PHYSIOLOGICAL RESPONSE OF UTERINE MUSCLE TO AQUEOUS ROOT EXTRACT OF *STEGANOTEANIA ARALIACEA HOCHST* (HERBAL''PITOCIN'') IN RATS

By

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LUSAKA

DECLARATION

The experimental work described in this thesis was carried out at The University of Zambia, School of Medicine in the Department of Physiological Sciences. This work has not been submitted for any other degree and therefore I **Lukubi Lwiindi** do hereby declare that this dissertation has been composed by myself, that it has not been accepted in any previous application for a degree, that the work of which it is a record has been done by me and that all sources of information have been specifically acknowledged by means of references.

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Dr. Lukubi Lwiindi, BVM.

CERTIFICATE OF COMPLETION OF DISSERTATION

I, **DR. FASTONE MATHEW GOMA**, having supervised and read this thesis is satisfied that this is the original work of the author under whose name it is being presented. I confirm that the work has been completed satisfactorily and is ready for presentation to the examiners.

Supervisor' signature……Date……

Head of Department

Signature……

Date……..

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CERTIFICATE OF APPROVAL

This thesis by **LUKUBI LWIINDI** has been approved as fulfilling the requirements for the award of the degree of Masters of Science in Human Physiology by the University of Zambia.

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Supervisor Signature

Examiner I ---------- Sign ---------- Date ----------

Examiner II ---------- Sign ---------- Date ----------

Examiner III ---------- Sign ---------- Date ----------

ABSTRACT

Background: The bark root of *Steganotaenia araliacea Hochst (*Umbelliferae) nicknamed "herbal pitocin" is used in the traditional circles in Zambia to induce and/or enhance labour. 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.

Objectives: To determine the physiological effect of SAE[a] on isolated pregnant and non-pregnant uterine muscle.

Methods: A gravid/non-gravid rat was sacrificed by cervical dislocation (decapitation) method and the abdomen 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. Acetylcholine (Ach) and Oxytocin (OT) were used as reference agonist with their corresponding antagonists; atropine (AT), and indomethacin and salbutamol (SBM) respectively. The effect of these reference agonists with their corresponding antagonist and that of aqueous extract of SAE on non-pregnant rat uterus pre-treated with 1 mg/kg stilboesterol for 24 h and on the pregnant rat uterus were investigated.

Results: *In vitro* studies of SAE[a] on uterine tissue showed contractile (uterotonic) activity on both isolated gravid and estrogenized non-gravid rat uterus at the concentration shown in this study. SAE [a] significantly increased the amplitude(from baseline value of 0.1471mN to 0.3003mN) and frequency of spontaneous uterine contractions. However, SBM significantly inhibited ($p < 0.05$) the frequency and amplitude of spontaneous uterine contractions on the isolated pregnant and non – pregnant rat uterus preparations but atropine and indomethacin could not modify the contractions produced by SAE[a], hence indicating a non-muscarinic and non-prostaglandin biosynthesis dependant respectively.

Conclusion: The inhibition of contractile effect of the crude aqueous extract of SAE[a] shown by Salbutamol ($p < 0.05$) suggests the probable stimulation of the Oxytocinergic 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,

DEDICATION

This Thesis is dedicated to:

My late mother, Florina Muloongo Lwiindi,

For the love, support and understanding she gave me (MHSRP).

My Wife and Daughter,

For believing in me

My Father, brothers and sisters

For making everything worthwhile

My friends

For being there for me

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INTRODUCTION

1.0 Overview

Several drugs are currently used to assist in the therapeutic induction and augmentation of labor. Synthetic oxytocin, the simulation of the natural hormone produced from the posterior pituitary, is widely used to induce and augment labour. Several synthetic forms of oxytocin have been used by the intravenous route for this purpose. Recently, the prostaglandins (F_2 and E_2) have also been used to induce and augment labour and for cervical ripening (Lippincott, 2005). Traditional health practitioners have likewise used herbal preparations for similar purposes.

Traditional medicines rely on the use of certain herbal plants and/or other remedies for beneficial effects during pregnancy, to induce labour, in the removal of retained products of conception and management of post-partum bleeding. However, some of these medicines have harmful side effects and when taken in large quantities can lead to the death of the unborn baby and /or uterine rupture, and other longer term effects on the mother or baby (Christian and Margaret, 2010).

1.1 Global Perspective

About 25 % of prescription drugs dispensed in the USA contain at least one active ingredient derived from plant material, or made from plant extracts, while others are synthesized to mimic a natural plant compound (Boye, 2010). The desire to exert more responsibility and control over one's body and life-style has led to resurgence in self - care practices. This self - treatment frequently reflects health-care practices influenced by folk remedies and the use of medicinal plants for maintaining health and treating common diseases. Most often, the biological effects elicited by these remedies are due to biomolecules (small molecules, peptide, and protein) that primarily act on the uterus. The nature of these actions may involve the modulation of uterine contraction at labour, resulting in either the stimulation ("uterotonic) or inhibition ("tocolytic") of myometrial muscle contraction (Christian and Margaret, 2010). 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) (Westfall,2001). Other traditionally used medicines, such as raspberry leaves (*Rubus idaeus* L.) (Whitehouse, 1941) castor oil (*Ricinus communis L*) (Kelly et al, 2001) and cotton bark root (*Gossypium hirsutum L*) (Nissim, 1996) are again receiving attention from midwives for application during pregnancy and labour (Born and Barron, 2005).

1.2 African perspective

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 (Hostettmann, 2000). Herbs have been reported to be the most widely used in traditional medicine (Orwa, 2002). Much of the medicinal use of plants seems to have been developed through observations of wild animal's uneventful use of herbs as food and also by trial and error (Boye, 2010). 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 (Gabriel *et al,* 2007). *Heliotropium indicum Linn* is used extensively among villagers and some urban dwellers in Ashanti, Ghana, in the treatment of various disease conditions (Irvine, 1930). Some of the uses include the external application of the poultice of the leaves to sores. In a study that was done by Agyare (2005), in the investigation of antimicrobial and uterine smooth muscle activities of *Albizia ferruginea* extracts in Ghana, reported that an ethyl alcohol extract of *A. ferruginea* is significantly active against bacteria (Gram positive and Gram negative) and on the fungi studied. It also has contractile effect on uterine smooth muscle (non-pregnant and pregnant) and hence should not be used by pregnant women.

The efficacy of a medicinal plant depends on the preparation of the plant material for consumption and/or remedy for various diseases. Some are taken raw, cooked, roasted, and applied as topical, while some have to go through a process of extract preparation before use. All the various methods and root of intake is to enhance the bioavailability

and hence, the efficacy of the plant material. However, if the method of preparation and intake of the plant material is wrongly applied, the desired result may not be achieved (Odey et al, 2012).

1.3 National perspective

In Zambia, herbal "pitocin" has been used by some women in traditional circles 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 herbal "Pitocin" extract in stimulating the isolated uterus using animal models.

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 (Kim et al, 2010). Oxytocin receptors have been identified in a variety of neural tissues, including the ventromedial hypothalamus (VMH), bed nucleus of the stria terminalis (BNST), central amygdala, anterior olfactory nucleus, lateral septum, ventral subiculum, and dorsal motor nucleus of the vagus (Freund et al, 1987). The amount of oxytocin in plasma is normal at the onset of labor. It is possible that the marked increase in oxytocin receptors at this time causes normal oxytocin levels to initiate contractions, setting up a positive feedback (Kim et al, 2010). Several aspects of oxytocin's action appear to be influenced by steroid hormones, including estrogen, progesterone, and the androgens. Both the gene for oxytocin and oxytocin receptor induction may be regulated by steroid hormones (Gainer et at. 1988). The best evidence indicates that oxytocin is important in the expulsive phase of labor in some species (for example,

rats); therefore, the timing of exogenous oxytocin administration is likely important (Heather, 2012). Using the rat as a model, factors responsible for the release of oxytocin have been studied extensively in the analysis of mechanisms underlying parturition (Fuchs, 1982).

Myometrial contraction is influenced by a variety of physiological mechanisms involving intracellular signaling, calcium, ion channels, cell membrane receptor peptides metabolites and neural factors and hormone (see appendix 6.4) (Sanborn, 1995). Many of the physiological pathways that mediate myometrial contractility and relaxation have been elucidated over the past 10–15 years and, although the mechanism of labour onset is reasonably well understood in mammalian and non-human primate systems, the precise sequence of events in people is unclear (Mitchell, 1984).

Oxytocin is known to act both directly and indirectly to stimulate smooth muscle contraction and it is widely used for artificial induction of labour (Owen and Hauth, 1992). The National women's health clinical Guidelines/Recommended best practice (2008) report shows that Oxytocin (syntocinon) augmentation is the mainstay of the pharmacological treatment for dysfunctional labour or to achieve induction of labour. The objective is to produce uterine contractions that effectively produce cervical change and descent of the presenting part.

Oxytocin is produced primarily in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus (Swanson et al, 1983). Oxytocin is a Nanopeptide characterized by a six amino acid ring structure with a three amino acid tail (Gainer et at, 1988). Arginine vasopressin (AVP) and oxytocin are closely related small peptides, each consisting of nine amino acid residues. Although AVP and oxytocin differ by only two amino acid residues, the structural differences are sufficient to give these two molecules very different hormonal activities. They are similar enough, however, for AVP to have slight oxytocic activity and for oxytocin to have slight antidiuretic activity (Lippincolt, 2005). Oxytocin and vasopressin are secreted into the circulation in conjunction with their carder proteins (neurophysins) (Gainer et at, 1988). In humans, oxytocin acts primarily on the breasts and uterus, though it appears to be involved in luteolysis as well. Oxytocin causes contraction of the smooth muscle of the uterus. The sensitivity of the uterine musculature to oxytocin is enhanced by estrogen and inhibited

by progesterone (Kim et al. 2010). The uterine myometrium contains receptors specific to oxytocin (Blakemore and Petrie, 1988). The inhibitory effect of progesterone is due to a direct action of the steroid on uterine oxytocin receptors (Kim et al. 2010). At the end of gestation, the uterus must contract vigorously and for a prolonged period in order to deliver the fetus. During the later stages of gestation, there is an increase in abundance of oxytocin receptors on uterine smooth muscle cells, which is associated with increased "irritability" of the uterus. Oxytocin secretion is increased during labour. After dilation of the cervix, descent of the fetus down the birth canal initiates impulses in the afferent nerves that are relayed to the supraoptic and paraventricular nuclei, causing secretion of sufficient oxytocin to enhance labour (ibid, 2010). In cases where uterine contractions are not sufficient to complete delivery, physicians and veterinarians sometimes administer oxytocin ("pitocin") to further stimulate uterine contractions and great care must be exercised in such situations to assure that the fetus can indeed be delivered and to avoid rupture of the uterus.

1.4 STATEMENT OF THE PROBLEM

Despite wide reported use of herbal medicine to induce and/or augment labour in Zambian communities, there is not much documentation in the available literature on their local use. However there are reports on the use of these, often with catastrophic results. There is no in-vitro evidence to validate the reported uterotonic effects of these herbal preparations. Furthermore, these reported “oxytocic” preparations are orally administered in contrast to the existing remedies which are mostly for parental use. This study is aimed at investigating the in-vitro influence of the herbal preparation on rat uterine muscle and also to elucidate possible physiological mechanistic pathways.

1.5 JUSTIFICATION

Information on physiological effect of *Steganoteania araliacea* in induction of labour would be useful as there are health and cost implications in the choice of therapy to use in management of labour. Data so obtained can be useful epidemiologically for the Obstetricians and Gynecologists, and may be a basis for further medical studies in order

to explore on the other physiological effects of *Steganoteania araliacea*. Documentation of traditional knowledge of medicinal plants is crucial, since it provides physiologists, chemists and pharmacologists with starting point for "targeted" analysis, discovery of natural drugs for treatment of various conditions including, pregnancy and childbirth-related problems.

The general public can be educated on the precautions to be taken when using *steganoteania araliacea* (Fyopola) and other Herbal plants, especially during pregnancy, in order to reduce on the risks of uterine ruptures and/ or other effects that are been reported among women who use *araliacea* (Fyopola).

1.6 GENERAL OBJECTIVE:

To determine the physiological effects of *steganoteania araliacea* aqueous root extract (Herbal ''Pitocin'') on isolated uterine muscle.

1.6.1 SPECIFIC OBJECTIVES:

1. To investigate the reported uterotonic activity of *steganoteania araliacea* aqueous root extract (Herbal ''Pitocin'') using the isolated rat uterus.
2. To identify the possible mechanism of action activated by *steganoteania araliacea* aqueous root extract on the rat uterine muscle.

Chapter 2

LITERATURE REVIEW

2.0 THE PLANT STEGANOTAENIA ARALIACEA HOCHST

*Steganotaenia Araliacea Hochst (*Family *Umbelliferae)* is a medicinal plant and has the following vernacular names in Zambia:

Chewa: Fyopola

Luvale: Mumono yakumapili

2.1 Morphology of Steganotaenia Araliacea Hochst

Steganotaenia araliacea is a small savannah tree 2-7 m tall. Bark is yellow green or grey, rather waxy and peeling off in papery strips or rectangles. Leaves are pinnate, crowded towards branch ends, aromatic; leaflets 2-3 pairs on a leaf stalk about 10 cm long with an expanded base around the stem, ovate, to 5 cm, sometimes stalked, margin toothed. Flowers are small, green-white, in rounded compound clusters at twig ends. 3-7 long stalks arise together; each further bears a crown of small heads (umbels) about 8 cm across. Stamens are longer than petals in male flowers. Fruit cream-brown, dehiscent, flat and heart shaped to 12 mm, winged each side with 3 ribs. The generic name is likely based on Greek 'stegnas' meaning covered and the Latin 'taenia' meaning band (*Orwa et al.2009).*

2.2 Ecology and Documented species Distribution

S. araliacea occurs over a wide range of altitude, but is abundant in low-altitude woodland or on rocky outcrops.

It is a native plant in the following countries: Angola, Benin, Botswana, Democratic Republic of Congo, Ethiopia, Kenya, Mozambique, Namibia, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe (*Orwa et al.2009).*

2.3 Folk Uses

2.3.1 Other medicinal Uses in Africa

The roots are used in treating snake bites and the tree trunk reported to have snake deterring activity, leaves are rubbed on wounds as a general disinfectant. Roots and bark are used to cure sore throat. Bark is chewed for fever. Twigs are used in dental care as toothbrushes and bark used in preparing a medication for a heart complication. The bark decoction, prepared by boiling the bark for one hour, is added to milk and administered orally to adults as a remedy for abdominal pain or dysentery. The roots are used in treating painful chest conditions. Plant materials are also used as medicine for dyspepsia. Saponins isolated from the leaves of *S. araliacea* have shown anti-leukemic activity (Chhabra *et al.,* 1993; Gessler *et al.,* 1995). Other reports show that the roots have also been used elsewhere to treat: rheumatism by rubbing the ash into scarifications, dysentery and flatulence by taking a decoction mixed with milk in East Africa (Hedberg *et al*., 1983).

2.4 Mammalian Uterine Muscle

2.4.1 General Uterine Muscle Physiology

The uterus of mammals is the central organ of reproduction. In its non-gravid form it is a thick, pear-shaped, muscular organ approximately, 7cm long, and 4-5 cm wide at its widest point. It is divided functionally and morphologically into three sections, namely the cervix the isthmus and the main body of the uterus (corpus uteri) (Symonds et at, 1998). The myometrium the middle muscular layer makes up the major proportion of the uterine body. Myometrial smooth muscle is arranged in undefined layers and contractile forces can occur in any direction enabling the uterus to assume virtually any shape (Alberts et al, 1989). Smooth muscle cells are embedded in an extracellular matrix composed principally of collagen fibres, which facilitate the transmission of contractile forces generated by individual cells. They are organized into sheets of closely opposed fibres +oriented at right angles to each other. These sheets form two

distinct layers, the "longitudinal layer" which consists of a network of bundle of smooth muscle cells generally oriented in the long axis of the organ and the "circular layer", in which the fibres are arranged concentrically around the longitudinal axis of the organ (Csapo, 1962).

2.4.2 Ionic basis for uterine smooth muscle contractility

The onset of labour is facilitated by phasic myometrial contractions that are driven by the development of action potentials across the plasma membranes, resulting from a transient increase in the cytosolic free Ca^{2+} concentration (Young, 2007). Depending on the activating stimulus, Ca^{2+} increase can be due to influx through voltage- or ligand-gated plasma membrane channels, efflux from intracellular stores through the ryanodine receptors (RyR), efflux from intracellular stores through the inositol triphosphate receptor (IP_3R) Ca^{2+} channels, or via a combination of these channels (Kim et al, 2010). Ca^{2+} binds to four binding sites of calmodulin causing a conformational change allowing the calmodulin-calcium complex to interact with inactive myosin light chain kinase (MLCK), thus activating its enzymatic properties (Olson et al, 1990). This enzyme catalyzes the phosphorylation of the myosin light chain on serine at position 19. The myosin is dephosphorylated by myosin light chain phosphatase in the cell. However, dephosphorylation of myosin light chain kinase does not necessarily lead to relaxation of the smooth muscle. Various mechanisms are involved. One appears to be a latch bridge mechanism, by which myosin cross-bridges remain attached to actin for some time after the cytoplasmic Ca^{2+} concentration falls. This produces sustained contraction with little expenditure of energy, which is especially important in vascular smooth muscle. Relaxation of the muscle presumably occurs when the Ca^{2+}-calmodulin complex finally dissociates or when some other mechanism comes into play (Kim et al. 2010).

A G-protein coupled serpentine oxytocin receptor has been identified in human myometrium, and a similar or identical receptor is found in mammary tissue and the ovary. It triggers increases in intracellular Ca^{2+} levels (Kim et al, 2010). Oxytocin is a hormone which activates transmembrane receptors that activate the enzyme phospholipase C attached to the inside projections of the receptors. This enzyme

catalyzes the breakdown of some phospholipids in the cell membrane, especially phosphatidylinositol biphosphate (PIP_2), into two different second messenger products: inositol triphosphate (IP_3) and diacylglycerol (DAG). The IP_3 mobilizes calcium ions from mitochondria and the endoplasmic reticulum, and the calcium ions then have their own second messenger effects, such as smooth muscle contraction as described above on the Ionic basis for uterine smooth muscle contractility (Morris and Malbon, 1999).

Many studies have indicated the existence of abundant cholinergic receptors in the uterine smooth muscle and that stimulation of myometrial muscarinic receptors by agonists such as acetylcholine causes contraction of the uterus (Pennefather et al, 1994). Oxytocin, the most potent of the endogenous oxytocics, acts on myometrial oxytocin receptors (OT1a) to directly cause uterine contraction and on endometrial oxytocin receptors (OT1b) to stimulate prostaglandins and cholinergic releases leading to uterine contraction (Dawood, 1995). Phospholipase C-mediated mobilization of mainly sarcoplasmic intracellular calcium via inositol triphosphate is the major intracellular mechanism after agonists initiate signal transduction by binding to G protein-coupled receptor in the cell membrane (Willets et al, 2008).

The presence of muscarinic, oxytocin and $PGF_{2\alpha}$ receptors in the uterus has been previously reported (Abdalla et al, 2004). These receptors were reported to be up-regulated by 17β-oestradiol (E_2) in late pregnancy particularly at term (Kimura et al, 1996) . In view of this, high dose E_2 administration to the rats prior to the experiment can result in an increase in the number of these uterotonin receptors, potentiating the effect of agonists on uterine contraction. Up-regulation of oxytocin receptor by E_2 has been reported in human (Brodt-eppley and Myatt, 1999), rat (Kobayashi et al, 1999) and mouse (Cook et al, 2003) uterus while muscarinic receptors expression has also been reported in rat (Munns and Pennefather, 1998), and human (Hay et al, 2010) uterus which were also being up-regulated by E_2 (Abdalla et al, 2004).

Atropine, a non-specific muscarinic receptor antagonist, relaxes smooth muscles and reduces the contractile effect of acetylcholine in the uterus (Kurtal et al, 1990). Salbutamol is known to be a β_2-receptor stimulating agent which has been reported to elicit marked decrease in uterine contractility even in dysmenorrheic women (Ie and

Zam, 2008). As a β_2-agonist, Salbutamol finds use in obstetrics. Intravenous Salbutamol can be used as a tocolytic agent to relax the uterine smooth muscle to delay premature labor (Rossi, 2004).

Major physiological pathways (**figure 2.0**) mediating myometrial contraction and relaxation result from the phosphorylation or dephosphorylation of myosin light chains (MLC), respectively. Phosphorylation, by the enzyme myosin light chain kinase (MLCK), in the presence of adenosine triphosphate (ATP), is regulated by intracellular calcium concentrations ($[Ca^{2+}]_i$), in conjunction with the intermediate protein calmodulin (Cam), which together form the calcium-calmodulin (Ca-Cam) complex. Calcium channels (voltage and agonist operated channels VOC/AOC), membrane endothelin (ET) receptors (ET_1, ET_2, ET_3), passive entry, membrane prostaglandin receptors (E_2, $F_{2\alpha}$) and stretch, all facilitate an increase in intracellular Ca^{2+} concentration ($\uparrow[Ca^{2+}]_i$) and result in smooth muscle contraction. Agonist-mediated activation of membrane acetylcholine (Ach) and oxytocin receptors stimulate 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. A byproduct of IP3 synthesis, the second messenger diacylglycerol (DAG) might promote cell contraction via intracellular prostaglandin synthesis from arachidonic acid (AA) by cyclooxygenase (COX) enzymes. The steroid hormone oestrogen promotes cellular contractility by up-regulating COX enzymes, particularly the COX-2 isoform. The active isoform of myosin phosphatase (MP) dephosphorylates MLC, promoting cell relaxation. Receptor-agonist binding and the formation or up-regulation of intracellular RhoA or Rho kinase could result in a shift in the equilibrium of intracellular MP in the direction of the inactive isoform, resulting in enhanced cell contraction—ie, calcium sensitization. Agonist binding of the a_1-adrenergic receptor stimulates inhibitory G-proteins (G_i), which inactivate the adenylate cyclase mediated 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 (Morrison et al, 1996).

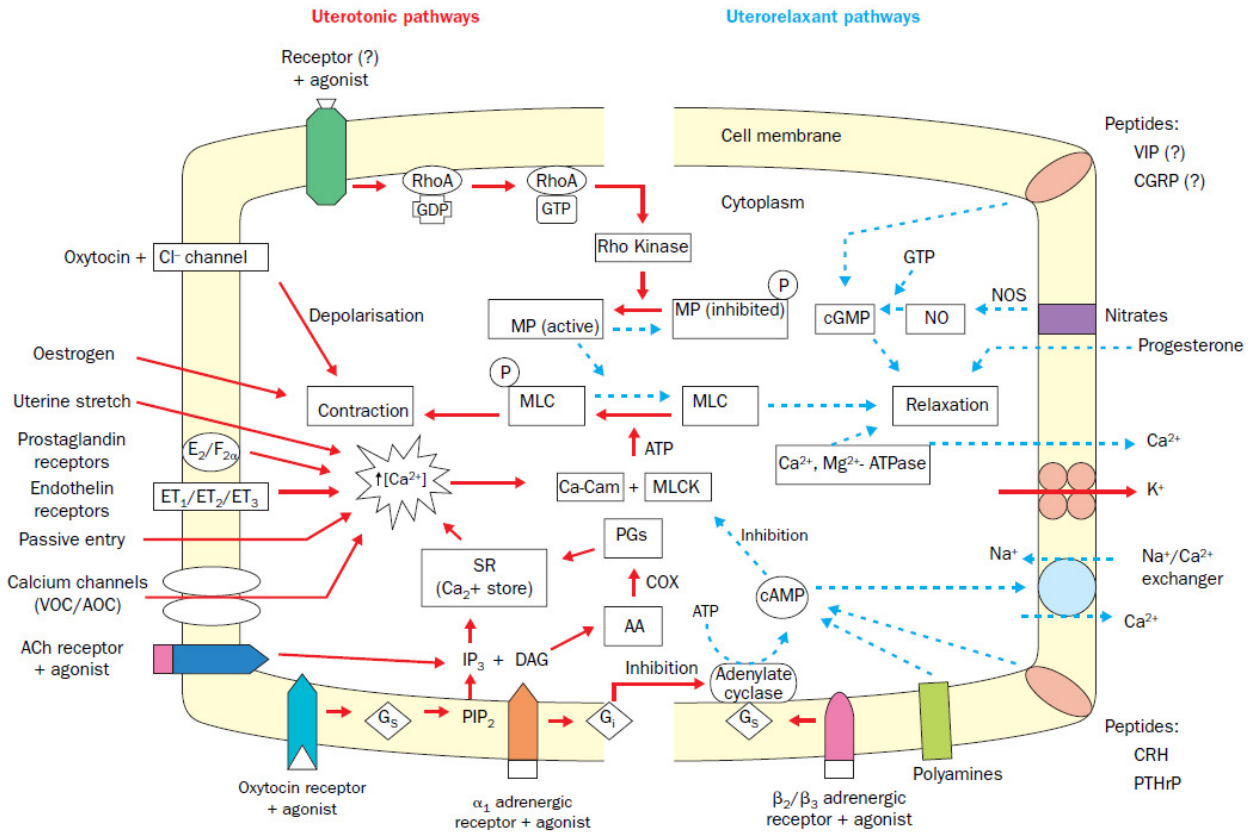


Figure 2. 1:Major physiological pathways mediating myometrial contraction and relaxation.

Chapter 3

METHODOLOGY

3.1 COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

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.

3.2 PREPARATION OF THE AQUEOUS EXTRACT OF STEGANOTEANIA ARALIACEA.

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 was then decanted, 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) as shown in figure **3.2b** 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} (Figure 3.3).

a)  b) 

Figure 3.2 1: The roots for Steganotaenia araliacea Hochst. (b) Concentration of the extract using a laboratory hot plate

Figure 3.3 A dark- brown solid aqueous extract of *Steganotaenia araliacea Hochst.*

Then the dark - brown solid extract of SAE was weighed and the reconstituted in a known volume of distilled water to come up with aqueous root extract of *steganoteania araliacea* (SAE[a]) in milligrams per kilogram (mg/kg).

3.3 EXPERIMENTAL 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 (National Institute of Health, USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002).

3.4 DRUGS (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), indomethacin (1 mg/ml) and Salbutamol (0.8mg/ml) respectively (NB: Salbutamol is not specific antagonist for Oxytocin receptors). Stilbestrol (0.1mg/ml) was administered subcutaneously to non-pregnant rats for uterine sensitization 24 hours before sacrifice.

3.5 ISOLATION AND MOUNTING OF GRAVID AND NON-GRAVID RAT UTERUS:

The isolation and mounting of both gravid and non-gravid rat uterus was by a modification of the procedure described by Boye (2010).

a) A gravid rat was sacrificed by cervical dislocation (decapitation) method and the abdomen 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 (appendix 6.1). 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 two pieces. A strip of the horn about 1 - 2 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 and the load on the tissue was 0.5 g (a tension of 5 mN). The tissue was aerated with ordinary air using an aquarium air pump (Model No: 9905). The temperature of the organ bath was maintained between 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 during which time the bathing solution was changed every 12 minutes.

b) In the second 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 sacrificed, dissected, and the uterine horns removed.

3.6 Influence of agonists on contractile action of uterine muscle

Effects of SAE[a] (12.4mg/ml), Acetylcholine (0.1 mg/ml) and oxytocin (10 IU/ml) were investigated on the mounted isolated pregnant and non-pregnant uteri in order to investigate their effect on rhythmic spontaneous contractions. 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.

3.7 THE EFFECT OF SAE[a]

After equilibration, the baseline (100%) amplitude and frequency of rhythmic spontaneous contractions were recorded in the first 10 minutes. This was followed by subsequent 5 min exposure of the tissue to increasing non-cumulative concentrations of 0.016 ─1.024 mg/mL of SAE.

3.7.1 Drug Administration.

In order to establish a complete dose - response tracings for Oxytocin, Acetylcholine and SAE[a] using the mounted isolated pregnant and non-pregnant uterus:

i. The starting doses for the reference agonist (oxytocin and acetylcholine) and SAE[a] were calculated using the dilution formula given below;

   $Conc_1 \times Vol_1 = Conc_2 \times Vol_2$

   Where;

   $Conc_1$ =Concentration of stock drug solution.

   Vol_1 = Volume of stock solution to be taken.

   Con_2 =Concentration you wish to prepare.

Vol_2 = Volume of the organ bath (25ml).

ii. Starting with the calculated dose, from (i) above as the starting dose, a suitable micro-pipette was used to put the calculated dose into the organ bath and tissue response was recorded at that final bath concentration.

iii. After 5 minutes the tissue was washed three (3) times and allowed appropriate recovery time until the amplitude returned to base line before putting a new dose which was always double the previous one.

iv. The procedures above, (ii) and (iii) were continued, always doubling the previous dose until there was no further increase in the amplitude with increasing dosage for each of the reference agonists and *steganoteania araliacea* aqueous root extract.

v. Then the responses for each of the reference agonist and *steganoteania araliacea* aqueous root extract at varying doses were used to plot dose-response curves.

3.8 DETERMINATION OF THE PHYSIOLOGICAL MECHANISM OF ACTION FOR SAE^a

The effects of atropine (1.8 x 10^{-4} mg/ml), indomethacin (1 mg/ml) and Salbutamol (9.6 x 10^{-3} mg/ml) on the contractile activity of SAE^a were compared with those of their respective agonists in an attempt to establish the possible mechanism(s) of action(s) of the plant extracts (SAE^a) from the observed response(s) as follows;

i. After stabilization of the uterine tissue, a dose of SAE^a that produces 50% of the contractions was tested 3 times on the isolated uterine tissue, then the uterine tissue was washed three times after each extract administration and allowed the baseline to be established each time before introducing a new dose.

ii. The procedure from above (i) was repeated but instead with earlier administration of a known antagonist (i.e. atropine, indomethacin or Salbutamol) into the organ bath 5 minute before adding a dose of SAE^a that produced 50% of the uterine contractions. The responses were compared with those of the agonist in the presence of their respective antagonists.

3.9 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 Dennett's Multiple Comparison Test and a value of $P < 0.05$ was considered significant in all cases.

Chapter 4

RESULTS

4.0 EFFECT OF SAE[a] ON ISOLATED SMOOTH MUSCLE PREPARATIONS

4.0.1 On amplitude and frequency of spontaneous uterine contractions

At baseline (fig.4a), the contractions were compared to those produced after addition of SAE[a] (fig.4b, 4c and 4d).

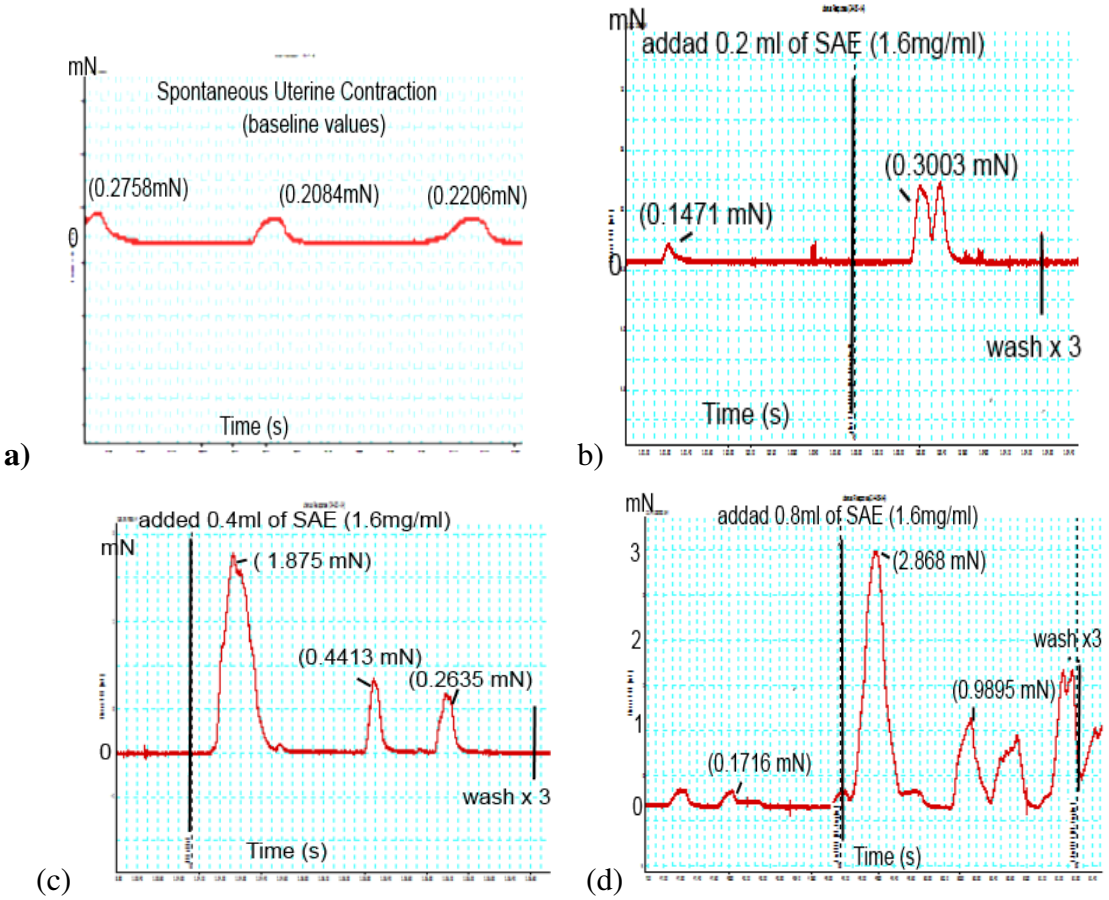


Figure 4. 1:(a) Tracing showing spontaneous uterine contraction (baseline values) and following administration of various doses (b, c and d) of SAE[a] (1.6mg/ml) on the isolated non-pregnant rat uterus.

Table 4. 1: Effect of SAE[a] (12.4mg/ml) on amplitude and frequency of spontaneous uterine contractions

Bath Concentrations of SAE[a] (mg/ml)	Frequency (sec)	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

KEY: 1, 2, 3, 4 and 5 describe the frequency of Uterine Contractions and the amplitude describes the strength of contractions.

Non-cumulative concentrations of SAE[a] increased both the amplitude (strength) and frequency of uterine contractions in a dose dependent manner.

4.1.1 Effect of ACh, OT and SAE[a] on Isolated Rat Uterus

SAE[a] produced dose - dependent myometrial contractions on both the isolated non - pregnant and pregnant rat uterus preparations similar to those produced by acetylcholine and oxytocin (Fig 4.1) and EC_{50} values OT, Ach and SAE[a] were obtained (Table 4.1).

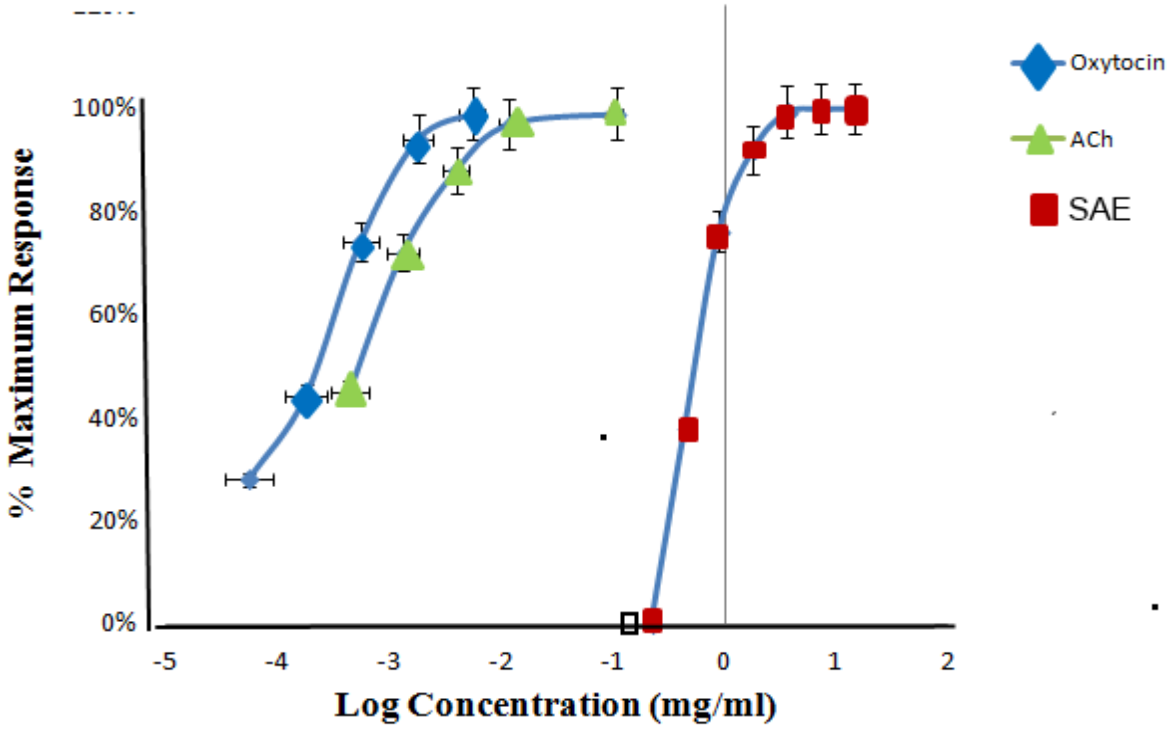


Figure 4. 2:Log dose-response curves of oxytocin (0.055 x10^{-3} — 5.5x 10^{-3}mg/ml), acetylcholine (0.44 x 10^{-3} — 0.107 mg/ml) and SAE[a] (0.11—14.32mg/ml) on the isolated non-pregnant rat uterus. Each point is the mean ± sem (n = 4).

Table 4. 2:EC50 values of ACh, Oxytocin and SAE[a] on the isolated rat uterine preparation

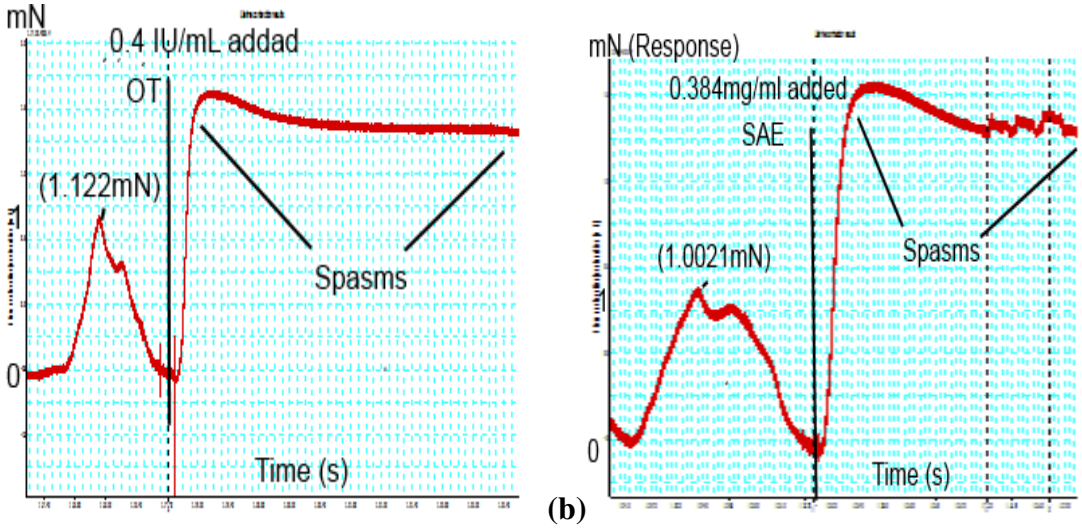
Stimulant/drug	OT	Ach	SAE[a]
EC_{50} (mg/ml)	0.0002	0.0005	0.6

Ach=Acetylcholine, **OT**= Oxytocin, **SAE[a]**=Aqueous extract of *Steganoteania araliacea*

Comment:ACh, Oxytocin and SAE[a] on the isolated rat uterine preparation were all efficacious but OT was the most potent followed by Ach and SAE was the list potent**.**

4.1.2 Effect of high concentration OT and SAE[a] on Isolated Rat Uterus

SAE[a] showed a similar effect to Oxytocin at a higher concentration (fig 4.2a and 4.2b).



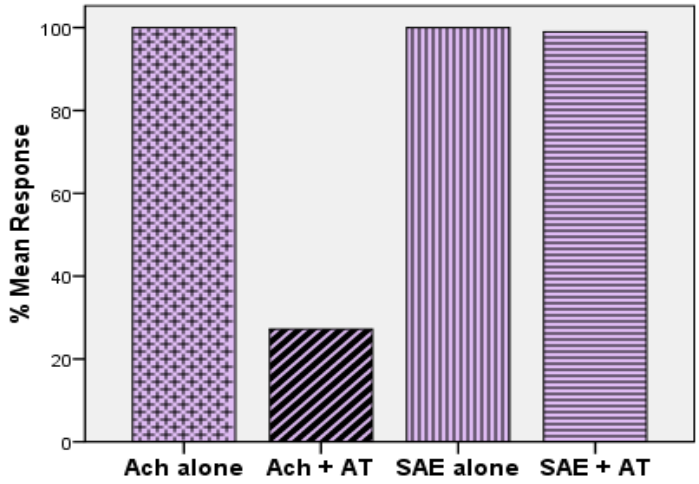
(a) (b)

Figure 4. 3:tracing of uterine contraction following administration of higher dose of (a) oxytocin (0.4 IU/ml) and (b) SAE[a] (0.384 mg/ml) on the isolated pregnant rat uterus

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

Table 4. 3:(a) Responses of ACh (1.8 x 10⁻4 mg/ml) and SAE[a] (0.2 mg/ml) in the absence and presence of atropine (4.8 x 10⁻4 mg/ml) on the isolated pregnant rat uterus.

Ach alone (mN)	Ach + AT (mN)	SAE alone (mN)	SAE + AT (mN)
1.891	0.528	1.753	1.716
1.889	0.491	1.749	1.709
1.890	0.670	1.701	1.701



AT=Atropine (1.8 x 10^{-4} mg/ml)
Ach= Acetylcholine (4.8 x 10^{-4} mg/ml)
SAE[a]= Aqueous Extract (0.4 mg/ml)

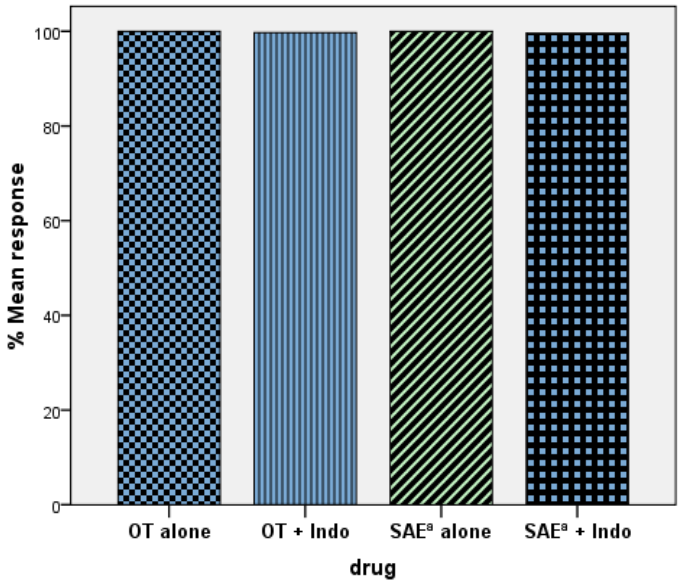
Figure 4. 4:(b) Effect of ACh (1.8 x 10⁻4 mg/ml) and SAE[a] (0.2 mg/ml) in the presence of atropine (4.8 x 10⁻4 mg/ml) on the isolated pregnant rat uterus. Each Each column represents the mean ± sem (n = 3 rats). Inhibitory responses are shown by P < 0.05 (very s

Atropine physiologically inhibited the myometrial contractions (amplitude) produced by acetylcholine by 72.8% ($P < 0.001$) but the same dose of atropine did not inhibit the contractile amplitude produced by SAE[a].

4.1.4 Effect of Indomethacin (Prostaglandin Synthetase Inhibitors) on contractions of the isolated rat uterus produced by SAE[a] extract and oxytocin

Table 4. 4(a) Responses of oxytocin and SAE[a] in the absence and presence of indomethacin on the isolated pregnant rat uterus.

OT alone (mN)	OT+Indo (mN)	SAE alone (mN)	SAE+ Indo (mN)
1.631	1.629	1.324	1.322
1.635	1.634	1.322	1.325
1.634	1.631	1.325	1.319



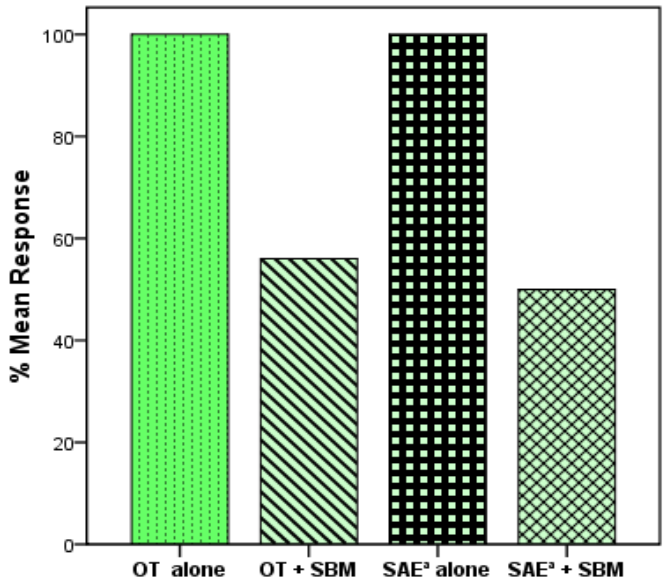
OT= Oxytocin (0.005 IU/ml)
Indo= Indomethacin (0.036 mg/ml)
SAE[a]= Aqueous Extract (0.2 mg/ml)

Figure 4. 5:(b) Effect of indomethacin (0.036 mg/ml) on the myometrial contractions produced by oxytocin (0.005 mg/ml) and SAE[a] (0.2 mg/ml) on the isolated pregnant rat uterus. Inhibitions of responses are shown by P < 0.05 (significant) and P>0.05 (non-significant

4.1.5 Effect of Salbutamol (B2-Adrenoceptor Agonist) On the Contractile Responses of the Isolated Rat Uterus to SAE[a] and Oxytocin

Table 4. 5:Responses of oxytocin and SAE[a] in the absence and presence of Salbutamol on the isolated pregnant rat uterus.

SAE alone (mN)	SAE+SBM (mN)	OT alone (mN)	OT+ SBM (mN)
1.875	1.088	1.704	0.835
1.884	1.017	1.637	0.881
1.873	1.059	1.722	0.819



OT= Oxytocin (0.008 IU/ml)
SBM= Salbutamol (0.0096 mg/ml)
SAE[a]= Aqueous Plant extract (1.04 m

Figure 4. 6: Effects of oxytocin (0.008 IU/ml) and SAE[a] (1.04 mg/ml) in the presence of Salbutamol (9.6 x10⁻3 mg/ml) on the pregnant rat uterus. Each column is the mean ± sem (n = 3 rats).

Salbutamol (0.0096mg/ml) inhibited the effect (amplitude) of oxytocin by about 50 % ($P < 0.05$) from average amplitude of 1.877mN to 1.055mN. The same dose of Salbutamol inhibited the effect (amplitude) of SAE[a] by 44 % ($P < 0.05$) from an average amplitude of 1.688mN to 0.833mN. See Table 4.5(a) and figure 4.5(b) above.

Chapter 5

D1SCUSSION

5.1 EFFECT OF SAE^a ON UTERINE SMOOTH MUSCLE

This study is aimed at demonstrating the physiological influence that the extract of SAE has on contractile activity of rat uterine muscle. A comparison was made with the influence of synthetic oxytocin (pitocin) and its behavior in the presence of some agonists and antagonists.

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. SAE^a had a lowest EC_{50} of 0.6mg/ml when compared to Oxytocin (0.0002mg/ml) and Acetylcholine (0.0005mg/ml) but however despite being the least potent, there were no statistical differences when the mean responses were compared (ANOVA) to the reference drugs, hence SAE is also efficatious.

Following binding of oxytocin to its G protein-coupled receptor, phospholipase C (PLC) is 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 induce extracellular Ca^{2+} influx (Shmygol et al, 2006), resulting in a further rise in the intracellular Ca^{2+} level. Ca^{2+} then binds to calmodulin, which activates the myosin light chain kinase leading to phosphorylation of myosin light chains, triggering contraction (Arthur et al, 2007). 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 (Salleh and Ahmad, 2013).

Oxytocin receptors: Boye (2010) reported that Salbutamol is not a specific antagonist for oxytocin; however its ability to inhibit oxytocin only suggests that it could be acting via similar receptor sites. The involvement of oxytocinergic receptors in myometrial contraction was investigated following administration of Salbutamol a selective β_2 - adrenoceptor agonists and Salbutamol inhibited the contractile action of both oxytocin and SAE almost equally. However its ability to inhibit both SAE[a] and oxytocin only suggests that SAE[a] and oxytocin could be acting via similar receptor sites.

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 uterotonic activity on both the isolated pregnant and non-pregnant rat uterus preparations, although the responses were higher on pregnant rat uterine tissue.

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. The myometrial contractions produced by SAE[a] at a higher concentration (i.e. 0.384mg/ml) on isolated pregnant rat uterus resulted in sustained contractions of the uterus which were comparable to those seen by oxytocin at final bath concentration of 0.4 IU/ml (i.e. 0.8 µg/ml). Alex (2010) reported that Salbutamol is not a specific antagonist for oxytocin; however its ability to inhibit both SAE[a] and oxytocin only suggests that SAE[a] and oxytocin could be acting via similar receptor sites.

Acetylcholine receptors: Atropine, a non-specific muscarinic receptor antagonist, relaxes smooth muscles and reduces the contractile effect of acetylcholine on the uterus (Kurtal et al, 1990). Acetylcholine caused myometrial contractions of rat uterus and when the tissue was pretreated with atropine, the myometrial contractions produced by acetylcholine which were earlier produced by the same concentration of acetylcholine were reduced by 72.8%. This demonstrated the influence of atropine on the possible

receptors where Ach would have been operating. In an attempt to investigate the involvement of SAE[a] on cholinergic receptors, the amplitude (SAE[a]-induced contraction) was not inhibited in the presence atropine. These findings suggested that SAE[a] -induced uterine contractions were not mediated via the acetylcholine receptors which was evidenced by the lack of inhibition of these responses 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). According to Vane and Williams (1973), uterine stimulating activity of oxytocic agents might be mediated through uterine prostaglandins release and uterine membrane sensitization. In this research Indomethacin (prostaglandin synthetase inhibitors) could not modify contractions produced by oxytocin and SAE[a] which suggests it may not be linked to prostaglandin biosynthesis.

The Overall observations in this research have 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.

Chapter 6

CONCLUSSIONS AND RECOMMENDATIONS

This in-vitro study using isolated rodent's uteri has provided the first scientific evidence to support the claim that *Steganoteania araliacea* stimulates uterine smooth muscle contraction. The SAE[a] had a significant effect on the amplitude and frequency of uterine contractions in both pregnant and non-pregnant uteri smooth muscle.

Pre-treating the tissue with atropine and indomethacin did not significantly decrease the uterine contractions elicited by SAE[a] indicating possible non-involvement of Muscarinic receptors and/or prostaglandin biosynthetic pathways respectively while pre-treatment with Salbutamol showed remarkable inhibition of uterine contraction indicating a possible involvement of Oxytocinergic receptors.

More work needs to be carried out, in order to fully understand how widely Fyopola is used in Zambia and also appreciate the physiological effects of SAE on uterine smooth muscle. There is need to further elicit its influence on the smooth muscle receptors especially in relation to the various intracellular contractile processes. This will lead to a further elucidation of the various contractile processes that are involved in the processes of parturition and how these could be manipulated pharmacologically.

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APPENDIX

6.1 COMPOSITION OF DE JALON'S PHYSIOLOGICAL SOLUTION

The physiological solution below will used to maintain the isolated rat uterus preparation. The solution is made up of:

PREPARATION OF PHYSIOLOGICAL SOLUTION	
Quantities of 10 liters of:	
Ingredients	**Grams (g)/ 10L**
Sodium chloride (NaCl)	90 g
Potassium chloride (KCl_2)	4.2g
D-Glucose ($C_6H_{12}O_6$)	5 g
Sodium hydrogen carbonate ($NaHCO_3$)	5g
Calcium chloride ($CaCl_2$)	0.8g
Aerating Gas	O_2

6.2 APPARATUS

POWERLAB 26T

- Model: ML 866
- Serial: T26-1766

UGO BASILE (4050 two Chamber Isolated organ bath)

- Biological Research apparatus, 21025 comerio varese Italy.

Specifications:

- Type: 4050
- Power: 220 V
- No: 32198 50Hz
- Fuse: 11.25 A1

AQUARIUM AIR PUMP-SONIC (IPX4)

- Model: 9905
- AC 230V, 50Hz, 2.9W

TEACHING FORCE TRANSDUCER

- Model: MLT0210/A
- Range: 5mg to 25g
- Serial No: 1002311

Made in Spain by panlab, S.I for ADinstruments Pty Ltd

ML 301 BRIDGE POD

- Serial No: PBH-5213
- Made in Australia by ADinstruments Pty Ltd

Specifications:

- Input impedance: ~470k||3pF differential
- Input range: 200µV-20mV in 1:2:5
- DC druft: 2µ V/°C
- DC excitation 2.5V normal
- Minimum load resistance: 30
- Amplifier noise: <1µVRMS (10 Hz band width)
- DC offset adjust range:+/- 200mV, potentiometer, software selectable.

LAB HOT PLATE

- Model No: 13474
- Power: 100V 3A
- 0.25 kW , 300°C Max
- IKEMOTO RIKAKOGYO CO. LTD

LAB-WARE DRYING OVEN

- Model No: DG-81
- Serial #: 206002
- Voltage: 220 V, 50Hz,
- Amps: 76A
- Made in Japan, Yamato Scientific Co. Ltd

HITACHI CENTRIFUGE

- Type: 05p-21
- Max. speed: 5000rpm
- Voltage: 100V CUR: 5A
- Freg: 50/60 Hz
- MFG No: 99698
- CAT No: 001472
- Hitachi Koki Co. Ltd

PH METER

- Model: M-8AD
- MFG No: 306009
- Power AC: 220V, 15 VA
- Freq: 50/60 Hz
- Date: 1985, HORIBA LTD
- Made in Japan

RKJ GRINDER MK

- Type: 40-525
- Source: AC 220V, 50/50 Hz, 0.2kW, 1A
- Seriai No: 21388
- Date: 1994
- IKEMOTO Scientific Technology Co.LTD
- Tokyo Japan

6.3 DRUGS

i. Acetylcholine Chloride (SIGMA)

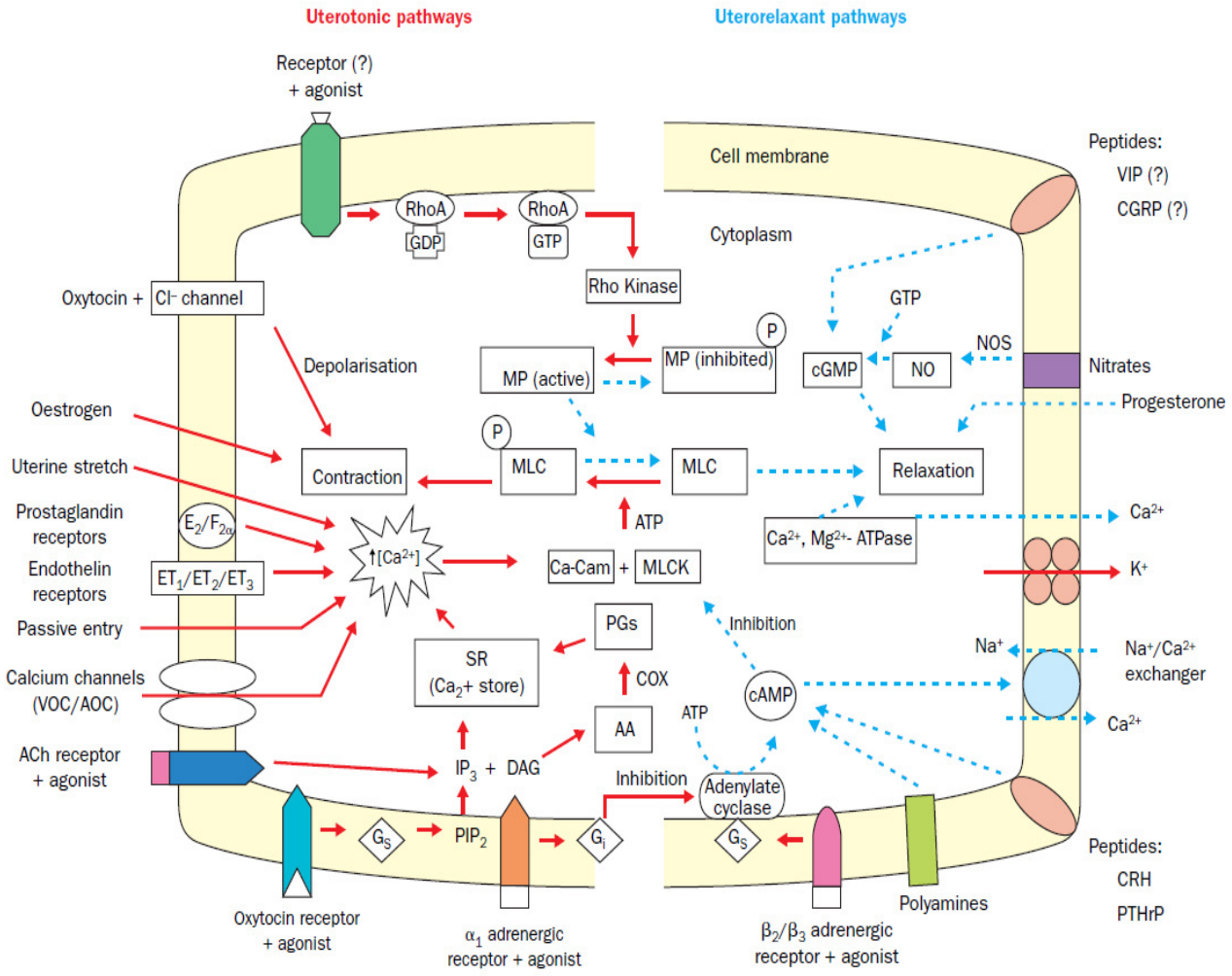
ii. Atropine sulphate 0.6mg/ml (SISHUI XIERKANG PHARMA)
License No: 269/008
Batch No: 110102

iii. Oxytocin 10 IU/ml (NCPC International Corp. Hebei China)
Batch No:120429,
MFG; Date:04/2012
EXP; Date:04/2015

iv. Salbutamol (Lebasma-4) (Leben Laboratories PVT Ltd, MUMBAI)
Batch No: T-2653
MFG Date: 10/2013
EXP Date: 09/2016

v. Indomathacin (Leben Laboratories PVT Ltd, MUMBAI)
Batch No: C-133
MFG Date: 06/2013
EXP Date : 02/2016

6.4 MECHANISM OF UTEROTONICS AND TOCOLYTICS



6.5 GHARTT CHART

	Aug 2013	Sept 2013	Feb 2014	March 2014	April 2014	June-Aug 2014	July-Aug 2014	Aug-Sept 2014	Sept 2014	Sept-Oct 2014	March 2015
Present to Department											
Submit proposal to Asst Dean (PG) office											
Present at GPPF											
Submit proposal to REC											
REC review and approval											
Enroll Lab animals and collect data											
Analyze data											
Write dissertation											
Submit final dissertation											

6.6 BUDGET

Item	Detail	Cost estimate ZMK
ERES submission fee	The revised fee for 2013	K 500
Stationery and printing	Specialist copyist/secretary to edit monographs from first post-data analysis to final submission	K 2,000
Personnel (research assistants)	Being payment of a research assistant for assistance in capturing individual data (see individual data set) K5OO/month x 2 people	K3,000
Digit camera	Standard camera of photography to meet international standard	K2,500
Lab animals	To be used as sample population, including feed costs	K3,000
Thesis Preparation costs	Professional printing and binding	K2,000
Transport costs	To and from place of data collection	K1,000
Drugs/materials	Oxytocin, Acetylcholine Salbutamol, syringes, glass slides, kymograph paper.	K5,000
Plant sample costs	The actual plant and plant extract preparation	K1,500
Other materials	Electric blender, electronic balance.	K1,500
Questionnaire cost	Colour Printing	K1,000
Plant Identification costs	For authentication	K1,000
Sub-Total		**K23,500**
Contingency @ 10%		K2,350
Grand Total		**K25,850**

6.7 Publication

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.

6.8 Others

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