals were maintained under standard conditions and had free access to standard diet (Ladokun Feeds Ltd, 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).

Preparation of Uterine Tissues

The animals were treated with diethylstilboesterol (0.2 mg kg^{-1} i.p.,) 24 h prior to the commencement of the experiment. Oestrus was confirmed by microscopic observation of vaginal smears and macroscopic observation of the vulva. The rats were sacrificed under chloroform anaesthesia and uterine segments, 2 cm in length, were rapidly dissected out and freed of adhering connective tissues and fat. The segments were mounted in 40 mL organ baths containing physiological salt solution of the following composition in 5 g L^{-1}: 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 a segment of the uterine 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 (Eferekeya and Nworgu, 1985). The preparations were equilibrated for 45 min at resting tension of 0.75 g before the start of the experiment.

High Concentrations of the Aqueous (aq) Leaf Extract of *F. exasperata* or Acetylcholine on Spontaneous Uterine Contractions

After equilibration, the baseline (100%) amplitude and frequency were recorded in the first 10 min (Perusquia and Navarette, 2005). This was followed by subsequent 10 min exposure of the

tissue to 2.5×10^{-2} mg mL^{-1} of the extract followed by increasing cumulative concentrations, Kurowicka *et al*. (2005) of 2.5×10^{-2} to 250×10^{-2} mg mL^{-1}. These were compared with the effect of acetylcholine (0.0005×10^{-2} to 0.5×10^{-2} mg mL^{-1}).

Salbutamol on the aq. Leaf Extract of *F. exasperata* or Acetylcholine Induced Uterine Contraction

Concentration - response relationships were done over a higher range of concentration of extract 2.5×10^{-2} to 500×10^{-2} mg mL^{-1} in the absence and presence of 10^{-6} mg mL^{-1} of salbutamol. The experiments were repeated with acetylcholine (0.0005×10^{-2} to 0.5×10^{-2} mg mL^{-1}) as the contractile agent.

Phytochemical Screening of the Extract

The aqueous leaf extract of *F. exasperata* was screened for glycosides, tannins, saponins, alkaloids, triterpenes and anthracenes by using the methods of Evans (2002).

Drugs

Diethylstilboesterol and acetylcholine were obtained from Sigma (UK). Salbutamol was obtained from Glaxo Smithkline (England). The drugs were prepared fresh on the day of the experiment by dissolving in physiological salt solution (composition stated above). Diethylstilboesterol for the induction of oestrus was constituted in 95% ethanol (Sigma, UK).

Statistical Analysis

All values were expressed as Mean±SEM and n represents the number of rats from which uterine segments were obtained. The EC_{50} (concentration which produced 50 % of maximum response) and E_{max} (maximum achievable response) were computed for each concentration-response experiment. Comparisons were made using one-way ANOVA with Dunnett multiple comparison test or Student's t-test. $p<0.05$ indicated statistical significance in all cases.

RESULTS

Effects of the aq. Leaf Extract of *F. exasperata* (AET) and Acetylcholine on Amplitude and Frequency of Spontaneous Contractions

The higher concentrations of AET and those of acetylcholine each significantly ($p<0.05$) increased the frequency but not the amplitude of spontaneous contractions (Table 1, 2).

Table 1: Effect of the aqueous leaf extract of *F. exasperata* (AET) on the frequency and amplitude of spontaneous uterine contractions

Concentration of AET ($\times10^{-2}$ mg mL^{-1})	Mean frequency (%)	Mean amplitude (%)
0 (Baseline)	100	100
2.5	115.3±5.6	101.8±0.6
5.0	136.3±9.4*	101.7±1.7
10.0	157.1±17.4**	101.6±1.6
25.0	174.5±16.9**	102.7±1.8
50.0	184.6±20.3**	103.7±1.8
100.0	195.8±18.0**	103.8±1.4
250.0	199.3±15.6**	103.7±1.5

*$p<0.05$ and **$p<0.01$ compared to baseline; n = 7 rats

Table 2: Effect of acetylcholine (ACh) on the frequency and amplitude of spontaneous uterine contractions

Concentration of ACh ($\times10^{-5}$ mg mL^{-1})	Mean frequency (%)	Mean amplitude (%)
0 (Baseline)	100	100
0.5	113.5±4.3	101.2±1.3
1	136.2±8.4*	102.8±1.2
2	168.1±9.2**	104.8±1.6
5	173.6±16.9**	104.8±1.9
10	186.8±20.3**	107.0±1.4
20	197.5±15.6**	107.7±0.9
50	212.8±11.3**	108.6±1.6
100	213.2±8.9**	107.6±1.3
200	215.7±17.8**	107.6±1.3
500	215.7±17.8**	106.4±1.1

*p<0.05 and **p<0.01 compared to baseline; n = 7 rats

Table 3: Concentrations of AET and ACh producing effects at 50% of maximal response (EC_{50}) alone and in the presence of salbutamol (Sbl)

Drugs (mg mL^{-1})	EC_{50} (mg mL^{-1}) ±SEM
AET alone	8.2×10^{-2}±0.22
AET+Sbl ($\times10^{-6}$ mg mL^{-1})	21.3×10^{-2}±0.15*
ACh alone	6.4×10^{-5}±0.32
ACh+Sbl ($\times10^{-6}$ mg mL^{-1})	4.3×10^{-5}±0.27**

*p<0.001 compared to Aet alone; **p<0.001 compared to ACh alone; n = 8 rats

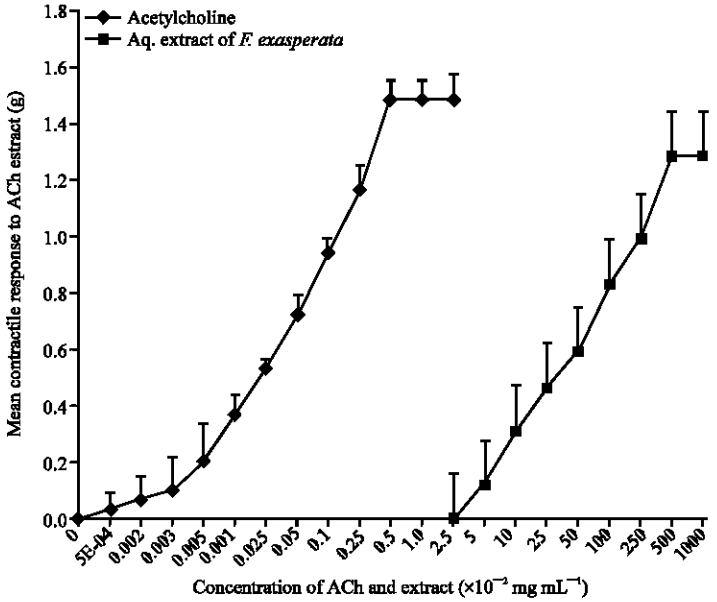

Fig. 1: Concentration-response curves showing the effects of acetylcholine and the aq. extract of *F. exasperata* on the isolated uterus. There was no significant difference in the E_{max} of both uterine stimulants n = 8 rats

Effect of Salbutamol on the Aq. Leaf Extract of *F. exasperata* (AET) and Acetylcholine Induced Uterine Contraction

AET and acetylcholine each caused a concentration-dependent contractile response in the isolated uterus. There was no significant difference in their E_{max} (Fig. 1). Salbutamol significantly ($p<0.05$) increased the EC_{50} of both extract and acetylcholine (Table 3) and significantly ($p<0.01$) depressed the E_{max} (Fig. 2).

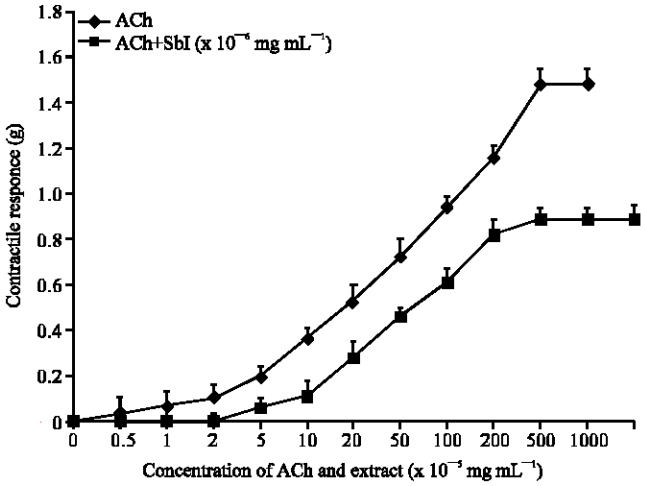

Fig. 2: Concentration-response curves showing the effects of salbutamol on acetylcholine induced contraction. Salbutamol significantly ($p<0.001$) depressed the E_{max} of ACh. n = 8 rats

Table 4: Phytochemical constituents of the aqueous leaf extract of *F. exasperata*

Constituents	Observation
Cardiac glycosides	Present
Cyanogenetic glycosides	Absent
Saponins	Present
Tannins	Present
Flavonoids	Present
Alkaloids	Absent
Anthracene derivatives	Absent

Phytochemical Constituents of the Extract

Results of the preliminary phytochemical analysis of the aqueous leaf extract of *F. exasperata* showed the presence of reducing sugars, cardiac glycosides, saponins and tannins (Table 4).

DISCUSSION

The observed increase in the frequency of spontaneous contractions of the rat uterine smooth muscle by the higher concentrations of the extract, may be due to an increase in the open state probability of the voltage-dependent calcium channels, allowing an influx of extracellular calcium thereby enhancing contractions (Aaronson *et al*., 2006) or the extract may regulate the opening of voltage-gated potassium channels which Aaronson *et al*. (2006) proposed as a major contributing factor to basal myometrial contractility. The extract may also promote directly or indirectly the production of prostaglandins *in situ* thereby increasing the inherent uterine contractility (Vane and Williams, 1973). However, this remains to be absolutely verified.

It was also observed that the higher concentrations of the extract which increased the frequency of spontaneous contractions did not have any effect on the amplitude. A probable explanation for this might be that the extract had no direct effect on the endogenous pacemaker cells, which was reported by Mackler *et al*. (1999), to reside in uterine tissues and promote increase in amplitude of uterine contraction. Thus, the extract would have no effect on gap junction assembly and would not enhance or inhibit cellular communication, culminating relatively in unaffected amplitude of uterine contraction. Otherwise, due to the increased sensitivity induced by the administration of diethylstilboesterol, the tissues were contracting at the maximum attainable amplitude of contraction and addition of a stimulant or an agonist would not produce any observable change.

The contractile effect of the extract was compared to that of acetylcholine (ACh), a stimulator of uterine smooth muscle contraction via activation of M_2 and M_3 receptors located within the myometrium (Pennefather, 1994). The extract and ACh appeared to contract the uterus similarly, though ACh was the more potent of the two producing a greater increase in the frequency of spontaneous contractions; a lesser concentration of ACh was required to elicit contractions and a shift in the concentration response curve of the extract, this was also confirmed from the potency ratio computation. Despite being crude, the higher concentrations of the extract exhibited a good potential as an oxytocic agent. It is hoped that further studies on the extract will produce a clinically useful oxytocic agent with minimal side effects for the facilitation of labour and as an abortifacient. The extract was also observed to be made up of tannins, flavonoids, saponins and cardiac glycosides which have been reported to have varied effects on the uterus. Sugimoto (1913) and Norris (1961) reported the stimulation of the uterus by cardiac glycosides. Calixto *et al*. (1986) reported that tannic acid affects calcium availability necessary for smooth muscle contraction and it dose-dependently and non-competitively antagonized contractions to several agonists in the rat uterus. Edward *et al*. (1996) reported that saponins inhibited the metabolism of prostaglandin E_2 and prostaglandin$F_{2\alpha}$. The culminative effect of these interacting phytoconstituents may contribute to the dual effect of the extract on the uterus.

CONCLUSION

This study has shown that higher concentrations of the aqueous leaf extract of *F. exasperata* stimulate contractions of the isolated rat uterus in a manner similar to acetylcholine and also increase the frequency of rhythmic spontaneous uterine contractions.

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