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Pharmacological Basis for the Use of the Antivenene Water Soluble Extract of *Diodia scandens* as a Laxative, Oxytocic Agent and a Possible Aphrodisiac in Traditional Medicine Practice in Eastern Nigeria†

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The effects of the antivenene fraction of an ethanol extract of *Diodia scandens* on some mammalian smooth muscles were investigated. On the guinea-pig ileum, the extract was shown to be a partial agonist acting via muscarinic receptors. Acetylcholine (ACh) was 2.5×10^5 times more potent. On the pregnant guinea-pig uterus, the extract induced concentration-dependent increases in the force of contraction and tonus. Oxytocin and ergometrine were respectively 10^6 and 10^3 times more potent.

The extract, at subliminal concentrations, potentiated ACh and adrenaline-induced contractions in the guinea-pig was deferens. It also induced dose-related vasodilatation in the rat hindquarters and depressed the blood pressure in the anaesthetized cat. It was concluded that these pharmacological actions offer some scientific explanation for the use of *Diodia scandens* in traditional medicine as a laxative and as an oxytocic agent. It is suggested that the extract could enhance erection and ejaculatory processes in the male, thus accounting for its regular use by some elderly males.

Keywords: Diodia scandens; antivenene ethanol extract; laxative; oxytocic activity.

INTRODUCTION

Onuaguluchi and Okeke (1989) showed that the water soluble fraction of an ethanol extract of *Diodia scandens*, a herb used in parts of Eastern Nigeria for the treatment of snake bites, had definite anti-thromboplastin activity against brain thromboplastin and *Echis carinatus* thromboplastin activity. It was also shown (Onuaguluchi, 1989) that although the extract at 1.5 mg/kg i.p partially protected mice from the lethal effects of *Echis carinatus* venom, there was no deleterious effect on mice even at 2 g/kg i.p.

Apart from its use in the treatment of snake bite, the herb, especially in Ikwerre and Etche local government areas of Eastern Nigeria, is also used as a laxative and as an oxytocic agent in the treatment of uterine inertia and postpartum haemorrhage. Many elderly men in these areas apparently regularly take water extracts of the plant in the belief that it facilitates micturition and improves their sexual performance.

In view of these uses of *Diodia scandens* in traditional medicine practice, it was decided to investigate whether there was any pharmacological basis for these therapeutic uses. The effects of the antivenene fraction of the ethanol extract of the herb on some mammalian non-vascular and vascular smooth muscles were therefore examined.

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MATERIALS AND METHODS

Extraction methods. The procedures were as described previously (Onuaguluchi, 1964, 1989). In brief, the chopped fresh herb was extracted with boiling ethanol in a soxhlet apparatus. The green residue obtained was dissolved in 50% aqueous ethanol and the solution was passed through a column of activated charcoal. The colourless filtrate after crystallization of a non-water soluble solid (0.1% w/w) was evaporated to dryness under reduced pressure and yielded a dark brown residue (1.3% w/w). This fraction was water soluble and is the antivenene fraction and the fraction used in the present study.

Isolated guinea-pig ileum preparation. The preparation was set up in the conventional manner according to the method described by The Staff of The Department of Pharmacology, University of Edinburgh (1970). After opening the abdomen, the caecum was lifted forward and the ileo-caecal junction was identified. The ileum was then cut at this point and a thread was tied at its jejunal end to serve as a marker. A length of ileum was then cut off starting from above the Peyers patch and transferred to a dish containing Tyrode's solution. The mesentery was trimmed away and a length of ileum was cut into segments about 3 cm long. The segments were suspended in Tyrode's solution (NaCl, 0.8%; KCl, 0.02%; $CaCl_2$, 0.02%; $MgCl_2$ 0.01%; NaH_2PO_4, 0.005%; and dextrose 0.1%). The bath maintained at 37 °C+0.5 °C was aerated

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with a mixture of 95% oxygen and 5% carbon dioxide. Contractions were recorded on smoked kymograph paper using a frontal writing lever. The load on the tissue was 0.5 g. Responses to increasing doses of the extract and acetylcholine (ACh) acting for 15 sec were obtained in four preparations. The time cycle was 3 min. When it was established that the extract caused dose-related contractions, the relative potencies of ACh and the extract were determined by obtaining responses in five preparations to increasing doses of ACh (4×10^{-9}–3×10^{-5} g/mL) until the tissue contracted maximally. Similarly, the responses due to increasing doses of the extract (4×10^{-3}–1.25×10^{-2} g/mL) were then obtained on the same preparation. The heights of contractions were expressed as % of height of maximum contraction induced by ACh. The log dose-responses were plotted and the ED_{50} determined.

When it was found that the intrinsic activity of the extract was low, four preparations were investigated to see whether the extract was a partial agonist. The first two contractions were obtained from ACh (4×10^{-6} g/mL) acting for 15 s. Then a contraction was obtained from the extract (1.28×10^{2} g/mL) acting also for 15 s. The time cycle was 3 min and the tissue was washed twice in between additions of drugs.

When the next dose of the extract was repeated, it was allowed to act for 2 min before the dose of ACh was repeated and allowed to act for 15 s. The effect of pretreatment with the extract on the agonist action of ACh was thus determined.

Lastly, the mechanism of the agonist action of the extract was investigated. In five preparations, the effects of pretreatment for 2 min with constant doses of atropine 2×10^{-9} g/mL on contractions induced by equipotent doses of ACh (2.5×10^{-7} g/mL) and the extract; diphenhydramine 2×10^{-9} g/mL on contractions induced by equipotent doses of histamine (1.28×10^{-7} g/mL) and the extract; hexamethonium 2×10^{-9} g/mL on contractions induced by equipotent doses of nicotine (4×10^{-6} g/mL) and the extract were compared.

The pregnant guinea-pig uterus preparation. Uterine horns from five pregnant guinea-pigs weighing between 486 and 495 g were suspended according to standard procedures in a 50 mL organ bath containing De Jalon's solution with the following composition: NaCl, 0.9%; KCl, 0.042%; $NaHCO_3$, 0.015%; $CaCl_2$, 0.03%; glucose, 0.05% aerated with a gas mixture of 95% oxygen and 5% carbon dioxide, and maintained at $31° \pm 1°C$. Contractions were recorded on smoked kymograph paper using a frontal writing lever. The load on the tissue was 1 g. The effects of increasing doses of the extract were first examined.

Next, the relative oxytocic potencies of the extract, oxytocin and ergometrine were then determined by obtaining responses to increasing doses of oxytocin until maximal contraction was obtained. Then on the same tissue, responses were obtained from increasing doses of the extract and ergometrine. The tissue–drug contact time was 3 min. The height of contraction expressed as % of height of maximum contraction induced by oxytocin was plotted against log-dose of the drugs. The ED_{50} of each agonist was then determined and the relative oxytocic potencies calculated.

Isolated guinea-pig vas deferens preparation. After opening the abdomen, the gut was lifted aside and the testes were pushed into the abdominal cavity by applying pressure on the scrotum. The vas deferens was identified and cut just above the epididymis and also at the point where it joins the urethra. A thread was then tied at each end and the preparation was set up according to standard procedures in a 50 mL organ bath containing Tyrode's solution maintained at $37° \pm 0.5°C$ and aerated with oxygen. The contractions were recorded with a frontal writing lever on smoked kymograph paper. The load on the tissue was 0.5 g. Responses to increasing doses of the extract were obtained. When it was found that up to 1×10^{-2} g/mL did not excite the tissue, the effect of pretreatment with a subliminal dose of the extract for 10 min on contractions induced by equipotent doses of ACh 1×10^{-5} g/mL and adrenaline 2×10^{-5} g/mL were then examined in four preparations.

The rat hindquarters preparation. Albino Wistar rats of either sex weighing between 200 and 250 g were used. The preparation was set up according to standard procedures. The abdominal aorta was connected to a reservoir containing Ringer-Locke solution (NaCl, 0.9%; KCl, 0.042%; $CaCl_2$, 0.024%; $NaHCO_3$, 0.05%; dextrose, 0.1%). The solution was aerated with a gas mixture of 95% oxygen and 5% carbon dioxide at $37° \pm 0.5°C$. The perfusion pressure was kept constant at 55 cm H_2O. The outflow of the perfusate was collected in a cylinder and measured every 5 min. A period of 40 min was allowed for equilibration by which time the flow had been constant over three consecutive readings. Then the effects of bolus injections into the cannula of 100 or 200 μg of the extract dissolved in 0.1 mL of the perfusion fluid were studied in four preparations for each dose. Similarly, in four preparations the effects of bolus injections of adrenaline 0.5 and 1 μg (0.1 mL) were also studied. Control experiments in which 0.1 mL of the perfusate was injected into the cannula were performed using four preparations and observations made for 30 min.

The blood pressure and respiration of the anaesthetized cat. Five cats weighing between 1.5 and 3 kg were anaesthetized with sodium thiopentone (50 mg/kg i.p.) and tied in a supine position in a Brown-Schuster myographic table. The trachea was cannulated with a metallic tracheal tube and connected to a tambour writing on smoked kymograph paper. One common carotid artery was cannulated in the conventional manner and connected to a mercury manometer writing on smoked kymograph paper. A femoral vein was exposed and cannulated for injection. The cannula was filled with heparinized normal saline and connected to a syringe using a three-way stop cock which allowed for the injections. The effects of various doses of the extract and of adrenaline were examined.

RESULTS

Isolated guinea-pig ileum preparation

The extract induced dose-related responses but the potency was low. No recordable contraction was induced when the concentration of the extract was below

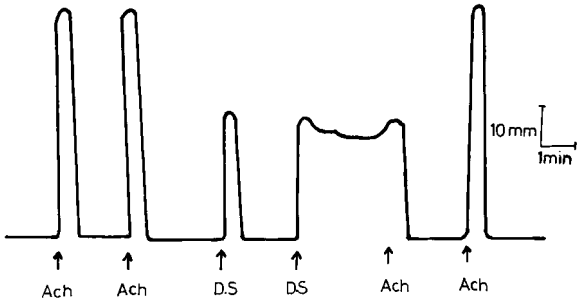

Figure 1. Guinea-pig ileum preparation showing partial agonist activity of *Diodia scandens* (DS) at high concentrations. ACh acetylcholine, 4×10^{6} g/mL; DS *Diodia scandens*, 1.28×10^{-2} g/mL.

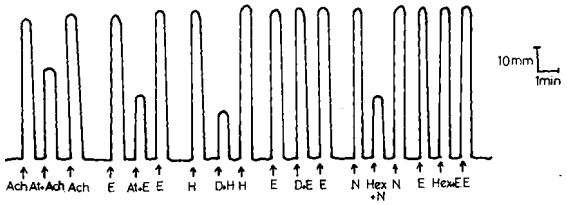

Figure 2. Guinea-pig ileum preparation. The effects of atropine (At) 2×10^{-9} g/mL on equipotent doses of acetylcholine (ACh) and the extract (E); diphenhydramine (D) 2×10^{-9} g/mL on equipotent doses of histamine (H) and the extract; hexamethonium (Hex) 2×10^{-9} g/mL on equipotent doses of nicotine (N) and the extract.

6×10^{-4} g/mL while ACh even at 6×10^{-9} g/mL induced appreciable contractions in all the preparations tested. It was found also that the maximum contraction achieved with the extract, usually at between 1–3 $\times 10^{-2}$ g/mL was 55%–70% of that achieved with ACh (often at between 4×10^{-6} and 1.5×10^{-5} g/mL). From the log dose-response curves, the ED_{50} for ACh and the extract were respectively 2.82×10^{-7} and 1.05×10^{-2} g/mL. Therefore ACh was about 2.6×10^{-5} times more potent. Figure 1 shows that pretreatment with the extract at 1.25×10^{-2} g/mL, antagonized ACh-induced contractions. Figure 2 shows that while atropine blocked to nearly the same degree the contractions induced by equipotent doses of ACh and the extract, diphenhydramine at a dose which substantially blocked histamine-induced contractions had no effect on contractions induced by an equipotent dose of the extract. Also, hexamethonium at doses which blocked nicotine-induced contractions had no effect on contractions induced by equipotent doses of the extract.

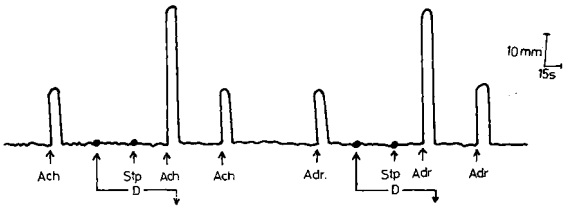

Figure 4. Guinea-pig vas deferens preparation. The effects of pretreatment for 10 min with *Diodia scandens* (D) 5×10^{-3} g/mL on the contractions induced by acetylcholine (ACh) 1×10^{-4} g/mL and adrenaline (Adr) 2×10^{-4} g/mL. Stp drum stopped for 9.5 min.

Isolated pregnant guinea-pig uterus preparation

The extract induced dose-related increases in the force of contraction. At lower concentrations (4×10^{-3} g/mL), relaxation was nearly complete in between contractions but as the concentration increased so did the tonus of the muscle increase and at 1.6×10^{-2} g/mL there was a marked increase in the force of contraction and tonus of the muscle. Figure 3 shows the effects of increasing doses of the extract, ergometrine and oxytocin on the force of contraction and tonus. From the log-dose response curves, the ED_{50s} of oxytocin, ergometrine and the extract were 2×10^{-8}, 5×10^{-5} and 2×10^{-2} g/mL respectively. Therefore, oxytocin and ergometrine in use at our hospital were respectively 1×10^{6} and 2.5×10^{3} more potent than the extract.

Isolated guinea-pig vas deferens preparation

Even at 1×10^{-2} g/mL, the extract did not induce any recordable contraction. However, pretreatment with the extract at 5×10^{-3} g/mL for 10 min, very markedly potentiated ACh and adrenaline-induced contractions (Fig. 4).

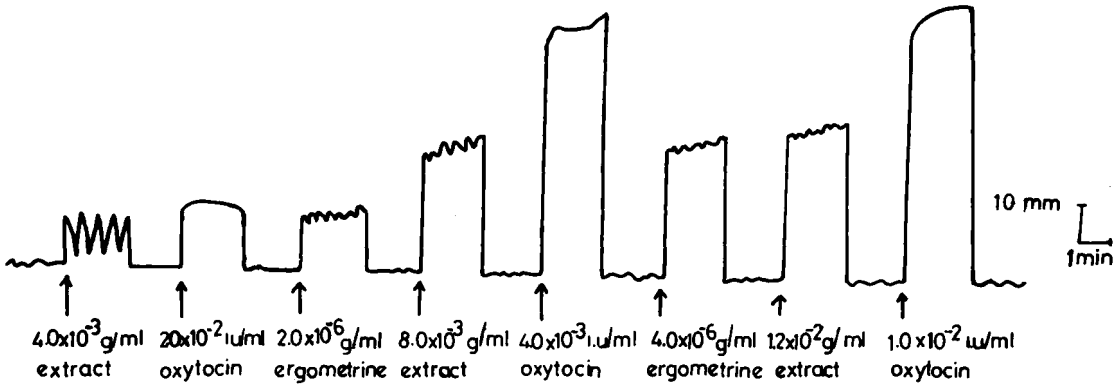

Figure 3. Pregnant guinea-pig uterus preparation. The dose-responses of the uterus to various concentrations of the extract, oxytocin and ergometrine.

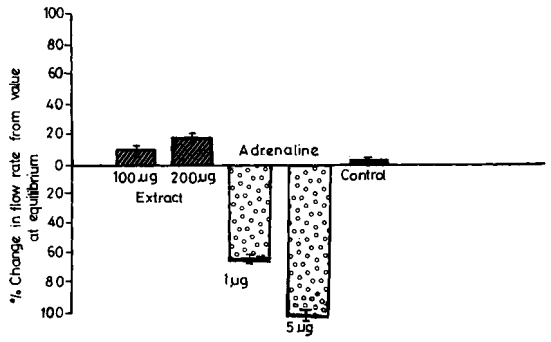

Figure 5. Rat hindquarters preparation. Comparative effects of bolus doses of extract and adrenaline.

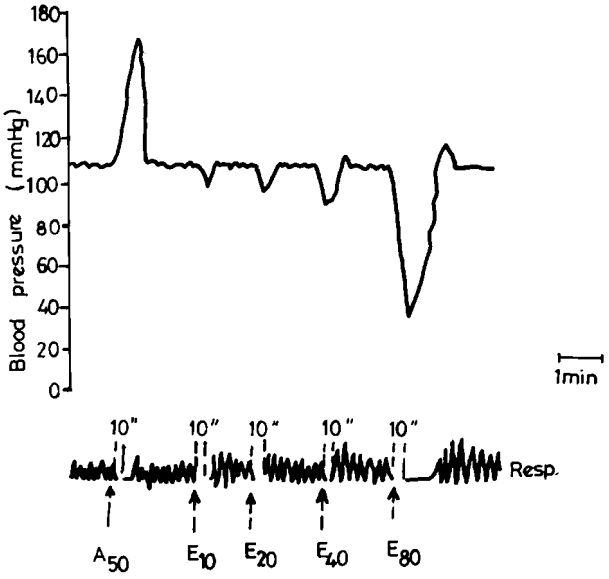

Figure 6. Effects of adrenaline (A) and the extract (E) on blood pressure of the anaesthetized cat. Subscript indicates dose of drug in mg/kg body weight.

Rat hindquarters preparation

Bolus injections of 100 and 200 µg of the extract caused increases in the flow rate. At peak activity (which occurred 20–30 min after injection), the percent increases in flow rate from values at equilibration were 9.62% ± 1.80% for 100 µg; 18.75% ± 1.27% for 200 µg and 0.39% ± 0.49% for controls. In contrast, 1 and 5 µg of adrenaline caused a decrease in flow rate of 65.18% ± 225% and 99.85% ± 0.9% respectively from values at equilibration (Fig. 5).

The blood pressure and respiration of the anaesthetized cat

The extract (10–80 mg/kg) caused dose-related falls in blood pressure. However, the pressure returned to the original level within 2 min. The respiratory volume was also increased at 10–40 mg/kg but a brief period of apnoea occurred at 80 mg/kg. Figure 6 shows the effects of various doses of the extract on the blood pressure and respiration.

DISCUSSION

The results from experiments on the guinea-pig ileum show that the extract stimulates the gut but only at high concentrations. ACh was 10^5 times more potent. Moreover, the maximum contraction induced by the extract was only 55%–70% of the maximum contraction induced by ACh on the same preparation. Figure 1 shows that the extract at the concentration that would produce about the maximum contraction inducably by the extract, antagonized ACh. The extract, therefore, may be a partial agonist. Figure 2 shows quite clearly that the agonist activity on the ileum is on the muscarinic receptors and not on H_1 receptors or on nicotinic receptors.

The yield of the antivenene fraction from the fresh herb was 1.3% although a yield of 3.5% had been obtained in a previous study (Onuaguluchi and Okeke, 1989). Therefore, 100 g of fresh herb is expected to yield between 1.3–3.4 g of the extract. In traditional medicine practice, about 50–100 mL of the boiled herb is administered on an empty stomach if a laxative or mild purgative action is required. The results of this study indicate that the concentration of the extract in the gut to stimulate the gut should be of the order of 10^{-3} g/mL (1 mg/mL). Therefore on the assumption that the gut fluid in which it is dissolved is between 200–500 mL, 200–500 mg of extract would be required.

That quantity of extract can be obtained from about 16–40 g of fresh herb if the yield is only 1.3% or 6–15 g of fresh herb if the yield is up to 3.4%. Even if 10 times these amounts of extract are required, that amount of extract should not produce any deleterious effect, as extract at 2 g/kg i.p. was shown not have any deleterious effect in acute studies in mice (Onuaguluchi, 1989).

The extract also has oxytocic activity on isolated pregnant guinea-pig uterus. Oxytocin and ergometrine were respectively, 10^6 and 10^3 times more potent. The dose of ergometrine for the treatment of postpartum haemorrhage is 0.5 mg administered orally or intramuscularly or intravenously (Clayton and Newton, 1983). This is equivalent to 0.5 g of the extract or 14.7–38.4 g of the fresh herb. Ergometrine because of its vasoconstrictive action is, however, contraindicated in cardiac or hypertenaive patients (Novy, 1982; Barbieri and Ferko, 1994).

In contrast, the extract induces vasodilatation and reduces blood pressure. Therefore, although the antivenene fraction of the ethanol extract of *Diodia scandens* is less potent than ergometrine (w/w), it should be a much safer agent than ergot preparations, in the treatment of postpartum haemorrhage. It would appear also, that unlike ergometrine, the extract at lower doses could be used to stimulate the uterus in delayed labour from uterine inertia.

The extract potentiates the action of ACh and adrenaline on the guinea-pig vas deferens. Although the effect of the extract on bladder smooth muscle was not investigated, it would seem reasonable to assume that the extract could enhance the activity of ACh on the detrusor muscle causing increased contractile response and in addition, relaxing the internal sphincter of the urinary bladder. What is probably more noteworthy, is that the potentiation of ACh action on the erectile tissue of the external genitalia would facilitate arterial vasodilatation and thus erection of the penis, while ejaculation

which is mediated through stimulation of alpha adrenoceptors in the smooth muscles of the seminal vesicles and vas deferens should be enhanced by the extract. It is conceivable that the elderly men in Etche and Ikwerre local government areas of Eastern Nigeria who regularly take *Diodia scandens* do indeed improve their sexual potency.

In conclusion, it would appear therefore, that there is some pharmacological basis for the use of *Diodia scandens* in traditional medicine practice as a laxative and an oxytocic agent to treat uterine inertia and postpartum haemorrhage. It is suggested that the antivenene fraction of the ethanol extract of the herb could aid micturition and most probably improve sexual performance in elderly males.

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