Evaluation of the oxytocic activity of the ethanol extract of the roots of *Alchornea cordifolia*

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Alchornea cordifolia has been used traditionally for the induction of labour as an abortifacient. This study is aimed at verifying the folkloric use of the plant by investigating the effect of ethanolic extract of the root bark on the isolated stilboestrol pretreated uteri of non-pregnant female rats. The extract (1, 10, 50 g/l), oxytocin (4×10^{-5} to 8×10^{-3} g/l), acetylcholine (4×10^{-6} to 8×10^{-4} g/l), atropine (4×10^{-3} g/l), phenoxybenzamine (4×10^{-3} g/l), diphenhydramine(2×10^{-1} g/l), and verapamil (12×10^{-2} g/l) were used. Log concentration response curves were plotted and EC_{50} and E_{max} were obtained. One-way analysis of variance (ANOVA) with Dunnet corrections using Graph pad Instat version 2.05a was used for statistical analysis. The extract produced dose-dependent contraction of the uterus. Its potency was less than that of oxytocin and acetylcholine ($P<0.05$), but the E_{max} showed no significant difference ($P>0.05$). The E_{max} values of the extract in the presence of all antagonists were significantly reduced ($P<0.01$). The EC_{50} in the presence of atropine showed no significant increase ($P>0.05$); however, in the presence of phenoxybenzamine, the increase was significant ($P<0.05$). The presence of diphenhydramine and verapamil produced an inhibition such that the EC_{50} was unattainable. *A. cordifolia* stimulates the uterus possibly by binding to alpha-adrenergic or histaminergic receptors or both. This indicates the existence of active principles in the plant, which may be responsible for some of the applications in traditional medicines as an abortifacient and in the induction of labour.

Key words: Folkloric medicine, root bark, smooth muscles, uterine contraction

INTRODUCTION

Medicinal and poisonous plants have always played an important role in African societies. However, among the more than 250,000 species of higher plants, only about 10% have been pharmacologically investigated.[1] The folklore knowledge of medicinal plants has significantly contributed in discovering many important drugs used in orthodox medicine. Most developing countries are endowed with vast resources of medicinal plants. In fact, modern pharmaceuticals still contain at least 25% drugs derived from plants.[2]

Alchornea cordifolia (Schum. and Thonn.) Muell. Arg. (Family: Euphorbiaceae) is widely distributed in tropical Africa, occurring in Senegal, Nigeria, Cameroun, amongst others.

It is an erect or scrambling, multi-stemmed shrub, woody climber or small tree growing up to 10 m in height, and is of 10 cm in diameter. Leaves are alternate, simple and with stipules absent.

Extracts from leaves of *A. cordifolia* have been found to inhibit the growth of bacteria such as *Staphylococcus aureus, Staphylococcus albus* and *Escherichia coli*.[3] The leaves and root bark extract have been shown to protect wistar albino rats against acetaminophen-induced liver damage and induce labour.[2] The uterus is a muscular organ whose function is to provide a nidus for the developing embryo. It is seen that the organ is periform. It is broader above and narrows down below. The uterus is about 7.5 cm in length. Its maximum width (near its upper head) is about 5 cm.[4] The uterus receives sympathetic and parasympathetic innervations. Alpha-adrenergic receptors mediate stimulation and beta-adrenergic receptors mediate relaxation. The parasympathetic effect is usually stimulatory and depends on the stage of menstrual cycle, amount of circulating oestrogen and progesterone, and other factors.

Drugs that cause contraction of the uterus include acetylcholine, oxytocin, ergometrine, and prostaglandins E and F2α.[5]

On the basis of its claimed folkloric use in the induction of labour, the present research is aimed at determining the effects of the ethanolic extract of *A. cordifolia* root on the uterine smooth muscle and to ascertain possible mechanism(s) of action.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats weighing 160±20 g (Grade II) were obtained from the animal house of the Department of Physiology, University of Ibadan, Nigeria.

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These rats were about 8 months old. They were housed in a single large cage in an environmentally controlled room provided with a 12:12 hour light:dark cycle for each 24 hour period at a temperature of approximately 26±1°C in the animal house of the Department of Pharmacology, University of Benin, Nigeria, for at least 7 days prior to the experiment. The rats were given free access to food and were fed on mice cubes obtained from Ewu feeds, Ewu, Edo State, Nigeria, and water.

All the experiments were carried out in a quiet laboratory setting with ambient illumination and temperature close to that of the animal house. All the experiments conformed to acceptable protocols for use of animals in the experiment and approval was given by the ethics committee on the use of animals, Faculty of Pharmacy, University of Benin, Nigeria. Animals were handled according to the standard protocols for the use of laboratory animals (National Institute of Health, USA: Public Health Service Policy on Humane care and use of Laboratory animals, 2002).

Drugs and Chemicals

Acetylcholine and oxytocin (Laborate Pharmaceuticals, Panipat, India), diphenhydramine (BOM Chemical limited, Poole, England), verapamil hydrochloride (Abbott Laboratories limited, Poole, England) and atropine (Martindale, Penrith England) were used. Diethylstilboesterol (Sigma Pharmaceuticals, Monticello, Utah) was constituted in 1:1 ethanol/water solution.

All stock solutions of these drugs and subsequent dilutions were made in distilled water just prior to use for the *in vitro* experiment.

Preparation of Plant Extract

Fresh roots of *A. cordifolia* were collected in Benin City in the month of June 2008 and authenticated by Dr. B.A. Ayinde of the Department of Pharmacognosy, University of Benin. Botanical identity of the plant was confirmed at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where a voucher specimen (No. FHT 108437) was deposited for future reference. The roots were chopped into little chips, air dried for 1 week and later oven dried for 3 days at a temperature of 50°C using a thermostat oven Gallenkamp, Australia. The dried roots were reduced to powder using an electric milling machine.

Extraction of Plant Material

About 445 g of the powdered plant was macerated in 2 l of absolute ethanol for 72 hours, a modification of the method of Olaleye *et al*. The ethanolic extract was separated from the mixture using Whatman No. 3 filter paper and the extract concentrated over a water bath. Thus, 33.46 g of the dried plant extract was obtained giving a percentage yield of 7.5% w/w. Everyday, during the experiment, fresh stock solution of this dried extract and subsequent dilutions were made in distilled water.

***In vitro* Assay for Uterotonic Activity**

Female non-pregnant rats were pretreated intraperitoneally with 0.2 mg/kg of diethylstilboesterol, 24 hours prior to the actual experiment. The rats were sacrificed under anaesthesia using chloroform and the lower abdomen was dissected out. The uterus was identified; the two horns were cut out and transferred to a Petri dish containing aerated De Jalon's solution having the following chemical composition: NaCl 154.1 mM, $NaHCO_3$ 5.95 mM, D-glucose 2.75 mM, KCl 5.36 mM and $CaCl_2.2H_2O$ 0.055 mM.[6] The horns were separated and freed from fat and the adhering blood vessels. Uterine segment measuring about 2 cm in length was cut out and threaded using surgical silk.[6] A loop was formed at one end of the tissue and attached to the tissue holder. The other end was connected to an isometric transducer connected to a unirecorder model 7050 (Ugo Basile, Comerio VA Italy). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection with a corresponding weight of 0.7 g. The tissue was mounted in a 50-ml organ bath containing continuously aerated De jalon's solution.[7]

The temperature of the organ bath was maintained at 37°C by means of an external jacket through which warm water was circulated, while the tension on the tissue was maintained at 7 N.[8] The tissue was allowed to equilibrate for 45 minutes.[6]

A pilot study was done to ascertain the effect of the ethanolic extract of *A. cordifolia* root on the uterus, and based on this study, doses of extract to be administered were selected (1, 10, and 50 mg/l). The effects of acetylcholine (4×10^{-6} to 8×10^{-4} g/l) and oxytocin (4×10^{-5} to 8×10^{-3} g/l) were also investigated using these selected doses. A time cycle of 3 minutes was allowed: 1 minute of contact time and 2 minutes of relaxation time. The effects of antagonists on the extract were investigated using atropine (4×10^{-3} g/l), phenoxybenzamine (4×10^{-3} g/l), and diphenhydramine (2×10^{-1} g/l). The effect of verapamil (12×10^{-2} g/l) on the extract was also investigated. These antagonists were administered separately; each one was allowed an equilibrium time of 10 minutes before subsequent administration of the extract. The effects of the individual antagonists in the presence of the extract and those of the extract alone were recorded. All doses were final bath concentrations.

Data Presentation and Statistical Analysis

Data were presented as mean±SEM (standard error of the mean). Dose response curves were plotted for the extract alone, acetylcholine, oxytocin, extract + diphenhydramine, extract + atropine, extract + phenoxybenzamine, and extract + verapamil. The concentration required to produce 50% of maximum (EC_{50}) and the maximum attainable (E_{max})

contraction were determined and compared using one-way analysis of variance (ANOVA) with Dunnet corrections using Graph pad Instat version 2.05a.

RESULTS

The crude extract of *A. cordifolia* had a stimilatory effect on the isolated uteri of stilboesterol pretreated rats. The uterine contractions elicited by the extract were dose dependent [Figures 1 and 2]. These responses when compared to those of oxytocin and acetylcholine showed significant difference in the EC_{50} (P<0.05), while there was no significant difference in the E_{max}. The contractions produced by the extract were less potent than those produced by oxytocin and acetylcholine [Figure 1]. It was observed that the log concentration-response curve of the extract was shifted more to the right. This means that a higher concentration of the extract will be required to obtain the response produced by a lower dose of oxytocin or acetylcholine.

The low potency effect may be due to the fact that the extract is not pure compared to the pure oxytocin and acetylcholine used. It is also possible that the extract does not stimulate uterine receptors as much as oxytocin and acetylcholine do.

All the antagonists used, i.e. atropine, phenoxybenzamine, diphenhydramine and verapamil showed significant inhibition (P<0.01) of the maximum attainable response by the extract [Figure 3].

The concentrations of the extract needed to elicit 50% of maximum response (EC_{50}) in the presence of atropine and phenoxybenzamine were significantly different from that required to elicit 50% of the maximum response in their absence (P<0.05) [Figure 3]. The inhibition effects produced by diphenhydramine and verapamil on the maximum response of the extract were so significant that it was not possible to attain the EC_{50} for comparison [Table 1 and Figure 3].

DISCUSSION

Oxytocin binds to oxytocin receptors on the uterus and causes release of inositol 1, 4, 5 triphosphate from the hydrolysis of phosphoinositide and release of prostaglandins, which further increases contraction.[9]

Table 1: The effect of atropine, phenoxybenxamine, diphenhydramine and verapamil on EC_{50} and E_{max} of the extract

Treatment	EC_{50} (mg/ml)	E_{max} (% response)
EE (0.004-0.4 mg/ml)	0.026±0.01	86.56±5.61
ATROP+EE (0.04 mg/ml)	0.049±0.02	56.56±6.06**
PHEN+EE	0.21±0.05**	56.40±7.21**
DIPHEN+EE	Not attainable	12.875±2.03**
VERA+EE	Not attainable	42.13±0.92**

**P<0.01 significantly different from the extract alone. atropine (atrop), phenoxybenzamine (phen), diphenhydramine(diphen), and verapamil (vera)

Acetylcholine binds to and activates muscarinic receptors on the uterus by initiating a second messenger mechanism that involves the activation of inositol 1,4,5 triphosphate and diacylglycerol.

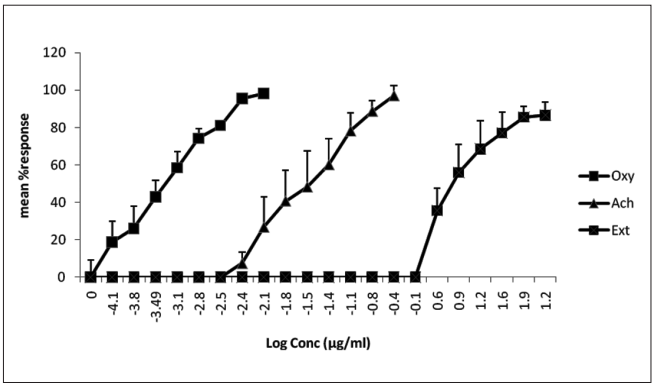

Figure 1: Log concentration response curves of extract, oxytocin and acetylcholine on the isolated diethylstilboesterol pretreared uteri of rats. The uterine contractions elicited by the extract were dose dependent, Ext: Aqueous extract of *Alchornea cordifolia*, Ach: Acetylcholine, Oxy: Oxytocin

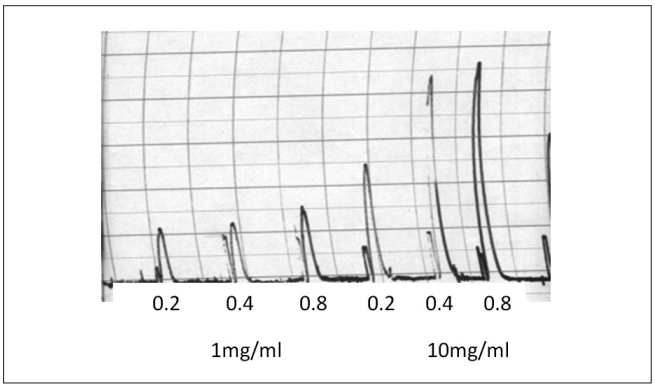

Figure 2: The effect of the crude extract of *A.cordifolia* on the isolated diethylstilboesterol pretreated uteri of rats. 1 mg/ml and 10 mg/ml of the extract of *Alchornea cordifolia* induce uterine contraction

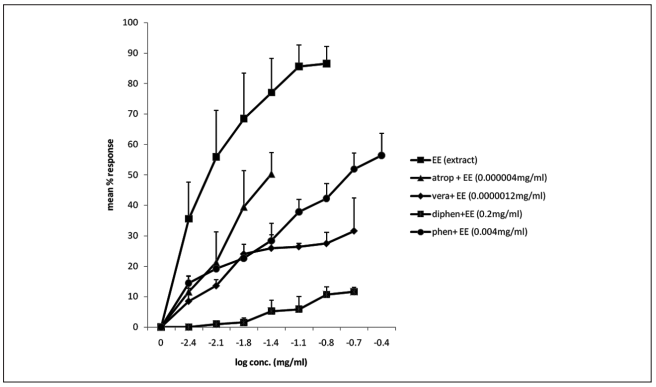

Figure 3: Log concentration response curve of the effect of the crude extract of *A. cordifolia* on the isolated stilboesterol pretreated uteri of rats in the presence and absence of antagonists. EE: Aqueous extract of *Alchornea cordifolia*, Atro: atropine, Vera: verapamil, Diphen: diphenhydramine, Phen: phenoxybenzamine, All antagonists employed atropine(atrop), phenoxybenzamine (phen),diphenhydramine(diphen), and verapamil (vera) showed significant inhibition(P<0.01) of the maximum attainable response by the extract

Atropine is a competitive antagonist at the muscarinic receptors. It binds to and prevents the action of muscarinic agonist at the receptor site. The fact that atropine is a competitive blocker means that its effect will depend on the ratio of antagonist to agonist present. If the amount of the agonist is higher, it will displace atropine and elicit its response again. From the result obtained, it can be seen that there was a significant difference in the E_{max} of the response produced by the extract in the presence of atropine (P<0.01). Higher doses of the extract (agonist) were unable to reverse this inhibition. This is indicative of the fact that the extract does not act through the muscarinic receptor. The reduction in E_{max} of the extract may be due to physiological antagonism by atropine. That is, atropine could have blocked the muscarinic receptors, thus preventing possible intrinsic contraction by endogenous acetylcholine.

The inhibitory effect of diphenhydramine on the extract-induced uterine contraction was significant both at the EC_{50} and E_{max}, suggestive of the fact that the uterine muscle contraction of the extract is probably by stimulation of the histaminergic receptor or the activation of the muscarinic and histaminergic receptors since diphenhydramine is a non-selective blocker of both the receptors.[10]

Phenoxybenzamine is an irreversible alpha-adrenergic receptor blocking drug. It has effect also on the serotonine and muscarine receptors. However, its pharmacological action is mainly due to blockade of alpha-adrenergic receptor.[9] The significant inhibition of the E_{max} and the increase in the EC_{50} suggests that the extract's contractile effect on the uterine smooth muscle may also be due to activation of the alpha receptor on the uterus.

Verapamil is a calcium channel blocking drug that acts by blocking the L-type calcium channels. In those phasic smooth muscles, such as the uterus, where action potentials occur, the resulting depolarisation and consequent opening of L-type Ca^{2+} channels make this the major source of Ca^{2+} for contraction.[11] Each phasic contraction is accompanied by a Ca^{2+} influx in the uterus, and both the influx and contractions are stopped if L-type channels are blocked.[12] Administration of the extract in the presence of verapamil produced contractions, though with significant reduction in the E_{max} and increase in EC_{50}. The observed contraction is suggestive of the fact that the extract does not cause calcium entry through the L-type calcium channels alone. There is some evidence that T-type Ca^{2+} channels may contribute to Ca^{2+} entry in the myometrium.[13]

The crude extract of *A. cordifolia* has stimulatory effect on the uterus and the response is dose dependent. This accounts for the folkloric use of the plant to induce labour.

The scope of this research showed that the plant extract exihibits its effect via alpha-adrenergic receptor or the histaminergic receptor (H_1).

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