Lwiindi L, Goma F, Mushabati F, Prashar L, Choongo K. (January 2015). Physiological response of uterine muscle to Steganoteania araliacea in rat models. *Jour of Med Sc & Tech*; 4(1); Page No: 40 – 45.

RA Publications
Journal of Medical Science & Technology

Original Article **Open Access**

Physiological response of uterine muscle to *Steganoteania araliacea* in rat models

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Abstract

The bark root of *Steganotaenia araliacea Hochst* (Umbelliferae) nicknamed "herbal pitocin" is used by traditional circle in Zambia to induce labour in pregnant women. This work was aimed at investigating the contractile stimulatory effects of the aqueous extracts of *Steganotaenia araliacea* (SAE[a]) on isolated smooth muscle preparations of the rat uterus to explain its reported local use. Objective of the study was to determine the physiological effect of SAE[a] root extract (nicknamed "herbal Pitocin'') on pregnant and non-pregnant uterine muscle. This work examined the effect of the aqueous extract of SAE[a] extract on rat uterus pre-treated with 1 mg/kg stilboesterol for 24 h and also on the pregnant rat uterus. The effects of reference agonists like oxytocin (OT) and Acetylcholine (Ach) were used and also antagonists like atropine (AT), indomethacin (Indo) and Salbutamol (SBM) on the uterine contractile effect of the extract were investigated. *In vitro* studies of SAE[a] on uterine tissue showed contractile activity which was dose dependant similar to OT and Ach while pre-treating the tissue with atropine, indomethacin or Salbutamol before administering the extract showed the inhibitory effects of the drug Salbutamol on the activity of the extract. The inhibition of contractile effect of the crude aqueous extract of *Steganotaenia araliacea* shown by Salbutamol ($p < 0.05$) suggests the probable stimulation of the Oxytocin-like receptors of the uterus by the extract. These physiological finding justify the traditional use of the plant for its uterotonic properties.

Keywords: *Steganotaenia araliacea*, uterine, Oxytocin, Atropine, Salbutamol

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Received: November 12, 2014 Accepted: December 25, 2014. Published: January 20, 2015.

Introduction

Plants have a long history of use on the African continent for the treatment of different diseases and complaints. In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicine [1]. Herbs have been reported to be the most widely used in traditional medicine [2]. Traditional healers and their plant medicines provide the only health care to the majority of the people in a curative rather than a preventive approach in the developing countries for common ailments [3].

The use of plant remedies is also documented in native Northern American, where herbal medicines are taken as tonics during pregnancy to prepare for labour (e.g., raspberry leaf, patridge berry and stinging nettle), to prevent miscarriage (e.g., black haw and false unicorn) and to induce labour (blue cohosh, black cohosh and beth root) [4]. Other traditionally used medicines, such as raspberry leaves (*Rubus idaeus* L.), Castor oil (*Ricinus communis L*) and cotton bark root (*Gossypium hirsutum L*) are again receiving attention from midwives for application during pregnancy and labour [5, 6-7]. The efficacy of a medicinal plant depends on the

preparation of the plant material for consumption and/or remedy for various diseases. However, if the method of preparation and intake of the plant material is wrongly applied, the desired result may not be achieved. [8]

It has been currently considered that herbal medication commonly known as herbal "pitocin" is uterotonic and has been used by some Zambian women in traditional circle in inducing and/or augmenting labour. There have been observation and reports made by health workers and traditional birth attendants that these herbal medications cause very strong contractions such that in some of the women with severe obstructing of labour it has been reported to cause uterine rupture (personal communication from midwives at UTH).

There is a vast array of information available on traditionally used herbs to treat gynaecological problems. However most of the studies that revealed uterotonic active plant compounds and their mode of action were conducted outside Zambia. Currently, no study has been carried out in Zambia to evaluate the stimulatory effect of this herbal "Pitocin" that is used by traditional communities on uterine activity.

In view of the above, this study aims at examining the potential of the aqueous back root extract of *steganoteania araliacea* (nicknamed "herbal Pitocin") in stimulating the isolated uterus using animal models.

Materials and Methods

Plant material

The fresh leaves and roots of *Steganotenia araliacea Hochst were* collected from the traditional birth attendants (TBA's) and knowledgeable local people in Chongwe, Mumbwa and Monze Townships of Zambia. Identification and authentication of the plant was done at The University of Zambia (UNZA), School of Natural Sciences, in the Herbarium section where a herbarium sample (voucher number of *LL2*) was prepared and deposited. A specimen voucher was also deposited in the Physiological Sciences department.

Fresh roots of *steganoteania araliacea* were washed clean, rendered free of adulterants and the earth remains or ground soil. From these the fresh barks were divested, chopped into bits and blended into fine semi-liquid paste using a LOGIK electric blender Model RSH-245611-018, and stored in air-tight containers. A 37.2 g quantity of the semi-liquid pest was mixed with 0.5 liters of distilled water and heated to boiling for 7 minutes. The infusion was centrifuged at 2500 rev/min for 10 minute using a centrifuge machine (type: 05p-21), and the supernatant filtered with Toyo No.2 filter paper (15 cm) to obtain a brown filtrate. The filtrate was concentrated using a laboratory hot plate (Model No: 13474) and later in a hot air drying oven (Model: DG-81) at 60°C to complete dryness until a constant weight was obtained to yield dark - brown solid extract which was named SAE[a]

Animals

Three sets of 6 healthy adult gravid female rats with 17-19 days of gestational period and non-gravid female rats (ranging from160-200g) were selected and housed in the animal unit of the Department of biomedical sciences, University of Zambia. The animals were maintained according to standard nutritional and environmental conditions and they had free access to standard diet (Bendel Feeds and Flour Mill,) and water ad libitum. Animal studies were conducted according to standard guidelines for use of laboratory animals [9].

Drug(s) : Acetylcholine Chloride (0.1mg/ml) and oxytocin (10 IU/ml) were used as reference agonist drugs with their corresponding antagonists; atropine (0.6mg/ml), and indomethacin (1 mg/ml) or Salbutamol (0.8mg/ml) respectively and Stilbestrol (0.1mg/ml) was used to induce oestrus in a non-gravid rat [10].

Extraction (Isolation) and Mounting of Isolated Rat Uterus:

Briefly, a gravid rat (Spraguen Dawley) was sacrificed by cervical dislocation (decapitation) method. The head was quickly severed from the trunk and the stomach opened to expose the internal organs. The two uterine horns were identified dissected out and transferred to a dish containing De Jalon's physiological solution. The two horns were separated and freed from fat and connective tissues and each horn was cut open longitudinally so that the uterine horn was now a sheet of muscle instead of a narrow tube. Each horn was further divided longitudinally to obtain four pieces. A strip of the horn about 2 - 3 cm was cut out. A thread was then attached to one end of the isolated strip of uterus and was tied to the aerator tube in the organ bath containing 25 ml De Jalon's physiological solutions. Another thread was attached to the other end of the isolated uterus and fixed to a lever system fitted on the transducer. The tissue was aerated with ordinary air using an aquarium air pump (Model No: 9905). The temperature of the organ bath was maintained at

32-33°C and the isolated strip of uterus was allowed to stay in the De Jalon's physiological solution for thirty minutes to one hour before use.

In another experiment, a non-gravid rat uterus was isolated (procedure as above) but with earlier injection of stilboestrol (0.1 mg/kg, S.C.) 24 h before the rat was euthanized, dissected, and the uterine horns removed.

Experimental Design

Effects of Acetylcholine (0.1 mg/ml), oxytocin (10 IU/ml) and SAE[a] (12.4mg/ml) were investigated on the mounted isolated pregnant and non-pregnant uterus and drugs were washed off the preparation using the overflow method and also the effects in the presence of atropine (1.8×10^{-4} mg/ml), and indomethacin (0.036 mg/ml) or Salbutamol(0.0096mg/ml) were obtained, in an attempt to establish the possible mechanism(s) of action(s) of the plant extracts from the observed response(s).The contractions of the longitudinal muscle of the isolated rat uterus were recorded on the graph displayed on lab chart on the computer revolving at a rate of 1k: 1, mm per minute. Responses to addition of standard drugs and extracts were recorded by a micro-dynamometer fitted to a transducer (Model: MLT0210/A). After each drug or extract addition, the tissue was washed three times with fresh physiological salt solution and allowed appropriate time to recover before subsequent additions of drugs or extracts.

Statistical Analysis: All values were expressed as the mean ± SEM (standard error of the mean) and **n** represents the number of rats from which uterine segments were obtained. The levels of significance were made using one-way ANOVA with Dunnettis Multiple Comparison Test (SPSS Version 16.0) and value of $P < 0.05$ (Significant) was considered in all cases. SAE[a] was used in all the experiments in this thesis because all the reported local uses involve aqueous preparations.

Results

Effect of SAE[a] on amplitude and frequency of spontaneous uterine contractions

- **On amplitude and frequency of spontaneous uterine contractions.**

Addition of non-cumulative concentration of SAE[a] (Figure 2) increased both the amplitude and frequency (Table 1) of uterine contractions when compared to those produced at baseline (see Figure 1).

Table 1: Effect of SAE[a] (12.4mg/ml) on amplitude and frequency of spontaneous uterine contractions. KEY: 1, 2, 3, 4 and 5 describe the frequency of Uterine Contractions and the amplitude describes the strength of contractions (mN). **NB**: Non-cumulative concentrations of SAE[a] increased both the amplitude (strength) and frequency of uterine contractions in a dose dependent manner.

Bath Concentrations of SAE[a] (mg/ml)	Frequency	Amplitude (mN)
0.11	1	0.5271
0.22	2	1.115
0.44	3	1.471
0.88	4	2.151
1.76	>5	2.1509

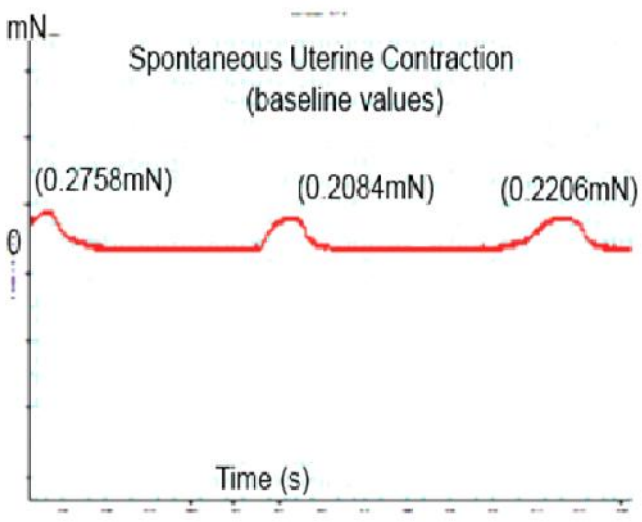

Figure 1: Tracing showing spontaneous uterine contraction (baseline values) on the isolated non-pregnant estrogenized rat uterus.

- **Effect of Ach, OT and SAE[a] on Isolated rat Uterus**

SAE[a] produced an increase in frequency and amplitude of myometrial contractions which were dose - dependent on the isolated non - pregnant estrogenized rat uterus preparations similar to those produced by acetylcholine and oxytocin. See Figure 3

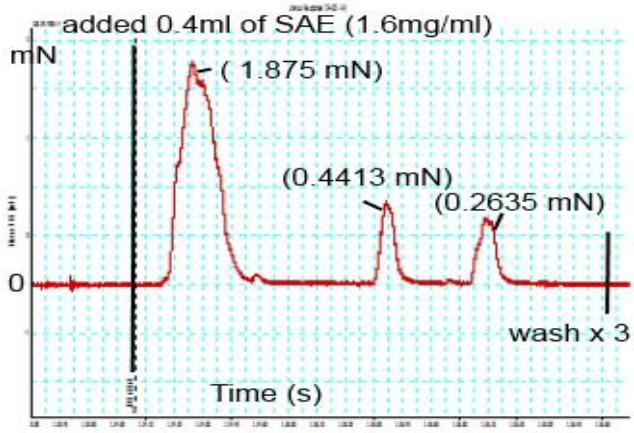

Figure 2: Tracing showing uterine contraction following administration of of SAE[a] (1.6mg/ml) on the isolated non-pregnant estrogenized rat uterus.

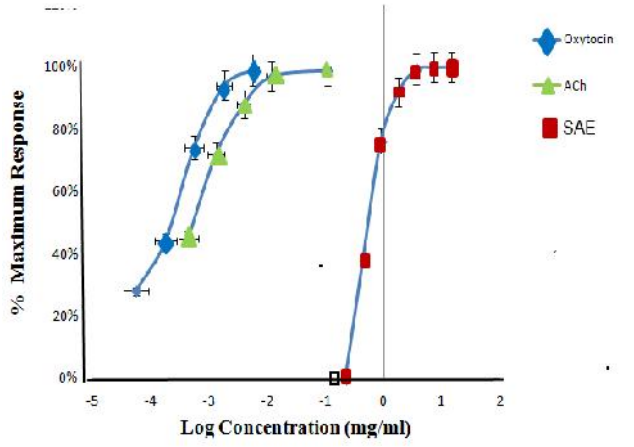

Figure 3: Log dose-response curves of oxytocin (5.5 $x10^{-5}$ — 5.5x 10^{-3} IU/ml), acetylcholine (4.4 x 10^{-4} — 0.107 mg/ml) and SAE[a] (0.11—14.32mg/ml) on the isolated non-pregnant rat uterus. Each point is the mean ± sem (n = 3).

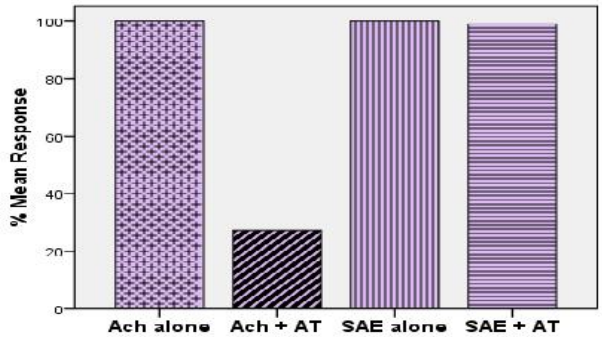

Figure 4: Effect of ACh (4.8 x 10^{-4} mg/ml) and SAE[a] (0.2 mg/ml) in the presence of AT (1.8 x 10^{-4} mg/ml) on the isolated pregnant rat uterus. Each Each column represents the mean ± sem (n = 3). Inhibitory responses are shown by P < 0.05 (significant)

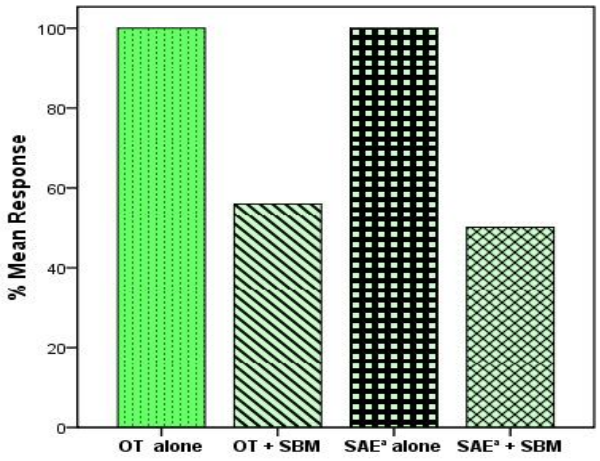

Figure 5: Effect of oxytocin (0.008 IU/ml) and SAE[a] (1.04 mg/ml) in the presence of Salbutamol (0.0096 mg/ml) on the pregnant rat uterus. Inhibitions of responses are shown by P < 0.05 (significant). Each column is the Mean ± SEM (n = 3).

- **Effect of Atropine on contractions of the isolated rat uterus produced by SAE[a] and acetylcholine (Ach).**

Atropine physiologically inhibited the myometrial contractions produced by acetylcholine by 72.8% (P < 0.001) and the same dose of atropine inhibited SAE[a] by 1 % (P > 0.05). See Fig 4.

- **Effect of Salbutamol On the Contractile Responses of the Isolated Rat Uterus to SAE[a] and Oxytocin**

Salbutamol (0.0096mg/ml) inhibited the effect of oxytocin by 50 % (P < 0.05). The same dose of Salbutamol inhibited the effect of SAE[a] by 44 % (P < 0.05). See Figure 5

Discussion and Conclusion

To the best of our knowledge, this study is the first to document in-vitro uterotonic effects of *Steganotaenia araliacea Hochst*, which justifies the claim that this plant assists in uterine contraction. From our experimental studies, SAE[a] was able to stimulate the isolated uterine smooth muscle suggesting its uterotonic activity on the uterine tissue. Following binding of oxytocin to its G protein-coupled receptor, phospholipase C (PLC) will be activated which causes an increase in inositol trisphosphate (IP_3) and diacylglycerol (DAG) levels. IP3 activates the IP3R receptor at the sarcoplasmic

reticulum membrane which causes the release of stored Ca^{2+} into the cytosol. Increased cytosolic Ca^{2+} will further induced extracellular Ca^{2+} influx [11], resulting in a further rise in the intracellular Ca^{2+} level. Ca^{2+} will then binds to calmodulin, which activates the myosin light chain kinase leading to phosphorylation of myosin light chains, triggering contraction. [12] Activation of beta-2 (β_2) and β_3 adrenergic receptors increases intracellular cAMP via G_s-mediated activation of adenylate cyclase which mediates production of cAMP from ATP. cAMP results in cell relaxation in many ways, including inhibition of MLCK and the efflux of $[Ca^{2+}]_i$ through sodium/calcium (Na^+/Ca^{2+}) exchanger channels. Chloride (Cl^-) channels, which might be activated by oxytocin, exert their uterotonic effect by depolarisation of the smooth muscle cell membrane [13].

In this study, the involvement of oxytocinergic receptors in myometrial contraction was investigated following administration salbutamol. Our findings indicate that SAE^a-induced uterine contraction depends mostly on oxytocin-like receptors and this was confirmed by Salbutamol (selective β2 - adrenoceptor agonists) which effectively inhibited oxytocin 50% ($P<0.05$), similarly inhibited the effect of SAE^a by 44% ($P<0.05$) on the pregnant rat uterus preparation. Salbutamol is not a specific antagonist for oxytocin [14]; however its ability to inhibit both SAE^a and oxytocin only suggests that SAE^a and oxytocin could be acting via similar receptor sites.

From these results, SAE^a has shown to interact with myometrial oxytocinergic receptors which stimulates the production of the second messenger D-myoinositol 1,4,5-triphosphate (IP_3), the latter through the action of the enzyme phosphoinositidase C (coupled to the oxytocin receptor by a stimulatory G-protein [G_s]), on the plasma membrane constituent phosphatidyl-inositol 4,5-bisphosphate (PIP_2). IP_3 releases Ca^{2+} from the sarcoplasmic reticulum (SR) thus increasing $[Ca^{2+}]_i$ and resulting in cell contraction.

SAE^a produced dose – dependent myometrial contractions on both the pregnant and non – pregnant isolated rat uterus preparations similar to the effects of oxytocin and acetylcholine, indicating non - specific activity on both the isolated pregnant and non-pregnant rat uterus preparations, although the responses were higher on pregnant rat uterine tissue, because in late pregnancy, the uterus becomes very sensitive to oxytocin coincident with a marked increase in the number of oxytocin receptors and oxytocin receptor mRNA [15]. In most preparations, phasic myometrial contractions produced by SAE^a were developed at lower concentrations (0.22-0.44 mg/ml) while a stronger tonic contraction was usually obtained with a final bath concentration of 1.76 mg/ml.

Our findings also suggested that SAE^a - induced uterine contractions were not mediated mainly via the acetylcholine receptor as evidenced by the lack of inhibition of these responses (Amplitude) by atropine. The cumulative non-inhibitory effect observed following concomitant administration of atropine ($0.4x10^{-3}$mg/ml) on the pregnant and non - pregnant rat uterus preparations ($P>0.05$), confirmed the lack of involvement of muscarinic receptors in mediating SAE^a-induced uterine contraction meanwhile, the same concentration of atropine was able to antagonize ($P<0.05$) acetylcholine-induced myometrial contractions (a muscarinic receptor agonist). Indomethacin (prostaglandin synthetase inhibitors) also could not modify contractions produced by oxytocin and SAE^a in this research which suggests it may not be linked to prostaglandin biosynthesis.

The observation in this research has shown that SAE^a could have been exerting some of its myometrial contractile effects on isolated pregnant and non - pregnant rat uterus preparations via Oxytocinergic receptors.

Acknowledgments: The authors would like to thank the University of Zambia for providing financial support by the Welcome Trust. This document is an output from a project funded by The Welcome Trust through SACORE for capacity building for the benefit of developing countries. The views expressed are not necessarily those of the Trust.

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