EFFECT OF METHANOL EXTRACT OF *NEWBOULDIA LAEVIS* STEM BARK ON THE UTERUS OF NON-PREGNANT RATS

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ABSTRACT

The plant *Newbouldia laevis* NEEM (P. Beauv), Bignoniaceae family is locally used in Nigeria for contracting the uterus to facilitate delivery. The stem bark was extracted using methanol via maceration and its effect on the uterus investigated. The effects of both the crude extract (0.8mg/ml) and the aqueous fraction on the uterus were examined against oxytocin (0.04-1.6x10-2) and acetylcholine (0.04-16 mg/ml) induced contractions. A significant reduction was noted in the force of contraction in the presence of the extract such that 0.51+ 0.12g produced by 0.04x10-2 oxytocin was significantly ($p<0.0001$) reduced to 0.059+0.013g in the presence of the extract. The aqueous fraction was observed to produce similar effects though not as significant as the crude extract. The aqueous fraction of the extract remarkably inhibited ($p<0.05$) acetylcholine induced contractions at all concentrations tested. The effect of both the crude extract and its aqueous fraction were comparable with those of salbutamol and atropine on oxytocin and acetylcholine induced contractions respectively. The phytochemical screening revealed the presence of alkaloids, saponins, tannins and flavonoids. In conclusion as opposed to the oxytocic effect claimed in ethno medicine, a tocolytic effect was observed. This effect may be mediated either via blockade of oxytocic receptors or muscarinic receptor blockade. Saponins and tannins may also contribute to its relaxant effect on a non-pregnant uterus.

Keywords: Oxytocin, Uterus, Herbal medicine, Tocolytic, Antagonism

INTRODUCTION

The use of plants is an ancient practice common to all societies and cultures. This practice continues to exist in both developing and developed nations. It is on this basis that researchers keep working on medicinal plants in order to develop the best medicines for physiological uses (Usman and Osuji, 2007).

Herbal formulations are prepared to combat various diseases and ailments varying from those caused by micro organisms to those brought by physiological activities in the body (Ayensu, 1978).

The plant *Newbouldia laevis* (P. Beauv) also known as boundary tree is used for therapeutic purpose against a number of diseases. It is locally called *Aduruku* in Hausa, *Ogirisi* in Ibo, *Ikhimi* amongst the binis and *Akoko* in Yoruba languages. In Ivory coast and Nigeria, the stem bark is used for the treatment of epilepsy and convulsion in children (Burkill, 1985). The bark is also used for the treatment of rheumatism especially painful arthritis of the knees in Senegal. In Nigeria the bark is chewed and swallowed for stomach pains, diarrhoea and toothache (Lewis and Manony, 1977). The stem bark has also found use in the treatment of ear ache, sore feet, chest pain (Akunyili, 2006), breast tumors and also to facilitate delivery during labour in Nigeria and Ghana (Burkill, 1985).

Based on its use during labour, the tar-

get organ is the uterus, which is a hollow thick muscular organ, situated in the mid line between the bladder and in front of the rectum. The uterus consists of a body and cervix. Uterine muscles have a high degree of spontaneous contraction and electrical activity. The contractions are myogenic in origin (Garfield and Yallanpalli, 1994). Earlier reports on the stem bark revealed the absence of flavanoids, saponins, quinine, terpenes or steroids (Oliver -Bever, 1986), however later phytochemical screening revealed the presence of alkaloids quinines and phenylpropanoid amongst others (Gafner , 1997).

This study was designed to ascertain firstly, the phytochemical constituents present in the stem bark and secondly the effects of the plant on the uterus and its probable mechanism of action.

MATERIALS AND METHODS

Plant material

The stem bark of *Newbouldia laevis* were collected in November, 2010 behind Federal Staff School from Ugbowo village Benin City, Edo State. The plant was identified by Mr Sunny Nweke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.

Extract preparation:

The stem bark of *Newbouldia laevis* was chopped into pieces, air-dried for four days and then pulverized into fine powder. About 250 g of the powdered stem bark was extracted with 2 L of methanol using maceration method for 72 hrs. The methanol extract was concentrated in a rotary evaporator after it was filtered and this gave a yield of 2.51 %. The extract obtained was kept in an air-tight container in the refrigerator till required. This gave the methanol extract.

Partitioning

About 20 g of the methanol extract was dissolved in aqueous methanol (1:1) and partitioned in a separating funnel with successive aliquots of chloroform 50ml (x4). The chloroform layer (lower phase) was collected after complete extraction, allowed to evaporate to dryness under room temperature and weighed. The aqueous phase (upper layer) was also collected, concentrated, weighed and stored in a refrigerator for further study.

Drugs and Chemicals:

Methanol (Sigma-Aldrich, UK), Chloroform (Sigma-Aldrich UK) and Diethylstilbesterol and De Jalon solution were used. All other chemicals and drugs used were of analytical grade.

Experimental animals:

Female adult albino rats weighing between 160-175 g were used for the study. The animals were obtained from Ibadan, Oyo State, Nigeria and maintained in the animal house of Department of Pharmacology and Toxicology of the University of Benin, Benin City. The animals were allowed an acclimatization period of one month before being subjected to the experimental protocols. They were kept in standard cages in a well ventilated room, and fed with standard growers mash (Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria) and allowed water *ad libitum*.

Ethical approval for the study was obtained from the Ethical Committee on the Use of Animals for Experiments, Faculty of Pharmacy, University of Benin. Animals were handled according to the standard protocols for the use of laboratory animals (National Institute of Health, USA, 2002).

Phytochemical Screening

Freshly prepared stem bark extracts of *Newbouldia laevis* were subjected to preliminary phytochemical screening for various constituents. The methods of analysis employed were as described by Evans (2002).

Experimental protocol

The animals were pre-treated with diethylstilbesterol (0.2 mg/kg i.p) 24 h prior to the experiment. The rats were sacrificed under chloroform anaesthesia. After opening of the abdomen, the uterine horns were rapidly dissected and placed in aerated physiological salt solution. Uterine segments 2cm long of 3 – 5 mm thickness were cut from the cervical section of the uterine horn and freed of adhering connective tissues. Longitudinal segments were mounted vertically in 50 ml organ baths

containing physiological salt solution (PSS, De Jalons) of the following 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.

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 ºC and continuously aerated with 95 % O_2 and 5 % CO_2. 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). Isometric tension studies were performed in a method similar to those of Oropeza *et al. (*2002).

Administration of drugs and extract

A concentration response curve for oxytocin was first obtained via administration of 0.2, 0.4 and 0.8 ml of oxytocin (0.1 -1 i.u). The same was done for acetylcholine (10 – 1000 µg/ml) 0.2, 0.4 and 0.8 ml, maintaining same dose cycle.

The concentration response curves for both oxytocin and acetylcholine were repeated separately in the presence of both the methanol extract (0.8 mg/ml) and the aqueous fraction (0.8 mg/ml). Finally the concentration response curves for oxytocin and acetylcholine were constructed in the presence of salbutamol (5µg/ml) and atropine (5µg/ml) respectively. This was for the purposes of comparison with the extract and the fraction.

Statistical analysis

All data were expressed as mean ±SEM and where applicable, the data were analysed statistically by Student's t-test using Graph pad instant version 2.05a. $P<0.05$ was taken as indicative of significant difference.

RESULTS

Phytochemical analysis

The results on the phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids and tannins (Table 1).

Effects of the methanol extract (ME) and the aqueous fraction (AF) on the uterus

Oxytocin administered alone was observed to produce remarkable uterine contractility with a corresponding increase in the contraction as the concentration increased (figure 1). A force of contraction of 0.93 ± 0.05 g was produced by $0.04x10^{-2}$ of oxytocin on the uterus, this gradually increased to 1.41± 0.05 g at the maximum dose. However in the presence of ME, there was a significant reduction ($p<0.05$) in oxytocin-induced contractions such that 0.51± 0.12 g produced by $0.04x10^{-2}$ was reduced to 0.059 ± 0.01 g in the presence of 0.8 mg/ml ME. This inhibitory effect increased and was more prominent at the 0.8 and 1.6 $x10^{-2}$ of oxytocin. The AF produced a similar significant reduction ($p<0.05$) of the contractions induced by oxytocin. However from the figure, it is evident that the effect of ME was more significant than AF. The effect of ME compares well with salbutamol, the standard uterine relaxant used.

In the acetylcholine (ACH) induced contraction, administration of ACH to the tissue produced remarkable contractions of the uterus, with a gradual increase observed in the force of contraction from 0.71±0.15 to 1.07± 0.01 g by 0.04ug/ml and 1.6 ug/ml of ACH respectively. However on administration of AF (0.8mg/ml) to the tissue in the presence of ACH, a significant reduction ($p<0.0001$) in uterine contractility was observed (figure 2). The first five initial doses of ACH were almost completely blocked by AF. Only the last dose 1.6 ug/ml gave a force of contraction of 0.3 g as opposed to 0.7 g obtained by same dose when administered alone. In comparison with atropine, a muscarinic receptor blocker, the effect of AF can be seen to be more effective as a complete blockade was observed on administration of AF.

Table 1: Phytochemical screening of the methanol extract of the stem bark of *Newboulda laevis*

Test	Results
Carbohydrate	Positive
Saponins	Positive
Tanins	Positive
Alkaloids	Positive
Anthraquinone glycosides	Negative

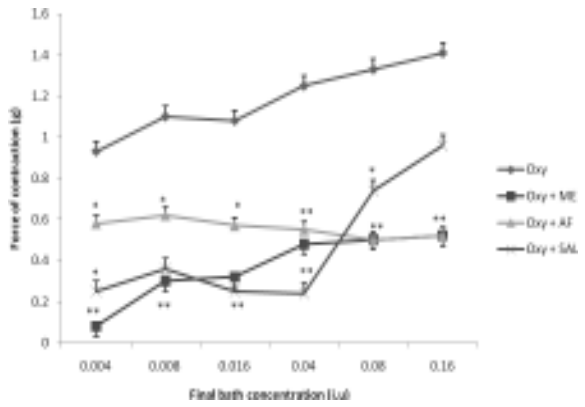

***Fig 1**: Inhibitory activities of the methanol extract and aqueous fraction of the stem bark of Newboulda laevis on oxytocin-induced contraction of the isolated rat uterus.*
Values are mean force of contraction ± SEM. (n = 5 animals).
$^{**}p<0.0001$ and $^{*}p<0.05$ significantly lower than the oxytocin treated group.

ME stands for the Methanol extract of the stem bark of *Newboulda laevis*, while AF is the aqueous fraction. Oxy is Oxytocin, Sal is Salbutamol (5µg/ml). The extract and fraction both at 0.8 mg/ml significantly reduced the force of contraction due to oxytocin

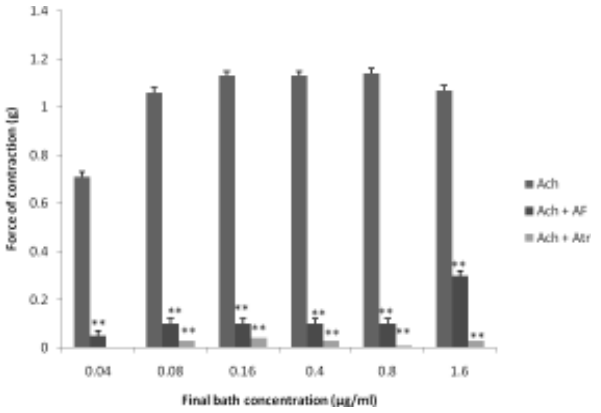

***Fig 2**: Inhibitory activities of the aqueous fraction of the stem bark of Newboulda laevis on acetylcholine-induced contraction of the isolated rat uterus.*
Values are mean force of contraction ± SEM. (n = 5 animals).
$^{**}p<0.0001$ significantly lower than the acetylcholine treated group.

AF is the aqueous fraction. Ach is Acetylcholine, Atr is Atropine (5µg/ml). The fraction at 0.8 mg/ml significantly reduced the force of contraction due to acetylcholine

DISCUSSION

On administration of oxytocin in the presence of the ME, a significant reduction of uterine contractility was observed. Oxytocin is a polypeptide hormone synthesized in several areas of the body such as the hypothalamus, uterus and foetal membrane. The uterus is a major site for oxytocin release. This is made possible by the presence of oxytocic receptors in the endometrial epithelium and myometrium of the uterus. This tends to increase during late stage of pregnancy (Rang, 2003). Hence oxytocin would normally produced strong rhythmic contractions of the uterus. This was seen when oxytocin at the different concentrations were administered alone. However the administration of ME in the presence of oxytocin significantly reduced the extent of the contractions, such that there was a significant reduction in the area under the concentration curve, secondly there was also a significant shift of the concentration response curve to the right and thirdly a significant increase in the EC50 was noted. The maximum response was unattainable, probably pointing to a non-competitive antagonism mediated via occupancy of oxytocic receptors. Features of non-competitive antagonism includes: increase EC_{50}, unattainable E-max (Rang, 2003), and these were the features noted on administration of ME. Though the administration of AF also reduced the extent of contractions, this was however not significant for the first 3 concentrations of oxytocin. Significance was only observed from the 4^{th} dose. Here again E-max was unattainable and EC_{50} was also increased. However the effect of ME was more significant than AF. Oxytocin binds to oxytocin receptors on the uterus and causes release of inositol 1, 4, 5 triphosphate from the hydrolysis of phosphoinositide, this leads to the release of calcium and thereafter contraction. Oxytocin-induced contraction is known to be mediated through specific G-protein-coupled membrane receptors. Oxytocin also increases local prostaglandin production which further stimulates uterine contractions (Rang, 2003). Inhibition of oxytocin-induced contractions may therefore result from blockade of this activation cascade directly or indirectly. On administration of sal-

butamol, a known β_2 agonist, a significant inhibitory effect on oxytocin-induced contractions were also observed. Salbutamol being a smooth muscle relaxant, antagonizes the contratile effect of oxytocin, however the effect of ME on the uterus appears to be more significant than that of salbutamol and hence better. In the presence of ME, a better blockade was noted, when compared with salbutamol.

Acetylcholine when administered alone also gave a dose dependent uterine contractility. This was however significantly inhibited in administration of AF. Interestly the first five doses of acetylcholine were completely blocked by AF, E-max was also unattainable as was seen with oxytocin. 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 (Ganong, 2005). Acetylcholine is known to produce a dose related increase in uterinecontractions by a direct interaction with specific muscarinic receptors in the uterine smooth muscles (Rang, 2003). Muscarinic receptors are G-protein-coupled. There is also an increase in cellular cyclic guanosine monophosphate (cGMP). Activation of muscarinic receptors also increases potassium influx across cell membrane (Ganong, 2005). AF significantly shifted the concentration response curve of acetylcholine to the right and the original Emax of acetylcholine was also unattainable. This indicates a non competitive antagonism by AF. In the presence of atropine, a blockade of Ach-induced contractions was also observed. However the blockade was reversible as E-max was re-attained by higher concentrations of Ach. The inhibitory effect of AF on Ach-induced contractions was similar to that of atropine, a known competitive muscarinic antagonist of acetylcholine. It is thus possible that the effect of AF may also be mediated via muscarinic blockade. The inhibitory effect of ME the crude extract was more significant than AF, indicating that partitioning may not result in enhanced activity. The phytochemical screening revealed the presence of saponins and tannins documented to contribute to tocolytic property of plants (ref).

CONCLUSION

This study permits the following conclusions: that the stem bark of *Newbouldia laevis* possesses relaxant effect and as such ideal in combating threatened abortion, preterm contractions and can thus be explored as an alternative to orthodox drugs.

The effect of *Newboulda laevis* is mediated via oxytocic and muscarinic receptors blockade.

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DECLARATION OF INTEREST:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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