EVALUATION OF CONTRACTILE EFFECTS OF *Uvariodendron kirkii* (Verdec.) EXTRACTS ON ISOLATED UTERINE STRIPS OF FEMALE WISTAR RATS

KINYUA ESTHER WAIRIMU

A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Animal Physiology of Egerton University

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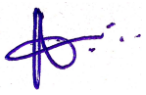
DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any institution.

Signature:  Date: 20th May 2021

Kinyua Esther Wairimu

SM21/20010/16

Recommendation

This thesis has been presented for examination with our approval as official university supervisors.

Signature:  Date: 20th May 2021

Dr. Charles Irungu Maina (Ph.D)

Egerton University

Signature:  Date: 20th May 2021

Dr. Catherine Kaluwa Kaingu (Ph.D)

University of Nairobi

DEDICATION

This thesis is dedicated to first of all God almighty. To my great and loving parents who never tired in giving themselves in countless ways. To my beloved brothers and sister who stood by me and came through when the going got tough.

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ABSTRACT

Uterotonic plants have been used to augment labour, induce labour and for abortifacient purposes since ancient times. However, current conventional therapies used as uterotonic agents in rural areas of developing countries to cause uterine contractions or in labour management are limited in terms of availability, their cost in terms of not being affordable and their preservation measures. They are also believed to produce some life disturbing side effects. These limitations associated with conventional treatments have made herbal medicines a feasible alternative for the management of these conditions. In turn this has led to the use of herbal plants to induce labour, enhance labour and for abortive purposes. Traditional Birth Attendants (TBAs) in Tana River County of Kenya historically use *Uvariodendron kirkii* to induce or enhance labour, in post-partum haemorrhage management, and expel retained after birth. This study evaluated the contractile effects of *Uvariodendron kirkii* extracts on diethylstilboestrol primed isolated uterine strips of Wistar rats. Isolated strips of Wistar rats' uteri were treated with 20, 40, 80 and 160 mg/ml concentrations of *Uvariodendron kirkii* aqueous extract. The plant extract concentrations were also tested against oxytocin and Prostaglandin F2α induced uterine contractions. In the absence or presence of oxytocin and Prostaglandin F2α, the effect on the frequency and amplitude of contraction was measured statistically using ANOVA. *p*- values < 0.05 were deemed significant. After exposure to the aqueous root bark extracts, all uteri displayed a heavy initial contraction across all concentrations. The extracts were able to specifically induce uterine tissue contractility and increase the response of the tissue to oxytocin and Prostaglandin F2α. The extract caused a dose dependent increase in uterine frequency and amplitude of contraction. The findings of this study are useful in generating a novel uterotonic agent that will be useful in augmenting labour or in expelling retained after birth. *Uvaridendron kirkii* aqueous extract showed oxytocic activity as it increased uterine contractions in a dose dependent manner, therefore, it is a potent uterotonic plant. In order to evaluate the active compound(s), potential modes of action and effectiveness of the plant extracts, more pharmacological studies are, however, needed.

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LIST OF ABBREVIATIONS AND ACRONYMNS

ACTH	Adrenocorticotropic Hormone
AF	Activation Function
ANOVA	Analysis of Variance
CAPs	Contraction Associated Proteins
CL	Corpus luteum
CRH	Corticotropin Releasing Hormone
DBD	DNA Binding Domain
DHEAS	Dihydroepiandrostenediene Sulphate
FSH	Follicle Stimulating Hormone
IUCN	International Union for Conservation of Nature
KNBS	Kenya National Bureau of Statistics
LBD	Ligand Binding Domain
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
OL	Obstructed Labour
OTRs	Oxytocin receptors
OXT	Oxytocin
PGE_2	Prostaglandin E2
PL	Prolonged labour
PPH	Postpartum Haemorrhage
PR	Progesterone Receptor
PSS	Physiological Salt Solution
RFM	Retention of Foetal Membrane
SEM	Standard Error of Mean
TBAs	Traditional Birth Attendants
WHO	World Health Organisation

CHAPTER ONE
INTRODUCTION

1.1 Background information

The utilization of herbal medicine keeps expanding rapidly over the world with different individuals straightforwardly falling back on these drugs for treatment, in different health related issues in various human organization settings. During the past decade, the acceptance and open interest for natural treatments is increasing both in developing as well as in developed countries. Uterotonic is a substance that causes uterine contractions. In both allopathic and traditional use of herbal medicine, substances that are called uterotonics often have laxative, purgative, diarrheagenic, cathartic and abortifacient effects (Roqaiya et al., 2015).

Uterine stimulants are those medications given to cause uterine surges, or to increase the force and frequency of uterine contractions. These drugs can be used to affect or augment labour, support uterine contractions upon a miscarriage to reduce haemorrhage, impel untimely birth or abortions, or to prevent post-partum haemorrhage. Three most frequently used uterotonics are oxytocin, prostaglandins and ergot alkaloids. Oxytocin, a biochemically produced hormone, utilizes distant hormone receptors that are activated and begins uterine contraction influences. These uterotonics are ordinarily administered towards the end of the gestation period or towards the onset of labour (Malpani et al., 2011).

Desensitization of the oxytocin receptors (OTRs) can arise after repeated exposure to oxytocin, which leads to uterine activity loss. This has been demonstrated clinically by use of intrauterine pressure catheter in women undergoing labour augmentation. Desensitization of the OTRs pauses a risk for uterine atony and haemorrhage in the peripartum period. Uterine hyperstimulation can also result after uterine infusion, and if uterine activity becomes excess and exceeds the ability of the foetal-placenta unit to compensate for blood supply to the placenta, oxygen supply to the foetus may decline and lead to intrapartum foetal hypoxemia or pain. Tetanic contractions that can induce foetal death can also result from the infusion of oxytocin (Arrowsmith & Wray, 2014).

A high number of traditional birth attendants (TBAs) provide herbal drugs to women during pregnancy or at near delivery, for intended uterotonic effects. Studies done in Nigeria and Kenya have documented that nearly 25% of TBAs utilize herbal preparations to expel retained placenta (Tripathi et al., 2013). More than 56 % of pregnant women in Kenya choose to deliver at home with the assistance of traditional birth attendants who use these herbal remedies to

complete the processes of child bearing (KNBS, 2011). This is due to reasons including poor maternity services, negative attitudes of the health workers, cost, accessibility of the health facilities and cultural preferences (USAID-Kenya, 2014).

Similarly, complications resulting from reproductive health related conditions such as maternal mortality and morbidity in Kenya is at 14.1% which account for second in number of the disease burden in Kenya. The national maternal mortality rates have unacceptably remained high with 488 deaths per 100,000 live births. These women suffer because of preventable problems such as haemorrhage, obstructed childbirth, retained placenta, unsafe abortion complications and elevated blood pressure that can be effectively avoided by using uterotonics (Maternal & Child health, 2012).

In the efforts to meet the standard development goal 3 of ensuring healthier lives for all ages and encourage well-being for all, and Kenya's vision 2030 that aims to offer all people fair, accessible and quality healthcare, the traditional medicines and indigenous expertise used by traditional birth attendants deserve the right to be recorded and the arguments duly affirmed under conditions that mimic indigenous methods of use through scientific confirmation. (Misonge et al., 2014).

Uvariodendron kirkii is a native medicinal plant in Tana River County, Kenya. It is reported to regulate fertility through its anti-fertility and anti-implantation properties (Kaingu, 2016). The plant probably causes uterine contractions and therefore leads to the anti-implantation effect. This study therefore, evaluated the contractile effects of *U. kirkii* on isolated rat uterine tissue. The study tested the functional responses to the plant aqueous extract of the uterine muscle strip. A large heated glass chamber capable of storing physiological saline of between 5 and 60 mL was used. In these chambers, strips typically need aeration with oxygen and large amounts of physiological salt solution (PSS).

1.2 Statement of the problem

Currently used interventional therapies to induce uterine contractions (uterotonic agents) are not available in rural parts of developing countries. Oxytocin has been used as the first line of therapy. However, there are barriers to its use in low-resource settings, such as to maintain its highest potency, oxytocin requires refrigeration. Safe and effective administration of oxytocin requires highly trained staff in intravenous or intramuscular administration techniques. Sterile needles, and safe disposal for injection equipment are also required and these are frequently unavailable or too costly during births in low-resource settings. Another uterotonic agent,

ergometrine has similar efficacy to oxytocin but has more side effects, which makes it the preferred option only when oxytocin is not available. Like oxytocin, its utility in low-resource settings is lessened by special storage requirements and also requires highly skilled staff to do the administration. Also, use of synthetic oxytocin, can lead to desensitization of the OTRs or hyperstimulation of uterine contractions which can lead to uterine atony. Common side effects of oxytocin include fast, slow or uneven heart rate, headache, slurred speech, hallucinations, severe vomiting, severe weakness, seizures, high blood pressure among others. Therefore, it is important to consider the use of alternative uterotonics such as *U. kirkii* extract prior to or immediately after delivery.

1.3 Objectives

1.3.1 General objective

To evaluate the contractile effects of plant aqueous extracts on isolated uterine strips of female Wistar rats.

1.3.2 Specific objectives

i. To determine the frequency and amplitude of uterine contractions caused by graded concentrations of *Uvariodendron kirkii* aqueous extracts.
ii. To determine the frequency and amplitude of uterine contractions caused by *Uvariodendron kirkii* aqueous extracts on oxytocin induced uterine contractions.
iii. To determine the frequency and amplitude of uterine contractions caused by *Uvariodendron kirkii* aqueous extracts on prostaglandin induced uterine contractions.

1.4 Hypotheses

i. There is no significant difference in the frequency and amplitude of uterine contractions caused by graded concentrations of *Uvariodendron kirkii* aqueous extracts.
ii. There is no significant difference in frequency and amplitude of uterine contractions caused by *Uvariodendron kirkii* extracts on oxytocin induced uterine contractions.
iii. There is no significant difference in the frequency and amplitude of uterine contractions caused by *Uvariodendron kirkii* extracts on prostaglandin induced uterine contractions.

1.5 Justification

Modern methods of managing prolonged labour, post-partum haemorrhage and retained after births, for example use of oxytocin, are known to result in undesirable side effects such as headaches and severe vomiting. On the other hand, traditional uterotonic medicines are known to have little to no side effects, making them a feasible alternative for labour management. In

addition, use of traditional medicine does not necessarily require highly trained staff or special equipment, especially in low-resource setting areas as they can be administered orally. Therefore, there is need for the study to find natural alternatives like herbal medicines (*Uvariodendron kirkii* extract) as they are cost effective and easily accessible as they are found locally. Furthermore, the study is in line with the sustainable development goal number 3 which aims to ensure healthy lives and promote well-being for all at all ages. It is also in line with one of the big four agenda Kenya which aims to provide affordable healthcare.

CHAPTER TWO
LITERATURE REVIEW

2.1 Physiology and pathophysiology of labour

Labour is the physiological process, which result to expulsion of the foetus from the uterus. It involves a series of events in the myometrium, decidua and the cervix that occur gradually over a period of days to weeks. These events require the presence of regular painful uterine contractions, which increase in frequency, intensity and duration leading to progressive cervical thinning and dilation (Asgari-safdar et al., 2013). This topic will review the uterine muscle, physiology of labour and its pathogenesis.

2.1.1 Uterine muscle

The uterus is a hollow organ consisting of thick, contractile walls designed for the purpose of implantation and growth of a sexually fertilized ovum. Its functioning is achieved by its muscular layer, which is largely made of fibres, each of which is arranged in a different order. Direction and differences are also defined in a particular way. The visible parts of the uterus include the body, an isthmus and the cervix (Escalante & Pino, 2017). Its anatomical constitution comprises three overlapping tunics from the outside to the inside: a serosatunic, a muscular tunic and a mucosa tunic. The muscular tunic is mostly composed of smooth muscle fibres, which together form the so-called myometrium. The myometrium is responsible for contractions during labour that efface and dilate the cervix and deliver the foetus. The myometrial contractions do not require hormonal or nervous input to contract since the contractions are spontaneous (Young, 2007).

There are five distinct physiological processes that constitute parturition: rupture of the foetal membrane, dilatation of the cervix, myometrial contractility, separation of the placenta and involution of the uterus; each of which is an individual physiological action. All five physiological events must function in unison in order for a prompt, natural birth to occur at term. Since the vast majority of the five physiological events is controlled by the regulation of myometrial contractility (Mijovic & Olson, 1996), then this will be used as the physiological reference point in most of the discussions on the management of parturition.

2.1.2 Phases of myometrial activity

The regulation of myometrial activity can be divided in various distinct phases which include; Phase 0: where the uterus is maintained in a quiescence state through the action of various inhibitors such as progesterone, prostacyclin, relaxin, parathyroid hormone-related peptide,

nitric oxide, calcitonin gene-related peptide and adrenomedullin. Phase 1 is the activation phase where uterus becomes activated by expression of a series of Contraction Associated Proteins (CAPs) which include the oxytocin and prostaglandin myometrial receptors. Specific ion channels are also activated as well as increase in connexin which increase gap junction formation (Young, 2007).

Phase 2 is the stimulatory phase where the uterus can be stimulated to contract by the action of uterine agonists such as Prostaglandin F2 alpha and oxytocin. Phase 3 is the involution phase where the uterus returns back to its pre-pregnant state and assumes its normal position in the pelvic cavity as well as gaining its muscular tone. The myometrial activity is regulated by the action of various hormonal regulation (Asgari-safdar et al., 2013).

2.2 Hormones involved in parturition

The endocrine profile of the foetus continues to adjust as pregnancy progresses. Stimulation of the foetus's hypothalamic-pituitary-adrenal axis is responsible for increased synthesis of the adrenocorticotropic hormone (ACTH) and the adrenal cortisol needed to induce childbirth. Additionally, placental hormone synthesis starts to change late in gestation. There is an increase in prostaglandin E2 (PGE2) synthesis that occurs after the 100th day of gestation (Dunlap, 2018).

Furthermore, PGE2 will stimulate the development of foetal ACTH and cortisol and initiate the breakdown of the matrix required for cervical maturation. The development of foetal cortisol continues as parturition becomes prevalent and progesterone decreases and oestrogen rises accordingly (Kindahl et al., 2002). In order to promote lubrication of the reproductive tract for delivery, elevated oestrogen increases secretions of the cervix and vagina and also helps to increase oxytocin receptors inside the uterine myometrium. The binding of oxytocin to its receptors will promote the requisite synchronized muscle contraction needed for the foetus and placental membranes to be expelled (Dunlap, 2018). This topic, therefore, reviews the hormones involved in parturition.

2.2.1 Oxytocin

Oxytocin is a neurotransmitter and a hormone that is produced in the hypothalamus. From there, it is transported to and secreted by the posterior pituitary gland, at the base of the brain. It plays a role in the female reproductive functions, from sexual activity to childbirth and breast feeding (Carter, 2014). During labour, oxytocin increases uterine motility, causing contractions in the muscles of the uterus, or womb. As the cervix and vagina start to widen for labour,

oxytocin is released. This widening increases as further contractions occur (Kabilan, 2014). Oxytocin also has social functions; it impacts bonding behaviour, the creation of group memories, social recognition, and other social functions (Gimpl & Fahrenholz, 2001).

Oxytocin is used as a prescription drug under the brand name Pitocin®. Side effects include unusual bleeding, nausea or vomiting, memory problems or confusion, runny nose, sore throat, severe headaches, hallucinations, vomiting, confusion, seizures and severe hypertension. If too much oxytocin is delivered too rapidly, it can lead to rupture of the uterus. Oxytocin can also be given to control bleeding after a delivery or a termination. It can be used medically to induce a termination or complete a miscarriage (Kabilan, 2014).

Oxytocin has been most widely used in labour but its relatively short half-life requires continual intravenous infusion, as is the case for labour augmentation. Syntometrine is an oxytocin/ergot alkaloid combination drug which is frequently used in vaginal deliveries. It has the rapid uterotonic activity of oxytocin combined with the prolonged effects of ergometrine, however, it is associated with more side effects due to the activity of ergometrine on other smooth muscles (Arrowsmith & Wray, 2014).

More recently, Carbetocin, a synthetic analogue of oxytocin with a suggested half-life approximately 4-10 times longer than that reported for oxytocin, has been developed for prevention of Post-partum haemorrhage (PPH) It has been shown to have a greater safety and tolerability profile compared to oxytocin with the added benefit that it can be administered as a single dose injection rather than long term infusion as required for oxytocin (Ortiz et al., 2014). Today, carbetocin is often the primary treatment administered after delivery to prevent PPH. It has been shown to be associated with less blood loss compared to syntometrine in women who have vaginal deliveries, and has significantly fewer adverse effects. It is also routinely administered prophylactically following caesarean section delivery to prevent severe blood loss and, therefore, reduce maternal morbidity and mortality (Theunissen et al., 2018).

In the liver and kidney, oxytocin is inactivated, but it is mostly degraded by placental oxytocinase during gestation. Oxytocin is the most potent endogenous uterotonic agent which, at intravenous infusion concentrations of 1 to 2 mU/min, is capable of inducing uterine contractions. The duration and amplitude of uterine contractions produced by oxytocin are similar to those that occur during spontaneous labour (Asgari-safdar et al., 2013).

2.2.2 Prostaglandins

The prostaglandins are a group of lipids which belong to a chemical group called prostanoids, part of an even larger group called eicosanoids that is derived from 20-carbon arachidonic acid. They are classified by variations of their ring structure as well as the number and location of side-chain double bonds (George, 2005). Prostaglandins are made at sites of tissue damage or infection that are involved in dealing with injury and illness. They control processes such as inflammation, blood flow, formation of blood clots and induction of labour.

Unlike most hormones, prostaglandins are not secreted from a gland to be carried in the bloodstream and work on specific areas around the body. Instead, they are made by a chemical reaction at the site where they are needed and can be made in nearly all the organs in the body (Ricciotti & FitzGerald, 2011)

The prostaglandins act as signals to control several different processes depending on the part of the body in which they are made. Prostaglandins cause inflammation, pain and fever as part of the healing process. When a blood vessel is injured, a prostaglandin called thromboxane stimulates the formation of a blood clot to try to heal the damage; it also causes the muscle in the blood vessel wall to contract (causing the blood vessel to narrow) in order to prevent blood loss. Another prostaglandin called prostacyclin has the opposite effect to thromboxane, reducing blood clotting and removing any clots that are no longer needed; it also causes the muscle in the blood vessel wall to relax, so that the vessel dilates. The opposing effects that thromboxane and prostacyclin have on the width of blood vessels can control the amount of blood flow and regulate response to injury and inflammation (Clark & Myatt, 2008).

Prostaglandins are also involved in regulating the contraction and relaxation of the muscles in the gut and the airways. They are known to regulate the female reproductive system, and are involved in the control of ovulation, the menstrual cycle and induction of labour. Indeed, manufactured forms of prostaglandins - prostaglandin E_2 and F_2 can be used to induce labour (Kelly et al., 2009).

The chemical pathway that synthesizes prostaglandins involves several steps; the first step is carried out by an enzyme called cyclooxygenase. There are two main types of this enzyme: cyclooxygenase-1 and cyclooxygenase-2. When the body is functioning normally, levels of prostaglandins are produced by the action of cyclooxygenase-1. When the body is injured (or inflammation occurs in any area of the body), cyclooxygenase-2 is activated and produces extra prostaglandins, which helps the body to respond to the injury (Smith et al., 2000).

Prostaglandins carry out their actions by acting on specific receptors; at least eight different prostaglandin receptors have been discovered. The presence of these receptors in different organs throughout the body allows the different actions of each prostaglandin to be carried out, depending on which receptor they interact with. Prostaglandins are very short-lived and are broken down quickly by the body. They only carry out their actions in the immediate vicinity of where they are produced; this helps to regulate and limit their actions (Moreno, 2017).

High concentrations of prostaglandins cause pain by direct action upon nerve endings. More typically, however, at low concentrations, they markedly increase sensitivity to pain. The pain threshold may be so altered that even normally painless stimuli may be painful. This effect of prostaglandins is long-lasting and cumulative, so that continued production of even small amounts can sensitize nerves to other irritants. This means that drugs, which specifically block cyclooxygenase-2, can be used to treat conditions such as arthritis, heavy menstrual bleeding and painful menstrual cramps and certain types of cancer, including colon and breast cancer (Lethaby et al., 2013).

New discoveries are being made about cyclooxygenases which suggest that cyclooxygenase-2 is not just responsible for disease but has other functions. Anti-inflammatory drugs, such as aspirin and ibuprofen, work by blocking the action of the cyclooxygenase enzymes and so reduce prostaglandin levels. This is how these drugs work to relieve the symptoms of inflammation. Aspirin also blocks the production of thromboxane and so can be used to prevent unwanted blood clotting in patients with heart disease (Ricciotti & FitzGerald, 2011).

2.2.3 Prostaglandins and labour

Prostaglandin suppositories are inserted into the vagina in the evening causing the uterus to go into labour by morning by making the cervix ripe or thinned out. They have been reported to have a high success rate of about 88% if used alone and 95% in combination with other uterotonic agents (Mousa & Alfirevic, 2007).

Prostaglandins act on the cervix to enable ripening by a number of different mechanisms. They alter the extracellular ground substance of the cervix, and PGE_2 increases the activity of collagenase in the cervix. They cause an increase in elastase, glycosaminoglycan, dermatan sulphate, and hyaluronic acid levels in the cervix. A relaxation of cervical smooth muscle facilitates dilation. Finally, prostaglandins allow for an increase in intracellular calcium levels, causing contraction of myometrial muscle (Tenore, 2003).

Risks associated with the use of prostaglandins include uterine hyperstimulation and maternal side effects such as nausea, vomiting, diarrhoea, and fever. Currently, two types of prostaglandins, dinoprostone gel (Prepidil®) and dinoprostone inserts (Cervidil®), are available for the purpose of cervical ripening. Prepidil® contains 0.5 mg of dinoprostone gel, while Cervidil® contains 10 mg of dinoprostone in pessary form (Hadi, 2000).

Misoprostol, a methyl ester synthetic analogue of natural prostaglandin E_1, can be administered orally, sublingually, buccally, vaginally or rectally. A Cochrane systematic review of randomised trials of misoprostol versus injectable uterotonics in management of the third stage of labour suggests that the drug is less effective than injectable uterotonics in the prevention of severe PPH (blood loss less than or equal to 1000 ml) and has more adverse effects, including nausea, vomiting and diarrhoea (Hofmeyr & Gülmezoglu, 2008; Tunçalp et al., 2012).

In most cases, uterotonic drugs will control postpartum bleeding, but if they do not, surgical intervention must be considered (Mousa et al., 2014). Prostaglandins are primarily paracrine/autocrine hormones, since they act on contiguous cells locally at their production site. A rise in the biosynthesis of uterine prostaglandin is a clear feature of the transition to labour and is possibly common to all animals (Asgari-safdar et al., 2013).

2.2.4 Progesterone

Progesterone receptor antagonist administration or elimination of progesterone receptor antagonist readily induces early pregnancy abortion (before 7 weeks of gestation), suggesting that progesterone is required for early pregnancy maintenance. Exogenous progesterone administration following early lutectomy prevents abortion, further supporting the theory that the development of ovarian progesterone is necessary for early pregnancy maintenance. Between 7 and 9 weeks, placental progesterone synthesis becomes important and the placenta is the dominant source of progesterone thereafter (Vladic, 2012).

The hormone progesterone is essential for the normal control of female reproductive functions, with the key biochemical functions of progesterone being in the ovary and uterus, that is in the stimulation of ovulation, facilitation of implantation and stabilization of early pregnancy. Other functions are lobular-alveolar formation in preparation for milk secretion in the mammary gland (Guennoun, 2020), neurobehavioural function linked with sexual responsiveness (Schumacher et al., 2012) and avoidance of bone loss in the brain and neck. Progesterone is a 21-carbon steroid that serves as a precursor to the biosynthesis of steroids (Al-Asmakh, 2007).

During the luteal process of the menstrual cycle, progesterone is predominantly released by the granulosa-lutein cells of the corpus luteum (CL) and the syncytiotrophoblast of the placenta during pregnancy. The adrenal cortex's zona fasciculata and zona reticularis, on the other hand, synthesise very little progesterone, less than 1 mg a day (Vladic, 2012).

Transcortin (corticosteroid-binding globulin) and albumin transport progesterone in the blood, with around 2% present in the active, unbound state. Progesterone has a half-life of around 5 minutes in the blood, and its main breakdown product, pregnanediol, is produced in the liver. During the follicular process of the menstrual cycle, plasma progesterone concentrations are usually less than 5 nmol/l (1.5 ng/ml). The plasma level increases to a high of about 40-50 nmol/l (12-16 ng/ml) during the luteal stage (Reshef et al., 2000).

Via the activity of progesterone receptors, progesterone regulates a number of biologically distinct mechanisms in a variety of tissues. The association of progesterone with two progesterone receptor isoforms, PR-A and PR-B, mediates the hormone's physiological effects. Both proteins are part of the nuclear receptor superfamily of transcription factors and are generated by the same gene. PR-B is structurally different from PR-A in that it has a 146-amino-acid stretch at the N-terminus (Conneely et al., 2002).

Both PR-A and PR-B are ligand activated transcription factors with a central DNA binding domain (DBD) flanked at the N terminus by an activation function-1 (AF-1) and a hinge region at the C terminus, as well as a ligand binding domain (LBD) containing a second activation function (AF-2). A third activation mechanism (AF-3) is found in the N terminal region of PR-B. PR-B has been shown to be a more powerful transcriptional activator of many PR-targeted genes that are regulated by both receptors. Furthermore, PR-A has the ability to inhibit PR- B's activity (Al-Asmakh, 2007).

During gestation, progesterone inhibits myometrial contractility. An increased resting potential and the prevention of electrical coupling between myometrial cells are two manifestations of this effect. Progesterone also inhibits the absorption of extracellular calcium, which is needed for myometrial contraction, by suppressing the expression of genes that code for voltage-dependent calcium channel subunits. The adrenergic system is involved in myometrial quietening during pregnancy. Progesterone enhances ß-adrenergic receptor transcription in late pregnant rats' myometrium, resulting in improved susceptibility to adrenergic agents (Zhang & Salamonsen, 2002).

The placental progesterone at maternal progesterone levels increases six to eightfold from the luteal phase to term. While progesterone is almost exclusively produced by the corpus luteum before 6 weeks of pregnancy, it is increasingly produced by the placenta after the 7th week. The placenta is the most reliable source of progesterone after 10 weeks (Reshef et al., 2000). Although the placenta produces a lot of progesterone, its ability to synthesize cholesterol from scratch is restricted. Therefore, low-density lipoprotein (LDL) cholesterol, which reaches the trophoblasts as maternal cholesterol, is the primary substrate for the placenta's biosynthesis of progesterone (Reshef et al., 2000).

Foetal progesterone plays a minor role in embryonic development. This is shown by the fact that progesterone levels remain elevated even after the death of the foetus. Moreover, oestrogen controls placental progesterone activity in nonhuman primates (Pepe & Albrecht, 1995), and the majority of progesterone released in the placenta is absorbed by the mother (Reshef et al., 2000).

2.2.5 Oestrogen

The main source of oestrogen biosynthesis during pregnancy is the placenta. Myometrial contractions are not triggered by oestrogens themselves, and maternal administration of oestradiol to rhesus macaques after 130 days of gestation has shown to have no effect on the duration of pregnancy. By controlling myometrial gap junctions and uterotonic receptors (including L-type calcium channels and oxytocin receptors), oestrogen, consequently increases the myometrium's ability to produce contractions (Kota et al., 2013).

Oestrogen therefore, has been hypothesized that it can promote uterine contractility and function synergistically with oxytocin in the control of uterine peristalsis and the mechanism of ova transport towards the corpus uteri and fallopian tubes (Mueller et al., 2006). Mueller et al. (2006) were also able to demonstrate the occurrence of uterine peristaltic waves with a cervico-fundal orientation when they perfused swine uteri with 17β-oestradiol.

Moreover, oestrogens are steroid hormones that control the growth and activity of male and female reproductive organs. Oestrogen production in the ovary starts with androgen synthesis in theca cells and ends with the enzyme aromatase converting androgens to oestrogens in granulosa cells. Oestrogens are produced in the male gonad by Leydig cells, Sertoli cells, and mature spermatocytes. Oestrogens, as other steroid hormones, join cells passively and bind to oestrogen receptors, which then regulate the transcription of oestrogen-responsive genes downstream (Barakat et al., 2016).

In mammals, 17β-oestradiol (oestradiol) is the most abundant and potent type of oestrogen among the many different types. For the first 5-6 weeks of pregnancy, the corpus luteum is the only source of 17β-oestradiol. The placenta is the main contributor of circulating 17 β-oestradiol during the first trimester. During gestation, the rate of oestrogen synthesis and the level of circulating oestrogens both rise dramatically, during the follicular phase of the development, 17β-oestradiol concentrations are less than 0.1 ng/mL and during the luteal phase, they are about 0.4 ng/mL (Reshef et al., 2000).

17β-oestradiol levels rapidly rise after fertilization, reaching a range of 6-30 ng/mL at term. The placenta is unable to convert progestogens to oestrogens due to a lack of 17-hydroxylase enzyme activity and 17-20 desmolase (lyase) activity. As a result, it depends on foetal and maternal adrenal glands to develop 19-carbon androgen precursors. LDL-cholesterol circulating in the foetal blood is the main contributor of foetal adrenal dihydroepiandrostenediene sulphate (DHEAS) (Bradshaw & Carr, 1986).

Pregnenolone, which is secreted by the placenta, is also a small contributor of foetal adrenal DHEAS. The maternal compartment is responsible for 20% of foetal cholesterol. The 19-carbon precursors are converted to oestrogen by the cytochrome P450 aromatase enzyme. Even in the presence of large quantities of aromatizable androgens, the efficiency of this enzyme protects the foetus from virilization (Simpson et al., 1994).

Other extragonadal organs manufacture oestradiol and they include the adrenal glands, liver, adipose tissue, skin, pancreas and other yet-to-be-identified locations. The discovery of extra-gonadal sites of oestradiol synthesis has considerably increased our understanding of oestrogen's novel functions outside of the reproductive system (Barakat et al., 2016).

2.2.6 Glucocorticoids

There are some acts in these hormones that can also help prepare the uterus for labour. Glucocorticoids function directly to control the synthesis of prostaglandin in the foetal membranes. It appears that cortisol promotes expression of Corticotropin Releasing Hormone (CRH) in vitro placental (but not hypothalamic). In addition, cortisol increases amnionic cyclooxygenase to increase synthesis of prostaglandins and inhibits the activity of chorionic prostaglandin dehydrogenase, preventing prostaglandin degradation (Asgari-safdar et al., 2013).

2.2.7 Peptide-related parathyroid hormone

The peptide associated with parathyroid hormone is a strong smooth muscle relaxant capable of inhibiting oxytocin-induced contractions in baboons. It is unclear if it has a physiologically significant role in the sustaining of uterine quiescence before labour starts (Mitchell et al, 2003).

2.2.8 Relaxin

Relaxin is a member of the protein family of insulin-like growth factors. At 8 to 12 weeks of gestation, the plasma levels are highest and subsequently decrease to low levels that remain until term. The corpus luteum is believed to be the main source of relaxin (Kota et al., 2013).

2.3 Physiology of placental maturation and separation

The expulsion of the foetal membranes consists of a complex hormonal process that occurs prior to parturition. When the foetal cotyledons surround the maternal caruncles, the placenta is formed, creating new structures called placentomes. Collagens play an important part in the isolation of these caruncles from cotyledons at the time of foetal expulsion in these attachment sites (Amin et al., 2013). Placental enzymes that convert progesterone into oestrogen activate the normal events of parturition. The elevated oestrogen level induces myometrium sensitivity of the oxytocin receptor as well as increased prostaglandin F2 alpha concentration.

This increased prostaglandin results in myometrium contraction and corpus luteum (CL) regression (Beagley et al., 2010). The luteolysis of CL results in the release of relaxin hormone along with a decrease in the progesterone levels. The activation of collagenase action is as a result of these two factors. Relaxin stimulates collagen lysis, softening of the cervix, and reproductive ligament growth. This operation of collagenase breaks down the connection between the maternal caruncle and foetal cotyledon. Placental separation then occurs between the mother and the foetal cotyledon, which leads to placenta expulsion (Abdisa, 2018).

2.4 Complications of labour and delivery

Once labour deviates from agreed rates of progression, it is considered abnormal, depending on the stage of labour and the parity of partuent (Taylor & Long, 2016). In three steps, labour begins and can certainly start weeks before the woman gives birth. When contractions begin, the first stage begins and lasts until the cervix is completely dilated, which means the cervix is at 10 centimetres or 4 inches dilated. In preparation for childbirth, this means that the cervix has opened fully. The second stage is the active stage, in which pushing downwards begins. Basically, it starts with full cervix dilation and ends with the baby's birth (MoH, 2007).

The third stage is known as the placental phase as well. This stage starts with the birth of the baby and ends with the placenta's full delivery. The majority of pregnant women go through these periods without having any issues. However, some women during one of the three stages of labour may experience irregular labour. During the three stages of labour, the following types of abnormal labour can occur at any point (Cirino, 2016).

2.4.1 Prolonged labour

Prolonged labour (PL) is a protraction disorder either slower than normal progression of labour or an arrest disorder which is complete cessation of the labour process. It is an important risk factor for birth-related complications, including foetal distress, which may lead to neurological damage or perinatal death, and maternal complications such as postpartum haemorrhage, uterine rupture, puerperal sepsis, and obstetric fistulae. In such cases, access to skilled birth attendants and use of the World Health Organisation's (WHO's) partograph are crucial to secure adequate surveillance and timely action, aiming for early detection of slow progress and preventing change from a normal labour to a prolonged labour (Nystedt & Hildingsson, 2014).

The causes of PL include poor power of contractions, malpresentation, malposition, and disproportion between the maternal pelvis and foetal head. In all other situations, a trial of labour is mandatory in order to diagnose PL. The first stage is the active phase of labour, and is prolonged when cervical dilation is less than 1 cm/hour, defined by the partograph's alert line. If this delay exceeds 4 hours, the action line is crossed, and immediate action must be taken. Prolonged labour in the second stage is defined as a fully dilated cervix for more than 1 hour in a multiparous woman and 2 hours in primigravidae (Garg et al., 2016).

Maternal risk factors that increase the chances of PL include primiparity and total maternal weight gain or high body mass index. Foetal risk factors include a heavy birth weight, large head circumference and occiput posterior presentation. A PL is also associated with worse labour pain than expected leading to greater use of epidural analgesic and risk of operative interventions (Nystedt & Hildingsson, 2014).

Use of oxytocin early enough for augmentation and early amniotomy are common management methods used to speed up labour progress and encourage dilation. The existing strategies of oxytocin augmentation prevent slow progress of labour from occurring, or to accelerate labour if the dilation rates become slower than the accepted minimum rate as defined by the diagnostic criteria. However, there are risk factors that can be associated with the use of oxytocin augmentation, and include emergency Caesarean section, hyper-stimulation and for the new

born, a low Apgar score, which is a test given to new-borns to check if an emergency health care is required (Berglund et al., 2010).

According to Bugg et al. (2013), the advantages of the use of oxytocin could be a reduction of length of labour, but its use does not increase the rate of normal births among women with slow progress. Both interventions, an unplanned Caesarean section and the use of oxytocin for augmentation, affect women's and infants' health.

2.4.2 Obstructed labour

Obstructed Labour (OL) is when the presenting part of the foetus cannot progress into the pelvis, despite strong uterine contractions due to physically being blocked. Obstructed labour affects approximately 3-6% of labouring women globally. In low-resource settings, OL is closely associated with severe maternal morbidity such as postpartum haemorrhage, uterine rupture, puerperal sepsis, genital fistula and maternal death. Obstructed labour also carries a high risk of intrapartum asphyxia, subsequent neonatal neurological damage, and perinatal death (Mgaya et al., 2016).

The most common cause of obstructed labour is cephalo-pelvic disproportion, which is a mismatch between the foetal head and the mother's pelvic brim. The foetus may be large in relation to the maternal pelvic brim, such as the foetus of a diabetic woman, or the pelvis may be contracted, which is more common when malnutrition is prevalent and lack of sufficient vitamin D (Neilson et al., 2003).

Some other causes of obstructed labour may be malpresentation or malposition of the foetus (shoulder, brow or occipital-posterior positions). In rare cases, locked twins or pelvic tumours can cause obstruction (WHO, 2008). Obstructed labour can also occur in adolescence as the pelvis is not fully grown. It is more common in humans than in primates, because the birth canal of a woman is not as straight and wide as in primates. Neglected OL is a major cause of both maternal and new born morbidity and mortality (Dolea & Abouzahr, 2000).

The obstruction can only be managed by means of an operative delivery, either caesarean section or other instrumental delivery such as use of forceps, vacuum extraction or symphysiotomy; surgical opening of the symphysis pubis. Maternal complications include intrauterine infections following prolonged rupture of membranes, trauma to the bladder and/or rectum due to pressure from the foetal head or damage during delivery, and ruptured uterus with consequent haemorrhage, shock or even death (Indra et al., 2017).

Injury to the bladder during vaginal or instrumental delivery may prompt pressure incontinence. By a wide margin, the most serious and troubling long-term condition following OL is obstetric fistula - a hole which forms in the vaginal wall imparting into the bladder (vesico-vaginal fistula) or the rectum (recto-vaginal fistula) or both. In developing countries, fistulae are commonly the result of prolonged obstructed labour and follow pressure necrosis caused by impaction of the presenting part during difficult labour (Dolea & Abouzahr, 2000).

2.4.3 Postpartum haemorrhage

Post-delivery of the foetus and placenta, forceful uterine contractions are required to clamp uterine blood vessels and stem bleeding. Postpartum haemorrhage (PPH) is commonly defined as at least 1,000 ml total blood loss or loss of blood together with signs and symptoms of hypovolemia within 24 hours after delivery of the foetus or intrapartum loss. Postpartum haemorrhage occurs in up to 3 to 5% of obstetric patients and represents one fourth of maternal morbidity and mortality worldwide (Evensen et al., 2017).

Excessive bleeding during labour or postpartum period in most cases is a result of uterine atony. Therefore, the prophylactic use of uterine contractile agents to enhance myometrial contraction and uterine retraction have long been used in the prevention of PPH. Thus, oxytocin's endogenous role in labour is also extended to reinforcing contractions after delivery (Arrowsmith & Wray, 2014). Other causes of PPH are tissue related and include; retained products of conception such as placenta, membranes and clots, trauma of the genital tract, that is, vaginal or cervical tears or haematoma, ruptured uterus, and broad ligament haematoma. Thrombin related issues such as abnormalities of coagulation and maternal bleeding disorders can also cause PPH (Mousa et al., 2014).

Administration of oxytocin (Pitocin®) just after delivery reduces rate of postpartum haemorrhage by 40 % (Bhattachar et al., 2013). This reduction also occurs if oxytocin is given after placental delivery. Oxytocin is the drug of choice for preventing postpartum haemorrhage because it is at least as effective as ergot alkaloids or prostaglandins and has fewer side effects. Misoprostol (Cytotec®) has a role in the prevention of postpartum haemorrhage but it has more side effects though it is affordable, heat- and light-stable, and requires no syringes (Anderson & Etches, 2007).

Risk factors for PPH include a prolonged third stage of labour, multiple delivery, episiotomy, foetal macrosomia, and history of postpartum haemorrhage. However, postpartum

haemorrhage can also occur in women with no risk factors, so birth attendants must be prepared to manage this condition at every delivery (Mousa & Alfirevic, 2007).

2.4.4 Retention of placenta (after birth)

This is described as failure of expulsion of the foetal membranes. It is also called Retention of foetal membrane (RFM). Retention of placenta occurs when foetal membranes fail to detach after parturition within 8 hours of parturition (Biner et al., 2015). Retention of placenta can create some problems or disorder in the reproductive organs by allowing growth of microorganisms inside the uterus thereby causing inflammation of the uterus, fever and weight loss. Other associated problems include decreased milk yield, longer calving intervals and in severe cases death of the animal (Abdisa, 2018).

Furthermore, animals with retained placenta can suffer from tetanus which is caused by a common organism *Clostridium tetani* found in faeces and in soil. Tetanus requires at least 1-3 months treatment therapy. This in turn affects the economy of the country by decreasing economic growth rate mainly due to decreased milk yield and calf drop (Amin et al., 2013). Although the actual causes of retained placenta are not clear, the condition usually follows dystocia, maternal hypo-immunity, malnutrition and unbalanced nutrition, stress, hereditary predispositions or infections (Hanafi et al., 2011).

2.4.5 Management of complications of labour and delivery

The purpose of labour is to promote vaginal delivery of a healthy child. A typical uterus is spontaneously contractile but changes overtime, so that it maintains its stable state to maintain the foetus. In the final 4 to 5 weeks of pregnancy, endogenous prostaglandins are released to enable maturation of the cervix, rendering it soft and extended, and increase uterus sensation to labour (Hofmeyr et al., 2008).

Different medications may be used to stimulate and improve labour, but they are typically associated with either the failure to achieve successful labour or uterine hyperstimulation. For decades, nonpharmacological cervical ripening strategies have been used, but there are not enough tests to show their effectiveness. They include herbal compounds, castor oil, hot baths, sexual intercourse, stimulation of the breast, acupuncture, acupressure, mechanical and surgical modalities, and transcutaneous nerve stimulation (Panesar, 2012).

2.4.6 Pharmacological techniques used in oxytocic drug discovery

Drug discovery aims to produce novel, potent and highly selective ligands that produce therapeutic effect by initiating or hindering cellular pathways. This requires an appropriate assay system for the testing of reliable and robust compounds. Pharmacological techniques such as ligand receptor binding and functional assays often incorporate heterologous cell-based systems designed to overexpress receptors that would otherwise not be present. While these techniques provide useful insights into receptor pharmacology and early development of drugs, the data collected may not reflect a true in vivo scenario (Arrowsmith et al., 2018). Therefore, it is vital that pharmacological data from cell-based assays are also affirmed in physiologically relevant models.

Several methods for the study of myometrial function and dysfunction have been developed, from *in vivo* internal and external tocography and intrauterine pressure catheters to generate myometrial-origin transformed or immortalized cells (Haran et al., 2012). While cell culture systems can detect whether a substance can act at the cellular level, tissue strips within organ baths can be used as instruments to measure the functional responses to pharmacological reagents of entire tissues. A large heated glass chamber capable of holding between 5 and 50 mL of physiological saline is typically the traditional organ bath (Arrowsmith et al., 2018). Strips normally require aeration with oxygen and large volumes of physiological salt solution (PSS) within these large chambers.

Within the bathing chamber, strips of tissue are dissected and suspended and connected to a force transducer that measures tension alterations, such as during a contraction. A number of research groups have used myometrium strips from both humans and animals, including guinea pig, mouse, rat, rabbit, and others (Lucovnik et al., 2011), to examine many questions relating to myometrial physiology and pathology, including preterm and dysfunctional labour. Myometrial strips, for example, have been used to identify factors that regulate and alter myogenic activity, determine organelle function such as SR22 and to investigate ion channel modulators, pumps and switches to determine their role in the physiology of myometrial contractions (Mccallum et al., 2011).

2.5 Medicinal plants

From approximately 422,000 flowering plants around the world, more than 5,000 of them are used for medicinal purposes (Kooti et al., 2015). The use of herbs to treat diseases is a common practice since ancient times. Use of natural remedies and herbal medicines is a beneficial cost-

effective method for treating various diseases. Plants have always played an important role in the health and treatment of human diseases. They have fewer side effects than synthetic drugs and due to their antioxidant properties, they reduce drug toxicity. The natural active ingredients in these medicinal plants cause biological balance and prevent drug accumulation in the body (Yuan et al., 2016).

All cultures have evolved herbal form of medicine dependent on the plants in their own environment (Gurib-Fakim, 2006). Some scholars have argued that this passed-down wisdom is the source of modern medicine and pharmacy. Hundreds of higher plants are grown throughout the world for medicinal and pharmaceutical purposes (Salmerón-Manzano et al., 2020). The plant's therapeutic effects is what has led to the development of pharmaceutical medicines derived from the specific plant compounds that have these therapeutic benefits (Jones et al., 2006).

The ability to isolate the active properties of medicinal plants was made possible by the origin of modern science, especially in the renaissance and particular in chemical analysis and related instrumentation such as the microscope. Since then, these active components have been synthesized in the laboratory to be used in the production of these herbal medicines (Atanasov et al., 2015). Conventional medicines use slowly increased and the direct use of medicinal plants seems to have been replaced in modern medicine. In many instances, pharmaceutical drugs today are mainly dependent on the active compounds of plants, which are used as raw materials in drug manufacturing industries (Salmerón-Manzano et al., 2020).

2.5.1 History of use of medicinal plants

Use of herbal drugs has been common since time immemorial. Many of the herbs and spices used for seasoning food often yields useful medicinal compounds. Angiosperms, also known as flowering plants, were the most important original source of plant medicines. Since prehistoric times, humans have used natural products, such as plants, animals, microorganisms, and marine organisms, in medicines to alleviate and treat diseases. According to fossil records, the human use of plants as medicines may be traced back at least 60,000 years (Yuan et al., 2016).

The use of natural products as medicines must, of course, have presented a tremendous challenge to early humans. It is highly probable that when seeking food, early humans often consumed poisonous plants, which led to vomiting, diarrhoea, coma, or other toxic reactions, and perhaps even death. However, in this way, early humans were able to develop knowledge

about edible materials and natural medicines. Subsequently, humans invented fire, learnt how to make alcohol, developed religions, and made technological breakthroughs, and they learnt how to develop new drugs (Kooti et al., 2015).

Initially, the trial-and-error approach was used to cure diseases or merely to feel better, and in this manner, valuable plants with beneficial effects were identified this way. The use of these plants has been perfected over centuries, and is still recognised as herbal medicine in many contexts. Traditional medicine is described as "the sum total of science", skills and practices based on ideas as well as values and experiences indigenous to various cultures. Whether explicable or not traditional medicine is used in the maintenance of health, as well as the prevention, diagnosis, improvement, or treatment of physical and mental illnesses (Salmerón-Manzano et al., 2020)

2.5.2 Modern study of plant medicines

At the beginning of the nineteenth century, modern drug era began. In 1805, the first pharmacologically-active compound, morphine, was isolated by a young German pharmacist, Friedrich Sertürner, from the opium plant (Joo, 2014). Subsequently, countless active compounds have been separated from natural products. Among them, some follow their traditional uses and others do not. Later, the development of synthetic techniques led to a significant reduction in the importance of natural products, and there were concerns that the use of some natural products for medicinal purposes might be completely banned. However, natural products are important for the development of new drugs, and these products have been in constant use. Some types of treatment, such as anticancer, antihypertensive, and anti-migraine, have benefited greatly from natural products (Newman & Cragg, 2016).

A majority of the population in the developing world is struggling to raise living standards and improve health-care delivery due to increasing poverty and population. About sixty percent of the world's population and about eighty percent of the developing world is dependent on conventional plant-obtained remedies (Mintah et al., 2019), as pharmaceuticals are expensive. Medicinal plants consumption has doubled in the west and the number of plant-derived medicaments or health foods has increased slowly to meet the rising demand. Currently, research is focused on the isolation of pharmacologically active compounds from natural sources in the area of those diseases where presently available drugs are not so effective. Also herbal medicines are experiencing greater resurgence as many people are turning their attention

from modern drugs towards parallel herbal systems which are also known as alternative medicine (Aslam & Ahmad, 2016).

Several drugs are directly or indirectly derived from plants including digoxin, taxol, vinblastine, nabilone and artemesin (Kigen et al., 2015). Medicinal plants have therefore become important source of research and development of new drugs (Kigen et al., 2013). Identifying medicinal plants and using them is imperative as it is essential for man's health and protects the environment throughout the world (Habib et al., 2016).

2.5.3 Medicinal plants phytochemical compounds

Medicinal plants are considered a repository of numerous types of bioactive compounds possessing varied therapeutic properties. The therapeutic potential of plants has been well explored over a long time period. The vast array of therapeutic effects associated with medicinal plants includes anti-inflammatory, antiviral, antitumor, antimalarial, and analgesic antimicrobial, antihelminth, oxytocic among others (Raina et al., 2014). These therapeutic effects are brought about by compounds known as phytochemicals. There are thousands of different phytochemicals based on the chemical structures like alkaloids, carotenoids, phenolics, flavonoids, coumarins, steroids, tannins and others (Abat et al., 2017).

Phytochemicals are classified depending on the role they play in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, chlorophyll, purines and pyrimidines. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides (Saxena et al., 2013).

These naturally occurring chemical compounds are responsible for colour, odour and therapeutic potential of plants. Plants synthesize these compounds as weapons for defence against biotic and abiotic stresses. Most of the phytoconstituents have antioxidant properties and protect cells against oxidative stress. Phytoconstituents also have commercial applications such as drugs, enzymes, preservatives, flavours, fragrances, cosmetics and fuels. Phytochemical screening is therefore an important tool in identifying chemical compounds of medicinal and industrial value (Abat et al., 2017).

2.5.4 *Uvariodendron kirkii*

Uvariodendron kirkii, common name "Msaidizi," in Giryama, is a species of flowering plants in the Annonaceae family, found in Kenya and Tanzania. It is among the seventeen well known

genera of the family. The plant is an evergreen shrub or a small tree (Figure 1) that usually grows from 1.5 - 6 metres tall, occasionally to 12 metres. It produces many of its flowers along the stems and bole. The tree is harvested from the wild for local use as a medicine and source of wood. It is also threatened by habitat loss due to forest loss and degradation activities such as agriculture, tourism, mining, fuelwood and charcoal. The plant is classified as vulnerable in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Eastern Arc Mountains and Coastal Forests CEPF Plant Assessment Project, 2015). The bark is used as an antidote for snake bites and as medicine for wounds and stomach-ache in Zanzibar. Other uses include the wood, used for building poles, wooden spoons and source of fuel (Useful Tropical Plants, 2018).

Figure 1: The plant *Uvariodendron kirkii* (Source; Author)

Uvariodendron kirkii aqueous extracts have been found to have anti-implantation effects which might either be at uterine level as a result of incompetent zygotes or blastocysts or interrupted hormonal milieu thereby compromising the implantation process (Kaingu et al., 2017). It also contains the following phytochemicals; alkaloids, flavonoids, Quinone's, terpenoids, tannins, saponins, sterols and phenols which may contribute to its anti-implantation effect (Kaingu et al., 2017). Its anti- implantation effect makes it a good candidate to generate a novel product for labour augmentation.

A study conducted by Kaingu et al. (2017) on acute oral toxicity of *Uvariodendron kirkii* aqueous extracts showed that the extracts did not cause any mortality of rats even at the highest concentration of 2000 mg/kg. It however, showed tremors and lethargy within 30 minutes of treatment. The rats had minimal activity for about 3 hours and then became active. Kenana et al. (2019) also suggested that *Uvariodendron kirkii* root bark extract is safe when used for a short time. However long-term use may warrant close monitoring of the liver and kidney functions especially when the plant extract is administered at high doses.

2.5.5 Uterotonic plants

Several plants have been found to have uterotonic activity and include; *Nymphaea alba* of the Nymphaeaceae family commonly known as European white waterlily, *Carica papaya* of the Caricaseae family commonly known as papaya pear, *Raphanus sativus*, *Ficus deltidea and Ficus asperifolia* of the Moraceae family, *Gloriosa superb* and *Agapanthus africanus* of the Liliaceae family, *Jussiaea repen* of the Onagracea family, *Harpagophytum procumbens* of the Pedaliaceae family, *Caesalpinia bonduc* of the Caesalpiniaceae family, *Clivia miniata* of the Amaryllidaceae family, *Ekebergia capensis* of the Meliaceae family, *Rhoicissus tridentate* of the Vitaceae family. *Sesamum radiatum* of the Pedaliaceae family, *Byrsocarpus coccineus* of the Connaraceae family and *Monechma ciliatum* of the Acanthaceae family. All have shown contractile response to isolated rat uterus (Roqaiya et al., 2015).

2.6 Use of rats in research

For animal tests around the world, a number of animal species are used, including rats, mice, rabbits, guinea pigs, hamsters, cats, dogs, mini-pigs, chimpanzees, goats, sheep, chickens, fish, among others. In biomedical science and testing, the use of animals in research is especially considered vital (Rodrigues, 2015). In addition to the study of what causes an illness, fundamental biological and medical science uses animals to study, for example, how the human body works and how its tissues and organ's function. As examples of medical advancements that has been enabled by animal studies, experimental therapies such as polio and diphtheria vaccination, insulin for diabetes and kidney transplants are cited (Fernandes & Pedroso, 2017).

In medical research, rats have a long history: they were the first specifically domesticated mammalian species to be used in the laboratory (Baumans, 2004). Rats have been used to address a broad variety of basic science questions ranging from physiology, immunology, pharmacology, toxicology, diet, behaviour and learning since their development as a laboratory animal (Phillips et al., 2013).

Researchers can, for a variety of reasons, prefer rats when it comes to their use in science. They are bigger in scale, making it easier to manage, sample and execute procedures. A lot is known about the responses and pathways in rats for physiological studies because of the abundance of data gathered in rats over years of research. That means much of the research on the foundation is already done (Baumans, 2004). Some work suggests that the rat reflects human physiology more accurately, producing more precise results in many instances. In addition, rats are second only to humans in biomedical research and share 90% of the genome with humans (Phillips et al., 2013). The Wistar Institute in America and its development of the Wistar albino strain have been linked to the success of the rat in research today.

2.6.1 Wistar rat

The Wistar rat is currently one of the most popular rat strains used for laboratory research. It is characterized by its wide head, long ears and having a tail length that is always less than its body length. Wistar rats are an outbred strain of albino rats belonging to the species *Rattus norvegicus* (Clause, 1993). This is notably the first rat strain developed to serve as model organism at a time when laboratories primarily used the common house mouse *Mus musculus* (Alexandru & Biosafety-Srl-d, 2011).

2.6.2 Wistar rat oestrus cycle

Wistar rat has been used as the main animal model in several studies involving reproduction. This is because their oestrus cycles occur without seasonal influence when submitted to environmental control under laboratory conditions. From the onset of sexual maturity up to the age of 12 months, the mean cycle length in the female rat is 4 days and this short cycle length makes the rat an ideal animal for investigation of changes occurring during the reproductive cycle. Oestrus cycles are characterized by morphological changes in ovaries, the uterus and the vagina which occur during different phases called proestrus, oestrus, metestrus and dioestrus. These phases are usually identified according to cell types observed in vaginal smears. Proestrus and oestrus phases last for twelve hours each, while metestrus lasts for twenty one hours and dioestrus lasts for fifty seven hours (Paccola et al., 2013).

During the oestrus cycle, prolactin, luteinizing hormone (LH) and follicle stimulating hormone (FSH) remain low and increase in the afternoon of the proestrus phase. Oestradiol levels begin to increase at metestrus, reaching peak levels during proestrus and returning to baseline at oestrus. Progesterone secretion also increases during metestrus and dioestrus with a decrease

afterwards. Then the progesterone value rises to reach its second peak towards the end of proestrus (Marcondes et al., 2002).

A proestrus smear consists of a predominance of nucleated epithelial cells, an oestrus smear primarily consists of anucleated epithelial cornified cells, a metestrus smear consists of the same proportion of leukocytes, cornified, and nucleated epithelial cells and a dioestrus smear primarily consists of a predominance of leukocytes as shown in Table 1 below (Hamid & Zakaria, 2013).

Table 1.

Behaviour and Vaginal Cytology with Oestrus Cycle Phases.

Cycle phase	**Duration (hrs)**	**Behaviour**	**Vaginal smear morphology**
Proestrus	12	Male acceptance at end of phase	Nucleated epithelial cells
Oestrus	12	Lordosis; male acceptance	Anucleated epithelial cornified cells
Metestrus	21	No male acceptance	Many leukocytes with nucleated epithelial and cornified cells
Dioestrus	57	No male acceptance	Mostly leukocytes

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

The plant material was collected from Tana River County, Kenya (Figure 2), specifically from Itsowe, Garsen and Ngao subdivisions, because they are the areas with high widespread use of herbal medicine and inaccessibility to health facilities (Kaingu et al., 2013). The County is found in the coast region of Kenya, covers an area of 38,862.20 km^2 and a population of 284,505 (KNBS, 2015). The administrative headquarter of the County is Hola. The County has three sub counties; Bura, Galole, and Garsen. It borders Kitui County to the West, Garissa County to the North East, Isiolo County to the North, Lamu County to the South East and Kilifi County to the South. The county lies between latitudes 00°53' and 20°41' South and longitudes 38°30' and 40°15' East (KNBS, 2015).

The major ethnic groups are the Pokomo, many of whom are farmers, and the Orma and Wardey. The county is generally dry and prone to drought. Rainfall is erratic, with rainy seasons in March to May and October to December. Flooding is also a regular problem, caused by heavy rainfall in upstream areas of the river Tana. The major physical feature in Tana River County is an undulating plain that is interrupted in a few places by low hills at Bilbil around Madogo and Bura divisions. The land in Tana River county generally slopes south eastwards with an altitude that ranges between 20 m to 200 m above sea level at the top of the Bilbil hills (Ngugi et al., 2013).

The most striking topographical feature is the river Tana that traverses the county from the Mbalambala in the north to the Indian Ocean in the South covering a stretch of approximately 500 km. Besides river Tana, there are several seasonal rivers in the county popularly known as *laghas*, which flow in a west-east direction from Kitui and Makueni counties draining into river Tana and eventually into the Indian Ocean. The river beds support livestock as well as wildlife during the dry season since they have high ability to retain water (KNBS, 2015).

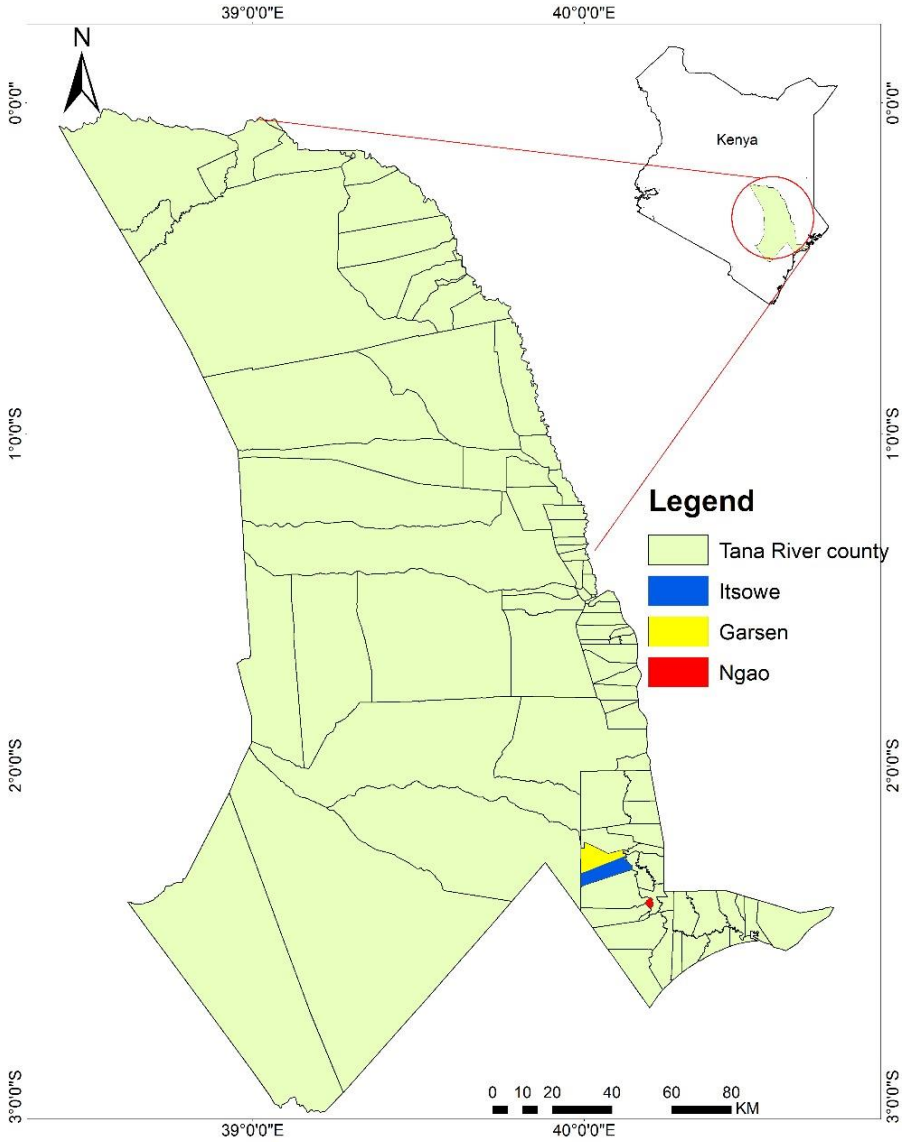

Figure 2: Map of Tana River County showing the study areas Itsowe, Garsen and Ngao subdivisions (Source: DIVA-GIS shape files modified using ArcMap version 10.3)

Laboratory work was carried out in the Departments of Veterinary Anatomy and Physiology, and Medical Physiology, at the University of Nairobi, Chiromo campus, Kenya. The university is located in Nairobi, the capital city of Kenya. The city stands at an altitude of 1,798 meters above sea level and lies between longitudes 36^{o} 45'East and latitudes 1^{o} 18'South (County Government of Nairobi, 2014).

3.2 Experimental animals

Female Wistar rats aged between 10-12 weeks and weighing 180-220 g from the Department of Biochemistry, University of Nairobi, were used in this study. They were kept in cages with

5 rats per cage. The cages were made of plastic material with metal wirings as the lids. Each cage was 12 cubic feet in terms of measurements. They were kept in an air-conditioned room at 22 ± 2^{o}C and 60-70% relative humidity. The cage floors were lined with untreated wood shavings as beddings which were changed every other day. The animals were exposed to the natural 12 hours light and 12 hours dark cycle, fed with commercial rat pellets (Unga Feeds Limited, Kenya) and had *ad libitum* access to clean water (Bozcheloei et al., 2017). All the rats were handled humanely in accordance with the institution's Animal Care and Use Committee guidelines and allowed to acclimatize for one week before beginning of the study.

3.3 Collection and preparation of the plant material

Uvariodendron kirkii roots were collected from Tana River County, and obtained using a panga and a hoe by cutting strips of the roots 2 cm wide and 2 cm long, with 4 cm between strips, for effective rates of root regrowth and for lower susceptibility to insect attack and diseases (Mariot et al., 2014). The roots were put in polythene bags and then transported in cool boxes to maintain their freshness, to the University of Nairobi, Department of Veterinary Anatomy and Physiology laboratory in Chiromo campus. They were washed carefully and thoroughly using tap water to remove foreign matter (Adongo, 2013).

Fresh root barks were removed and cut into small pieces using a knife. The root barks were kept under shade and dried at room temperature for a period of two weeks. They were then ground into powder using a Cunningham grinder machine. This was done in a fume chamber. The plant powder was weighed using an analytical balance (Aczet CY 205C USA) and packed in 200 g sachets and stored in cool and airy cupboards away from direct sunlight ready for extraction (Kaingu et al., 2017).

3.4 Preparation of the plant extract

In order to obtain an aqueous extract similar to the traditional recommendation, 200 g of *Uvariodendron kirkii* root bark powder was weighed. The root bark powder was macerated in warm distilled water at a ratio of 1 to 6 (weight/volume) in a volumetric flask. The suspension was rotated on a shaker for 24 hours at room temperature and left to soak for 48 hours. Filtration was then carried out using Whatman filter paper (number 4). Filtrate obtained was freeze dried for 48 hours to ensure all the distilled water had evaporated (Kaingu et al., 2017). Extract was then weighed to determine yield. 200 g yielded 6 g of extract hence the percentage yield was calculated as follows;

$$Percentage\ extract\ yield\ \left(\frac{W}{V}\right) = \frac{dry\ extract\ weight}{dry\ starting\ material\ weight} \times 100$$

$$= \frac{6}{200} * 100 = 3\%\ yield$$

3.5 Aqueous stock solution

Aqueous *Uvariodendron kirkii* stock solution of 160 mg/ml was prepared by weighing 1600 mg of freeze-dried extract and reconstituting with 10 millilitres of distilled water. Similarly, double dilution of stock solution was undertaken to generate 80, 40 and 20 mg/ml extract concentrations respectively to constitute the four working doses (Kaingu et al., 2012).

3.6 Experimental design

A completely randomized design was adopted. A total of sixty-five female rats were used. They were divided into groups of five. Five rats were used as positive control (Oxytocin). Twenty rats were then used to study each extract concentration, that is, 20, 40, 80, and 160 mg/ml (5 rats for each concentration). Twenty rats were used to study effect of extract on oxytocin induced contraction and another twenty to study effect of extract on prostaglandin induced contractions.

3.7 Preparation of the uterine tissues

Non-pregnant female rats were used and their oestrus cyclicity closely monitored every day between 9-10 a.m. for 15 days. Vaginal smears were obtained and prepared. With the aid of a Pasteur pipette, 0.1 mm of the vaginal smear was collected, and observed using a light microscope (×10 objective lens) in order to ascertain the oestrus stage (Marcondes et al., 2002). Rats in proestrus and oestrus stages of the oestrus cycle were selected. These oestrus stages are when the uterus is most receptive to contraction. The rats were subcutaneously injected with 2 mg/kg diethylstilboestrol 24 hours prior to extract exposure (Misonge et al., 2014). Diethylstilboestrol increases the sensitivity of the uterus to contractile agents.

The rats were humanely sacrificed using diethyl ether (Goodies et al., 2015). The uterine horns were immediately harvested and placed into a Petri dish containing previously warmed and aerated de Jalon solution. The uterine horns were trimmed of all connective tissue. The uterine horns were cut into 2 cm strips (Watcho et al., 2010) and each strip was mounted in an organ bath containing 20 ml De Jalon's solution (Kaingu et al., 2012; Pakoussi et al., 2015).

De Jalon solution was maintained at a temperature of 37± 0.50°C and continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide gas (Misonge et al., 2014). Each strip was mounted vertically within the organ bath (Figure 3). The upper end of the segment was hooked to an isometric force transducer (ML500/A, AD Instrument) coupled with Power Lab data acquisition system (Power Lab 8/30) (Figure 4). The frequency of contraction (number of peaks recorded) and amplitude of contraction (in microvolts) were recorded and analysed using Chart5 software for windows.

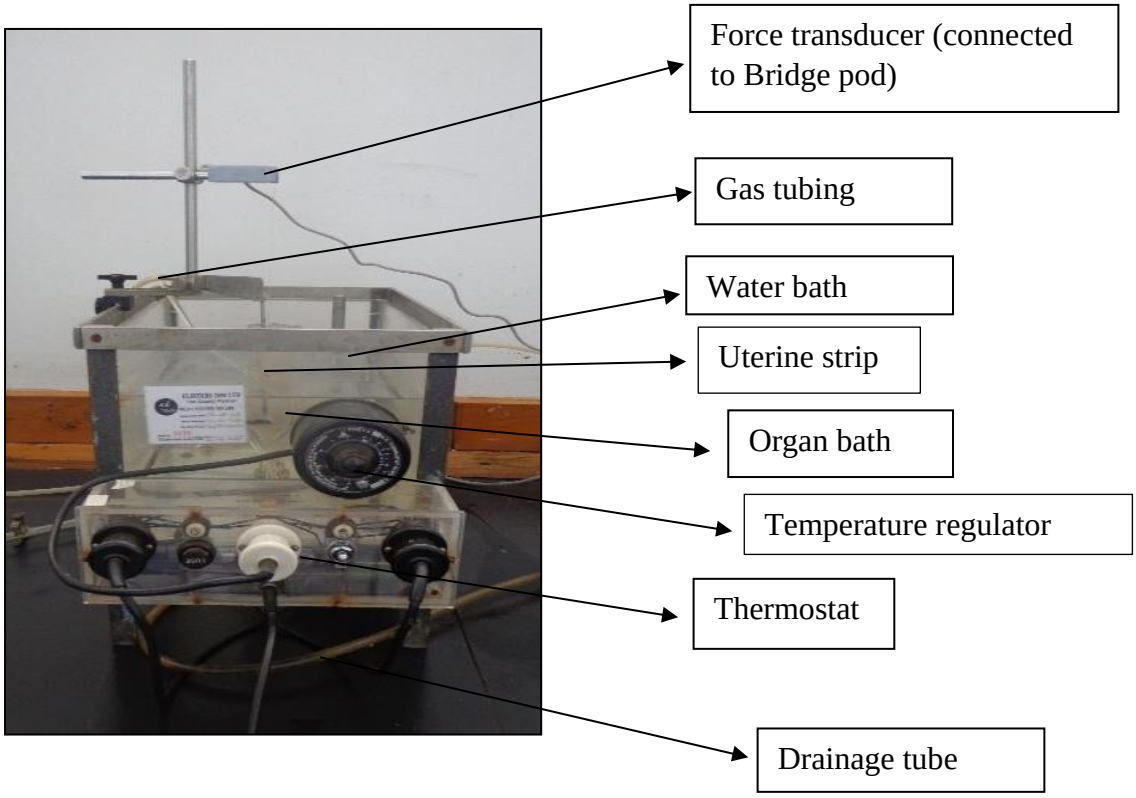

Figure 3: Organ bath and its associated components (Photo; Author)

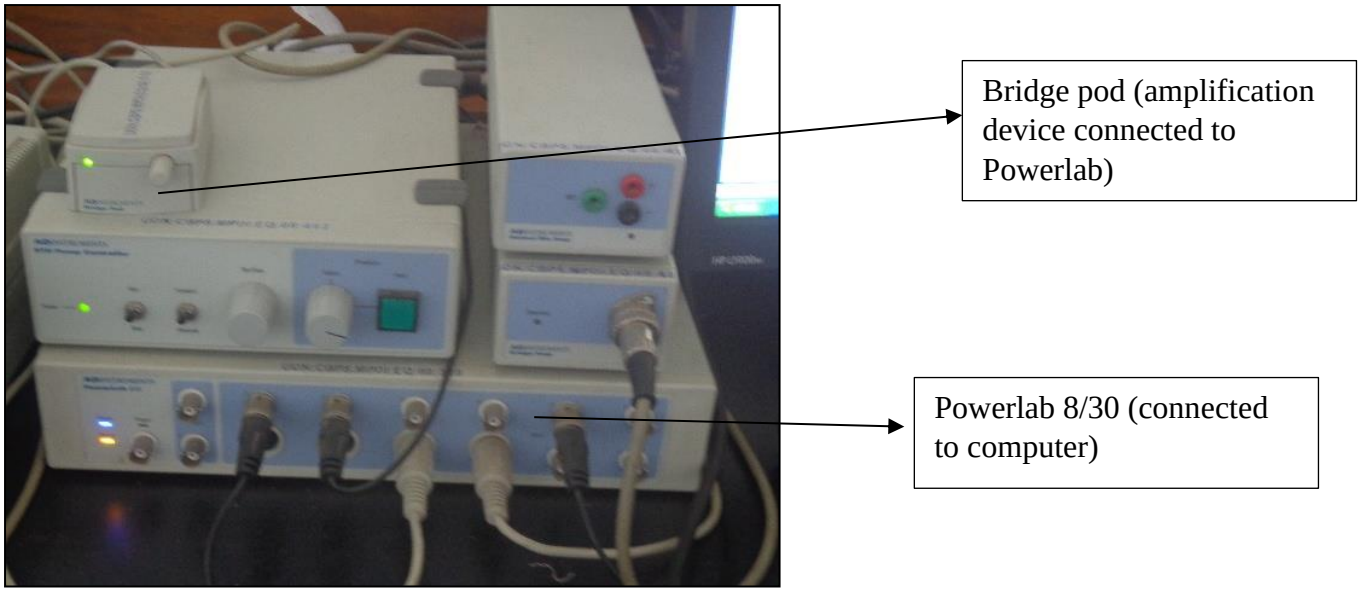

Figure 4: Powerlab data acquisition system (Photo; Author)

3.8 Positive control contractions

Non-pregnant uterine strips were harvested and prepared as described in Section 3.7 above. They were mounted and allowed (one at a time) to equilibrate for 30 minutes in de Jalon solution alone, after which its contractions were recorded for 10 minutes. These initial contractions were taken as the negative control recordings. After the 10 minutes of negative control contractions the tissue was exposed to 1.0 ml Oxytocin which contained 10 IU synthetic oxytocin (Bafor et al., 2017). The isometric contractions were recorded for 10 minutes, recording was taken as the positive control.

Frequency and amplitude (force) of uterine contractions was analysed from the chart recordings, whereby, frequency was taken as the number of contractions recorded over the 10-minute period, that is, the number of peaks recorded. The amplitude or force was taken as the mean height in microvolts (µV) of the peaks produced over the 10-minute period. By comparing the test group frequency and amplitude of contraction to the negative control groups, one was able to determine whether the extract increased, reduced or had no effect on frequency and amplitude of uterine contraction.

3.8.1 Effect of *Uvariodendron kirkii* aqueous extracts on non-pregnant rats' uteri

Fresh uterine horns were prepared as described in Section 3.7. Mounting of the non-pregnant uterine tissue was done and immediately after the 10 minutes of negative control contractions, the tissue was exposed to 1ml of 20 mg/ml *Uvariodendron kirkii* aqueous extract which was

added to the organ bath. The isometric contractions were recorded for 10 minutes. Each extract concentration challenge was repeated using five rats.

Fresh de Jalon solution was used to wash the inner compartment (organ bath chamber) and a fresh uterine strip mounted. The fresh uterine tissue was exposed to 40 mg/ml extract concentration (Kaingu et al., 2012). The process was repeated for the 80 and 160 mg/ml extract concentration. Each extract concentration challenge was repeated using five rats. Frequency and amplitude of uterine contractions was obtained as described in Section 3.8

3.8.2 The effect of *Uvariodendron kirkii* aqueous extracts on oxytocin induced uterine contractions

Fresh uterine tissues prepared as described in Section 3.7 were mounted, one at a time, and after 10 minutes of negative control contractions was exposed to 1.0 ml Oxytocin which contained 10 IU synthetic oxytocin. The isometric contractions were recorded for 10 minutes (taken as positive control), after which the strip was not rinsed and organ bath contents not altered. The uterine strip was then exposed to 1ml of 20 mg/ml of *Uvariodendron kirkii* aqueous extract and isometric contractions recorded for 10 minutes. The extract concentration was added into the organ bath and process was repeated 5 more times using fresh strips (Bafor et al., 2017; 2018). Similarly, 40, 80 and 160 mg/ml extract concentration challenge was conducted. Frequency and amplitude of uterine contractions was obtained as described in Section 3.8.

3.8.3 The effect of *Uvariodendron kirkii* aqueous extracts on prostaglandin induced uterine contractions

Fresh uterine tissues prepared as described in Section 3.7 were mounted, one at a time, and after 10 minutes of negative control contractions was exposed to 1.0 ml prostaglandin F2α which contained 250 μg active ingredient, cloprostenol. The isometric contractions were recorded for 10 minutes, after which the strip was not rinsed and organ bath contents not altered. The uterine strip was then exposed to 1ml of 20 mg/ml of *Uvariodendron kirkii* aqueous extract and isometric contractions recorded for 10 minutes. The extract concentration was added into the organ bath and the process was repeated 5 more times using fresh strips (Bafor et al., 2017; 2018). Similarly, 40, 80 and 160 mg/ml extract concentration challenge was conducted. Frequency and amplitude of uterine contractions was obtained as described in Section 3.8.

3.9 Data analysis

All values were expressed as percentage increase or decrease in mean ± standard error of mean (SEM) relative to the controls, using the following formula.

$$\%\ contraction = \frac{frequency\ or\ amplitude\ after\ treatment - control\ contractions}{control\ contractions} * 100$$

GraphPad prism 8.0.1(244) was used to analyse the data, that is frequency and amplitude of uterine contractions using one-way ANOVA. P-values ($P < 0.05$*; $P < 0.01$**; $P < 0.001$*** and $P<0.0001$****) were considered significant. A post hoc Tukey's multiple comparison test was conducted to analyse statistical differences among groups.

CHAPTER FOUR

RESULTS

The tissues exhibited contraction patterns that resembled (more or less) those formed by exogenous oxytocin upon exposure to the plant aqueous extract. There was a heavy initial and prolonged contraction in almost all tests, during which isometric contractions resumed. Analysis and contrast were made and the data was expressed as mean ± standard error of mean (SEM) as a percentage increase or decrease in contraction frequency and amplitude relative to the control.

4.1 Positive control

Figure 5 shows the effect of oxytocin (OXT) on frequency and amplitude of uterine contractions. Upon addition of 1ml of oxytocin the frequency and amplitude of contraction increased by 11.29 ±0.774 % and 18.48 ±4.363%, respectively, compared to the negative control with De Jalon solution only.

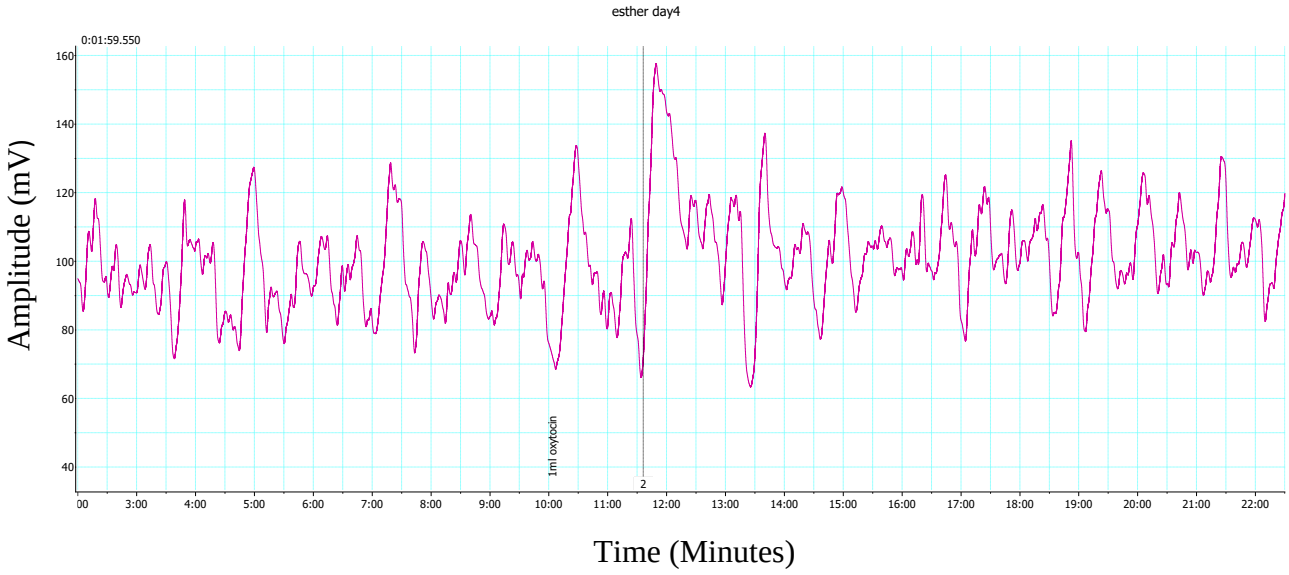

Figure 5: The effect of oxytocin on frequency and amplitude of uterine contraction (positive control)

4.2 Effect of *Uvariodendron kirkii* aqueous extract on frequency and amplitude of uterine contraction

Figures 6, 7, 8 and 9 show representative tracings of the effect of varied concentrations of *Uvariodendron kirkii* extracts on frequency and amplitude of uterine contractions. Figure 6 shows contraction pattern of negative control versus 20 mg/ml of *Uvariodendron kirkii* extract, Figure 7 shows the contraction pattern of negative control versus 40 mg/ml of *Uvariodendron*

kirkii extract, Figure 8 shows contraction pattern of negative control versus 80 mg/ml of *Uvariodendron kirkii* extract, and Figure 9 shows contraction pattern of negative control versus 160 mg/ml of *Uvariodendron kirkii* extract.

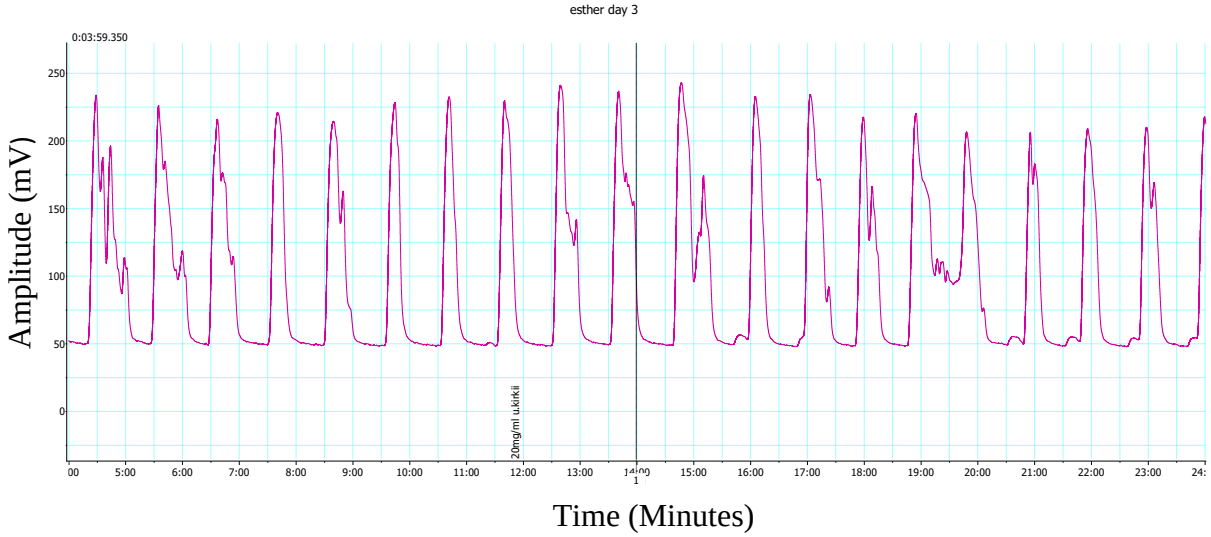

Figure 6: The effect of 20 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of uterine contractions.

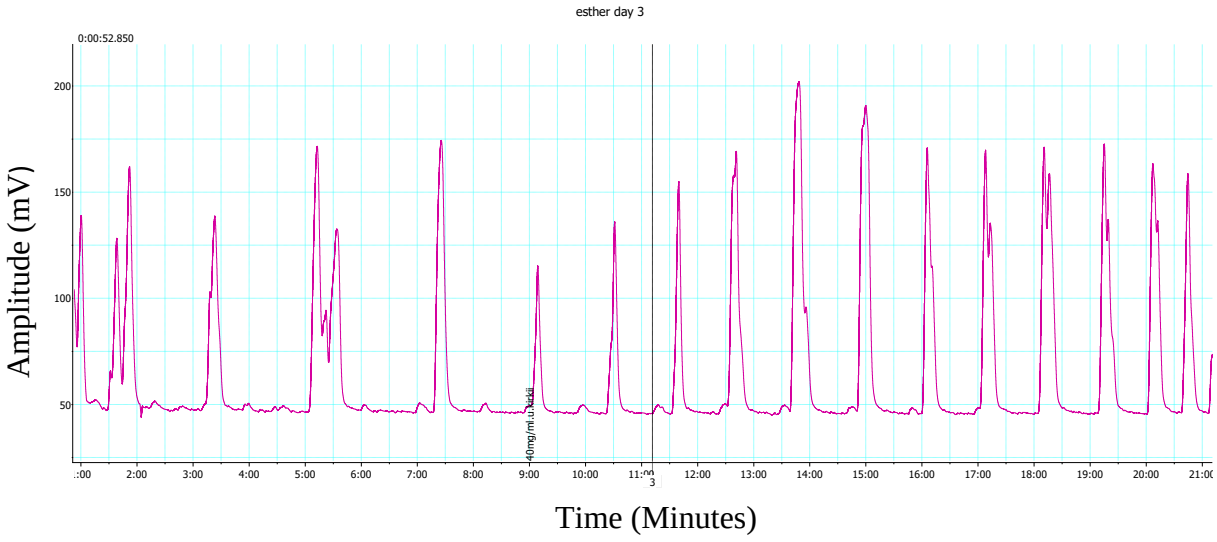

Figure 7: The effect of 40 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of uterine contractions.

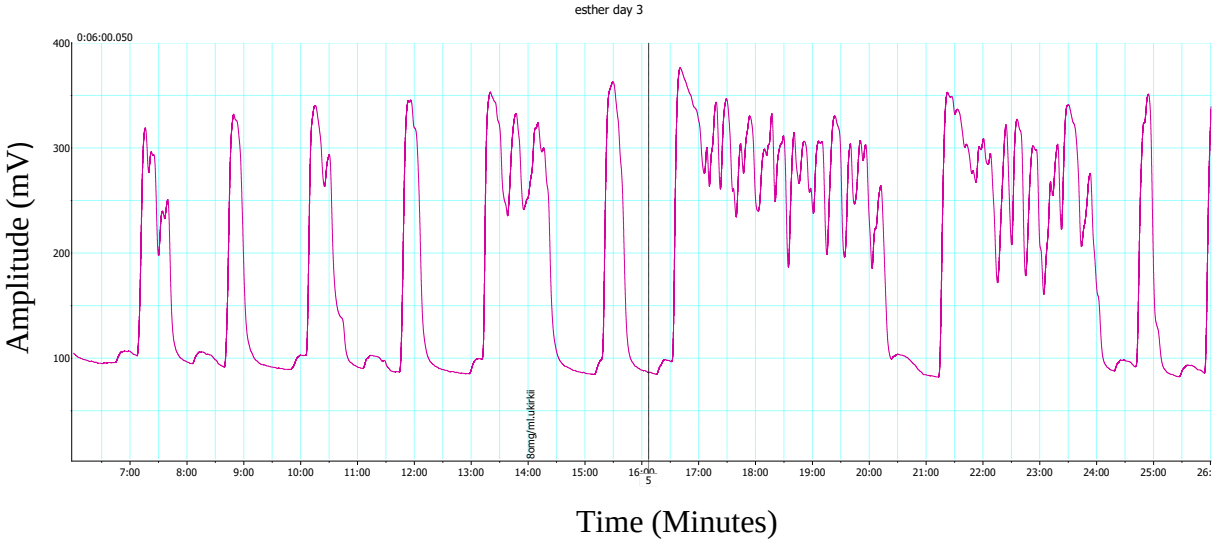

Figure 8: The effect of 80 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of uterine contractions.

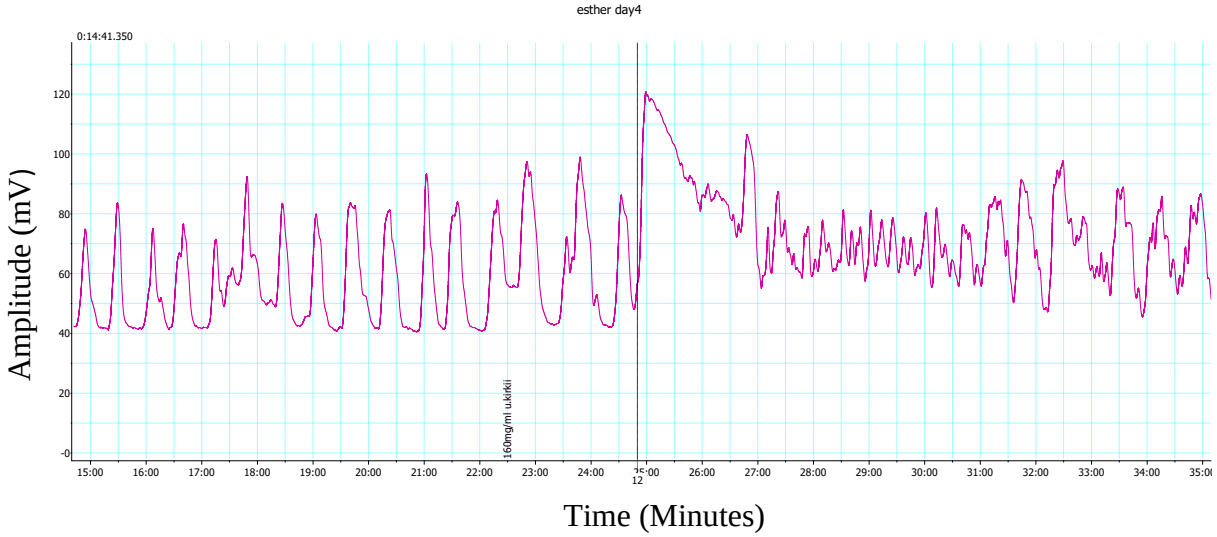

Figure 9: The effect of 160 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of uterine contractions.

Before extract addition, the frequency of uterine contractions with De Jalon solution alone was taken as control. Upon addition of 20 mg/ml of the extract, the uterine contraction frequency increased by 16.53 ±2.400 % at 20 mg/ml, 25.12 ±4.875 % at 40 mg/ml; 33.48 ±7.370 % at 80 mg/ml and 56.39 ± 9.815% at 160 mg/ml. The mean uterine contraction frequencies were significant ($P<0.05$) at 40 mg/ml, at 80 mg/ml ($P< 0.01$), and at160 mg/ml ($P< 0.0001$) (Figure 10).

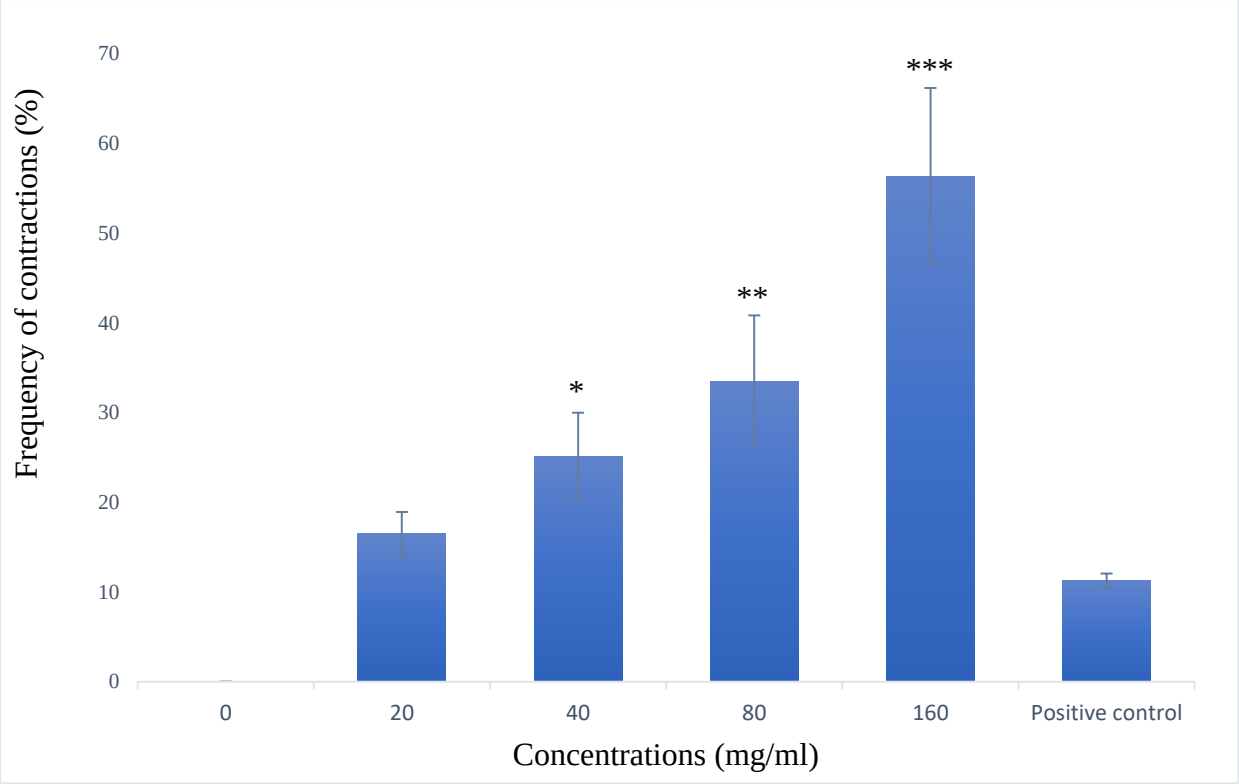

Figure 10: The effect of *Uvariodendron kirkii* extract concentrations on uterine frequency of contractions.

Upon addition of 20 mg/ml, 40 mg/ml and 80 mg/ml *Uvariodendron kirkii* extract, the amplitude increased by 2.87 ±0.333%, 9.218 ±3.779% and 16.37 ±5.802%, respectively. The three extract concentrations did not cause any significant difference in amplitude of contraction at $P < 0.05$ level of significance. At 160 mg/ml, however, there was a significant ($P<0.01$) difference in amplitude of contraction as contraction amplitude increased by 24.32 ±5.997 % (Figure 11).

There was no significant difference in frequency of uterine contractions between 20 mg/ml, 40 mg/ml and 80 mg/ml; and oxytocin (positive control) at $P<0.05$. However, there was a significant difference in frequency of uterine contractions between 160 mg/ml and oxytocin at $P<0.0001$. In terms of amplitude of uterine contractions there was no significant difference between all the varied concentrations and oxytocin at $P<0.05$ level of significance.

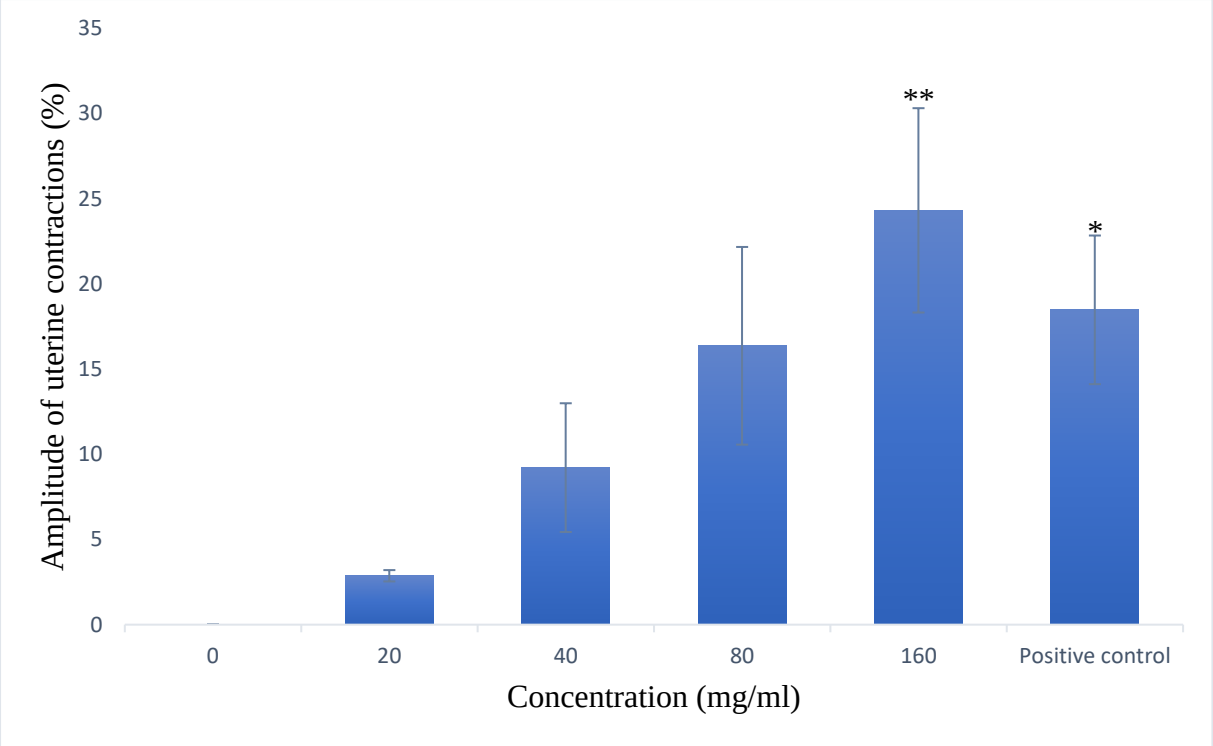

Figure 11: The effect of *Uvariodendron kirkii* extract concentrations on amplitude of uterine contraction.

4.3 Effect of *Uvariodendron kirkii* aqueous extract on frequency and amplitude of oxytocin- induced uterine contraction

Figures 12, 13, 14 and 15 show representation of effect of *Uvariodendron kirkii* extracts on oxytocin induced uterine frequency and amplitude of contractions.

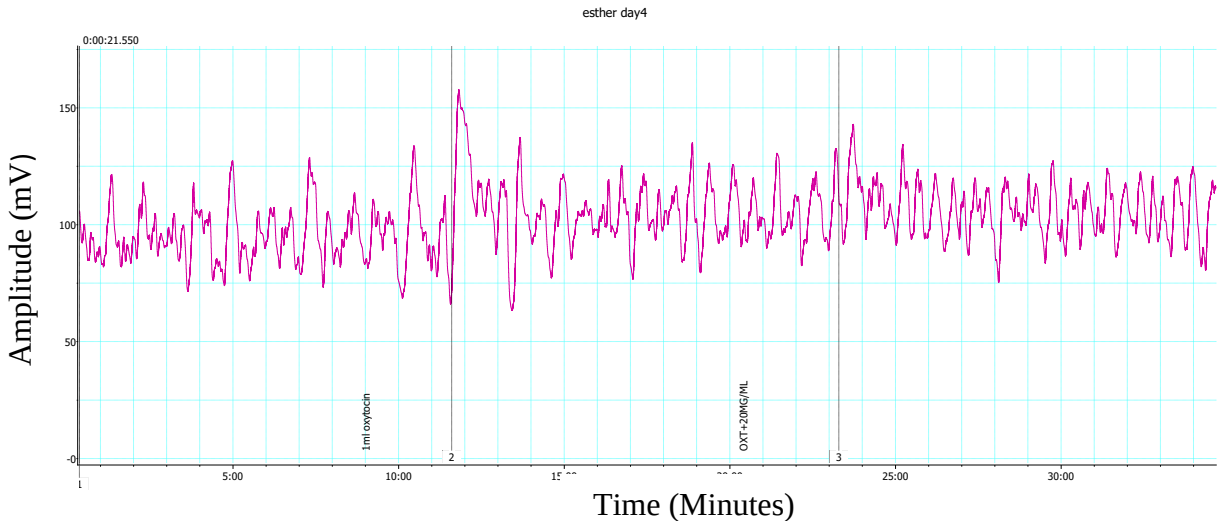

Figure 12: The effect of 20 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of oxytocin induced uterine contractions.

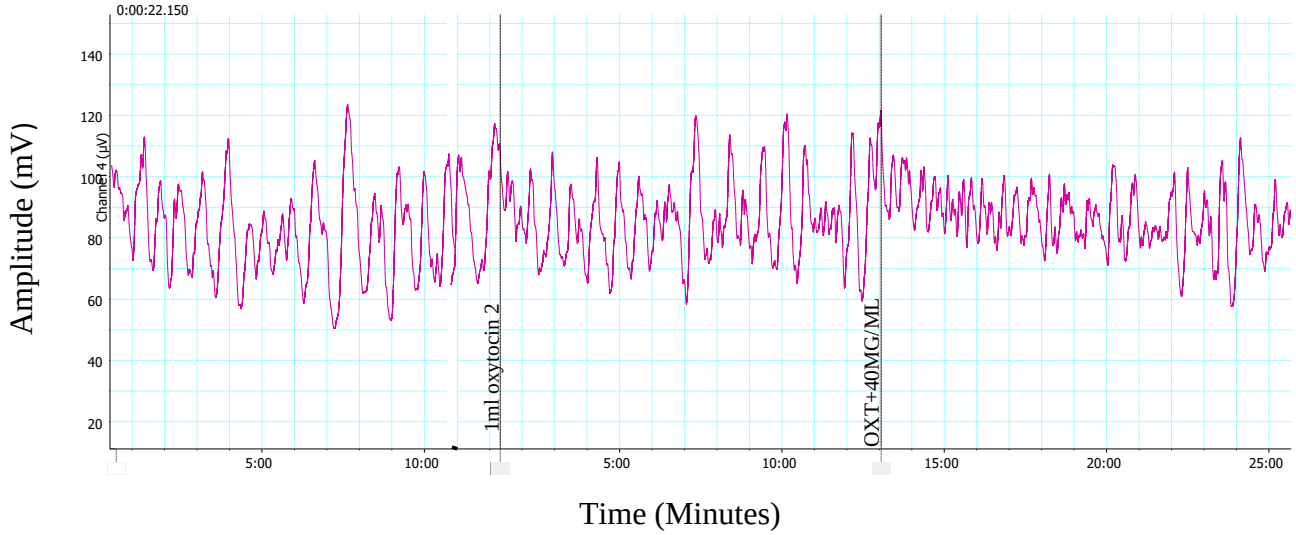

Figure 13: The effect of 40 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of oxytocin induced uterine contractions.

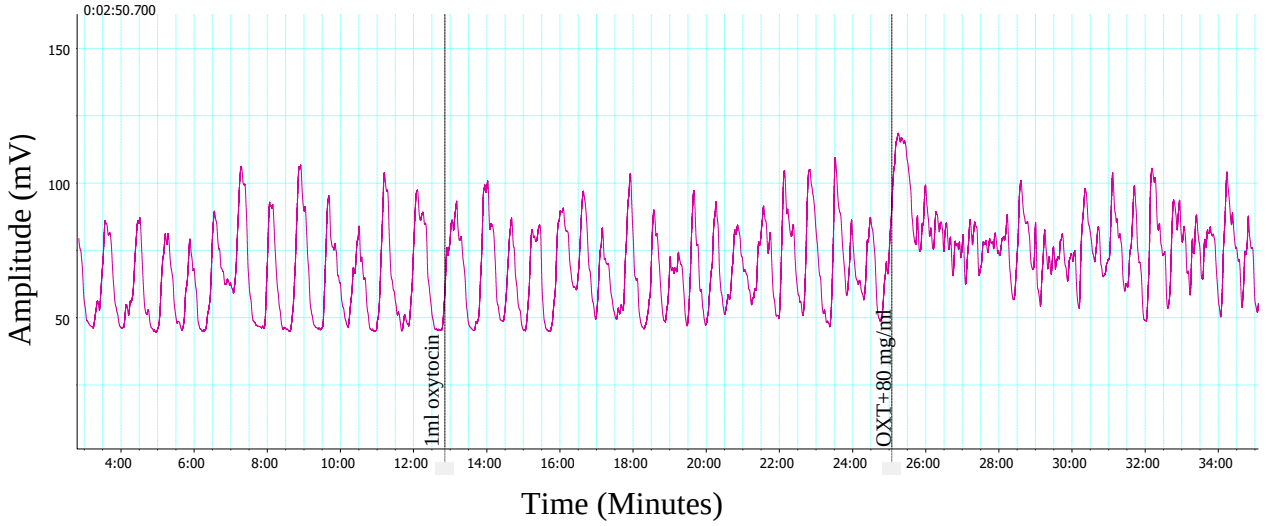

Figure 14: The effect of 80 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of oxytocin induced uterine contractions.

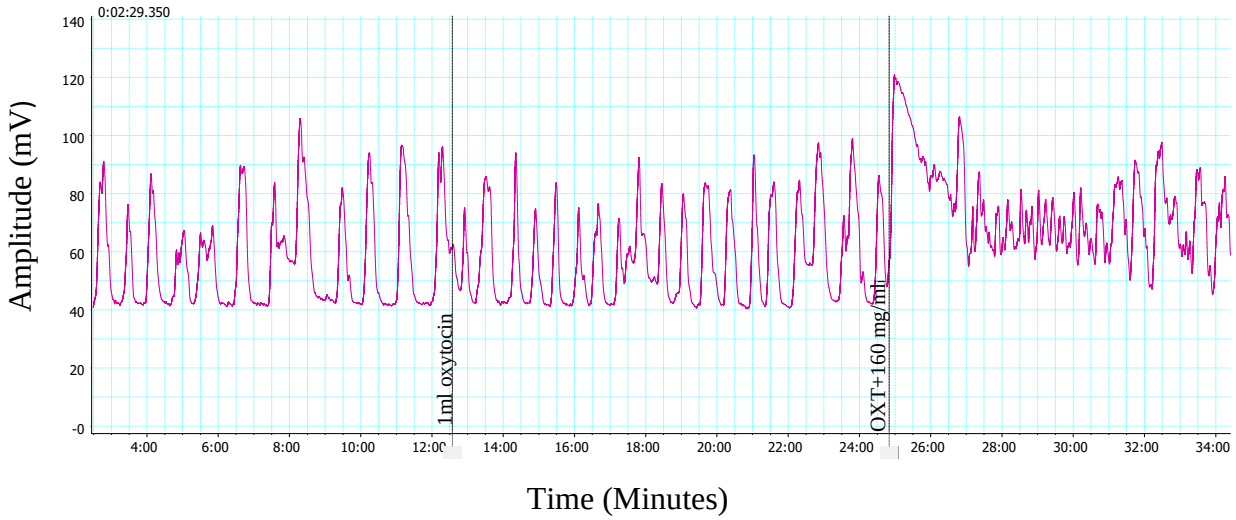

Figure 15: The effect of 160 mg/ml *Uvarıoɑenaron kırkii* extract on frequency and amplitude of oxytocin induced uterine contractions.

Oxytocin frequency and amplitude of uterine contractions was taken as control. The uterine contraction frequencies of oxytocin induced contractions increased by 6.92 ±5.582 % at 20 mg/ml, 28.31 ±7.193 % at 40 mg/ml, 47.06 ±9.684% at 80 mg/ml and 58.78 ±11.75% at 160 mg/ml. The significant difference was P<0.01 at 80 mg/ml and P< 0.001 at 160 mg/ml (Figure 16).

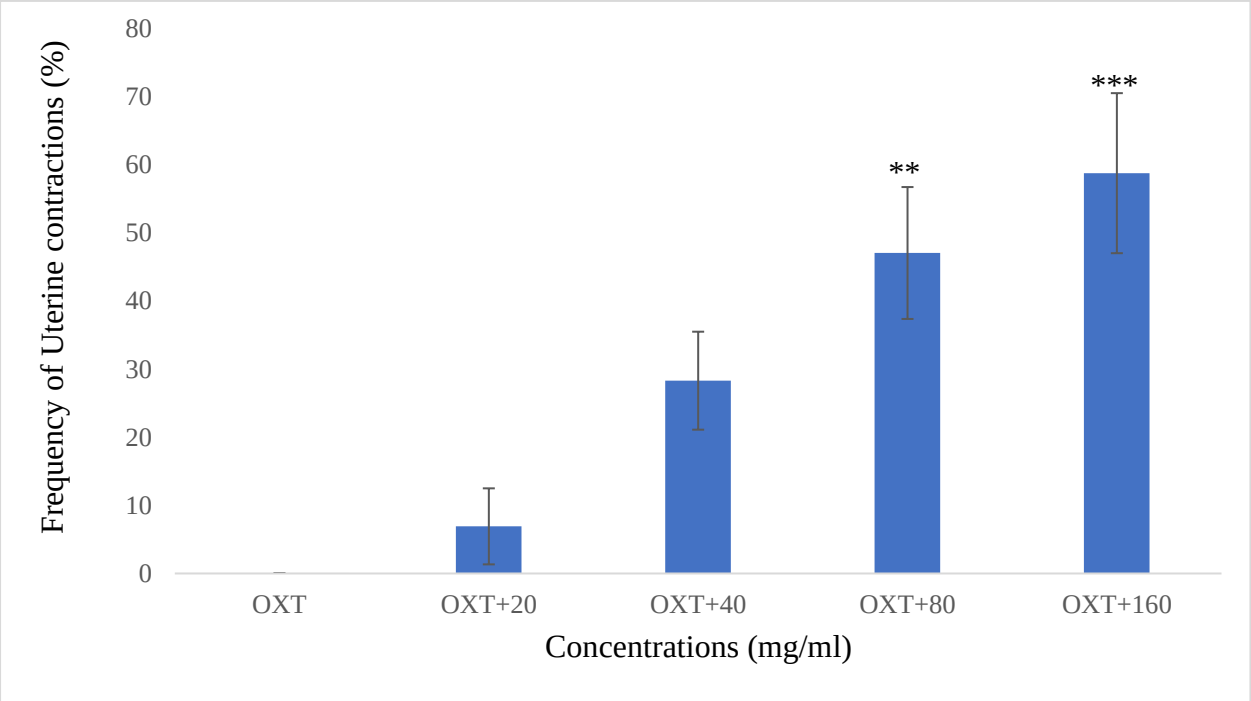

Figure 16: The effect of *Uvariodendron kirkii* extract concentrations on frequency of oxytocin induced uterine contractions.

At 20, 40, 80 and 160 mg/ml, the mean amplitude of oxytocin induced uterine contractions increased by 6.07 ±1.617 %; 9.40 ±2.087%; 15.19 ±1.250% and 23.56 ±3.096 %, respectively. The increase in amplitude was significant at P<0.05 at 40 mg/ml, P<0.001 at 80 mg/ml and P<0.0001 at 160 mg/ml (Figure 17)

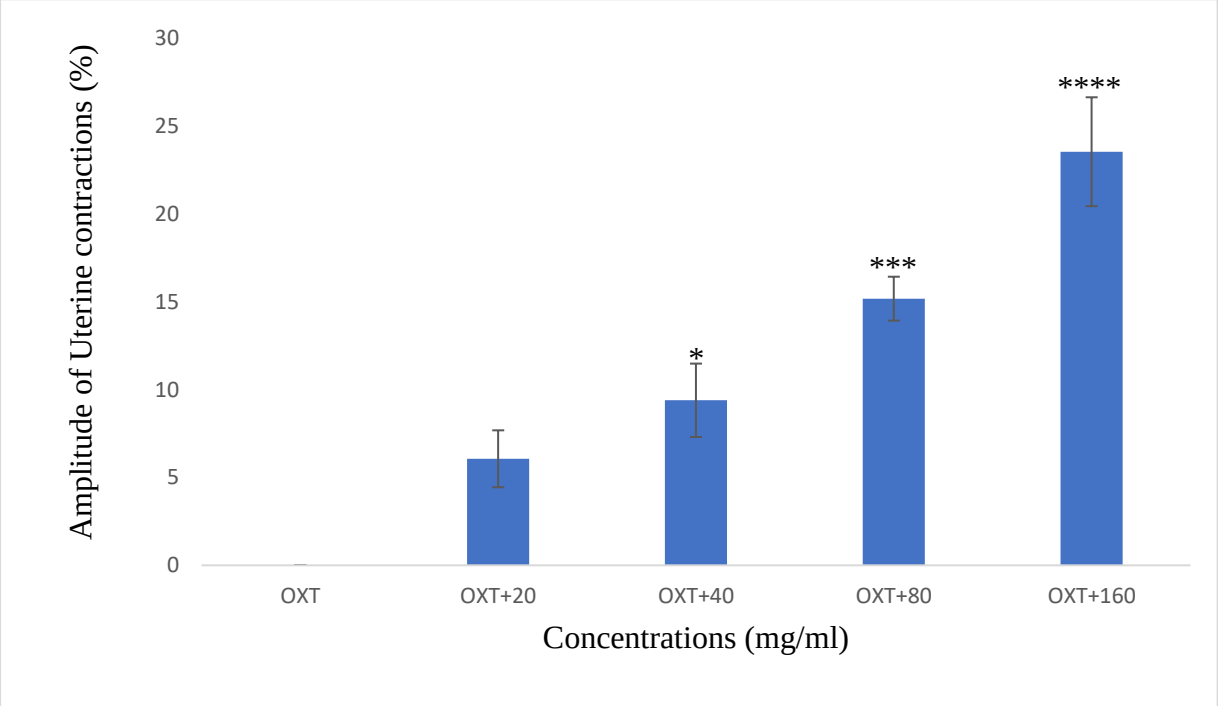

Figure 17: The effect of *Uvariodendron kirkii* extract concentrations on amplitude of oxytocin induced uterine contractions.

4.4 Effect of *Uvariodendron kirkii* aqueous extract on prostaglandin-induced uterine contraction

Figures 18, 19, 20, and 21 show representatives on frequency and amplitude of prostaglandin induced uterine contractions. Prostaglandin frequency and amplitude of uterine contraction was taken as control.

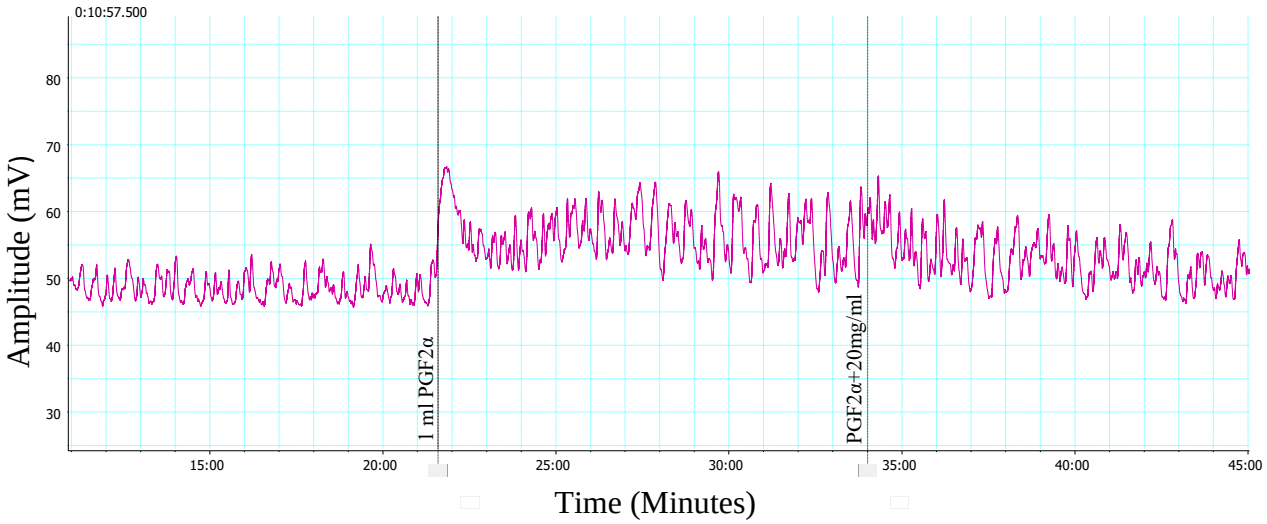

Figure 18: The effect of 20 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of prostaglandin induced uterine contractions.

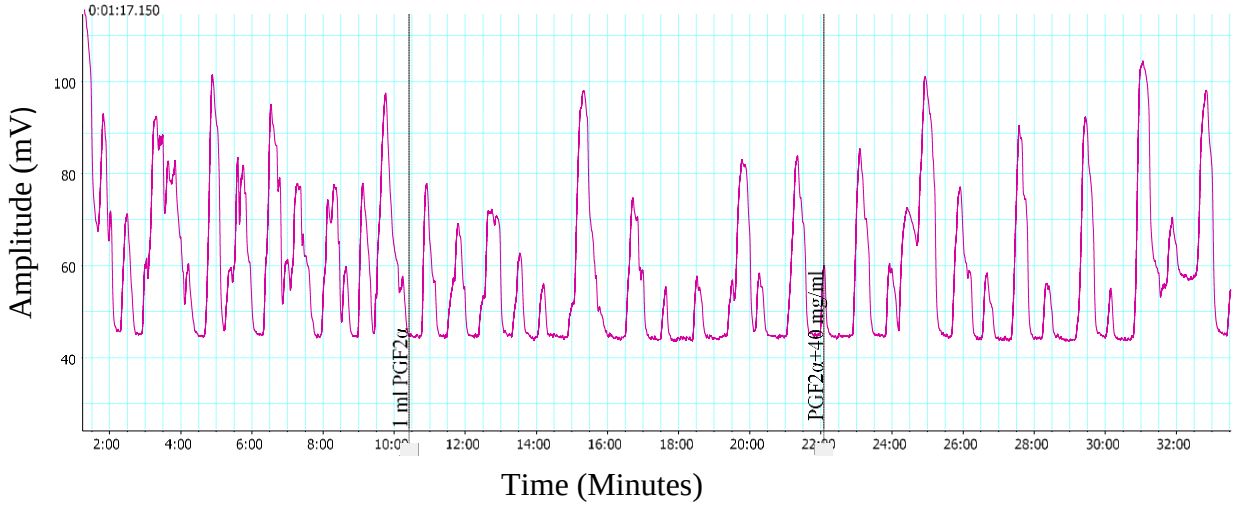

Figure 19: The effect of 40 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of prostaglandin induced uterine contractions.

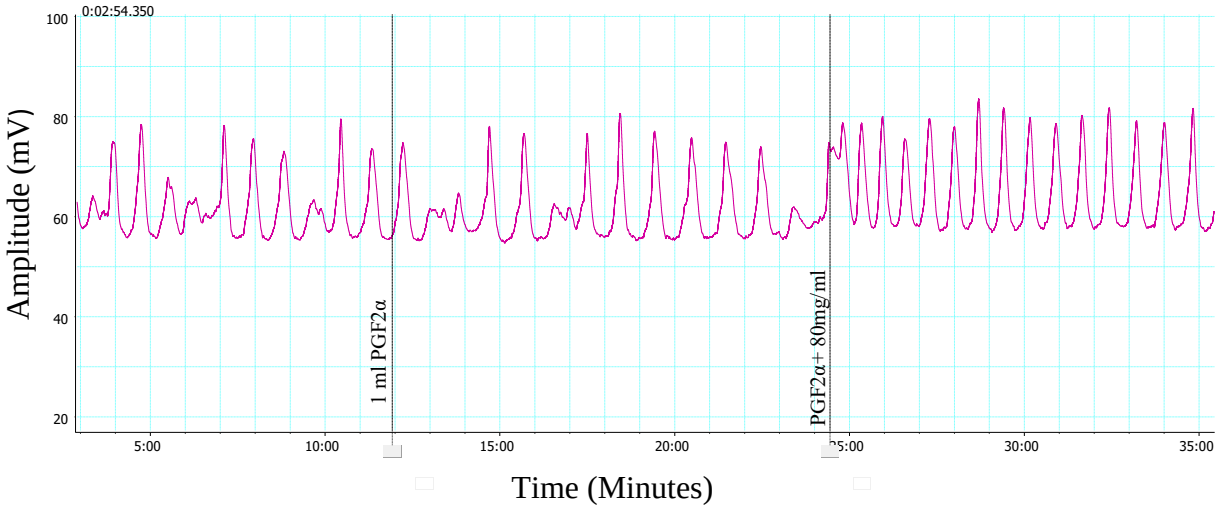

Figure 20: The effect of 80 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of prostaglandin induced uterine contractions.

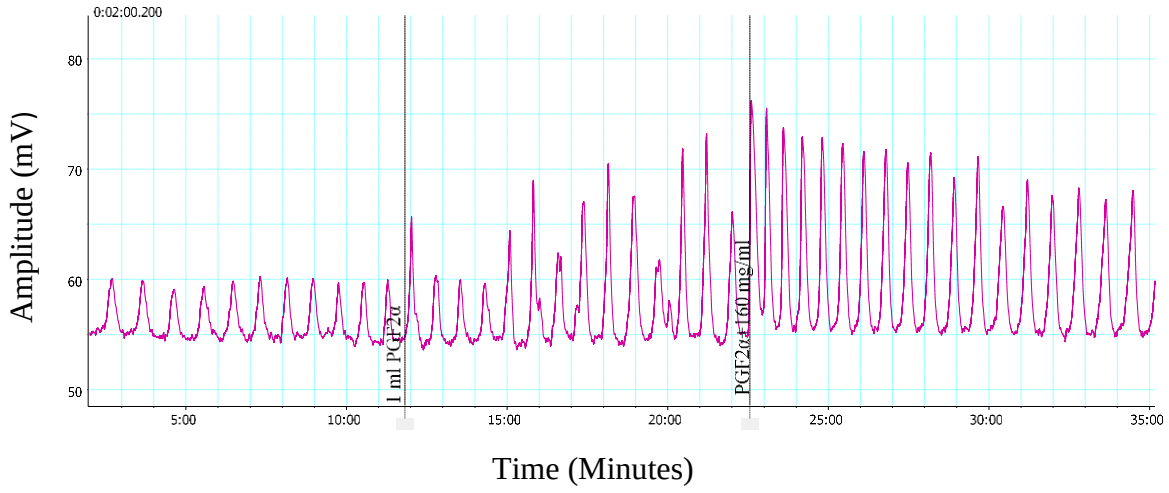

Figure 21: The effect of 160 mg/ml *Uvariodendron kirkii* extract on frequency and amplitude of prostaglandin induced uterine contractions.

The percentage increase in frequency of uterine contraction was 11.44 ±2.626 % at 20 mg/ml, 8.916 ±1.257% at 40 mg/ml, 20.65 ±1.462 % at 80 mg/ml and 35.71 ±8.821 % at 160 mg/ml. There was a significant difference (P< 0.05) at 80 mg/ml and (P< 0.0001) at 160 mg/ml (Figure 22).

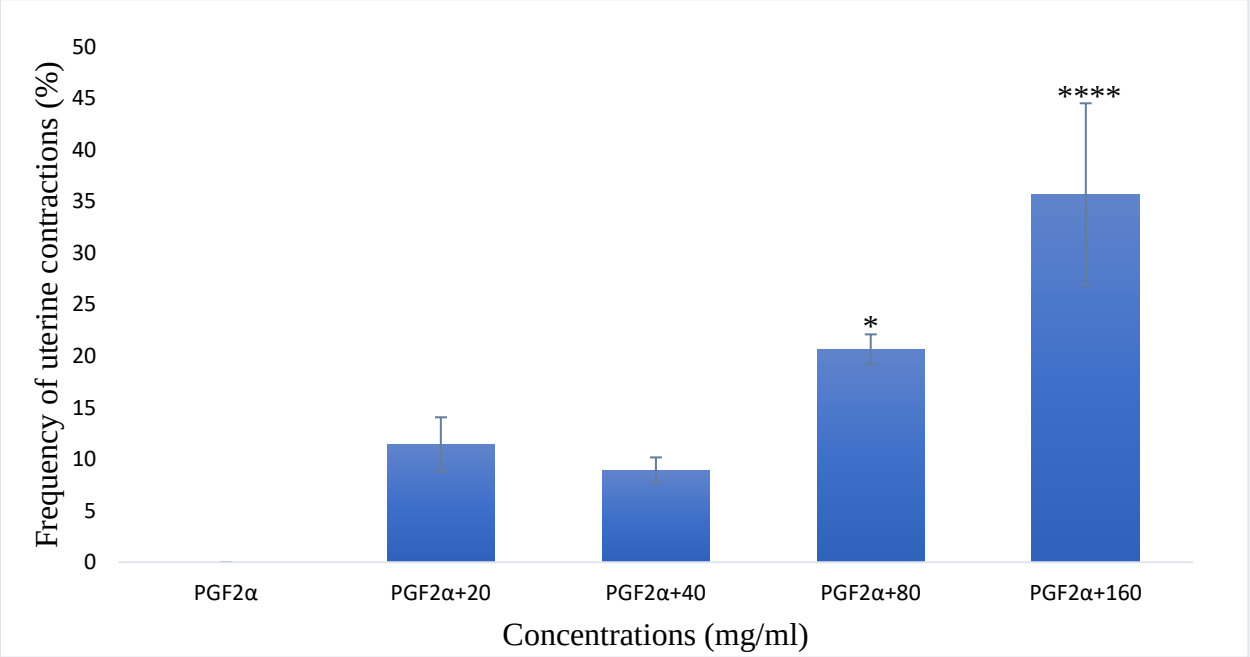

Figure 22: The effect of *Uvariodendron kirkii* extract concentrations on frequency of prostaglandin induced uterine contractions.

The mean amplitude of uterine contractions increased by 4.75 ±0.694% at 20 mg/ml, 3.89 ±0.639% at 40 mg/ml, 8.29 ±0.766 % at 80 mg/ml and 15.91 ±3.46% at 160 mg/ml. The significance difference was at P< 0.05 at 80 mg/ml and P< 0.0001at 160 mg/ml (Figure 23).

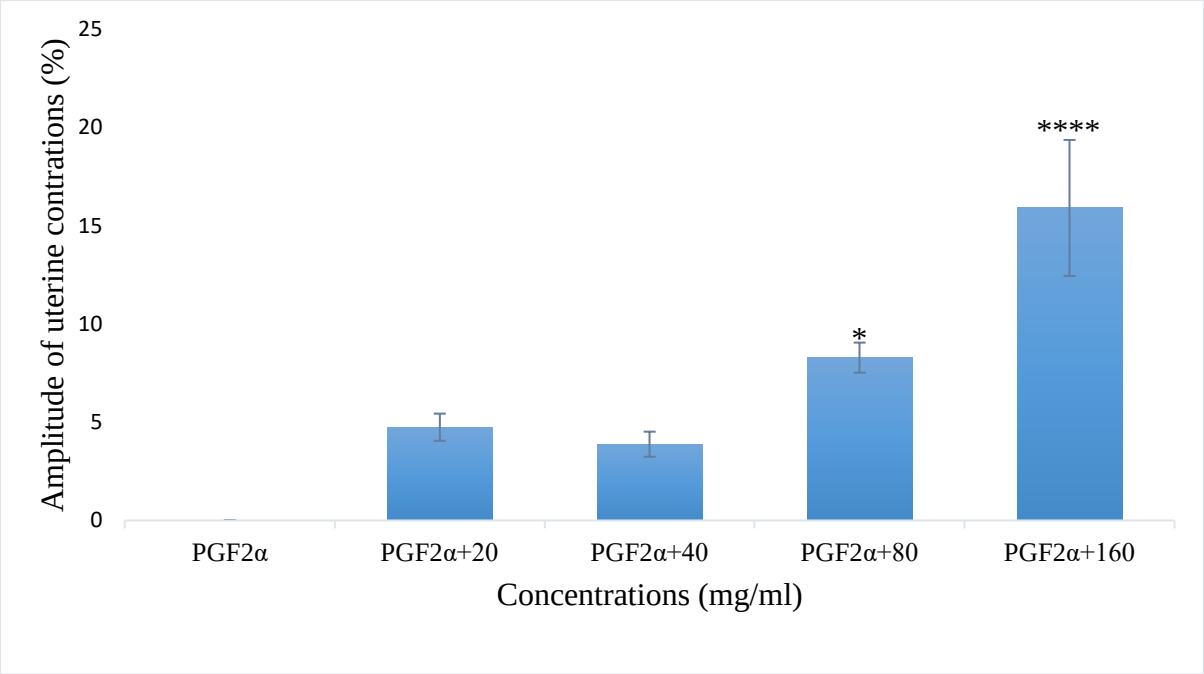

Figure 23: The effect of *Uvariodendron kirkii* extract concentrations on amplitude of prostaglandin induced uterine contractions.

CHAPTER FIVE
DISCUSSION

5.1 Effect of *Uvariodendron kirkii* extract on uterine smooth muscle

The most significant finding of this study was the increase in frequency (rate) and amplitude (force) of uterine contractions caused by graded concentrations of *Uvariodendron kirkii* aqueous extract, as shown in Figures 10 and 11. The plant has been used in Tana River County to expel the placenta, regulate fertility and also as an abortifacient. To cause infertility, the root bark is dissolved in water and the decoction consumed every day, and the leaves are placed post-delivery in the vagina to expel the placenta.

The findings of this study are in agreement with other plant studies which have been reported to have similar effects like *Uvariodendron kirkii* on the uterus. For instance in 2010, Ahangarpour & Oroojan reported that aqueous extract of *Cassia italica* leaves increased uterine contractions in rats, with the highest doses of the extract having the most contractile effect on the peak and frequency of uterus contraction. Another study by Kaingu et al. (2012) reported that aqueous extracts of *Euclea divinorum* and *Ricinus communis* enhanced uterine contractility directly. Pakoussi et al. (2018) also reported that *Spondias mombin* leaves extract induced a spontaneous increase in uterine contraction amplitude by inducing prostaglandins release. Bafor et al. (2009) also found out that the aqueous extract of the leaves of *Ficus exasperata* increased the frequency of rhythmic uterine contractions in Sprague-Dawley rats, thereby justifying its use in easing childbirth.

In the neighbouring country of Tanzania, Nikolajsen et al. (2011) have reported several plants to have an abortifacient effect by induction of strong and frequent uterine contractions in Sprague-Dawley rats. The plants that increased both the force and frequency of uterine contractions included *Bidens pilosa*, *Commelina africana*, and *Desmodium barbatum*,. Ibrahim et al. (2018) reported that the intensity of the contractions in presence of *Ficus deltoidea* var. Angustifolia aqueous extract increased moderately following the administration of the extract in a dose-dependent manner which is similar to the findings of this study. These results support the traditional claim of use of these plants in assisting labour, improving menstrual circulation, removal of retained placenta and treating post-partum hemorrhage.

The effect of *Uvariodendron kirkii* on uterine contractions can be explored in terms of treating or managing retained placenta as one of the causes is failed uterine contractions by the retroplacental myometrium (Urner et al., 2014). Retained placenta is defined as failure of the

foetal placenta (tufts) to separate from the maternal placenta (crypts). It is a serious problem in dairy cattle as is the main cause of cattle infertility and decreased milk yield (Zubair and Ahmad, 2014). Several studies have reported the use of medicinal plants in expelling retained placenta in cattle. They all work by means of causing an increase in the uterine contractions. The leaves of *Vernonia amygdalia* has been used to treat retained placenta by mixing with table salt, then administered to cow orally, and after some time the animal is relieved from retained placenta (Birhanu & Abera, 2015; Tekle, 2014).

A study on *Ricinus communis* has suggested its use to treat retained foetal membranes (Birhanu & Abera, 2015). Other plants that have been suggested by the traditional medical practitioners for treating retained foetal membrane include the leaves of *Ensete ventricosum* which are given to cattle during parturition (Mesfin et al., 2016). *Grewia ferrugina* extracts are useful for ease of expulsion of the placenta (Yadav et al., 2014). Bamboo leaves *Bambusa vulgaris* used together with black pepper *Qunda barbaree* assist in placenta expulsion (Abdisa, 2018). Raspberry leaves when consumed during the last 45 days of gestation period have low chances of development of peri-parturient diseases, that is, prolonged labour and retained placenta (Simpson et al., 2001). Whole 100g of *Argemone mexicana* plant when used in feeds together with any available local grass once a day can be used for removal of retained placenta in cows (Yadav et al., 2014). Leaves of both *Opuntieficus indica* and *Urera hypselodendron* are used for expulsion of retained placenta. The leaves are chopped, then mixed with water and administered to the animal orally (Bobaso et al., 2019). *Achyranthes aspera L*. has been reported to be used by people of Kalahindi district, Orissa, India, where the plant is applied on the genital part and also through inhalation to aid removal of the placenta (Sadangi & Sahu, 2004).

However, the findings of the present study are contrary to Kaaria et al. (2019) who reported that *Asparagus racemosus* caused a relaxation instead of uterine contraction. Similarly, Pio et al. (2019) showed that *Rhaphiodon echinus* (Re) crude ethanol extract, Lyophilized-Re and Hexane-Re relaxed utero fragment rats, pre-contracted in presence of potassium ions with concentration-dependent response. Peppermint oil antispasmodic effect on uterine muscle is by blockage of the calcium channels (Bahmani et al., 2015). Kauser et al. (2016) also reviewed on *Saraca asoka* bark and reported that it is used as a uterine tonic drug where it treats disorders associated with dysmenorrhea, abnormal bleeding, menorrhagia and threatened abortion.

Plants are known to produce secondary metabolites also known as phytochemicals. These phytochemicals are important agents that contain different roles and they differ in potency (Saxena et al., 2013). The chemicals, such as tannins and flavonoids, contained in the extracts of uterotonic plants act as antioxidants, antimicrobial and initiate the contractility of the uterus; thus placenta is easily detached and removed from the uterus (Abdisa, 2018). Tannin, for instance, produced by the plant, has an astringent property that act by shrinking the small blood vessels which in turn lessens the capillary pressure and in turn lead to separation of the foetal membranes from the uterus. Tannin also enables the availability of calcium ions to uterine tissues that brings about the contraction response (Amiera et al., 2014). Flavonoids on the other hand has antioxidant properties and also assist in oestrogen activation. Oestrogen production leads to activation of contraction proteins, connexins, which in turn aid in placenta removal by increasing uterine contractions (Abdisa, 2018).

A study by Kenana et al. (2019) on *Uvariodendron kirkii* phytochemical analysis reported the presence of flavonoids in high concentrations and tannins. These may cause uterine contractility and, therefore, aid in the removal of the placenta. *Vernonia amygdalia* contains secondary metabolites such as tannins and flavonoids which act by increasing uterine contraction through the reduction of progesterone concentration. The enhanced uterine contractions in turn lead to removal of retained placenta (Yeap et al., 2010). Similarly, phytochemical analysis of *Ficus deltoidea* aqueous extract reported presence of flavonoids and tannins that may have caused the uterine contraction response in adult female Sprague Dawley rat (Amiera et al., 2014).

5.2 Effect of *Uvariodendron kirkii* extract on oxytocin and prostaglandin F2*α* induced uterine contractions

Physiologically, during labour and the immediate post-partum period, levels of oxytocin and prostaglandins are high within the uterus. During parturition, the levels of oxytocin and prostaglandin gradually increase to facilitate parturition (Kaingu et al., 2012). The findings of the present study also showed that *Uvariodendron kirkii* increased the frequency and amplitude in both oxytocin and prostaglandin-induced rat uterus as shown in Figures 16 and 17 and Figures 22 and 23, respectively.

These findings corroborate those of Katuura et al. (2018), who reported that leaf extracts of *Vernonia amygdalina*, *Maesa lanceolata* and *Rhus natalensis* caused high frequency and amplitude of contractility in isolated rabbit uterus strips similar to, and in sometimes higher

than that observed in oxytocin alone. Similarly, Eze et al. (2016) reported that addition of increasing concentrations of oxytocin in the presence of 80 mg/ml aqueous extract of *Sida acuta* produced significant highest uterine contraction response compared to oxytocin and extract alone which is similar to the response stimulated by *Globimetula braunii* extract as studied by Ie & Zam. (2008). Both the plants potentiated the effect of oxytocin, which is similar to this study.

Oxytocin enhances uterine contractions by two main modes of action in the uterus. Oxytocin binds the myometrium oxytocin receptors to cause contractions and it also binds endometrium oxytocin receptors to increase prostaglandin production (Amiera et al., 2014). Once oxytocin is stimulated, there is a markedly increase in intracellular concentrations of calcium ions and inositol triphosphate. The release of calcium ions stimulates calcium dependent calmodulin which activates the myosin light chain kinase which in turn catalyzes the contraction response (Arrowsmith & Wray, 2014). Prostaglandin F2α on the other hand is produced by the uterine decidual cells. It enhances parturition by induction of luteolysis via the F prostanoids receptors (FP) in the luteal cells. Luteolysis results in cessation of progesterone production and luteal cell apoptosis. A decrease in levels of progesterone leads to the expression of the myometrium genes required for successful parturition (Sugimoto et al., 2015).

This study is also corroborated by Kaingu et al. (2012), where aqueous *Euclea divinorum* and *Ricinus communis* aqueous extracts enhanced prostaglandin and oxytocin induced uterine contractility on isolated uterine strips of Swiss rabbits. *Vitex doniana* bark aqueous extract induced graded uterine contractions and potentiated the effects of prostaglandins, ergometrine and oxytocin in rats (Ladeji et al., 2005). *Trichosanthes kirilowii* (Maxim.) root extract similarly strengthened spontaneous contractions and enhanced response of rabbit uterus muscle to prostaglandin F2 α and that of oxytocin (Gruber & Brien, 2011). Similarly, in this study *Uvariodendron kirkii* potentiated prostaglandin and oxytocin induced contractions as shown in Figures 16, 17, 22 and 23.

However, PGF2α induced uterine contractions were significantly reduced by ginger oil (*Zingiber officinale*) as reported by Buddhakala et al. (2008). Other species of plants that have shown smooth muscle relaxation effect with anti-prostaglandin effect under laboratory conditions include *Citrus orantiifolia, Rosmarinus officinalis* and *Psidium guajava* (Andel et al., 2014). *Echinophora platyloba* is reported to reduce uterine contractions (Bahmani et al.,

2015), and *Foeniculum vulgare* inhibited prostaglandins induced uterine contraction (Mirabi et al., 2014).

Uterotonic plants can also be used to prevent or manage postpartum haemorrhage (PPH) (Gruber & Brien, 2011). As earlier stated, PPH is caused mainly by uterine atony, and therefore, conservative management techniques such as use of uterotonic medications are used as the first line of therapy. This is done to counteract uterine atony and, therefore, assist in retained placenta expulsion and removal of blood clots. One of the commonly used uterotonics is oxytocin (Likis et al., 2015). This study has shown *Uvariodendron kirkii* to be a uterotonic agent similar to oxytocin and therefore suggests its use in treatment of PPH.

Attah et al. (2012) suggests use of *Commelina africana*, *Vernonia amygdalina*, *Sida corymbose, Ocimum gratissimum, Hyptis suaveolens, Duranta repens, Calotropis procera, Sclerocarya birrea,* and *Saba comorensis* in treatment of PPH. *Commelina africana*, *Vernonia amygdalina* and *Sida corymbose,* however, yielded the biggest increase in uterine contractility in a pharmacological uterine model. The pharmacological results are in agreement with the results of this study as *U. kirkii* aqueous extracts increased uterine contractility. It is therefore of vital importance to pursue alternative sources of remedies for labour management.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

i. *Uvariodendron kirkii* root bark possess contractile effects towards isolated rats' uteri. When exposed to the plant aqueous extracts, the tissues displayed contraction patterns that mirrored (more or less) those produced by exogenous oxytocin. In nearly all experiments, there was a strong initial and sustained contraction, after which isometric contractions resumed. Therefore, it is concluded that there is a significant difference in the frequency and amplitude of uterine contractions caused by graded concentrations of *Uvariodendron kirkii* aqueous extracts.

ii. The plant aqueous extract also augmented the action of oxytocin and prostaglandin F2α. This contractile effect of the plant explains its use in augmentation of labour, treatment of retained placenta and postpartum haemorrhage, by women in Tana River County. Therefore, there is a significant difference in frequency and amplitude of uterine contractions caused by *Uvariodendron kirkii* extracts on oxytocin and prostaglandin F2α induced uterine contractions.

iii. The determination of uterotonic activity of crude extract of *Uvariodendron kirkii* provides a starting point towards its potential use in human as a novel uterotonic agent.

Recommendations

i. Further studies should be carried out to elucidate the mechanism of action of *Uvariodendron kirkii* in stimulating uterine contractions, that is, the pathways into which it uses in stimulation of contractions.

ii. The mode in which the plant extract used to augment the activity of oxytocin and prostaglandin F2α was not studied in this study therefore, there is need to further study the specific channels the plant compound(s) uses to establish this fact.

iii. Also, there is need for further studies on isolation of the active compound that is responsible for its uterotonic effect.

iv. Use of collagen gel uterine contractility assays to study the long-lasting uterotonic effect of *Uvariodendron kirkii* extract is also recommended

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APPENDICES

Appendix A: Abstract of paper published

TANG [HUMANITAS MEDICINE]

Original article

EVALUATION OF OXYTOCIN LIKE EFFECTS OF *Uvariodendron kirkii* (Verdec.) EXTRACTS ON ISOLATED UTERINE STRIPS OF WISTAR RATS

Esther Wairimu Kinyua[1*] Charles Irungu Maina[1], Catherine Kaluwa Kaingu[2], David Kayaja Wafula[3]

[1]Department of Biological Sciences, Egerton University, Nairobi, Kenya, [2]Department of Veterinary Anatomy and Physiology, University of Nairobi, Nairobi, Kenya.[3]Department of Medical Physiology, University of Nairobi, P.O Box 30197-00100, Nairobi, Kenya.

ABSTRACT

Uterotonics have the ability to contract uterus. Such plants might be useful in augmenting or inducing labour, expelling retained afterbirth and for abortifacient purposes. Limitations associated with conventional treatments have made herbal medicines a feasible alternative for the management of these conditions. The aim of this study was to evaluate the contractile effects of *Uvariodendron kirkii* extracts on isolated uterine strips of female Wistar rats. Isolated strips of Wistar rats' uteri were treated with 20, 40, 80 and 160 mg/ml concentrations of *Uvariodendron kirkii* aqueous extract. The plant extract was also tested against prostaglandin and oxytocin induced uterine contractions. *Uvariodendron kirkii* extract concentrations (20, 40, 80 and 160 mg/ml) increased the frequency of uterine contraction (16.53, 25.12, 33.48 and 56.39 percentages respectively) compared to the control. The graded extract concentrations caused a significant increase in amplitude (force) of uterine contractions by 2.87, 9.22, 16.37 and 24.32 percentages respectively. The concentrations significantly increased the frequency of oxytocin induced uterine contractions by 6.92; 28.31; 47.06, 58.78 percentages respectively. The graded extract concentrations also significantly increased the amplitude of oxytocin induced uterine contractions by 6.07; 9.40; 15.19 and 23.56 percentages respectively. *Uvariodendron kirkii* extract concentrations significantly increased the frequency and amplitude of prostaglandin induced contractions. The percentage increase in frequency was 11.44, 8.92, 20.65 and 35.71 at 20, 40, 80 and 160 mg/ml respectively. The mean amplitude of prostaglandin induced uterine contractions also increased (4.75, 3.89, 8.29 and 15.91% at 20. 40, 80 and 160 mg/ml respectively). The extract caused a dose dependent increase in uterine frequency and amplitude of contraction. The findings of this study are useful in generating a novel uterotonic agent that will be useful in augmenting labour or in expelling retained after birth in cattle. More studies at molecular level will further elucidate the plant mechanism of action.

Keywords: *Uvariodendron kirkii*, Oxytocin induced, Prostaglandin induced, Uterine contractions

Appendix B: Key Data analysis outputs

Descriptive statistics of Effect of extract on frequency of uterine contractions

	0	20	40	80	160	Positive control
Number of values	5	5	5	5	5	5
Mean	0.000	16.53	25.12	33.48	56.39	11.29
Std. Error of Mean	0.000	2.400	4.875	7.370	9.815	0.7742

Ordinary one-way ANOVA of Effect of extract on frequency of uterine contractions: ANOVA results

	Data Sets				
Table Analysed	frequency extracts				
Data sets analysed	A-F				
ANOVA summary					
F	12.83				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)	Yes				
R square	0.7278				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	9667	5	1933	F (5, 24) = 12.83	P<0.0001
Residual (within columns)	3616	24	150.6		
Total	13282	29			

Data summary Number of treatments (columns) Number of values (total)	 6 30				

Ordinary one-way ANOVA of Effect of extract on frequency of uterine contractions: Multiple comparisons

	Data Sets	
Number of families	1	
Number of comparisons per family	15	
Alpha	0.05	
Tukey's multiple comparisons test	Significant?	Summary
0 vs. 20	No	ns
0 vs. 40	Yes	*
0 vs. 80	Yes	**
0 vs. 160	Yes	****
0 vs. Positive control	No	ns
20 vs. 40	No	ns
20 vs. 80	No	ns
20 vs. 160	Yes	***
20 vs. Positive control	No	ns
40 vs. 80	No	ns
40 vs. 160	Yes	**
40 vs. Positive control	No	ns
80 vs. 160	No	ns

80 vs. Positive control	No	ns
160 vs. Positive control	Yes	****

Descriptive statistics of Effect of extract on amplitude of uterine contractions

	control	20	40	80	160	Positive control
Number of values	5	5	5	5	5	5
Mean	0.000	2.870	9.218	16.37	24.32	18.48
Std. Error of Mean	0.000	0.3334	3.779	5.802	5.997	4.363

Ordinary one-way ANOVA of Effect of extract on amplitude of uterine contractions: ANOVA results

Data Sets					
Table Analysed	amplitude extracts				
Data sets analysed	A-F				
ANOVA summary					
F	5.215				
P value	0.0022				
P value summary	**				
Significant diff. among means (P < 0.05)?	Yes				
R square	0.5207				

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	2239	5	447.9	F (5, 24) = 5.215	P=0.0022
Residual (within columns)	2061	24	85.89		
Total	4301	29			
Data summary					
Number of treatments (columns)	6				
Number of values (total)	30				

Ordinary one-way ANOVA of Effect of extract on amplitude of uterine contractions: Multiple comparisons

	Data Sets	
Number of families	1	
Number of comparisons per family	15	
Alpha	0.05	
Tukey's multiple comparisons test	Significant	Summary
control vs. 20	No	ns
control vs. 40	No	ns
control vs. 80	No	ns
control vs. 160	Yes	**
control vs. Positive control	Yes	*
20 vs. 40	No	ns
20 vs. 80	No	ns

20 vs. 160	Yes	*
20 vs. Positive control	No	ns
40 vs. 80	No	ns
40 vs. 160	No	ns
40 vs. Positive control	No	ns
80 vs. 160	No	ns
80 vs. Positive control	No	ns
160 vs. Positive control	No	ns

Descriptive statistics of Effect of extract on frequency of prostaglandin induced uterine contractions

	0	20+PGF2a	40+PGF2a	80+PGF2a	160+PGF2a
Number of values	5	5	5	5	5
Mean	0.000	11.44	8.916	20.65	35.71
Std. Error of Mean	0.000	2.626	1.257	1.462	8.821

Ordinary one-way ANOVA of Effect of extract on frequency of prostaglandin induced uterine contractions: ANOVA results

	Data Sets				
Table Analysed	Prostaglandin induced frequency				
Data sets analysed	A-E				
ANOVA summary					
F	10.39				
P value	0.0001				
P value summary	***				
Significant diff. among means (P < 0.05)?	Yes				
R square	0.6751				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	3675	4	918.7	F (4, 20) = 10.39	P=0.0001
Residual (within columns)	1768	20	88.42		
Total	5443	24			
Data summary					
Number of treatments (columns)	5				
Number of values (total)	25				

Ordinary one-way ANOVA of Effect of extract on frequency of prostaglandin induced uterine contractions: Multiple comparisons

	Data Sets	
Number of families	1	
Number of comparisons per family	10	
Alpha	0.05	
Tukey's multiple comparisons test	Significant	Summary
0 vs. 20+PGF2a	No	ns
0 vs. 40+PGF2a	No	ns
0 vs. 80+PGF2a	Yes	*
0 vs. 160+PGF2a	Yes	****
20+PGF2a vs. 40+PGF2a	No	ns
20+PGF2a vs. 80+PGF2a	No	ns
20+PGF2a vs. 160+PGF2a	Yes	**
40+PGF2a vs. 80+PGF2a	No	ns
40+PGF2a vs. 160+PGF2a	Yes	**
80+PGF2a vs. 160+PGF2a	No	ns

Descriptive statistics of Effect of extract on amplitude of prostaglandin induced uterine contractions

	0	20+PGF2a	40+PGF2a	80+PGF2a	160+PGF2a
Number of values	5	5	5	5	5
Mean	0.000	4.746	3.882	8.288	15.91
Std. Error of Mean	0.000	0.6937	0.6394	0.7661	3.462

Ordinary one-way ANOVA of Effect of extract on amplitude of prostaglandin induced uterine contractions: ANOVA results

	Data Sets				
Table Analysed	prostaglandin induced amplitude				
Data sets analysed	A-E				
ANOVA summary					
F	13.36				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)	Yes				
R square	0.7276				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	719.3	4	179.8	F (4, 20) = 13.36	P<0.0001
Residual (within columns)	269.3	20	13.46		
Total	988.6	24			

Data summary Number of treatments (columns) Number of values (total)	 5 25				

Ordinary one-way ANOVA of Effect of extract on amplitude of prostaglandin induced uterine contractions: Multiple comparisons

	Data Sets	
Number of families	1	
Number of comparisons per family	10	
Alpha	0.05	
Tukey's multiple comparisons test	Significant?	Summary
0 vs. 20+PGF2a	No	ns
0 vs. 40+PGF2a	No	ns
0 vs. 80+PGF2a	Yes	*
0 vs. 160+PGF2a	Yes	****
20+PGF2a vs. 40+PGF2a	No	ns
20+PGF2a vs. 80+PGF2a	No	ns
20+PGF2a vs. 160+PGF2a	Yes	***
40+PGF2a vs. 80+PGF2a	No	ns
40+PGF2a vs. 160+PGF2a	Yes	***
80+PGF2a vs. 160+PGF2a	Yes	*

Descriptive statistics of Effect of extract on frequency of oxytocin induced uterine contractions

	OXT	OXT+20	OXT+40	OXT+80	OXT+160
Number of values	5	5	5	5	5
Mean	0.000	6.922	28.31	47.06	58.78
Std. Error of Mean	0.000	5.582	7.193	9.684	11.75

Ordinary one-way ANOVA of Effect of extract on frequency of oxytocin induced uterine contractions: ANOVA results

	Data Sets				
Table Analysed	OXT FREQ				
Data sets analysed	A-E				
ANOVA summary					
F	10.08				
P value	0.0001				
P value summary	***				
Significant diff. among means (P < 0.05)	Yes				
R square	0.6685				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	12693	4	3173	F (4, 20) = 10.08	P=0.0001
Residual (within columns)	6295	20	314.7		
Total	18988	24			

Data summary Number of treatments (columns) Number of values (total)	 5 25				

Ordinary one-way ANOVA of Effect of extract on frequency of oxytocin induced uterine contractions: Multiple comparisons

	Data Sets	
Number of families	1	
Number of comparisons per family	10	
Alpha	0.05	
Tukey's multiple comparisons test	Significant	Summary
OXT vs. OXT+20	No	ns
OXT vs. OXT+40	No	ns
OXT vs. OXT+80	Yes	**
OXT vs. OXT+160	Yes	***
OXT+20 vs. OXT+40	No	ns
OXT+20 vs. OXT+80	Yes	*
OXT+20 vs. OXT+160	Yes	**
OXT+40 vs. OXT+80	No	ns
OXT+40 vs. OXT+160	No	ns
OXT+80 vs. OXT+160	No	ns

Descriptive statistics of Effect of extract on amplitude of oxytocin induced uterine contractions

	OXT	OXT+20	OXT+40	OXT+80	OXT+160
Number of values	5	5	5	5	5
Mean	0.000	6.070	9.404	15.19	23.56
Std. Error of Mean	0.000	1.617	2.087	1.249	3.096

Ordinary one-way ANOVA of Effect of extract on amplitude of oxytocin induced uterine contractions: ANOVA results

	Data Sets				
Table Analysed	Effect of extract on amplitude of oxytocin induced contraction				
Data sets analysed	A-E				
ANOVA summary					
F	22.29				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)	Yes				
R square	0.8168				
		DF	MS	F (DFn, DFd)	P value
ANOVA table	SS	4	403.8	F (4, 20) = 22.29	P<0.0001
Treatment (between columns)	1615	20	18.11		
Residual (within columns)	362.3	24			
Total	1977				

Data summary Number of treatments (columns) Number of values (total)	 5 25				

Ordinary one-way ANOVA of Effect of extract on amplitude of oxytocin induced uterine contractions: Multiple comparisons

	Data Sets	
Number of families	1	
Number of comparisons per family	10	
Alpha	0.05	
Tukey's multiple comparisons test	Significant	Summary
OXT vs. OXT+20	No	ns
OXT vs. OXT+40	Yes	*
OXT vs. OXT+80	Yes	***
OXT vs. OXT+160	Yes	****
OXT+20 vs. OXT+40	No	ns
OXT+20 vs. OXT+80	Yes	*
OXT+20 vs. OXT+160	Yes	****
OXT+40 vs. OXT+80	No	ns
OXT+40 vs. OXT+160	Yes	***
OXT+80 vs. OXT+160	Yes	*

Appendix C: Ethical clearance

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line, 4448648

Ms. Esther Wairimu Kinyua
Egerton University
Dept of Biological Sciences

REF: FVM BAUEC/2019/190

02/02/2019

Dear Ms. Kinyua,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Evaluation of contractile effects of *Uvariodendron kirkii (Verdec)* extracts on isolated uterine strips of female winstar rats.

By Ms. Esther Kinyua (SM21/20010/2016).

We refer to your MSc proposal submitted to our committee for review and your application letter dated 10/12/2018.

We have reviewed your MSc. proposal, particularly section 3.2 that involves use of laboratory animals for estrus cyclicity, mating success and isolated uterine strips studies.

We are satisfied that the proposed treatment and care of the animals meets acceptable standards for animal welfare. Furthermore, the numbers proposed are reasonable.

We have also noted that Dr. Catherine Kaluwa (KVB 1373) will supervise the animal experiments and humane end points.

We hereby give approval for you to proceed with the experiments as outlined in the submitted proposal.

Yours sincerely

Kaluwa.

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine.

Appendix D: Research Permit

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/09248/30730** Date: **25th June, 2019.**

Esther Wairimu Kinyua
Egerton University
P.O. Box 536-20115
NJORO.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Evaluation of contractile effects of uvario dendron kirkii (Verdec.) Extracts on isolated uterine strips of female Wistar rats."*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi and Tana River Counties** for the period ending **24th June, 2020.**

You are advised to report to **the County Commissioners, the County Director of Health Services, and the County Directors of Education, Nairobi and Tana River Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

DR. ROY B. MUGIIRA, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.