CHEMICAL ANALYSIS AND CONTRACTILE (OXYTOCIC) ACTIVITY OF HEXANE EXTRACT OF Newbouldia laevis ON ALBINO RAT UTERUS


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ABSTRACT

Newbouldia laevis (life plant) is a medicinal plant used to facilitate birth among traditional healers in Nigeria. In this study, extraction was maintained in N-Hexane solvent using soxhlet extractor and solvent remove via rotary evaporator at less than 50°C. Phytochemical screening were performed on the extract by standard methods. Oxytocic (contractile activity) of the hexane extract was investigated in female albino rat’s uterus using the Ugo Basile channel recorder machine. Comparatively two standard drugs, oxytocin and Acetylcholine were used in this study. Phytochemicals present were tannins and steroid only. From the graphical plot, the percentage response to log dose of Newbouldia laevis (hexane extract) supplemented with oxytocin indicated an anticontractile effect of the uterus in rats.

Keywords: Newbouldia laevis, Phytochemicals contractile (oxytocic) effects.

INTRODUCTION

The use of plants to facilitate birth or to protect the young embryo appears to be a common practice among traditional healers in Nigeria. Newbouldia laevis is one of such plants and their leaves are used in southern Nigeria by herbalist to hasten parturition and to expel the placenta after delivery (Obute, G.C., 2002). Many herbal remedies are traditionally used as contraceptives (to prevent ovulation or fertilization), abortifacients (to prevent implantation), to stimulate uterine flow or oxytocics (to stimulate uterine contractions, particularly to promote labour) (Ritchie, H.E, 2001). Contractions of the uterus (during child birth) are the periodic tightening and relaxing of the uterine muscle, the largest muscles in a woman’s body. During labour, the pituitary gland is triggered to release a hormone called oxytocin which stimulates the uterine tightening.

Newbouldia laevis (life plant) family Bignoniaceae, Akoko-Yoruba, Ikhimwin-Binis, Aduruku by Hausas and Ogirisi-Ibos is a tropical plant growing to a height of about 10-12metres. It’s evergreen, though its leaves turn somewhat dark purple during the cold seasons (Okeke, A.O., 2003).

Plate 1: showing Newbouldia laevis leaves and the tree

The decoctions of its leaves are used by herbalists to purify menstruation in young women. The bark of Newbouldia laevis (Akoko), a whole snail are burnt into powder and taken by pregnant women in Edo state of Nigeria to weaken the cervix and hasten labour (Bisiriyu and Akanji, 2010).

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In Nigeria, the plant serves as boundary for land dispute, to induce labour, used by rainmaker to stop rain and as honour plant during chieftaincy title in Yorubaland. The chemical analysis and contractile activity in albino rat uterus of the hexane extract are being examined in this study.

MATERIALS AND METHODS
Collection/Identification of plant
Fresh leaves samples were collected in Ikirun, in Ifelodun Local Government Area of Osun State of Nigeria between the months of September-October, 2010. They were identified and confirmed by Dr. J.F. Bamidele in Biology and Biotechnology Department, University of Benin, Benin City.

Extraction
About 299.00g of the powdered leaves was extracted using n-hexane solvent in a soxhlet extractor for eight hours. The extract was then concentrated by rotary evaporator (model: RE200) at less than 50°C. The extract were then transferred into a sample bottles stored in a desiccators for further analysis.

Animal sourcing
Non-pregnant female albino rats were obtained from the Department of Animal and Environmental Biology, University of Benin. The animals were maintained under standard environmental conditions and had free access to standard rat’s feeds (broiler mash) and water. The rats were allowed an acclimatized period of two weeks before they were used for the experiment.

Preparation of physiological salt solution (De-Jalon)
Sodium chloride (45.0g), KCl (2.10g), NaHCO₃ (2.50g), D-glucose (2.50g) were weighed and made into of solution of 3.5L distilled water. Calcium chloride (0.40g) was made into a solution of 1.5L distilled water in a separate beaker. Both solution were then added to give 5L.

Phytochemical Screening
Phytochemical screening of the hexane-extract was performed by standard procedures prescribed by Sofowora (1993), Trease and Evans (1989) and Odebiyi and Sofowora (1978). The extract was screened for: tannins, saponins, flavonoids, terpenoids, steroids and reducing sugar.

Determination of invivo contractile activity of n-hexane extract of Newbouldia laevis on non-pregnant albino rats
Five female non-pregnant rats (200-220.0g) were pretreated/primed with 0.2mg/kg of Diethylstilbesterol intraperitoneally for 24hours. The rats were anaesthetized in CHCl₃ inhalation and sacrificed. The uterus was identified and the two horns of the uterus cut out and transferred to a petri-dish containing Physiological salt solution (P.S.S.). Two centimetres (2cm) in length of the uterus was cut out and threaded using a surgical silk or thread. The channel recorder of Ugo basile was calibrated, threaded with the uterus and connected to a transducer. The uterus tissue was mounted in 25ml of organ bath containing De-Jalon solution and allowed to acclimatize for 30minutes till spontaneous contraction were abolished, before administration of drugs (+ve control) and extract.

Two standard drugs for uterine contraction (positive control) obtained were oxytocin and Acetylcholine. For oxytocin, concentration of 0.10i.u/ml and 1.0i.u/ml were prepared and volumes at 0.2ml, 0.4ml and 0.8mls were taken. A contact time of 60 seconds was allowed after which the tissue was washed and allowed to relax for 60seconds before the administration of the next dose.

Acetylcholine (Ach) of 1.0µg/ml, 100µg/ml and 1mg/ml were used at volumes of 0.2ml, 0.4ml and 0.8mls. A contact time of 60 seconds was allowed after which the tissue was washed and allowed to relax for 60 seconds before administration of the next dose.

About 0.2mg and 0.5mg of Newbouldia laevis n-hexane extract (in DMF) were administered to the tissue for 10minutes while oxytocin of 0.1i.u and 1.0 i.u were added to the extract.

RESULTS
The results of phytochemical screening and invivo contractile activity of hexane extract of Newbouldia laevis on non pregnant rats are shown below:
Chemical Analysis and Contractile (Oxytocic) Activity of Hexane Extract of *Newbouldia Laevis* on Albino Rat Uterus

Table 1: Results of phytochemical analysis of n-hexane extract of *Newbouldia laevis*

<table>
<thead>
<tr>
<th>s/n</th>
<th>Constituents</th>
<th>n-Hexane extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Phlobatanins</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Anthraquinone</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Reducing sugars</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Present, - = Absent

RESULTS OF INVIVO CONTRACTILE ACTIVITY OF *Newbouldia laevis* EXTRACT OF NON PREGNANT RATS

**Fig 1:** Effect of the n-hexane extract of *Newbouldia laevis* on oxytocin induced contractions in the non-pregnant rat uterus

Values are mean percentage responses ± SEM (n = 5 per group). *P<0.05 significantly different from oxytocin induced contractions alone.

OXY: Oxytocin alone
Oxy+NL30: Oxytocin and 30 mg of *Newbouldia laevis*
Oxy+NL60: Oxytocin and 60 mg of *Newbouldia laevis*
Oxy+Sal: Oxytocin and 5 µg of Salbutamol
**DISCUSSION**

The results points to the fact that the n-hexane extract of *Newbouldia laevis* produced a significant decrease in both oxytocin and acetylcholine-induced contraction of the rat uterus. Acetylcholine is known to produce a dose related contraction of the uterus by stimulating muscarinic receptors in the uterine smooth muscles (Rang H.P, Dale M.M, Ritter J.M, Moore P, 2003). There is also an increase in cellular cyclic guanosine monophosphate (CGMP). Activation of these receptors also increases potassium in influx across cell membrane (Ganeg F.W., 2005).

Since both doses of the extract inhibited Acetylcholine induced contractions, the extract might act on a muscarinic receptors. Secondly, the e-max could not be attained in the presence of the extract pointing to a non-competitive inhibition by the extract. Inhibition of oxytocin-induced contractions by the extract at both doses, seems to be non-competitive in nature, since the e-max of oxytocin could not be attained in the presence of the extract. Oxytocin-induced contraction is known to be mediated via a-protein-coupled receptors. Upon activation it leads to sensation of Imrho 1,4-5 triphosphate, there is release of calcium, depolarization-induced activation of voltage sensitive calcium ion channels (Lauscence L, Brunton J.S, Lazo K.L, Parker Goodman and Gilman’s, 2001) and cause contraction of the uterus.

Inhibition by the extract may this be via blockade of this activation cascade that results in mobilization of calcium. With calcium mobilization inhibited, a relaxant effect is seen. Salbutamol is a known physiologic antagonist of oxytocin. Hence it normally will produce a dose-dependent effect on oxytocin-induced inhibitory contraction as seen in this study. The effect of salbutamol was more significant than the extract, this is understandable as the extract is crude, unrefined and probably contains other materials.

The effect of *Newbouldia laevis* on uterine contraction from results obtained is suggestive of a non-competitive and physiologic antagonism.

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**Fig 2**: Effect of the n-hexane extract of *Newbouldia laevis* on acetylcholine induced contractions in the non-pregnant rat uterus

Values are mean percentage responses ± SEM (n = 5 per group). *P*<0.05 significantly different from acetylcholine induced contractions alone.

Ach: Acetylcholine alone
Ach+NL30: Acetylcholine and 30 mg of *Newbouldia laevis*
Ach+NL60: Acetylcholine and 60 mg of *Newbouldia laevis*
Ach+Atr: Acetylcholine and 5 µg of Atropine
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The n-hexane extract of *Newbouldia laevis* leaves decreased both oxytocin and acetylcholine induced contractions. Thus inhibition was non-competitive. This activity may thus account for its use as a tocolytic agent by traditional birth attendants.

From the graphical plot, the percentage response to log dose of *Newbouldia laevis* extract supplemented with oxytocin indicated an anticontractile effect of the uterus of rats at 15%: -1.99, 10%: -1.398 and 12%: -1.097 (oxy+ NL 30mg) and at 8%: 0.6989, 10%: -0.3979, 4%: -0.0909, 2%: 0.301 and 5%: 0.602 (Ach NL 30mg). While for an increase dosage of *Newbouldia laevis* extract, the uterus was relaxed (anticontractile) at 0%: -1.699, 6%: 1.398 and 15%-1.097 (oxy+NL 30mg) and 0%: -0.6989, 0%: -0.3979, 0%: -0.6989, 0%: -0.3979, 0%: -0.0969, 4%: 0.301 and 12%: 0.602 (Ach + NL 30mg).

*Newbouldia laevis* extract has shown uterine relaxant activity in dose dependent manner (Graphs 1 and 2) complete inhibition of contraction was noted at 30mg and 60mg dose of *Newbouldia laevis* extract when compared to the standard uterine relaxant (Atropine and Salbutamol) P. values was highly significant (p<0.5 for oxytocin) and (p < 0.01 for Ach). This indicated that *Newbouldia laevis* extract had similar activity as Atropine but differ in the dose.

This contractile activity corroborates local use of *Newbouldia laevis* as medicinal plants relaxing the uterus of pregnant women.

ACKNOWLEDGMENT

Appreciation goes to Dr. S.O. Okpo, Head of Department Pharmacology and Toxicology University of Benin, for the technical assistance and permission to use their facilities.

REFERENCES


