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Original Research

# Uterine Contractile Effect of Ethanol Extracts of *Sida acuta* Burm F. Leaves

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## ABSTRACT

**Background:** The study evaluated the ureotonic potentials ethanol extracts leaves of *Sida acuta*. **Methods:** Preliminary phytochemical tests were carried out on the ethanol extract of *Sida acuta* Burm F. leaves. The presence or absence of phytochemicals such as saponnins, flavonoids, alkaloids, tanninis, terpenoids, steroids, carbohydrates, hydrogen cyanide, cardiac glycosides, phenols and reducing sugars were observed. Two pregnant Wistar albino rats were used for the study. The rats were killed by stunning and abdomens were cut open. The uterus were carefully isolated and transferred into dejalon solution which was continuosly bubbled with air and maintained at 37°C and 7.0 pH. **Result:** Phytochemical screening revealed high content of flavonoids mg/100g (6.172 ± 0.003), phenols (4.643 ± 0.006), alkaloids (4.643 ± 0.006), cardiac glycosides mg/100g (3.813 ± 0.003), and moderate contents of hydrogen cyanide (2.695 ± 0.003) while low concentrations were observed in steroids (0.512 ± 0.005), saponins (0.246 ± 0.003) and terpenoids (0.813 ± 0.003). Resins, carbohydrates, reducing sugars, tannins, proteins, fats and oil were not detected. The results of the uterine contractility test showed effects compared with the normal rhythm of the uterus. Atropine did not block the extract-induced contraction. Ergomentrine in doses that blocked adrenaline induced-contraction reduced the extract-induced contraction by half. Indomethacin blocked oxytocin-induced contraction and reduced the extract-induced contraction slightly. **Conclusions:** These results suggest that ethanol extract of *Sida acuta* leaves possesses oxytocic effects and this portends danger among pregnant women who might take the leaf infusion. The investigation substantiates its use in hastening of labour.

KEY WORDS: Acetylcholine; Adrenaline; Atropine; Contractility; Ergomentrine and Ureotonic.

## INTRODUCTION

Herbal preparations are use in pregnancy problems to induce labour, removed retained placenta and in the management of post-partum bleeding [1]. Some of these preparations have side effects and can lead to the death of the unborn baby and/or uterine rupture, and other long-term effects on the mother or baby [2]. Biological effects generated by these preparations are due to biomolecules that act primarily on the uterus [3]. These actions may be the regulation of uterine contractions during labour, resulting in either stimulation (uterotonic) or inhibition (tocolytic) of myometrial muscle contractions [4]. flavonoids have effect on calcium availability for the contraction of smooth muscle and cardiac muscle while cardiac glycosides have effect on the uterus of animals [5,6]. Uterotonic plants are plants that stimulate uterine contraction. They are use to assist labour, remove retained placenta, treat post-partum bleeding, and as an abortifacient [1]. Substances that are called uterotonics are suggested to have laxative, purgative, diarrheagenic, cathartic, abortifacient, and emmenagogic effects [7]. Oxytocin as an uterotonics agent is biochemically synthesized hormones that act on distant hormone receptors or upstream from other hormones in the body to induce uterine contractions. Labour is facilitated by phasic myometrial contractions that are herd by the development of action potentials across the plasma membrane which result from a transient increase in the cytosolic free $Ca^{2+}$ concentration [4]. Calcium-dependent smooth muscle contractions are released from the sarcoplasmic reticulum (SR) and extracellular stores through voltage-gated calcium channels. Smooth muscle contractility by different agonists or by electrical depolarization which results in a rapid increase in $[Ca^{2+}]i$ [8]. When $Ca^{2+}$ binds to four binding sites of calmodulin, it causes a conformational change that allows the calmodulin-calcium complex to interact with inactive myosin light chain kinase (MLCK), to activate the enzyme [9]. MLCK phosphorylates the 20kDa myosin light chain (MLC). Phosphorylation of MLC causes the conformational changes in the myosin head that leads to activation of myosin ATPase which results in force generation and/or shortening (contraction) of the muscle fibres [10]. Dephosphorylation of MLC by MLC phosphatase (MLCP) results in smooth muscle relaxation [11]. Other physiological mechanisms that influence myometrial contraction include interactions with cell membrane receptors, peptides, metabolic and neuronal factors and hormones [12]. In Nigeria, the infusion of the leaves of *Sida acuta* Burm F. are use in folk medicine to induce labour. Therefore this work is aimed at investigation of *in-vitro* contractility effect of ethanol extract of *Sida acuta* leaves on isolated rat uterus

## MATERIALS AND METHODS

### Plant materials

The leaves of *Sida acuta* Burm F. (*Iseketu*) were collected from Nsukka in Nsukka Local Government Area of Enugu State, Nigeria. A taxonomist, Mr Alfred Ozioko of

Bioresouces Development and Conservation Programme (BDCP) Research Centre, University of Nigeria Nsukka taxonomically identified the plant and a voucher specimen "SAF-2013 of *Sida acuta* leaves was deposited at the Herbarium.

### Animals

Two pregnant Wistar albino rats were used for the study. The rats were obtained from the University of Nigeria Teaching Hospital (UNTH) Enugu, Nigeria. All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985), as expressed [13]. The study was approved by institutional animal ethical committee and the norms were observed strictly. The animals were acclimatized for two weeks under standard conditions with 12 hour light and dark conditions, and had free access to food and water until the end of the experiments.

### Chemicals

All chemicals used in this study were of analytical grade. They were products of May and Baker, England and Merck, Darmstadt, Germany.

### Phytochemical analysis

Preliminary phytochemical tests were carried out on the ethanol extract of *Sida acuta* using the methods of [14], [15] and [16]. The presence or absence of phytochemicals such as saponnins, flavonoids, alkaloids, tanninis, terpenoids, steroids, carbohydrates, hydrogen cyanide, cardiac glycosides, phenols and reducing sugars were observed.

### Determination of Uterotonic Activity

The uterine contractile activity was determined as described by [17]. The rats were killed by stunning and abdomens were cut open. The uterus were carefully isolated and transferred into dejalon solution which was continuosly bubbled with air and maintained at 37°C and 7.0 pH

**Reagent:** Physiological solution (Dejalon) containing 90 g NaCl, 42 ml KCl 10 % solution, 5 g $KH_2PO_4$ 10 % solution, 5 g glucose, 5 g $NaHCO_3$, 2.7 ml $CaCl_2M$ solution and $O_2$+5% $CO_2$ aerating gas.

**Procedure:** A known volume (50 ml) of Dejalon solution (physiological solution) was measured into the inner vessel of the organ bath. The temperature of the outer bath was set at 35° C and not exceeding 37° C. Fine stream of air bubbling through the solution in the inner bath was ensured. Then 2-3 cm segment of the uterus was isolated into a tissue plate containing physiological solution while aerating. The uterine tissue was prepared by making a tiny hook using a strand of thread in a needle to pierce the hollowed tissue from the inner wall and end with a rift-knot at both ends. Two thread strands were allowed on one end and one strand at the other end, while in aeration. The end was attached with two equal strands of thread to the neck of the aerating glass tube by knotting the thread at the point, and allowing the loop to hold the tissue only, taking it across the stem of the tube twice to secure it tightly. The lever was balanced with plasticized such that it moved with small increase in tension. The lever was adjusted to a horizontal position in the first instance. The tissue was held up gently and parallel with the aerating tube, the preparation was suspended with the neck of the tube downward into the inner vessel of the organ bath. The tissue end was secured with the long strand of thread by plasticized on a horizontal writing lever with the writing point resting on the drum. The preparation was allowed to rest in the solution with continuous aeration for 30 min at an ambient temperature. The Kymograph was set at the lowest speed 0.0 mm/s and a multiplier of 30 min and was also recorded for a normal spontaneous rhythm for 1 min. It was repeated continuously with time.

### Statistical Analysis

The data obtained from the laboratory tests were subjected to one-way analysis of variance (ANOVA). Differences between mean at $p \leq 0.05$ were accepted as significant. The results were expressed as mean ± stardand deviation (SD). The analysis was estimated using computer software known as statistical product and service solution (SPSS), version 21.

## RESULTS

Phytochemical analysis of the ethanol extract leaves of *Sida acuta* showed the presence of phytochemicals such as alkaloids, saponins, cardiac glycosides, steroids, flavonoids, terpenoids, Hydrogen Cyanides and phenol. Other constituents like resins, tannins, reducing sugars, carbonhydrates, proteins, fats and oil were absent. Table 1 and 2 shows the contraction of uterus for the various additions of drugs at different concentrations and time intervals. On the control rats' uterine contraction, normal rhthyme uterine contraction was taken before addition of 0.01iu oxytocin.

**Table1.** *In-vitro* Contractile Effects of *Sida acuta* Burm F. Leaves on Isolated Uterine Rats

| | FBC (µg/ml) | MeanBasal Amplitude (mm) | Amplitude Response (mm) | % Rise in Amplitude |
|---|---|---|---|---|
| Oxytocin | 0.20 | 1.20 ± 0.00 | 2.57 ± 0.05 | 114.17 |
| Acetylcholine | 0.40 | 1.20 ± 0.00 | 3.00 ± 0.04 | 150.00 |
| Adrenaline | 0.40 | 1.20 ± 0.00 | 2.40 ± 0.14 | 100.00 |
| Extract | 0.40 | 1.20 ± 0.00 | 2.30 ± 0.13 | 91.67 |

**Table2.** *In-vitro* Relaxation Effects of *Sida acuta* Burm F. Leaveas on Isolated Uterine Rats

| | FBC (µg/ml) | Basal Amplitude (mm) | Amplitude response (mm) | % Inhibition |
|---|---|---|---|---|
| Oxytocin and Indomethacin | 1.20 | 2.00 ±0.01 | 0.90 ±0.02 | 55.00 |
| Extract and Indomethacin | 1.40 | 1.20 ±0.03 | 0.70± 0.05 | 41.67 |
| Acetylcholine and Atropine | 0.80 | 2.40 ±0.06 | 1.60± 0.11 | 33.33 |
| Extract and Atropine | 1.00 | 2.10 ±0.04 | 1.30 ±0.10 | 38.10 |
| Adrenaline and Ergometrine | 0.60 | 2.30 ±0.05 | 1.10 ±0.02 | 52.17 |
| Extract and Ergometrine | 0.80 | 1.50 0.06 | 1.00 ±0.03 | 33.33 |

## DISCUSSION

The effect of ethanol extract of *Sida acuta* leaves on isolated uterine rat was able to produce rhythm and spontaneous contraction with uterotonic-like activity Table 1. It was able to activate the pathways that are involved in stimulation of uterine contraction. From the result, acetylcholine, oxytocin and adrenaline were the most potent agent that induced uterine contraction compared to ethanol extract of *Sida acuta* leaves. This is probably because acetylcholine, oxytocin and adrenaline are pure compounds [18] while the extract is crude. Oxytocin binds to the oxytocin receptor (OTR), a classical membrane receptor with seven transmembrane domains linked through a G-protein complex to a phospholipase C-protein kinase C signal transduction system [19]. After oxytocin stimulation, there are markedly increase intracellular concentration of inositol trisphosphate and Calcium ion ($Ca^{2+}$). The higher $Ca^{2+}$ and calmodulin increase the myosin light chain kinase which catalyses the contraction response. Oxytocin performs two actions in the uterus: Oxytocin stimulates uterine contractions by binding to myometrium oxytocin receptor (OT1a). It stimulates prostaglandin release by acting on oxytocin receptor (OT1b) at the endometrium [20]. The ability of the extract to produce uterine contraction is still unclear. Study showed that *Musanya cecropioides* stem back aqueous extract produce oxytocin-like effect towards uterine contraction through activation of muscariine receptor [21] unlike atropine, a muscariine receptor antagonist which did not block extract-induced contraction. Aqueous extract of *Ficus exasperate* leaves was able to stimulate uterine contraction via calcium channel, histamine (HI) receptor and also activation of α1-adrenergic receptors [22]. Different pathways involved in stimulation of uterine contraction by uterotonic plants are dependent on the active compounds present in the plants. The response rhythm of the extract-induced uterine contraction in the presence of oxytocin proved that ethanol extract of *Sida acuta* leaves has the potentiating effect. The *Sida acuta* extract used in this study probably enhanced the binding of the oxytocin to OTR on myometrium tissues, thereby producing the observed greater response of uterine contraction. Study showed that the aqueous extract of *Globimetula Braunii* (Loranthaceae) has similar potentiating effects [18]. Addition of increasing concentrations of oxytocin in the presence of 80 mg $ml^{-1}$ aqueous extract of *Globimetula braunii* produced significant highest uterine contraction response compared to oxytocin and extract alone which is similar to the response stimulated by *Sida acuta* extract. [23] Suggestively justify that *Uvariodendron anisatum verdec* has motility effects on isolated rats use by traditional attendants for assisting labour and also removal of after birth if it get retained which are in accordance with this study.

## CONCLUSION

This study suggested, the ethanol extract of *Sida acuta* leaves possesses oxytocin effects but mighty portends danger among pregnant woman who take the leaf infusion to induce labour.

Probably, the investigation substantiates its use in hastening of labour.

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## CONFLICT OF INTEREST STATEMENT

Authors explained, there are no competing interests for the study.

## REFERENCES

1. Sewran V, Raynor MW, Raidoo DM, Mulholland DA. Coupling SFE to uterotonic bioassay: an on-line approach to analyzing medicinal plants. Int J Pharm and Biomed Anal 1998; 18: 451-55.
2. Martinez GJ. Traditional practices, beliefs and uses of medicinal plants in relation to maternal-baby health of Criollo woman in Central Agentina. J Midwif 2008; 24: 490-502.
3. Gruber CW and O'Brien M. Uterotonic plants and their bioactive constituents. Advance online publication. 2010; DOI http://dx.doi.org/10.1055/s-0030-1250317.
4. Young RC. Myocytes, myometrium, and uterine contractions. J Aca Sci 2007; 1101: 72-84.
5. Catherine KK, Jemimah AO, Titus K. Preliminary investigation of contractile activity of *Ricinus communis* and *Euclea divinorum* extracts on isolated rabbit uterine strips. J ethnopharmacol 2012; 142:496-502
6. Omodamiro OD, Ohaeri OC, Nweke IN. Oxytocic effect of aqueous, ethanolic n-hexane and chloroform extracts of *Xylopia aethiopica* (annonaceae) and *Ocimum gratissium* (Labiatae) on guinea pig uterus. Asian J Plt Sci and Res 2012; 2: 73-78

7. Lopez BA. Preterm labour: mechanisms and management. BMC J Preg Childbirth 2007; 7: 1- 2.
8. Somlyo AP, Somlyo AV. Smooth muscle structure and function. In: Fozzard HA (ed). The heart and cardiovascular system. Raven Press; New York, pp 1295–1324, 1991.
9. Olson NJ, Pearson RB, Needleman DS, Hurwitz MY, Kemp BE Means AR. Regulatory and structural motifs of chicken gizzard myosin light chain kinase. PNAS 1990;87:2284–88.
10. Stull JT, Tansey MG, Word R A, Kubota Y, Kamm KE. Myosin light chian kinase phosphorylation: regulation of the $Ca^{2+}$ sensitivity of contractile elements. J Adv Exp Med and Biol 1991; 304:129-38.
11. Sakurada K, Seto M, Sasaki Y. Dynamics of myosin light chain phosphorylation at Ser19 and Thr18 Ser19 in smooth muscle cells in culture. American J Physiol 1998; 274:1563–1572.
12. Sanborn BM. Ion channels and the control of myometrial electrical activity. J Sem Perinatol 1995; 19:31–40.
13. Sofowora A. Medicinal plant in Nigeria, University of Ibadan Press, Ibadan, Nigeria, pp 224 – 226, 1993
14. Harborne JB. Phythochemical Methods: A Guide to modern technology of plants analysis. (3rd Edition). Champman and Hall, New York, pp 188-185, 1998.
15. Trease GE Evans MC. A Textbook of Pharmacognosy. 13th Edition. Bailiere. Tindall, pp 700-775, 1996.
16. Uchendu CN. Role of $Ca^{2+}$ on the uterine force stimulated by glycoside from the root of *Dalbergia saxatilis*. Indian J physiol and pharmacol 1999; 43 (2): 171-178.
17. Ie O, Zam N. Oxytocin properties of the aqueous extract of *Globimetula braunii* (Loranthaceae). Parkistan. J pharmacol Sci 2008; 21: 356-360.
18. Phaneuf S, Europe-Finner GN, Varney M, MacKenzie IZ, Watson FP Lopez BA. Oxytocin-stimulated phosphoinositide hydrolysis in human myometrial cells: involvement of pertussis toxin-sensitive and in-sensitive G-proteins. J Endocrinol 1993; 136:497–509.
19. Dawood MY. Pharmacologic stimulation of uterine contraction. J Sem Perinatol 1995; 19: 73-83.
20. Ayinde BA, Onwukaeme DN Nworgu ZAM. Oxytocin effect of the water extract of *muscanya aeropioides* R. Brown (moraceae) stem bark. Afri J Biotechnol 2006; 5: 1350-1354.
21. Misonge JO, Ogeto JO, Sengera,GO, Mwalukumbi JM, Mwaura AM Juma SD. Evaluation of phytochemistry and uterotonic activity of root aqueous extract of Uvariodendron anisatum verdec used in childbirth in Eastern/Central Kenya. J Pharm 2014; 4 (12): 48-53


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