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OXYTOCIN INHIBITING EFFECT OF THE AQUEOUS LEAF EXTRACT OF *FICUS EXASPERATA* (MORACEAE) ON THE ISOLATED RAT UTERUS

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Abstract: The leaves of *Ficus exasperata* Vahl Enum. Pl. vahl (Moraceae) are used by traditional healers in Southern Nigeria and some parts of Africa to avoid preterm births. However, previous reports showed that the plant also exhibited uterine contractions at specific concentrations. This study is therefore aimed at investigating the purported uterine inhibitory aspect of the plant on the isolated rat uterus. The aqueous extract (AET) was tested on rhythmic spontaneous uterine contractions. Concentration–response relationships were obtained for oxytocin (OT), acetylcholine (ACh) and ergometrine (EGM), in the presence or absence of fixed concentrations of AET. Salbutamol (SBL) and verapamil (VER) were used as positive controls. AET, at 1.0×10^{-2} mg/mL, significantly increased ($p < 0.05$) the EC_{50} of oxytocin-induced contractions but had no significant effect on ACh, EGM and spontaneous uterine contractions. However, SBL and VER significantly increased ($p < 0.01$) the EC_{50} of OT, ACh and EGM and significantly inhibited ($p < 0.01$) the frequency and amplitude of spontaneous uterine contractions. The aqueous leaf extract of *F. exasperata* inhibits oxytocin-induced uterine contractions at the concentration shown in this study. This observation may explain its folkloric use in counteracting preterm contractions and alleviating dysmenorrhoea.

Keywords: *Ficus exasperata*; rat uterus, oxytocin

Plants have a long history of use on the African continent for the treatment of different diseases and complaints. In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicine (1). The plant *Ficus exasperata* Vahl Enum. Pl. vahl is commonly used by traditional healers in Nigeria. The plant belongs to the family Moraceae (2).

In Edo State of Nigeria, by personal communication with traditional medicine healers, the leaves of the plant are either macerated fresh in water or the dried powdered leaves boiled and the extract used as a tocolytic to prevent preterm delivery. Adjanahoun et al. (3) reports the use of the aqueous macerated powdered leaves in Togo for the treatment of dysmenorrhea. In Ivory Coast, the leaves are also used to treat dysmenorrhoea (4).

From the foregoing, it would appear that the leaves of *F. exasperata* also possess uterine relaxant activities. Previous pharmacological reports on *F. exasperata* showed that it protected rats from aspirin-induced ulcerogenesis, delayed intestinal transit, increases pH and decreased both the volume and acidity of gastric secretion; it also has antioxidant, palm oil stabilizing effects, and hypotensive actions (5, 6). However, no study has been carried out to evaluate its inhibitory effect on uterine activity. In view of the above, this present study aims at examining the potential of the aqueous leaf extract of *F. exasperata* in relaxing the isolated non-pregnant uterus using animal models.

EXPERIMENTAL

Preparation of plant material

The collection and identification of the plant was as already described (7). Briefly, the leaves of *Ficus exasperata* were collected in September from the premises of the University of Benin, Benin City, Nigeria. The plant was authenticated by Mr. Felix Usang of the Forest Research Institute of Nigeria, Ibadan, where a herbarium sample with voucher number of F.H.I. 107312 was prepared and deposited. A specimen voucher was also deposited in the Department of Pharmacognosy, University of Benin. The fresh leaves were cleaned, rendered free of adulterants and ground. The aqueous leaf extract

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Table 1. Concentration of OT producing 50 % of maximum response alone and in the presence of AET, SBL and VER on isolated uteri of non pregnant rats.

Drugs (mg/mL)	EC_{50} (mg/mL)
OT Alone	0.00019 ± 0.4
OT/AET (0.25×10^{-2})	0.00024 ± 0.23[c]
OT/AET (0.5×10^{-2})	0.00021 ± 0.41[c]
OT/AET (1.0×10^{-2})	0.00061 ± 0.33 [b]
OT/SBL (0.6×10^{-6})	0.0019 ± 0.25[a]
OT/SBL (6.2×10^{-6})	–
OT/VER (0.13×10^{-6})	0.0016 ± 1.92[a]
OT/VER (1.32×10^{-6})	–

[a]$p < 0.01$; [b]$p < 0.05$; [c]$p > 0.05$ compared to OT alone; (–) indicates that due to significant inhibition, 50% response could not be reached.

Table 2. Concentration of ACh producing 50% of maximum response alone and in the presence of AET, SBL and VER on isolated uteri of non pregnant rats.

Drugs (mg/mL)	EC_{50} (mg/mL)
ACh Alone	6.4×10^{-5} ± 0.4
ACh/AET (0.25×10^{-2})	5.7×10^{-5} ± 0.23[c]
ACh/AET (0.5×10^{-2})	5.1×10^{-5} ± 0.41[c]
ACh/AET (1.0×10^{-2})	4.6×10^{-5} ± 0.33 [b]
ACh/SBL (0.6×10^{-6})	5.1×10^{-4} ± 0.25[a]
ACh/SBL (6.2×10^{-6})	–
ACh/VER (0.13×10^{-6})	5.7×10^{-4} ± 1.92[a]
ACh/VER (1.32×10^{-6})	–

[a]$p < 0.01$; [b]$p < 0.05$; [c]$p > 0.05$ compared to ACh alone; (–) indicates that due to significant inhibition, 50% response could not be reached.

Table 3. Concentration of EGM producing 50% of maximum response alone and in the presence of AET, SBL and VER on isolated uteri of non pregnant rats.

Drugs (mg/mL)	EC_{50} (mg/mL)
EGM Alone	4.5×10^{-5} ± 0.4
EGM/AET (0.25×10^{-2})	4.0×10^{-5} ± 0.23[c]
EGM/AET (0.5×10^{-2})	3.9×10^{-5} ± 0.41[c]
EGM/AET (1.0×10^{-2})	3.8×10^{-5} ± 0.33 [b]
EGM/SBL (0.6×10^{-6})	3.7×10^{-4} ± 0.25[a]
EGM/SBL (6.2×10^{-6})	–
EGM/VER (0.13×10^{-6})	3.6×10^{-4} ± 1.92[a]
EGM/VER (1.32×10^{-6})	–

[a]$p < 0.01$; [b]$p < 0.05$; [c]$p > 0.05$ compared to EGM alone; (–) indicates that due to significant inhibition, 50% response could not be reached.

was obtained by macerating the ground leaves in distilled water for 24 h. The decoction was then decanted, filtered and concentrated under reduced 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 was kept in a freezer until required.

Drugs

Diethylstilbestrol, acetylcholine (Ach) and ergometrine (EGM) were obtained from Sigma (UK) and oxytocin (OT) from Laborate Pharmaceuticals India Ltd.

Animal/Uterine tissue preparation

The animals were prepared and the isolated rat uterus was set up as described by Bafor et al. (7). Briefly, adult female Sprague–Dawley rats (160–200 g) were housed in the animal unit of the Department of Pharmacology and Toxicology, University of Benin. The animals were maintained according to standard nutritional and environmental conditions (8) and had free access to standard diet (Bendel Feeds and Flour Mill, Ewu, Nigeria) and water as approved by the Faculty of Pharmacy, University of Benin Committee on the Use of Experimental Animals. University of Benin and 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).

Prior to experiments on the inhibitory activity of the plant, diethylstilbestrol (0.2 mg/kg *i.p.*) was injected into each animal 24 h prior to the commencement of the experiment. Confirmation of estrus was by microscopic observation of vaginal smears and macroscopic observation of the vulva. The rats were sacrificed under anesthesia and 2 cm length of uterine horns were rapidly dissected out and placed in previously warmed and aerated physiological salt solution. Uterine segments, 2 cm in length were cut and freed of adhering connective tissues and fat. Briefly, the segments were mounted vertically in 40 mL organ baths containing physiological salt solution (PSS) of the following composition in g/5 L: NaCl 45.0, $NaHCO_3$ 2.5, D-glucose 2.5, KCl 2.1, and $CaCl_2 \times 2H_2O$ 1.32. The lower ends of the tissue were attached to tissue holders by means of silk suture and the upper ends to Ugo Basile isometric force-displacement transducer (Model 82145) connected to Ugo Basile unirecorder (Model 7050). The PSS was maintained at 37°C and continuously aerated. Each uterine segment was placed under optimum resting tension of 0.75 g and equilibrated for 45 min before the start of the experiment. During the equilibration period, the preparations were washed with the PSS every 10 min (Perez-Hernandez et al., 2008). Details of isometric tension studies have been previously described by Bafor et al. (7).

Study on the effect of AET on spontaneous uterine contractions

After equilibration, in order to investigate AET's effect on rhythmic spontaneous contractions, the baseline (100%) amplitude and frequency were recorded in the first 10 min (9). This was followed by subsequent 10 min exposure of the tissue to 0.25×10^{-2} mg/mL of AET followed by increasing cumulative concentrations (10) of 0.25×10^{-2} to 2.5×10^{-2} mg/mL.

Study of the effect of AET on oxytocin-, acetylcholine- and ergometrine-induced uterine contractions

The effects of single concentrations of OT (10^{-4} IU/mL), ACh (10^{-5} mg/mL) and EGM (10^{-5} mg/mL) were investigated alone and in the presence of 0.25×10^{-2} to 1.0×10^{-2} mg/mL of AET. The tissue was incubated with AET for 5 min before addition of the respective agonists.

Study on the effect of SBL and VER on spontaneous uterine contractions

The effects of SBL (0.6×10^{-6} mg/mL; 6.2×10^{-6} mg/mL) and VER (0.13×10^{-6} mg/mL; 1.32×10^{-6} mg/mL), which were used as controls in this study, were investigated on spontaneous contractions as described above.

Study on the effect of SBL and VER on OT-, Ach- and EGM-induced uterine contractions

SBL (0.6×10^{-6} mg/mL; 6.2×10^{-6} mg/mL) and VER (0.13×10^{-6} mg/mL; 1.32×10^{-6} mg/mL) were also tested on OT, ACh, and EGM and compared with those of AET.

Statistical analysis

All values were expressed as the mean ± SEM (standard error of the mean) and n represents the number of rats from which uterine segments were obtained. Comparisons were made using one-way ANOVA with Dunnett's Multiple Comparison Test. A value of $p < 0.05$ was considered significant in all cases.

RESULTS

Effect of extract on amplitude and frequency of spontaneous contractions

AET at 0.25×10^{-2} to 1.0×10^{-2} mg/mL did not significantly increase or decrease the frequency and amplitude of spontaneous uterine contraction when compared with baseline values.

Effect of extract on OT-, Ach- and EGM-induced uterine contractions

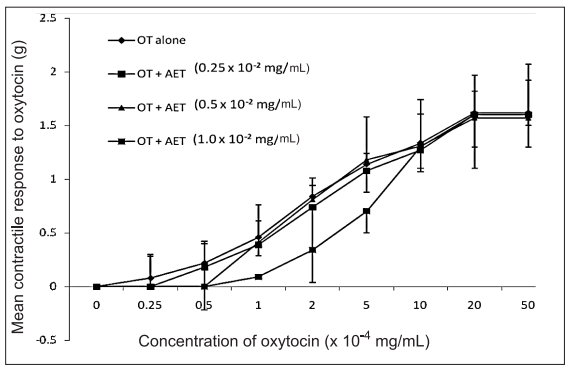
Concentrations of 0.25×10^{-2} to 0.5×10^{-2} mg/mL of AET did not significantly inhibit OT-induced contractions but the highest of the concentrations used, 1.0×10^{-2} mg/mL, caused a rightward shift in the concentration response curve of OT without a change in slope (Fig. 1). However, AET at all concentrations used in this study had no significant effect on Ach- (Fig. 4) or EGM-induced (Fig. 7) contraction.

Effect of SBL and VER on spontaneous uterine contractions

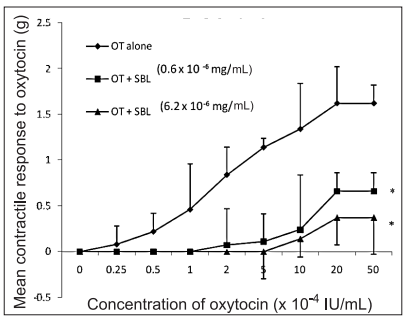
SBL and VER caused a significant decrease ($p < 0.01$) of both the 100% control frequency and amplitude of spontaneous uterine contractions and completely abolished contractions at maximum concentrations used, which were not regained by washing. SBL at 6.2×10^{-6} mg/mL produced a mean frequency and amplitude inhibition of 8.4 ± 1.4% and 5.7 ± 2.2 %, respectively, while VER at 2.7×10^{-6} mg/mL produced a mean frequency and amplitude inhibition of 5.5 ± 2.3% and 2.6 ± 2.9%, respectively.

Effect of SBL and VER on OT, ACh and EGM-induced contractions

SBL significantly increased the EC_{50} of OT, ACh and EGM ($p < 0.01$) (Table 1, 2 and 3, respectively) and significantly depressed the E_{max} ($p < 0.05$) of OT (Fig. 2), ACh (Fig. 5) and EGM (Fig. 8). Similarly, VER significantly increased the EC_{50}



1a



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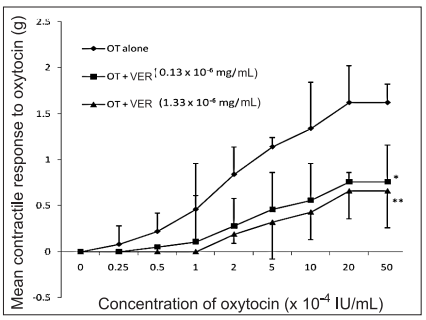


1c

Figure 1. Concentration-response curves showing the effects of AET (1a), SBL (1b) and VER (1c) on OT-induced uterine contractions. n = 5 rats

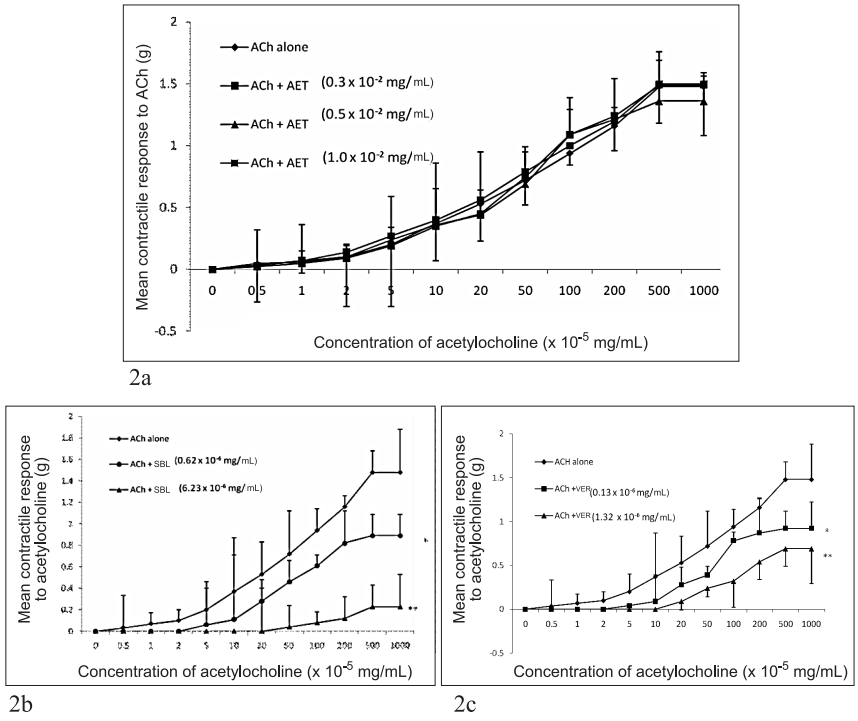


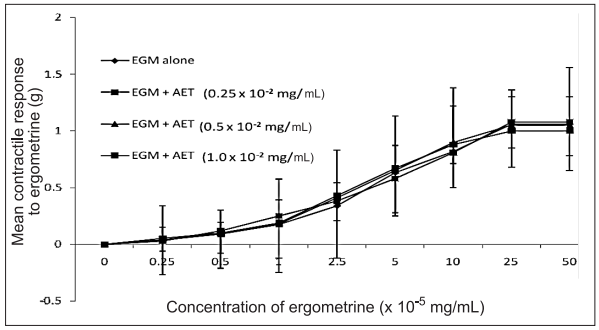
Figure 2. Concentration-response curves showing the effects of AET (2a), SBL (2b) and VER (2c) on ACh-induced uterine contractions. n = 5 rats

of OT, ACh and EGM ($p < 0.01$) (Table 1, 2 and 3, respectively) and significantly depressed the E_{max} ($p < 0.05$) of OT (Fig. 3), ACh (Fig. 6) and EGM (Fig. 9).

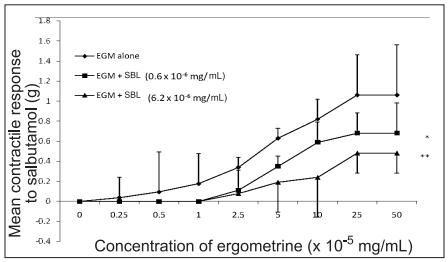
DISCUSSION AND CONCLUSION

It was noted that at the concentrations used in this study, AET did not significantly inhibit the frequency or amplitude of spontaneous uterine contractions. This effect was compared with the effects of salbutamol and verapamil on spontaneous contractions. Salbutamol is a ß-adrenoceptor agonist that binds to ß adrenergic receptors in the myometrium and activates the GTP binding protein G_s. The G_s protein activates the membrane bound enzyme adenylyl cyclase, resulting in increased intracellular cyclic adenosine monophosphate (cAMP). The result is a generalized relaxation of the myometrial smooth muscles (11). Verapamil, on the other hand, is a phenylalkylamine calcium channel antagonist. Increased concentrations of cytosolic calcium causes increased contraction of smooth muscle cells. The release of calcium from intracellular storage sites is more important in initiating uterine smooth muscle contraction than extracellular calcium. Verapamil inhibits voltage-dependent calcium channels in smooth muscles, thus causing relaxation (12). Salbutamol and verapamil, in this study, clearly relaxed the myometrial preparations by methods described above. This was observed in the significant reduction of the frequency and amplitude of spontaneous contractions.

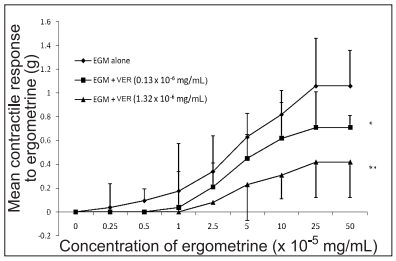
However, the observation that AET at a concentration of 1.0×10^{-2} mg/mL inhibited OT induced uterine contraction without inhibiting acetylcholine induced contractions (a muscarinic receptor agonist), ergometrine induced contractions (an ergot alkaloid) or even spontaneous contractions suggests that whilst SBL and VER can be referred to as uterine relaxants, AET may not be a uterine relaxant but rather a direct oxytocin inhibitor. Oxytocin is a polypeptide hormone which is endogenously produced. It directly and indirectly initiates myometrial contractions when it binds to its receptor, located in the myometrium (13). From the results, AET may



3a



3b



3c

Figure 3. Concentration-response curves showing the effects of AET (3a), SBL (3b) and VER (3c) on EGM-induced uterine contractions. n = 5 rats

possibly interact with myometrial oxytocinergic receptors. This hypothesis seems supported by the observation that AET at this concentration had no significant effect on basal spontaneous myometrial contractions. The shift of the concentration response curve of oxytocin to the right by AET, is suggestive of competitive antagonism, though the maximum concentration was restored. It is also possible that AET may be interacting or inhibiting a pathway in the signal transduction mechanism of oxytocin's response, which may not be common to the other agonists used in this study. However, these explanations remain to be absolutely verified by future studies.

There was also observed a marked significant difference ($p < 0.01$) between the EC_{50} of oxytocin in the presence of salbutamol and AET and between verapamil and AET. This suggests that the ability of AET to inhibit oxytocin induced uterine contraction is not as potent as those of salbutamol and verapamil. Ross and Kenakin (14), noted that depression of E_{max} of agonists by antagonists is an indication of non competitive antagonism and may possibly indicate physiologic antagonism. The depression of E_{max} by both verapamil and salbutamol were significant ($p < 0.05$) compared to AET. This may lend further credence to possible competitive inhibition by AET on oxytocin receptors.

It is pertinent to mention that in the pathogenesis of preterm labor it has been suggested that the early idiopathic activation of the normal labor process might be amongst the many causes of preterm labor (11) and it has been reported that the powerful contractions of the uterus during labor needed to expel the foetus are primarily stimulated by OT, amongst others (15). One of the pathways in the signal transduction mechanism of OT involves stimulation of the synthesis and action of prostaglandins which have been reported to play a significant role in dysmenorrhoea (16). It is suggested that an in-depth investigation into these possible mechanisms performed using fractions and isolates of the extract may give more insight into these mechanisms.

In conclusion, this study has shown that the aqueous leaf extract of *F. exasperata* inhibits oxytocin induced uterine contractions without significantly affecting acetylcholine- or ergometrine-

induced uterine contractions. This study also showed that the extract had no significant effect on the amplitude and frequency of spontaneous contractions. This effect of the aqueous leaf extract of *F. exasperata*, being a crude extract containing several phytochemicals, may account for its use by traditional healers in counteracting preterm labour and dysmenorrhoea. Further studies are underway to isolate and characterize specific constituents responsible for this observed activity.

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