ORIGINAL ARTICLE

Effect of the ethanolic extract of *Nauclea latifolia* (Family: Rubiaceae) on the isolated uterus of non-pregnant rats

Nworgu Z. A. M., Owolabi O. J., Atomah J. E.
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin city, Edo State, Nigeria

The plant *Nauclea latifolia* has been reported to be used by traditional healers to arrest pre-term labour. The ethanolic extract of the root of *N. latifolia* was screened for activity via agonist–induced contractions of uterine smooth muscles in non-pregnant female albino rats. The extract, at 0.1 and 0.2 mg/ml (final bath concentration), was tested against oxytocin (4×10^{-5} to 8×10^{-2} I.U/ml: final bath concentration), acetylcholine (0.04 to 40 µg/ml: final bath concentration) and ergometrine (0.05 to 100 µg/ml: final bath concentration) induced contractions invitro. The effect of the extract was compared to that of (0.004 µg/ml: final bath concentration) salbutamol and (0.004 µg/ml: final bath concentration) atropine. Both concentrations of the extract significantly shifted the concentration response curves of oxytocin ($P<0.01$), acetylcholine ($P<0.0001$) and ergometrine ($P<0.0001$) to the right with a slight depression of the Emax. This shift was more with the 0.2 mg/ml concentration, thus suggesting the possibility of a dose dependent action. There was no statistical significant decrease in Emax by 0.1 mg/ml of the extract, while the 0.2 mg/ml produced a significant depression ($P<0.05$) of the Emax, which like salbutamol could not be overwhelmed by higher concentrations of oxytocin. Similarly a significant reduction of the Emax of acetylcholine induced contractions was produced by 0.2 mg/ml, while both concentrations (0.1 and 0.2 mg/ml) produced significant ($P<0.0001$) reduction in Emax of ergometrine. It can thus be concluded that *N latifolia* root extract reduces oxytocin, acetylcholine and ergometrine-induced uterine contractions. These inhibitions were non-competitive. The result indicates an anti-abortifacient property.

Key words: Antagonism, *Nauclea latifolia*, root extract, uterine-contraction, uterus

INTRODUCTION

Herbal medicine is a practice that involves the use of natural plant substances (botanicals) to treat and prevent illness.[1] Herbal medicine is sometimes called botanical medicine or phytotherapy (from Greek *phytos:* plant). Herbal medicine is the use of plants, plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other form of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems; or, in a more conventional medical sense, to treat, cure, or prevent disease in animals or humans.[2]

Nauclea latifolia Family: Rubiaceae is a small evergreen tree or straggling shrub with leaves rounded ovate, apex shortly acuminate, rounded or cuneate base and stipules ovate.[3] Parts used include leaves, roots, stem and fruits.

Analysis of the root has shown the presence of sugar, saponins, and flavonoids.[4] Indole alkaloids have been found in the heartwood and trunk-bark, stem bark and root.[5] The presence of monoterpenes in the epicarp of the fruit has also been reported.[6]

It has medicinal use in Igbo land; the decoction of the leaves is recommended for stomach upset, especially in children.[7] The infusion of the root is also used as a remedy for stomach upset in adults. The dose is one tea cup twice daily.[7] The decoction of the leaves along with the leaves of *Rauwolfia vomitoria* is used to bathe children with measles.[7] The decoction of the root with alligator pepper is given for cough, cold, and general weakness of the body.[7] The fruit is recommended for piles, dysentery, colic, emetic and menstrual disorders. The root is chewed as chew-stick.[7] Other ethno botanical uses of *N. latifolia* include malaria, leprosy, piles, gonorrhoea, debility, dyspepsia and enteritis.[3]

Traditional birth attendants in Nigeria have used the ethanolic extract of *Nauclea latifolia* stem and root bark in arresting preterm contractions in pregnant women.[3]

The uterus is a hollow, thick-walled muscular organ located in the female pelvis between the bladder and rectum.[8] It lies between the bladder in front and the pelvic or sigmoid colon and rectum behind, and is completely within the

Address for correspondence: Mrs. Owolabi J. Omonkhelin, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. E-mail: josphineomo@yahoo.com
Received: 17-03-2009; **Accepted:** 06-10-2009; DOI: 10.4103/0973-8258.62162

pelvis so that its base is below the level of the superior pelvic aperture.[9]

The uterus, both *in situ* and when excised, contracts rhythmically. These spontaneous contractions and relaxation originate from the uterine smooth muscle itself with the myometrial cells of the fundus as pacemakers.[10] The frequency and force of contraction vary greatly with different conditions of the sex cycle perhaps due to the complex hormonal changes associated with these conditions.[11]

This study aims to provide a scientific basis for the use of *Nauclea latifolia* in arresting pre-term labour.

MATERIALS AND METHODS

Collection of Plants

Fresh root parts of *Nauclea latifolia* were collected from the premises of the University of Benin Ugbowo campus Benin City, Nigeria. Botanical authentication was confirmed at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where a herbarium specimen (No. FHI16938) was deposited for future reference.

After collection, washing and cutting into small pieces, the plant material was first air dried and then dried in an oven at 40°C for 48 hours. The dried sample was then reduced to coarse powder, weighed and then stored in an airtight container for use in analysis.

Extraction

The powdered plant material (500 g) was macerated with two litres of 70% ethanol for 48 hours during which the mixture was stirred six-hourly using a sterile glass rod. The extract was then filtered and the filtrate evaporated to dryness using rotary evaporator and thereafter the percentage yield was calculated. The concentrated extract was stored in air tight containers, labelled and refrigerated at -4°C prior to use.

Preparation of Stock Solution of the Extract

On each day of the experiment, one gram of the plant extract was weighed and dissolved in one ml of dimethylsulphoxide (DMSO) to obtain a one g/ml stock solution. Serial dilutions of the stock using distilled water were done to obtain 50 mg/ml and 25 mg/ml concentrations of the extract.

Animals

Female non pregnant wistar albino rats weighing between 150 g and 250 g were used for the procedure and handled according to standard protocol. The rats were obtained from the Animal House of the College of Medicine, University of Ibadan, Oyo State, Nigeria and allowed to acclimatize for two weeks before being subjected to the experimental procedure. The rats were maintained on a standard diet (Grower's mash) and had access to food and water. They were housed, four in a cage, with a 12-hour light-dark cycle.

Research was conducted in accordance with the internationally accepted principles for laboratory animal use and care.

Drugs and Solvents

Diethylstibosterol {Sigma-Aldrich Inc, USA}, Oxytocin {Laborate Pharmaceuticals, India}, Acetylcholine {Sigma-Aldrich Inc, USA}, Ergometrine {Rotex Medica, Germany}, Salbutamol {Glaxosmithkline USA}, Atropine sulphate {Sisbiu Xierkang}, Ethanol {Sigma-Aldrich, Inc, USA}, Dimethylsulfoxide {Sigma-Aldrich Inc, USA}.

Organ Bath Experiment

The rats were pre-treated with 0.2 mg/kg diethylstilboestrol, intra peritoneally, 24 hours before the experiment. Vaginal smear was carried out to ascertain if the rats were in oestrous on the day of the experiment. This was confirmed by the presence of squamous stratified epithelial cells when viewed under a light microscope.

The rats were sacrificed under anaesthesia using chloroform. The abdomen was cut open and one of the uterine horns rapidly isolated with the surrounding fat and loose connective tissues trimmed off. The tissue was transferred into a petri dish containing aerated De Jalons' physiological salt solution comprising 45.0 g NaCl, 2.5 g $NaHCO_3$, 2.5 g D-glucose, 2.1 g KCl and 0.4 g $CaCl_2.2H_2O$ per 5.0 litres distilled water.

The isolated tissue was suspended vertically in an organ bath containing 50 ml De Jalons' solution. The uterine horn about two cm long was attached to the tissue holder at one end with a thread while the other end was connected to the transducer. The bath was maintained at a temperature of 37°C and well aerated continuously with air. The isometric tension generated by the tissue was measured using an isometric force transducer and recorded with Ugobasile single channel recorder system. The tissue was then originally set at a 0.50 g resting tension and was allowed to equilibrate with De Jalons' solution for 10 minutes.

The effects of (4×10^{-5} to 8×10^{-2} I.U/ml: final bath concentration), of oxytocin on the uterine smooth muscle were investigated. The same was done for acetylcholine (0.04 to 40 μg/ml: final bath concentration) and ergometrine (0.05 to 100 μg/ml: final bath concentration) induced contraction. In each case a time cycle of approximately two minutes was allowed: contact time one minute and relaxation time one minute.

Preliminary studies revealed that 0.1 mg/ml and 0.2 mg/ml of the extract produce a moderate inhibition of the uterine smooth muscle contraction, hence each of these concentrations were used on the uterus against oxytocin-, acetylcholine- and ergometrine-induced contractions of the uterine smooth muscles. The tissue was left to equilibrate with 0.1 mg/ml extract for 10 minutes and, without washing, a dose of oxytocin was added and the effect determined. A time cycle of two minutes was allowed. Thereafter the effects of the various doses of oxytocin in the presence of this dose of the extract were determined. The above procedure was repeated in the presence of 0.2 mg/ml extract, and effects of the positive control 0.004 μg/ml salbutamol were determined.

Using the same procedure, the effects of acetylcholine in the presence of 0.1 mg/ml and 0.2 mg/ml extract and in the presence of 0.004 μg/ml atropine were determined. The effect of 0.1 mg/ml and 0.2 mg/ml of the extract were also tested against the various concentrations of ergometrine.

Data Presentation and Statistical Analysis

The results are expressed as the mean percentage response of five experiments±standard error of mean (SEM). Concentration response curves were plotted for acetylcholine, oxytocin and ergometrine alone and in the presence of extract using the same concentrations used when the agonist were administered alone; also for acetylcholine (same concentration when alone) in the presence of atropine and for oxytocin (same concentration when alone) in the presence of salbutamol. The Emax were determined and the data were analyzed using one way ANOVA with Dunnett multiple comparison test for mean percentage responses and one way ANOVA with Turkey-Kramer multiple comparison post test for Emax. The level of significance was $P<0.05$.

RESULTS

The extract produced a reduction in oxytocin-induced contraction [Figure 1]. This inhibition was greater at lower concentrations of oxytocin and with the higher concentration 0.2 mg/ml of the extract than with 0.1 mg/ml. The concentration response curve of oxytocin was shifted to the right by 0.1 mg/ml ($P<0.05$) and 0.2 mg/ml ($P<0.01$) of the extract. The curve was shifted more to the right with salbutamol ($P<0.01$) than with 0.1 mg/ml of the extract but 0.2 mg/ml of the extract produced a further shift to the right than that produced by sabutamol [Figure 1].

The extract also depressed the Emax [Figure 2] of the concentration-response curve of oxytocin at 0.1 mg/ml ($P>0.05$) and at 0.2 mg/ml ($P<0.05$). Salbutamol (the standard antagonist) also produced a slight depression in Emax though this was considered not statistically significant ($P>0.05$). The extract showed no intrinsic activity on the uterus but shifted the concentration-response curve of acetylcholine to the right [Figure 3]. The shift was more with the 0.2 mg/ml ($P<0.01$) than with the 0.1 mg/ml ($P<0.05$) concentrations. Atropine also produced a significant shift ($P<0.01$) in the curve to the right but the inhibition was almost completely overcome at higher concentrations of acetylcholine. Like atropine, the Emax [Figure 4] produced in the presence of 0.1 mg/ml extract was considered not statictically significant ($P>0.05$). However, unlike atropine there was a very significant depression in the Emax ($P<0.01$) of the concentraton-response curve of acetylcholine by 0.2 mg/ml of the extract [Figure 3].

The extract shifted the concentration response curve to the right indicating inhibition in ergomentrine-induced contraction [Figure 5]. The inhibition was greater with 0.2 mg/ml than with 0.1 mg/ml of the extract [Figure 5]. There was also a very significant depression in the Emax [Figure 6] at 0.1 mg/ml ($P<0.001$) and at 0.2 mg/ml ($P<0.001$) extract.

DISCUSSION

The results show that at 0.1 mg/ml and at 0.2 mg/ml the ethanolic extract of *N.latifolia* produced a significant ($P<0.05$) decrease in oxytocin-induced contraction of the rat uterus in a dose dependent manner. Oxytocin-induced contraction is known to be mediated through specific G-protein-coupled membrane receptors. In the human myometrium, these receptors are coupled with Gq and G11. Upon activation it leads to the generation of inositol- 1,4,5-triphosphate IP_3 from phosphonositide hydrolysis; subsequent mobilization of calcium from intracellular stores; and depolarization-induced activation of voltage sensitive calcium ion channels.[12] Oxytocin also increases local prostaglandin production which further stimulates uterine contractions.[10]

Inhibition of oxytocin-induced contractions may therefore result from blockade of this activation cascade directly or indirectly. At 0.2 mg/ml, the extract produced a statistically significant decrease in Emax of oxytocin ($P<0.05$) unlike at 0.1 mg/ml, where the decrease in Emax was considered statistically not significant ($P>0.05$). This indicates non-competitive antagonism of oxytocin at 0.2 mg/ml. The higher concentrations of oxytocin could not produce the initial maximum response in the presence of the extract. This suggests that the extract produces inhibition by a mechanism other than receptor antagonism.

The extract also produced a significant decrease in acetylcholine-induced uterine contraction at 0.1 mg/ml

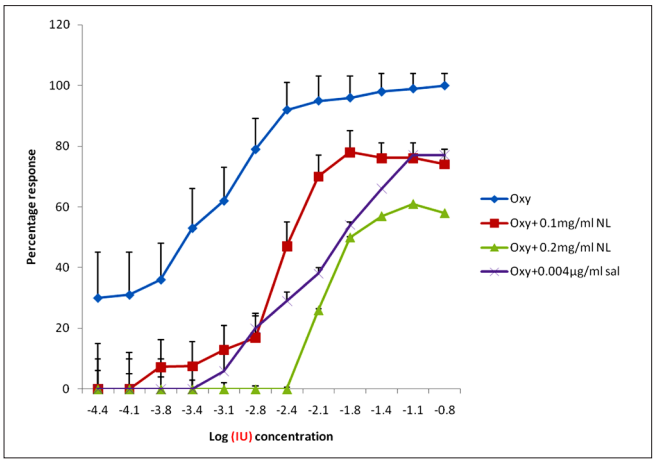

Figure 1: Concentration response curves showing the effects of oxytocin (oxy) on isolated rat uterus in the presence and absence of the ethanolic extract of *N. latifolia* or salbutamol (sal). The extract at 0.2 mg/ml significantly (P<0.05) reduced the percentage response due to oxytocin; however the extract's effect at 5mg/ml was insignificant (n=5)

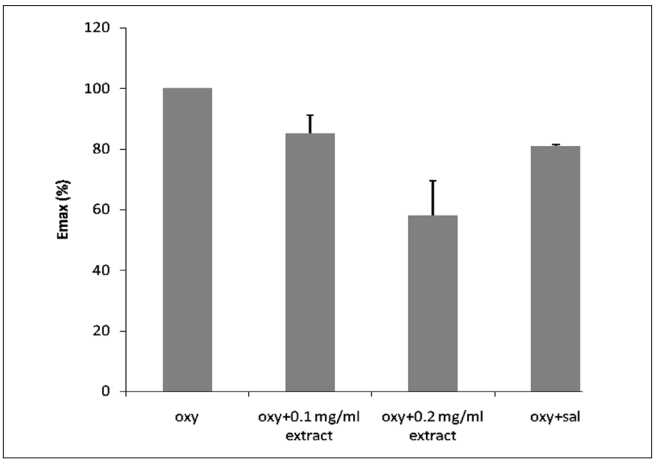

Figure 2: Bar chart showing the Emax of oxytocin alone, in the presence of extract, and in the presence of salbutamol (P=0.03887). The extract at 0.2 mg/ml significantly (P<0.05) reduced the Emax of oxytocin (n=5)

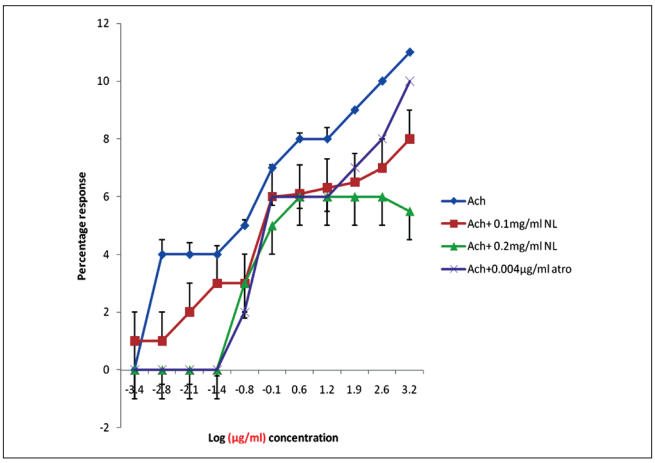

Figure 3: Concentration response curves showing the effects of acetylcholine (ACh) in the absence and presence of ethanolic extract of *N. latifolia* extract or atropine. The extract at both concentrations significantly (P<0.005) reduced the percentage response due to acetylcholine.(n=5). NL: Ethanolic extract of *Nauclea* latifolia

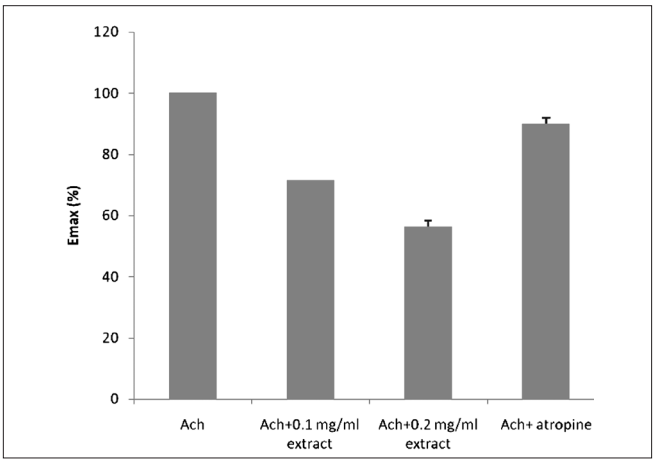

Figure 4: Bar chart showing the Emax of acetylcholine alone, in the presence of extract and in the presence of atropine. The extract at 0.2 mg/ml significantly (P<0.05) reduced the Emax of acetylcholine (n=5)

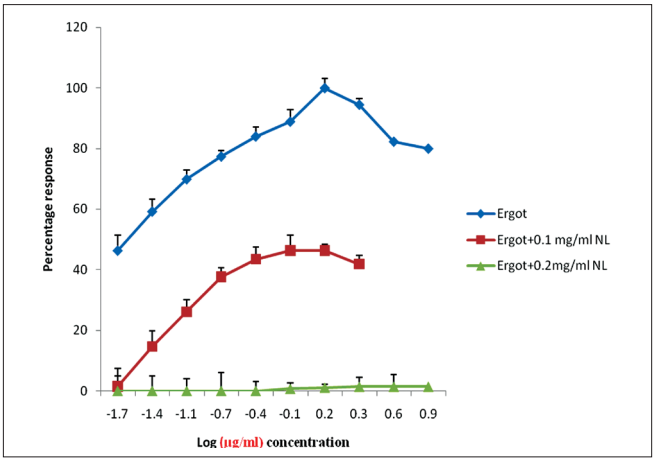

Figure 5: Concentration response curves showing the effects of ergometrine (ergo) in the absence and presence of ethanolic extract of *N.latifolia*. The extract at both concentrations significantly (P<0.05) reduced the percentage response due to ergometrine. (n=5) NL: Ethanolic extract of *Nauclea* latifolia

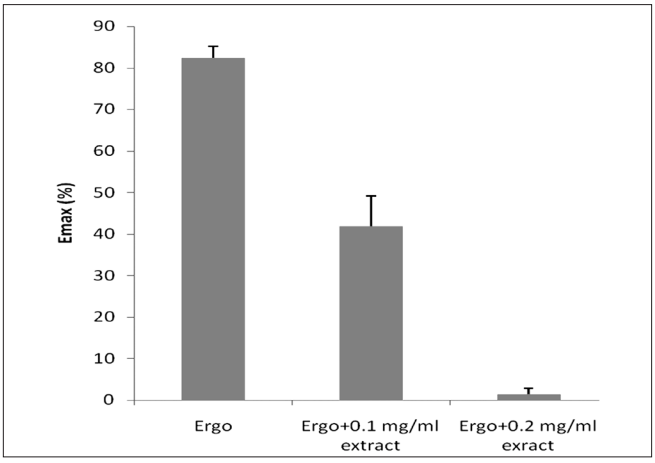

Figure 6: Bar chart showing the Emax of ergomentrine alone and in the presence of extract. The extract at both concentrations significantly (P<0.0001) reduced the Emax of ergometrine (n=5)

(P<0.05) and at 0.2 mg/ml (P<0.001). There was a significant decrease in Emax (P<0.01) of acetylcholine by the extract at 0.2 mg/ml (P<0.01) unlike at 0.1 mg/ml (P>0.05) where the decrease was considered not significant. Acetylcholine is known to produce a dose related increase in uterine contractions by a direct interaction with specific muscarinic receptors in the uterine smooth muscles.[10] Muscarinic receptors are G-protein-coupled, and activation results in IP_3 and diacylglycerol (DAG) cascade.[10] There is also an increase in cellular cyclic guanosine monophosphate (cGMP). Activation of muscarinic receptors also increases potassium influx across cell membrane.[11]

Both concentrations of the extract shifted the concentration-response curve of acetylcholine to the right and the original Emax of acetylcholine could not be reached even at higher concentrations of acetylcholine. This indicates a non-competitive inhibition by the extract.

The results indicate that the extract at 0.1 mg/ml and at 0.2 mg/ml produced a highly significant decrease in ergometrine-induced uterine contraction (P<0.001). Ergometrine among the ergot alkaloids is the most active uterine α-adrenoceptor agonist.[13] Generally, ergot alkaloids exert agonistic and partial agonistic and antagonistic effects on several receptors viz: α-adreneceptors, 5-HT, and dopamine receptors.[14]

Ergometrine contracts the uterus, an effect that partially depends on the state of the uterus. At smaller doses, contractions increase in force and frequency with complete relaxation in between but as the dose is increased contractions become more forceful and prolonged with resting tonus increased. It can result in sustained contracture.

A reduction in the level of ergometrine-induced contraction by the extract may either be through a direct interaction with α-adrenoceptors or just a physiological antagonism. However, higher doses of ergometrine could not overcome the blockade as the initial maximum response could not be reached. So the inhibition can be said to be non-competitive possibly because it is not interacting with α-adrenoceptors..

Atropine is a competitive muscarinic receptor antagonist of acetylcholine.[10] Thus, as shown by the results, atropine produced a competitive blockade of acetylcholine with no significant difference in the Emax (P>0.05). Inhibition of oxytocin-induced contraction was observed in the presence of salbutamol. Salbutamol is a selective β_2-adrenoceptor agonist with a known uterine relaxant activity and other blood pressure lowering and metabolic effects. By binding to G-protein coupled β_2-receptors, adenylate cyclase is activated resulting in increased intracellular cyclic adenosine monophosphate (cAMP) which inhibits the action of MLCK, thus preventing the phosphorylation of myosin and myosin-actin interaction that usually results in contraction.

Salbutamol, therefore, is not a receptor antagonist of oxytocin but producer of physiological antagonism. It produced an apparently dose-dependent significant inhibition of oxytocin-induced contraction (P<0.05). Like salbutamol, 0.1 mg/ml and 0.2 mg/ml extract shifted the dose-response curve of oxytocin to the right and slightly depressed the maximum response indicating non-competitive antagonism.

Comparison shows that the dose-response curve of oxytocin in the presence of salbutamol was shifted more to the right than that obtained with 0.1 mg/ml extract unlike 0.2 mg/ml extract which shifted the curve more to the right than salbutamol. Also, there was no statistically significant decrease in Emax (P>0.05) in the presence of salbutamol as in the presence of 0.1mg/ml extract. However, 0.2 mg/ml of the extract produced a significant depression (P<0.05) of the Emax of oxytocin than did salbutamol and this inhibition could not be overwhelmed by higher concentrations of oxytocin. This indicates non-competitive antagonism of oxytocin at 0.2 mg/ml of the extract.

The extract produced an apparently similar effect as atropine on the contractile effect of acetylcholine as they both shifted the dose response curve to the right. However, the inhibitory activity of atropine was almost completely overcome by higher doses of acetylcholine indicative of a receptor competitive antagonism. The higher depression of the maximum response to acetylcholine observed with the 0.1 mg/ml extract (P<0.05) and 0.2 mg/ml extract (P<0.001) than with atropine (P>0.05) is suggestive of a non-competitive inhibition of acetylcholine-induced contraction by the extract. And since the initial maximum response could not be reached at higher doses of acetylcholine, it suggests non-receptor antagonism, possibly just physiological antagonism.

The ethanolic extract of *Nauclea latifolia* root reduces the contraction induced by oxytocin, acetylcholine and ergometrine in a dose-dependent manner. This inhibition was non-competitive. However, the exact mechanism for this action requires further investigation. This activity of the extract may account for its use by traditional birth attendants to prevent premature labour.

ACKNOWLEDGMENT

The authors are grateful to Mr. Paul Osigbeme for providing the drugs and his commitment towards the completion of this work.

REFERENCES

1. Tyler VE, Foster S. Tyler's Honest Herbal. The Physician's Desk Ref. for Herbal Medicines (annual). The Columbia Encyclopedia. Copyright © 2001-08. Columbia University Press; 1999. p. 1-2.
2. Blumenthal M. Herbal Medicine. Available from: http://www.amsa.org/ICAM/C7.doc (last accessed on Sep 17, 2008).
3. Duke JA. Ethnobotanical uses of *Nauclea latifolia*. Dr. Duke's Phytochemical and Ethnobotanicaldatabases.Availablefrom: http://www.ars-grin.gov/cgi-bin/duke/ethnobot.pl?ethnobot.taxon=Nauclea%20latifolia (last accessed on Aug 23, 2008).
4. Nworgu ZAM, Onwukaeme DN, Afolayan AJ, Ameachina FC, Ayinde BA. Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. Afr J Pharma Pharmacol 2008;2:37-41.
5. Brown RT, Chapple CL, Lashford AG. Isolation of strictosidine (Isovincoside) Lactam from *Nauclea latifolia*. Phytochemistry 1977;16:1619-20.
6. Abreu P, Pereira A. A new Indole alkaloid from *Sarcocephalus latifolius*. Heterocycles 1998;48:885-91.
7. Gill LS. Ethnomedical uses of plants in Nigeria. UNIBEN Press; 1992. p. 172.
8. Arthur V, Sherman J, Luciano D. Human Physiology. 7th edition 1998. p. 649-80.
9. Gray H. The uterus. Anatomy of the Human Body 2007. Available from: http://www.bartleby.com (last accessed on Oct 15, 2008).
10. Rang HP, Dale MM, Ritter JM, Moore P. Pharmacology. 5th edition, Churchill Livingstone: Edinburgh London; 2003. p. 441-3.
11. Ganong FW. Review of Medical Physiology 22nd Edition, 2005. p. 640-5.
12. Laurence L, Brunton JS, Lazo KL, Parker. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th edition. 2001. p. 231-351, 853-60.
13. Katzung BG. Basic Clinical Pharmacology 8th edition. McGraw-Hill: New York; 2001. p. 134-5
14. Wilson A, Schild HO, Walter M. Applied Pharmacology. 11th edition, Churchill: Livingstone; 1975. p. 55-62,248-57, 261-7.

Source of Support: Nil, **Conflict of Interest:** None declared.