EFFECT OF ETHANOL EXTRACT OF THE AERIAL PARTS OF *PHYLLANTUS AMARUS* ON THE UTERINE SMOOTH MUSCLE REACTIVITY AND PREGNANCY OUTCOME IN FEMALE WISTAR RATS

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN-CITY, EDO STATE.

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CERTIFICATION

This is to certify that this work was carried out by ETUKPERE KIRSTEN OGHENEFEJIRO in the department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria, in partial fulfillment for the award of the PharmD degree of the University.

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Dr. F.C Amaechina **Date**
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(HOD, Department of Pharmacology)

DEDICATION

I dedicate this work to the almighty God who has greatly sustained and blessed me. I also dedicate this work to my supportive parents, whom have been my basic source of strength and support, morally and financially throughout my stay in this school.

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ABSTRACT

Preterm labor and miscarriages are reproductive complications that often require various pharmacological and non-pharmacological interventions such as bed rest, hormonal therapy and tocolytic agents. Various cultures have also exploited the use of herbs and some medicinal plants to manage these gynaecological problems. *Phyllanthus amarus* is a medicinal plant widely used in traditional medicine in the treatment of hypertension and also for its female health benefits. While *Phyllanthus amarus* has been reported to be used in ethno-medicine as an abortifacient agent, it has also been reported to have toxic in female reproductive health.

This work investigated the effect of the ethanol extract of the aerial parts of *Phyllanthus amarus* on uterine smooth muscle reactivity, and on pregnancy outcome in female Wistar rats. The ethanol extract of *Phyllantus amarus* was prepared and tested using both in vivo and in vitro models. In the in vivo study, the female wistar rats were administered graded doses of the extract (6.25, 12.5, 25 and 50 mg/kg) orally for 14 days and each groups were mated with two male wistar rats on the first day of administration. For the in-vitro study, isolated uterine tissues were used to evaluate the effect of the extract on spontaneous contraction of the uterus as well as oxytocin, and KCl-induced pre-contracted uterus. The extract produced a dose-dependent relaxation of uterine smooth muscle. The extract also had a positive outcome on conception in some treated groups,

This study suggests that *Phyllanthus amarus* possesses utero-relaxant, and positive pregnancy oucomes in female wistar rats.

CHAPER ONE

INTRODUCTION

1.0 REPRODUCTIVE HEALTH

Reproductive health is an essential element of total well-being which incorporates the physical, mental, and social dimensions of the reproductive system and functions. The World Health Organisation (WHO) defines reproductive health as a state wherein individuals may engage in a responsible, fulfilling and satisfying sexual life, as well as retaining the capacity for reproduction, and asserting the independence on reproduction. (WHO, 2022). Ensuring good reproductive health is crucial for sustaining fertility, facilitating safe pregnancies, and enhancing the quality of life for individuals of reproductive age.

1.0.1 Infertility

Infertility constitutes a significant global reproductive health issue, impacting around 10 – 15% of couples globally (Mascarenhas *et al.,* 2012). In many societies, women disproportionately face the social and emotional consequences of infertility. Notwithstanding advancements in technologies for assisted reproduction like in vitro fertilization (IVF), access is still constrained by cost and availability, particularly in impoverished nations. Consequently, attention has shifted toward exploring safe, effective, and affordable plant-based alternatives for managing reproductive disorders and supporting conception.

1.0.2 Conception and Its Challenges

Conception marks the initiation of pregnancy and it depends on coordinated interactions between hormonal regulation, ovulation, fertilization, and uterine receptivity (Aplin and Ruane, 2017). Due to a variety of physiological, environmental, and behavioural variables, conception and fertility rates have decreased globally over the past few decades, especially in low and middle-income nations (Sengupta *et al.,* 2018). Exposure to environmental pollutants,

delayed childbearing, malnutrition, and stress have been identified as significant contributors to reduced conception rates. In sub-Saharan Africa, conditions such as pelvic inflammatory disease, sexually transmitted infections, and untreated reproductive tract infections continue to affect uterine and tubal health, thereby impeding conception (Okonofua *et al.,* 2022).

1.0.3 Role of Medicinal Plants in Reproductive Health

Traditional medicine makes extensive use of medicinal plants and they are widely used to manage reproductive disorders. They include a variety of bioactive substances that can directly affect reproductive organs or effect on hormonal pathways, including alkaloids, flavonoids, saponins, and tannins. (Nwankpa *et al.,* 2016). Some of the countries that are known to use herbal plants for reproduction health and activity include china, India, and Thailand. In china, for example, herbal plants have been used for centuries to support reproductive health, herbs like ginseng and chaste berry are commonly used to help balance hormones and increase fertility.

1.1 THE REPRODUCTIVE SYSTEM

The reproductive system is made up of internal and external organs involved in sexual intercourse, fertilization, ejaculation (in men), and menstruation (in women). This system also controls the production of sex hormones and its primary function is for conception. The reproductive system also helps the reproduction process itself and supports pregnancy.

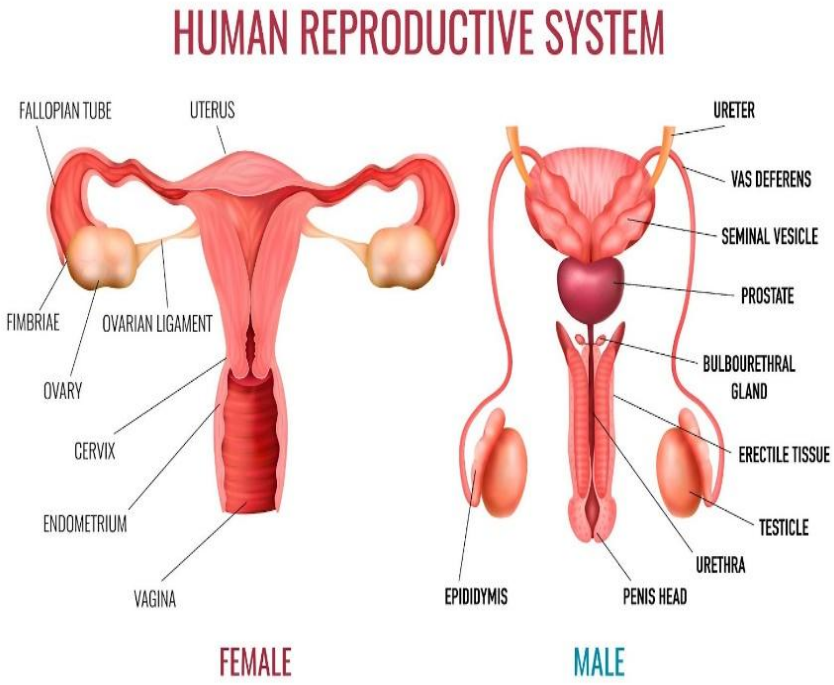

Fig 1.1: Image of the Human Reproductive System (Adopted from freepik.com)

1.1.1 The Female Reproductive System

The female reproductive system is a biological system that produces, transports, and stores eggs while also facilitating the meeting of eggs and sperm for fertilisation. The female reproductive system comprises of both internal and external components (organs). The reproductive hormones which regulates the female reproductive system are produced by the pituitary gland and ovaries, such as oestrogen, progesterone, and gonadotropins. (Andina, 2023).

1.1.2 Organ system of the Female Reproductive System

The female sex organs are composed of both internal and external genitalia. They both make up the female reproductive system, which facilitates sexual and reproductive functions. The vulva, or external genital organs, are located in the female perineum. The vestibule, vestibular bulb, clitoris, mons pubis, labia majora and minora, and glands are all affected.

The vagina, uterus, ovaries, and uterine tubes are examples of internal genital organs. (Drake *et al.,* 2015)

External Genitalia (Vulva)

The external genitalia, which is also known as the vulva, consist of the mons pubis, labia majora, labia minora, clitoris, and vestibule.

Mons Pubis: This is a fatty tissue pad that is located above the pubic bone.

Labia Majora: These are fleshy outer folds that protect the remaining external genitalia.

Labia Minora: These are thin inner folds that surround the vaginal and urethral openings.

Clitoris: This is a tiny, sensitive organ located at the labia minora's anterior junction. It consists of nerve endings and erectile tissue and it plays an important role in sexual arousal and pleasure. When aroused, it can get engorged with blood and enlarge, as does the male penis.

Vestibule: This is the area between the labia minora where the urethra and vaginal apertures are located. (Nguyen *et al*.,2025)

Internal Genitalia

The ovary: The ovaries are paired, oval-shaped gonads that are situated on either side of the uterus in the pelvic cavity. Each ovary is around 3-5 cm long and are made up of an outer cortex and an inner medulla. The cortex includes ovarian follicles, each bearing an immature ovum, and the medulla is made up of loose connective tissues and main blood arteries. The ovaries have two primary functions: gametogenesis, or the creation and maturation of oocytes (eggs), and hormone production, which includes the synthesis and secretion of sex steroids (mostly oestrogens and progesterone) and tiny amounts of androgen. (Gibson and Mahdy., 2019).

The Uterus: This is a hollow, pear-shaped organ, aids in pregnancy, menstruation, and labour. It is located in the female pelvis, prior to the rectum and posterior to the bladder. This organ is divided into four anatomic pieces, sorted from superior to inferior: the fundus, which is a broad curved region where the fallopian tubes link, and the corpus, which begins below the level of the tubes and constitutes the major uterine body. It also includes the isthmus, a constricted portion of the lower uterine neck, and the cervix, which descends into the vagina from the isthmus. The uterus facilitates implantation and foetal development. Following fertilisation, the ovum travels down the fallopian tube and is implanted in the endometrium, which supplies nourishment via specialised blood vessels. (Ameer *et al.,* 2022)

The fallopian tubes: Fallopian tubes are canals on both sides of the female pelvis that connect the ovaries to the uterus. They facilitate the movement and fertilisation of oocytes. They are 11 to 12 centimetres in length and have a lumen diameter of less than 1mm. There are four bodily parts in the fallopian tube: uterine, isthmus, ampulla, and infundibulum. The uterine portion is mostly medially composed of the uterine ostium and a short segment near the uterine horn. The isthmus is located next to the uterine portion. The ampulla is located laterally to the isthmus and it is the most common location of fertilisation. The infundibulum finishes most distant from the uterus with an abdominal ostium that opens into the peritoneal cavity and fimbriae, which, during each menstrual cycle, collect the released oocyte. The fallopian tubes transport ovums or gametes from the ovary to the uterus. (Han and Sadiq, 2023).

The vagina: This is a 7–10 cm elastic muscular tube that extends from the vulva to the cervix of the uterus, where the anterior and posterior fornixes mark its termination. The vaginal cells have oestrogen receptors and are sensitive to the hormone, resulting in a reaction critical to the maintenance of the vaginal wall. The vagina serves several functions, including

menstruation, immune defence, reproductive functions, and sexual activities. (Gold and Shrimanker, 2023).

1.1.3 The Female Reproductive Cycle

The reproductive cycle is the recurring series of physiological events that prepare a female organism for ovulation and potential pregnancy. In mammals there are two broad types commonly discussed: the menstrual cycle, found in humans and some primates and the oestrous / estrous cycle, found in most non-primate mammals. Both cycles coordinate the hypothalamus–pituitary–ovarian (HPO) axis, gonadotropins (GnRH → FSH & LH) and ovarian steroid hormones (estradiol, progesterone), but they differ in uterine/endometrial responses and behavioural receptivity to mating. (Nowak, 2018).

The menstrual cycle: Menstruation is the regular, cyclical shedding of the uterine lining that occurs in response to hormone interactions from the hypothalamus, pituitary, ovaries, and uterus. The menstrual cycle is separated into two phases: the follicular/ proliferative phase and the luteal/ secretory phase. The number of days between the first day of menstrual flow in one cycle and the start of menstruation in the subsequent cycle is the length of a menstrual cycle.. A menstrual cycle has a average duration of 28 days, with most of the cycles lasting 25-30 days. (Reed and Carr, 2018).

Stage 1: The follicular and proliferative phase

The initial stages of menstruation begins with the follicular and proliferative phases, which correlate to ovarian follicle development and endometrial proliferation, respectively. **Follicular Phase events:** This phase, although varies in length, always begins on the first day of the menstrual cycle, when menstrual bleeding occurs, and ends with the initiation of ovulation. In a typical 28-day cycle, this phase spans from day 1 to day 14. During the follicular phase, FSH induces the primordial follicles to mature into Graafian follicles,

promoting the production of; 17-β oestradiol and inhibin B within the ovary. Negative feedback from 17-β oestradiol and inhibin B reduces FSH levels, causing nondominant follicle degeneration. (Thiyagarajan *et al.,* 2024).

Proliferative phase events: This phase in the uterus, begins after monthly bleeding ends and lasts until ovulation. Growing follicles produce 17-β oestradiol, which deepens the spiral arteries that supply the endometrium and promotes the growth of the endometrial stroma and glands from the decidual basalis (basal layer of the endometrium). By the end of the proliferative period, the endometrium has reached its maximum development. Following ovulation, these changes prime the endometrium for a potential pregnancy. which occurs around the ending stage of the proliferative phase. (Thiyagarajan *et al.,* 2024).

Ovulation: Ovulation usually happens 14 days before menstruation starts. Thus, on day 14 of a normal 28-day cycle. Oestradiol levels increase during the follicular phase, and near the end, 17-β oestradiol switches from negative to positive feedback in the anterior pituitary gland. In response to this hormonal mileu, the mature follicle secretes plasminogen activator and other cytokines which causes the follicle to break and release the egg. After ovulation, 17-β oestradiol levels fall. (Thiyagarajan *et al.,* 2024).

Stage 2: The Luteal and Secretory Phases

The corpus luteum in the ovary and the mature endometrium in the uterus are represented by the subsequent luteal and secretory phases of the menstrual cycle. Ovulation marks the beginning of this phase, which concludes when menstrual bleeding starts. The luteal or secretory phase, which lasts roughly 14 days, is often consistent in each individual, in contrast to the follicular or proliferative phase's variable duration. Throughout this period, progesterone is the main hormone, and it is stimulated by LH. In the absence of a fertilised ovum, the pituitary gland's natural rise in progesterone prevents the release of LH through

negative feedback, which causes a sharp decline in progesterone and oestradiol levels near the end of the phase. Hypothalamic GnRH is released from feedback inhibition as these hormone levels fall resulting in increased production in preparation for the next cycle. (Thiyagarajan *et al.,* 2024).

Normal menstruation: The sharp decrease in the progesterone and oestradiol levels at the conclusion of the luteal phase causes endometrial shedding, as a result of no longer being sustained without these hormones. This shedding is referred to as menstruation. Menstruation takes place in the early stages of the follicular phase, as indicated by the term "day 1 of the menstrual cycle," which refers to the first day of monthly bleeding. (Thiyagarajan *et al.,* 2024).

The oestrous cycle: This cycle is very essential for reproduction. However, the cycles differ amongst species, with animals adopting the cycle that is best suited to their environment and reproductive strategy. (Sato, Nasu, and Tsuchitani, 2016). The rodents's oestrous cycle consists of four phases: proestrous, oestrous, metestrus, and diestrus, and it lasts 4 to 5 days. (Ajayi, 2020).

Proestrus phase: This stage is characterized by follicular growth, rising estradiol, epithelial cells in vaginal cytology predominate (nucleated epithelial cells). Female may become attractive to males but usually not yet receptive.

Estrus phase: ovulation and peak receptivity, cornified epithelial cells dominate vaginal smears (high level of anucleate keratinized cells). This is the "in heat" period when mating commonly occurs.

Metestrus phase: post-ovulatory; early corpus luteum formation, mixed cell cytology and increase in leukocytes.

Diestrus phase: luteal phase with relatively high progesterone (corpus luteum function). Then the cycle returns to proestrus. (Ajayi, 2020).

1.1.4 Anatomy & Physiology of the Female Reproductive System

The uterus is the muscular organ that sustains and nourishes the growing embryo. When the female is not pregnant, it is usually around 5 cm wide by 8 cm long (2 in by 3 in).. (Ameer *et al*, 2022). It consists of three parts. The area of the uterus above the uterine tube opening is called the fundus. The corpus is the main part of the uterus. The cervix is the small inferior portion of the uterus that extends into the vagina. The cervix secretes mucus that facilitates sperm passage through the reproductive canal when systemic plasma oestrogen concentrations are high.

The secretions turn stringy and thin. The uterus is held in place inside the abdominal and pelvic cavities by a number of ligaments. The main support for the uterus is the broad ligament, a peritoneal fold that runs laterally from both sides of the uterus and connects to the pelvic wall. The round ligament eventually reaches the labia majora after attaching to the uterus around the uterine tubes. Lastly, the uterus is supported posteriorly by the utero-sacral ligament, which connects the cervix to the pelvic wall. There are three layers to the uterine wall. The outermost layer of the uterus is called the serous membrane, or perimetrium, and it is made of epithelial tissue. Uterine contractions are caused by the myometrium, a thick layer of smooth muscle in the centre. Myometrial tissue, which makes up the majority of the uterus, comprises horizontal, vertical, and diagonal muscle fibres that enable both intense contractions during delivery and lesser contractions (often referred to as cramps) that help a woman's monthly blood evacuate during her period. Near ovulation, anteriorly directed myometrial contractions are hypothesised to facilitate the passage of sperm through the female reproductive system.

The lamina propria, an endometrial connective tissue lining, is covered by the epithelial tissue that lines the lumen. Stratum basalis and stratum functionalis, sometimes referred to as the basal and functional layers, are the two structural layers that make up the endometrium. Menstruation does not cause the stratum basalis layer, which is a component of the lamina propria and is situated next to the myometrium, to shed. The thicker stratum functionalis layer contains the endothelial tissue lining the uterine lumen and the glandular portion of the lamina propria. The stratum functionalis thickens and expands in reaction to elevated levels of progesterone and oestrogen. Spiral arteries, unique branches of the uterine artery, feed the thicker stratum functionalis during the luteal phase of the menstrual cycle. Only the stratum functionalis layer of the endometrium sheds during menstruation if fertilisation is unsuccessful. This inner functional layer guides the fertilised egg to the proper spot. Recall that throughout the follicular phase of the ovarian cycle, tertiary follicles develop and release oestrogen.

In preparation for implantation, the stratum functionalis of the endometrium is thickening. Maintaining a thick stratum functionalis depends on the luteal phase, which is characterised by a post-ovulatory rise in progesterone. The endometrial lining is ready for implantation if the corpus luteum of the ovary is operating normally. In actuality, the corpus luteum gets signals to keep secreting progesterone in order to maintain the endometrium and, consequently, the pregnancy if an embryo implants. The corpus luteum shrinks and gets no signal if an embryo does not implant, which stops progesterone synthesis and ends the luteal phase. While prostaglandins cause the endometrial spiral arteries to spasm and burst, limiting the flow of oxygenated blood to the endometrial tissue, progesterone causes the endometrium to shrink. Because of this, endometrial tissue dies during menstruation, or the menstrual cycle, and white blood cells, blood, and pieces of endometrial tissue pass through the vagina. The

first menstrual cycle during puberty, known as menarche, can happen before or after the first ovulation.

Although the uterus of a rat and a human differs in structure, they are fundamentally similar in terms of their basic tissue layers (endometrium, myometrium, and perimetrium), functions in supporting foetal development, hormonal regulation during the menstrual/estrous cycle, and the ability to contract during labour. The primary difference is that the rat uterus has two distinct "horns" as opposed to the human uterus's pear-shaped single uterine chamber, which makes it ideal for large litters, whereas the human uterus is best suited for single births. (Raine *et al*., 2024).

Important Similarities:

Tissue Layers: Both human and rat uteri have three layers of tissue: the outer serosa (perimetrium), the muscular middle layer (myometrium), and the inner endometrium (lining).

Hormonal Regulation: They possess similar reproductive hormones, such as progesterone and oestrogen, which control the oestrous cycle in rats and the menstrual cycle in humans, have an impact on both uteri.

Placentation: Although there may be minor variations in placental structure, both species produce a placenta to support the foetus during pregnancy.

Muscle Contractions: During labour, the myometrium in both uteri contracts to push the foetus out. (Iwanaga *et al.*, 2016)

Rats are frequently employed as animal models in investigations pertaining to human uterine function and pregnancy because of these parallels. This informed the course of this study.

1.1.5 Hormones of the Female Reproductive System

Gonadotropin releasing hormones: Human reproduction depends on gonadotropin-releasing hormone (GnRH), a decapeptide that is a part of the hypothalamic-pituitary-gonadal axis. Hypothalamic neurons produce GnRH, which triggers the production of sex hormones in the gonads. In females, this hormone eventually affects ovulatory cycles, sexual development, and the start of puberty. (Casteel, 2023). GnRH stimulates the secretion of FSH. GnRH is produced by the hypothalamus and acts on G-protein-coupled receptors on gonadotropic cells in the anterior pituitary after being released into the hypophyseal portal circulation. FSH and luteinizing hormone (LH), which are discharged into the peripheral circulation, are produced by these gonadotropic cells. (Orlowski, 2023).

Follicle stimulating hormones: Gonadotropin-releasing hormone (GnRH) from the hypothalamus causes the anterior pituitary gland to release follicle-stimulating hormone (FSH). In both males and females, FSH controls sexual development and reproduction. (the induction and maintenance of spermatogenesis) and females (oestrogen production, follicular development, and the menstrual cycle). The granulosa cells of the ovaries have female FSH receptors. FSH receptors are found in the testis' Sertoli cells in males. (Orlowski, 2023).

Lutenizing hormones: The growth and development of follicles is strictly regulated by two pituitary gonadotropins: follicle-stimulating hormone (FSH) and luteinizing hormone. Luteinizing hormone stimulates follicle growth by promoting follicle maturation. It stimulates androgen synthesis within ovarian follicles and appears to help speed and improve the transition from the primordial to antral stages of folliculogenesis. (Longo *et al.*, 2025).

Oestrogen: The development and preservation of the endometrium, or uterine lining, are principally attributed to oestrogen. It encourages cell division and prepares the uterus for potential implantation of a fertilised egg. Some of the functions of oestrogen includes

a) In the Uterus, oestrogen plays an important part in preparing the uterus for pregnancy by increasing endometrial lining thickening via endometrial cell proliferation during the menstrual cycle's follicular phase.
b) In the Breasts, this hormones is essential for breast development during puberty, promotes the growth of mammary ducts during adolescence and pregnancy, and contributes to breast milk production in the postpartum period.
c) In the Vagina, oestrogen controls the proliferation of epithelial cells in the vagina mucosa, with its presence or absence influencing vaginal tissue health, as seen in conditions like menopause.
d) In the bones, it supports bone growth by aiding in the development of long bones and the fusion of epiphyseal growth plates. Additionally, it inhibits osteoclast activity, helping to prevent osteoporosis.
e) In contraception, Ovulation is delayed by oestrogen because it decreases the hypothalamus's release of gonadotropin-releasing hormone (GnRH), which in turn prevents the release of follicle-stimulating hormone (FSH) and lutenizing hormone (LH). (Delgado *et al*., 2023).

Progesterone: Progesterone and oestrogen cooperate to control the menstrual cycle. To prepare the uterine lining for implantation, it thickens it. Progesterone supports the placenta's growth, which helps sustain the pregnancy if fertilization takes place.

In the brain, the pituitary gland secretes two hormones: luteinizing hormone (LH) and follicle-stimulating hormone. They act by stimulating the ovaries to generate more oestrogen and progesterone, which regulates the uterus' activity during the menstrual cycle.

The effect these hormones have on the uterus and overall, well-being is significant, in cases like hormonal balance and the healthy operation of the uterus are essential for both general health and reproductive health. Hormone imbalances or disturbances can result in several illnesses and problems, such as:

Menstrual irregularities: Heavy or protracted periods, missing periods, or frequent irregular bleeding are all examples of irregular menstrual cycles that can be caused by hormonal imbalances.

Infertility: Ovulation can be impacted by hormonal abnormalities, which reduce fertility and make conception more difficult.

Menopause: Hormone levels alter as women get closer to menopause, causing a range of symptoms include mood swings, vaginal dryness, and hot flashes.

Miscarriages: The female sex hormone progesterone that helps maintain the uterine lining and support the placenta. If the body doesn't produce enough progesterone, it can lead to miscarriage. Also, High or low levels of prolactin can increase the risk of miscarriage.

1.2 TOCOLYTIC (UTERINE RELAXATION) EFFECT

Uterine relaxation describes a state in which the uterine muscles are at rest and not contracting, which is usually induced to stop preterm labor and miscarriages. A miscarriage occurs when a pregnancy ends on its own before the foetus is viable. It occurs when a baby dies in the first few weeks of pregnancy (before 20 weeks), before the unborn child can live on its own. According to WHO approximately one in four pregnancies end in miscarriage,

generally before 28 weeks. Ten to twenty percent of known pregnancies terminate in miscarriage, although the true rate is likely greater. This is because many miscarriages happen before a person discovers they are pregnant. Women at risk of miscarriage have been treated with uterine muscle relaxants, which are thought to reduce the likelihood of miscarriage by relaxing the uterine muscle. It is the most common and most feared complication in pregnancy.

This can be a painful loss for expecting parents. Carers and the society may not regard this as a devastating loss as foetal death later in pregnancy, yet it frequently leads in a comparable level of mental agony and sorrow for the mother and her husband. When there is vaginal bleeding and occasionally pain during the first part of pregnancy but the cervix stays closed, it is frequently referred to as a threatening miscarriage. Pain may or may not be connected to this. Miscarriage is inevitable after the cervix begins to dilate. A threatened miscarriage might cause minor blood loss or severe shock. (McBride, 1991).

Increased uterine activity is linked to impending miscarriage, though it is often unclear whether this is a cause or effect. Relaxin is a hormone generated by the ovaries and placenta that plays an important role in the reproductive system, particularly during pregnancy. Its main job is to relax the uterus and prevent contractions. Studies has shown low relaxin levels in women who experience preterm labour, However, there is no convincing evidence that relaxin (administered intravenously or intramuscularly) can prevent premature birth in women in labour (Bain *et al*., 2013). Morphine was introduced in the nineteenth century with the notion that it would suppress uterine activity and therefore prolong the pregnancy (Charpentier, 1883).

However, two types of medicines were believed to have a more particular impact in relaxing the uterine smooth muscle many years ago, morphine is no longer utilised to treat impending

miscarriages. The earliest were hyoscine and other antispasmodic medications. Myometrial relaxants, often known as tocolytic drugs, gained popularity later. Among these were salbutamol, ritodrine, and isoxsuprine. Beta2-adrenoreceptor stimulants, or beta-agonists, are myometrial relaxants, whereas belladonna-derived atropine-like compounds are antispasmodics. Beta 2-adrenoreceptor stimulants are hypothesised to reduce the frequency and severity of uterine contractions, hence decreasing the risk of miscarriage. The side effects include palpitations, flushing, nausea, vomiting, low serum potassium, and high blood glucose levels, which also affect smooth muscle in other sections of the body.Rare but severe side effects have been documented in cases of these medications used intravenously or intramuscularly to prevent preterm birth.

An ancient class of medications known as antispasmodics is used to treat common clinical conditions such intestinal, renal, and hepatobiliary colic. They should be used cautiously during pregnancy due to their serious adverse effects, which include dry mouth, thirst, difficulty swallowing, flushing, and cardiac arrhythmias. Because these drugs also cross the placenta, they may have a number of adverse effects, such as an increased foetal heart rate.

1.2.1 Abortifacient and Oxytocic Agents

These medications acts by stimulating uterine contractions thus increasing uterine activity. (Bamber and Elder, 1990). Over the past 10 to 20 years, there has been a significant shift in the types of drugs used to regulate uterine function. Older agents such as pill ergot and buccal-pitocin have gradually been replaced by more effective, safer alternatives like prostaglandins alternatives. Commonly used oxytocic agents include misoprostol, dinoprostone, ergometrine, ergotamine, and oxytocin. Additionally, even medical conditions such as hypotension, which is not uncommon, have been linked to spontaneous abortion.

1.2.2 Tocolytic agents (uterine relaxants)

Tocolytic drugs are agents used to reduce uterine contractions, thereby preventing premature labor and preterm birth. Magnesium sulphate, beta-adrenergic agonists, oxytocin antagonists, calcium channel blockers, and adrenergic beta-receptor antagonists are examples of some medications that can help delay premature uterine activity. Previously, intravenous alcohol was used as a tocolytic, but this practice has now become obsolete.

Calcium Channel Blockers (CCBs – Nifedipine)

These drugs function by blocking L-type calcium channels, which are influenced by calcium-activated potassium channels, beta-adrenergic receptors, and sex hormones. Among them, nifedipine is the most widely used in clinical settings due to its comparable effectiveness to beta-agonists and oxytocin antagonists, while also demonstrating a better safety profile. However, its known side effects include headache, hypotension, and tachycardia.

NSAIDS (Indomethacin)

A prostaglandin synthesis inhibitor, indomethacin has been found to be more effective than beta-sympathomimetic nylidrin as a tocolytic agent. However, its frequent association with neurodevelopmental side effects makes it less favorable for use in delaying labor, similar to other nonsteroidal anti-inflammatory drugs (NSAIDs). Additionally, cervical ripening plays a critical role in the labor induction process.

Oxytocin Receptor Antagonists (Atosiban)

Atosiban is an oxytocin receptor antagonist (Romero, 2005). It primarily functions as an arginine vasopressin (AVP) V1a receptor blocker, with a lower affinity for oxytocin receptors. Its method includes a dose-dependent suppression of oxytocin-induced increases in intracellular calcium., which leads to the closure of voltage-gated calcium channels, thereby

preventing calcium influx. As a result, uterine contractility is reduced, and prostaglandin release is inhibited (Beck *et al.*, 2019).

Magnesium Sulfate

The mechanism of action of magnesium sulfate is multifaceted, involving both vascular and neurological effects. As a calcium antagonist, it promotes vascular smooth muscle relaxation and vasodilation, which likely contributes to a decrease in total peripheral resistance (Altman *et al.*, 2007). They also provide neuro-protection of the fetus and relax the uterine smooth muscles.

Beta-adrenergic agonists (Terbutaline)

Beta-mimetic drugs work by targeting receptors on myometrial cell membranes, activating adenylate cyclase, which converts ATP to cyclic AMP (cAMP). Increased cAMP levels then activate the cAMP-dependent protein kinase, which subsequently reduces intracellular calcium levels, leading to decreased myometrial contractility (Wilkins & Creasy, 1986). Drugs within this class include salbutamol, terbutaline, and ritodrine. However, their use is associated with side effects. Examples include maternal hypotension, tachycardia, cardiac arrhythmias, myocardial ischaemia, chest discomfort, and pulmonary oedema. In addition, chronic exposure to beta-adrenergic agonists might produce receptor down-regulation and desensitisation to therapy. (Caritis *et al.*, 1988)

1.2.3 Contrainidications for the use of tocolytics

Tocolytic medications are not recommended in certain clinical situations where the risks of prolonging pregnancy outweigh the potential benefits. In many cases, immediate delivery is preferred over attempting to delay labor. The key contraindications include:

1. Gestational age beyond 34 weeks, where the risks of preterm birth are minimal compared to the potential complications of prolonging pregnancy.
2. Intrauterine fetal demise, where the fetus has already passed away in the womb.
3. Lethal fetal anomalies, where the fetus has congenital conditions that are incompatible with life.
4. Non-reassuring fetal status, indicating fetal distress or compromised well-being.
5. Severe preeclampsia or eclampsia, conditions that pose a significant threat to maternal and fetal health.
6. Maternal hemorrhage with hemodynamic instability, where excessive bleeding endangers the mother's life.
7. Chorioamnionitis, a serious infection of the fetal membranes that necessitates immediate delivery.
8. Preterm premature rupture of membranes (PPROM)—tocolytics are generally avoided unless there is no maternal infection, and there is a need for maternal transfer, corticosteroid therapy, or both to support fetal lung development

Specific Contraindications for Certain Tocolytic Agents

In addition to general contraindications, certain medical conditions make the use of specific tocolytic drugs inappropriate:

1. Advanced cervical dilation (greater than 5 cm), where labor is already in an advanced stage and unlikely to be reversed.
2. Maternal heart disease, where tocolytics could exacerbate cardiovascular complications.
3. Hyperthyroidism, as beta-adrenergic agonists can worsen thyroid-related symptoms.

4. Uncontrolled diabetes, where some tocolytics may interfere with glucose regulation.
5. Mild placental abruption, where partial detachment of the placenta increases risk
6. Stable placenta previa, where the placenta is covering the cervix but without active bleeding.
7. Intrauterine growth restriction (IUGR), where the fetus is already experiencing poor growth, and delaying delivery may not be beneficial.

1.4 PHYLLANTUS AMARUS

Fig. 1.2: Aerial Parts of *Phyllantus amarus* obtained from its natural habitat in the University of Benin, Ugbowo Campus, Benin City.

Phyllanthus amarus is a leafy herbal plant found in tropical areas of Southeast Asia, the Americas, Africa, China, India, and Sri Lanka. Gale of the wind, carry me seed, seed on the leaf, pick-a-back, stonebreaker, and dukung anak are some of the common names for this plant. P. amarus is an erect, glabrous, annual herb that reaches a height of 10–60 cm. The herb's main stem is either simple or branching, and it can be smooth or scabridulous in younger areas. Because of its remarkable medical effects, this plant has been appreciated in

many countries for a range of diseases. The plant's fruits and seeds are high in carbohydrates, low in fat, ash, and crude fibre, and have a minor amount of protein, according to a study that examined the nutritional and phytochemical components of the plant. Other ingredients include magnesium, calcium, potassium, ascorbic acid, iron, zinc, thiamine, niacin, and riboflavin. P. amarus has been listed under many names in multiple published research and review articles, and it has been mistaken with Phyllanthus niruri. Linnaeus and other early taxonomists designated several other species, including P. amarus, as P. niruri. (Ghosh *et al.,* 2022).

1.4.1 Scientific Classification

Kingdom: Plantae

Phylum: Tracheophytes

Class: Angiosperm

Clade: Rosids

Order: Malpighiales

Family: Phyllanthaceae

Genus: *Phyllantus*

Species: *Amarus*

1.4.2 Phytochemistry

Phytochemistry is a discipline of chemistry that studies the chemical properties of plants or plant products. Many chemical elements found in plants are either therapeutically active or inactive, including carbohydrates, triterpenoids, alkaloids, glycosides, tannins, flavonoids, essential oils, and other related secondary metabolites. *P. amarus* produces a variety of

chemical substances, including secondary metabolite groups such as lignans, flavonoids, alkaloids, hydrolysable tannins (Ellagitannins), polyphenols, triterpenes, sterols, and volatile oils. In addition to the fact that the herb is a centre of a wide variety of secondary metabolites found in its numerous sections, these compounds have been regarded as having substantial medicinal importance.

Lignans:

They are a frequent class of phenylpropanoids that are made from phenylalanine using the conventional phenylpropanol pathway, which dimerises modified cinnamic alcohols called monolignols to a dibenzylbutane skeleton. Lignans such as the bitter element, phyllanthin and the non-bitter constituent, hypophyllanthin have been identified from P. amarus and are highly significant because of their numerous medicinal uses, including hepatoprotection, antitumor, antimitotic, antiviral, and antioxidant activities. The largest concentrations of phyllanthin (0.7% w/w) and hypophyllanthin (0.3% w/w) have been found in leaves, while they are present in trace amounts in the stem. (Ghosh, 2022).

Flavonoids:

These are polyphenolic chemicals that are found as secondary metabolites of plants. Flavanone, flavones, flavonols, isoflavones, catechins, chalcones, and their derivatives are among the several groups. The flavonoids are synthesised via the phenylpropanoid route, which converts phenylalanine into 4-coumaroyl-CoA before entering the flavonoid biosynthesis pathway. The first enzyme, chalcone synthase, in the flavonoid pathway to generate chalcone scaffolds, which are the building blocks of all flavonoids. The primary flavonoid production pathway is known to be preserved in plants. Flavonoid subclasses vary by species and are formed by enzymes including Fe2+/2-oxoglutarate-dependent dioxygenases, hydroxylases, reductases, and isomerases that modify the flavonoid skeleton.

The various flavonoids serve a variety of biological roles, including flower colouration, tress responses, auxin transport, and defence against UV radiation and phytopathogens. Furthermore, flavonoids' positive roles in human health, as well as their usage in the prevention and treatment of many illnesses, have been extensively documented. This family of chemicals also accounts for P. amarus' varied bioactivities. Some of the key flavonoids found in this strong herb include rutin, astragalin, kaempferol, and quercetin. (Ghosh, 2022).

Alkaloids:

These are one of the most diverse classes of secondary metabolites known, with a wide range of structural types, biosynthesis processes, and pharmacological effects. Alkaloids are nitrogenous chemicals with cyclic structures and a low molecular weight. The Angiospermae, or blooming plants, have been the primary source of alkaloids, accounting for approximately 20% of the total. Aside from its role in plant defence against herbivores and pathogens, it has long been known for its many pharmacological actions, particularly in mammals such as humans. *P. amarus* is also known to contain many alkaloids such as securinine, epibubbialine, isobubbialine, and others as part of its wide class of secondary metabolites, which are also responsible for the herb's various purported therapeutic qualities. (Ghosh, 2022).

Terpenes and terpenoids:

Terpenes and terpenoids are an important component of plant secondary metabolites, as well as a huge and diversified group of chemical molecules. Terpenoids, also known as modified terpenes, are terpene-like compounds. On the other hand, other authors use the term "terpenes" in a broader sense to encompass terpenoids. This type of secondary metabolite is important in plant-insect, plant-pathogen, and plant-plant interactions. Furthermore, They provide applications in the food and pharmaceutical industries as well as chances to address a variety of societal and human health challenges. This class of chemicals has strong

therapeutic promise, including Immunomodulatory, anticancer, antiparasitic, antibacterial, antiallergenic, antispasmodic, antihyperglycemic, and anti-inflammatory properties

Immunomodulatory, anticancer, antiparasitic, antibacterial, antiallergenic, antispasmodic, antihyperglycemic, and anti-inflammatory properties. They are often found in vegetative tissues, flowers, and occasionally roots, and are more abundant in higher plants. P. amarus is one of several plant species known to contain this class of phytoconstituents, including lupeol, phyllanthenol, and phyllanthenone. (Ghosh, 2022)

Tannins:

Chemically, it is difficult to identify because the name tannin refers to a variety of polymers and oligomers that are distinct. The tannins are a broad class of high molecular weight polyphenolic substances that can combine to generate both irreversible and reversible complexes with alkaloids, minerals, proteins (mostly), polysaccharides (cellulose, hemicellulose, pectin, etc.), and nucleic acids (Yousaf *et al.,* 2013). Tannins can be classified into four types depending on their structural properties: gallotannins, ellagitannins, complicated tannins, and condensed tannins.

1.4.3 Secondary Metabolites of *Phyllantus amarus*

Over the past few years, various phytochemical investigations in *P. amarus* have been conducted worldwide using various analytical techniques to identify the powerful medicinal herb's various classes of secondary metabolites. Studies and analyses have been conducted on the flavonoids and tannins of many *Phyllanthus* species, including *P. amarus* (Nara *et al.*, 1977, Ueno *et al.*, 1988). To separate and identify tannin-class components such geraniin, ellagic acid, and gallic acid, the herb's MeOH eluate was chromatographed and analysed (Ueno *et al*., 1988). A hexane extract of the strong plant was used to extract an acyclic triterpene and analyse its structure. (Singh *et al*, 1989). Following extraction from P. amarus,

an X-ray analysis confirmed the structure and absolute stereochemistry of the alkaloid compound ent-norsecurinine (Joshi et al, 1986). The sensitive and accurate method of high-performance liquid chromatography (HPLC) was employed to recover the two primary lignans, phyllanthin and hypophyllanthin, from various parts of the *P. amarus* plant at a rate of about 98% (Sharma *et al*., 1993).

Chemical derivatisation and GC-MS analysis with *P. amarus* extract were used to test the antibacterial activity of the herb's lignan phyllanthin (Ribeiro *et al.,* 2019). Recent research on phytochemical screening, quantitative, and gas chromatography-mass spectrometry (GC-MS) studies employing an ethanolic leaf extract of *P. amarus* has found major bioactive components (Ajayi *et al.,* 2020; Oshomoh EO *et al.,* 2020). Recently, a new green extraction and purification method was devised to quickly produce the tannin corilagin from *P. amarus* and four other species in the same genus. The experiment used precipitation and preparative high-performance liquid chromatography (prep-HPLC) in combination with an aqueous ionic liquid (Hou *et al.,* 2020).

P. amarus has a higher concentration of lignans than the other species utilized in the study, according to the GC–MS technique, which provides an accurate identification and quantification of lignans discovered in several *Phyllanthus* spp. (Novellino *et al.*, 2020). Over the years, several analytical methods have been used with this medicinal herb, and research is still being done globally to find several classes of biomolecules with therapeutic significance. To promote commercial production of *Phyllanthu*s species, including *P. amarus,* a recent study developed and validated a simple reversed-phase HPLC-PDA method for profiling the lignan classes: phyllanthin, hypophyllanthin, nirtetralin, and niranthin in Phyllanthus extracts. The study's approach was designed to be utilised for quality control in herbal formulations containing *Phyllanthus* species plants (Patel *et al.,* 2021). Another team of researchers identified and measured the phytoconstituents in *P. amarus* leaf and root extracts, and they

found three bioactive chemicals that are significant for medicine. Among these was 9-Octadecenoic acid, which was found to be present in the plant's leaves and roots at percentages of 92.23% and 82.46%, respectively. It was followed by n-Hexadecanoic acid and Tetradecanoic acid, which were discovered to be present in the leaves and roots with percentages of 7.7% and 17.54%, respectively. (Ameen *et al*, 2020).

1.4.4 Pharmacological Properties / Medicinal uses of *Phyllantus amarus*

Antihypertensive Activity:

Although the study investigating the chloroform extract of this plant showed that it had no notable effect on systolic blood pressure however, it did generate a non-dose dependent significant drop in diastolic blood pressure and mean arterial blood pressure. Meanwhile, the aqueous extract was discovered to produce a dose dependent considerable reduction in both diastolic and systolic blood pressure, as well as the mean arterial blood pressure in normotensive white Wistar rats. (Amaechina and Omogbai, 2007).

Antimicrobial Activities:

When the hot water and ethanol extracts of *Phyllanthus amarus* were tested against Salmonella Typhi using the agar cup diffusion method and compared to standard antibiotics tested using the disc diffusion method, the test results was shown and it was observed that the ethanolic extract was the most potent against the test bacteria, with a growth inhibition zone diameter of approximately 8.0mm. This investigation confirmed one of the major traditional applications of Phyllanthus amarus for typhoid illness.

Another study in which hexane, petroleum ether, chloroform, acetone, and methanol extracts of Phyllanthus amarus were used on the following bacteria cells: Klebsiella pneumoniae, Proteus mirabilis, Streptococcus Faecalis, Serratia marcesces, Staphylococcus aureus, Eschierichie Coli, and Enterobacter species. These were examined using the agar well

diffusion method, and the results revealed that the methanol extract produced the best inhibitory efficacy against these species. (Oluwafemi and Folashade, 2008).

In another study, its antibacterial potential was tested using the agar well diffusion method against many drug-resistant organisms, including Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and even Klebsiella species. The results showed a minimum inhibiting effect, which is highly impressive. (Saranraj and Sivasakthivelan, 2012).

Antiamnesic Activity:

This plant has been reported to help those experiencing a partial or total loss of their memory. In this work, Joshi and Parle, 2007, the aqueous extract of P. amarus leaves and stems was examined in male Swiss albino mice for its nootropic effects and brain cholinesterase activity. Scopolamine (an anti-muscarinic) and diazepam (a benzodiazepine) were used in inducing amnesia, with raised plus maze and passive avoidance paradigms serving as models for assessing cognitive processes and the results showed that diazepam and scopolamine-induced amnesia were reduced in a dose-dependent manner, as well as brain cholinesterase activity. Since the decrease in cholinesterase is well related with an increase in acetylcholine concentration in the brain, which is also responsible for memory improvement and cognitive impairments. (Joshi and Parle, 2007).

Antioxidant Activity:

This activity is determined by the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) test, which involves reducing the stable radical, DPPH to yellow diphenyl picryl hydrazine. Thus, the capacity of the test items to reduce this radical is indicative of their antioxidant qualities. A recent study compared phyllanthin and Phyllanthus amarus. It was discovered that the DPPH free activity was concentration dependant, peaking at 20mol/ml for phyllanthin and 300g/ml

for Phyllanthus amarus extract. Thus, phyllanthin has very high antioxidant properties, as seen by its low IC50 value of 7.4mol/ml. (Murtijaya and Lim, 2007).

Anti-leptospiral Activity:

Leptospirosis is a well-known disease that occurs primarily when humans come into contact with the urine of diseased animals, infected animals, or urine-contaminated settings. This plant's antileptospiral activity via micro dilution tests and tube dilution techniques resulted in a well-emphasized inhibitory action of the methanolic and aqueous extract of the complete plant against Leptospira. (Ismail *et al*, 2021).

Anticonvulsant Activity:

Epilepsy is a serious neurological illness characterized by the occurrence of recurring seizures. Meanwhile, two generally hypothesised causes for these seizures include voltage-dependent ion channel changes, such as a reduction in inhibitory GABA-mediated inputs. This chronic progressive CNS condition affects a large proportion of the global population. Aqueous and ethanol extracts of *P. marus* were tested for anticonvulsant activity against pentylenetetrazol (PTZ) and maximum electroshock-induced seizures (MES) in Swiss albino rats. The findings of this study clearly demonstrated that the ethanol and aqueous extracts of *Phyllanthus amarus* leaves and stems are considerably effective in inhibiting hind limb extension generated by MES as well as PTZ-induced seizures. (Amaechina and Omogbai, 2013).

Antidiabetic Effect:

Diabetes is a well-known metabolic illness involving carbs, lipids, and proteins that is regarded as the world's greatest endocrine disease. The antidiabetic potential of this plant was investigated in an experiment using fasted rats who were made diabetic by a single intraperitoneal injection of 120mg/kg of alloxan monohydrate, followed by two doses of the

aqueous and hydroalcoholic extract *of P. amarus* administered orally, which were then compared to the normal control group that received only distilled water. After 15 days of treatment, the results demonstrated that these extracts considerably reduce blood glucose levels.

Serum analysis of the treated experimental animals revealed a rise in insulin and a decrease in malondialdehyde levels. As a result, these *P. amarus* extracts have been shown to have antidiabetic properties. Another study demonstrated that the methanolic extract of this plant inhibited lipid peroxidation and scavenged hydroxyl and superoxide radicals. Because free radicals are connected with diabetes, quenching free radicals may be one of its mechanisms of action. (Moshi *et al.,* 2001).

Anti-inflammatory Activities:

The plant's potential was assessed using several approaches, including Kupffer cells, macrophages RAW264.7, human whole blood in mice, and so on. The anti-inflammatory properties of two distinct extracts, hexane and ethanol/water extracts, were investigated against the lipopolysaccharide-stimulated test cells listed above. Furthermore, the anti-inflammatory impact was assessed in mice that had previously been treated with galactosamine/lipopolysaccharide to induce acute toxic hepatitis. In the long term, this evaluation criteria represent the production of nitrate and prostaglandin E2 by radioimmunoassay and, later, by enzyme linked immunosorbent assay. (Kiemar *et al,* 2003).

Hepatoprotective activity:

The study demonstrated the hepatoprotective activities of the lignans phyllanthin, hypophyllanthin, and triacontanol in primary cultured rat hepatocytes against carbon-tetrachloride and galactosamine-induced cytotoxicity. Although therapeutic uses of *P. amarus* have been documented in traditional (Ayurvedha and Siddha) literature for over a

century, scientific research on the subject has only been conducted in the last 50 years. The efficiency of *P. amarus* in ethanol-induced fatty liver in rats was shown after administering its herbal powder. The hepatoprotective mechanism was thought to be attributable in part to the protective effect on the depletion of hepatic reduced glutathione, as well as its antioxidant activity, particularly its radical scavenging and iron chelating properties. In addition to its hepatoprotective properties, P. amarus actively inhibits the hepatitis B virus. Several research have demonstrated the herb's anti-hepatitis properties over time. (Ghosh *et al*., 2022).

Antitumor activity:

A 1:1 blend of phyllanthin and hypophyllanthin, both *P. amarus* extracts, shown anticancer activity against Ehrlich Ascites Carcinoma in Swiss albino rats. This occurred after the rats were pre-treated orally with these extracts at doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg body weight, followed by EAC administration after an 18-hour fast. When the animals were euthanised, the decreased tumour volume, packed cell volume, and viable cell count seen clearly indicates an anticancer property when compared to the control group. (Mazumder *et al,* 2008).

1.5 STATEMENT OF PURPOSE (JUSTIFICATION)

The use of *Phyllanthus amarus* has become increasingly popular, owing to its reputed medicinal and therapeutic benefits. This growing trend of its use is such that, different parts of the plant which includes; the leaves, stem, and roots are widely utilized in traditional medicine to manage several ailments. Although previous research has examined the pharmacological activities of *Phyllanthus amarus* on different organs and physiological systems, there remains limited evidence regarding its specific effects on the female reproductive system, particularly the uterus and its ability to influence conception outcomes. Considering its extensive use, it is important to evaluate whether the plant possesses

uterotonic and uteroprotective effects as well as influence conception outcomes.The lack of extensive scientific data in this area underscores the need for further research, forming the basis of this study. Hence, the purpose of this review is to do so.

1.6 AIMS & OBJECTIVES OF THE STUDY

Aim:

To investigate the effect of *Phyllanthus amarus* extract on the uterine smooth muscle reactivity, and conception outcome in female wistar rats.

Objectives:

To assess the effect of ethanol extract of *Phyllanthus amarus* on influencing uterine reactivity at resting tension.

To investigate the relaxant effect of ethanol extract of *Phyllantus amarus* on oxytocin pre-contracted uterine smooth muscle.

To investigate the relaxant effect of ethanol extract of *Phyllantus amarus* on 80mmol KCL pre-contracted uterine smooth muscle.

To investigate the effect of ethanol extract of *Phyllantus amarus* extract on influencing conception outcomes in gravid Female Wistar rats.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Apparatus

The study employed the following apparatus; a weighing balance, Data Capsule (Model 17400), an organ bath (Ugo Basile), an aerator, beakers, surgical silk, dissecting kit, a Petri dish, porcelain dish, a spatula, a stirrer, micropipettes, syringes, markers, a mercury-in-glass thermometer, tissue papers, dispensing bottles, oro-gastric tube, distilled water and cages.

2.1.2 Chemicals and Drugs

The substances utilized included:

1. Oxytocin
2. Diethylstilbestrol, dissolved in a 1:1 ethanol-water solution
3. Potassium chloride (KCl 80 mMol)
4. Ethanol extract of *Phyllanthus amarus*

2.1.3 Solvent for extraction

Ethanol

2.1.4 Physiological Salt Solution (PSS)

Kreb's solution was used as the physiological salt solution (PSS), with the following salts:

1. Sodium chloride (Nacl) pharmatrend Nig. Ltd.
2. Potassium chloride (BDH chemical Ltd People England).
3. Magnesium sulphate (MgSO4.7H20).
4. Sodium Bicarbonate. (NaHCO3) May and Baker Ltd.
5. Potassium dihydrogen phosphate (KH2PO4).

6. D-Glucose (BDH chemical Ltd People England).
7. Calcium chloride(CaCl2) May and Baker Ltd.

2.1.5 Preparation Of Kreb's Physiological Salt Solution

A precise quantity of each salt was measured using an electronic weighing balance: Sodium chloride (6.9g), Potassium chloride (0.35g), Magnesium sulphate (0.29g), Potassium dihydrogen phosphate (0.16g), D-Glucose (2.0g). These components were dissolved in 800 ml of distilled water contained in a 1 litre beaker. Separately, calcium chloride (0.37g) was weighed and dissolved in 100ml of distilled water in a 100 ml beaker. The calcium chloride solution was then quantitatively transferred into the solution of the mixture of salts with continuous stirring to ensure uniform mixing. The resulting physiological solution was then made up to 1 litre volume using distilled water.

2.2 PLANT EXTRACT

Ethanol leaves extract of *Phyllantus amarus* was used for the study.

2.2.1 Plant Collection And Extract Preparation

The leaves of *Phyllanthus amarus* were collected from the University of Benin, Ugbowo Campus, Benin City, Edo State, Nigeria, and authenticated and identified by the Department of Pharmacognosy, University of Benin. The plant material was carefully cleaned to remove any debris and foreign particles, then air-dried for two weeks. Subsequently, it was oven-dried at 45°C for approximately 60 minutes to ensure complete dehydration.

After drying, the leaves were finely ground using a Kenwood electric blender at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin. A Soxhlet apparatus was then used to extract 250 g of the pulverized plant material with 1 liter of ethanol at 70°C. This extraction process was performed twice, and the combined extracts were concentrated using a rotary evaporator until a solid, dark-black mass was obtained. The

resulting extract was carefully sealed in an airtight container and stored in a refrigerator until it was ready for use.

2.3 EXPERIMENTAL ANIMALS

Non-gravid female adult Wistar rats, weighing between 160-220 g, were obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Nigeria, where they were also housed and maintained. A total of forty (40) female rats were used for the study. The animals were kept in standard rat cages and fed commercial rat feeds, with unrestricted access to clean, fresh water provided in bottles ad libitum. Before the experiment, they underwent an acclimatization period of two weeks under standard laboratory conditions. Animal care and all experimental procedures adhered to internationally accepted guidelines, including the National Institute of Health (NIH, USA) Public Health Service Policy on Humane Care and Use of Laboratory Animals (2002). Additionally, all protocols complied with the Ethics Committee on Research in Animals, University of Benin.

2.4 METHOD

2.4.1 Invivo Evaluation of Conception

Twenty five female Wistar rats were randomly divided into five groups (n=5) to receive 6.25, 12.5, 25 and 50mg/kg of the extract and the control group. *Phyllanthus amarus* extract was prepared by ethanol extraction from the dried whole plant. Each rat was weighed, and the required dose was calculated. The doses of extract was administered orally to the experimental animals once daily for 14 days. The control group received vehicle (distilled water) only.

Each groups experimental animals were mated with male wistar rats on day one (1) of treatment and coitus was confirmed with vaginal smear. Observation done under the microscope. Conception was assessed by the presence of pregnancy and subsequent litter

production. The litter weights were recorded seven (7) days after delivery. Animals from various groups were observed throughout the period of gestation up to thirty one (31) days after coitus was confirmed.

2.4.2 In-Vitro Evaluation of Uterotonic Activity (Animal Preparation for Uterine Studies)

Uterine segments were isolated and prepared for in vitro experiments. Briefly, diethylstilbestrol (0.1mg/kg, intra-peritoneal) was administered to the animals 24 hours before the procedure to prime the uterus. The estrus stage was confirmed through microscopic examination of vaginal smears and macroscopic observation of the vulva. The rats were then sacrificed by cervical dislocation, and the uterine horns were quickly dissected out and transferred into pre-warmed and aerated Kreb's physiological salt solution (PSS). Segments of the uterus, approximately 2 cm in length, were carefully trimmed to remove any adhering connective tissues and fat. Each segment was then mounted longitudinally in a 20ml organ bath containing the PSS mentioned above. The lower end of the tissue was attached to tissue holders using surgical thread(silk), while the upper end was attached to an isometric force-displacement transducer (Model 17400, Ugo Basile, Monvalle VA, Italy) connected to a unirecorder for data recording. The PSS was maintained at 36°C and continuously aerated throughout the experiment. Each uterine segment was subjected to a resting tension of 1.0 g and allowed to equilibrate for 30 minutes before the commencement of the study.

2.4.2.1 Protocol 1 (Study on the effect of the extract on spontaneous contractions)

The uterine tissue was allowed to spontaneously contract for 30 minutes before cumulatively administering 25µL, 62.5 µL, 125 µL and 250 µL of *Phyllanthus amarus* extract at concentrations of 0.1mg/ml, 1mg/ml, 10 mg/mL, 100 mg/mL, and 200 mg/mL to generate a concentration-response curve for spontaneous contractions. A contact time of 4 minutes was

maintained for each administration. This procedure was repeated two times, ensuring a final sample size of n = 6 for all experimental conditions.

2.4.2.2 Protocol 2 (Study on the Effect of the Extract on Oxytocin Induced Contractions)

The uterine tissue was allowed to spontaneously contract for 30 minutes, after which, 0.001 IU/mL, 0.01IU/mL, 0,1IU/mL, 1IU/ mL was introduced until a sustained/ increased contraction each for about 4 minutes before cumulatively administering 25µL, 62.5 µL,125 µL and 250 µL of *Phyllanthus amarus* extract at concentrations of 0.1mg/mL, 1mg/ mL, 10 mg/mL, 100 mg/mL, and 200 mg/mL to generate a concentration-response curve for Oxytocin induced contractions. A contact time of 5 minutes was maintained for each administration. This procedure was repeated two times, ensuring a final sample size of n = 6 for all experimental conditions.

2.4.2.3 Protocol 3 (study on the effect of the extract on potassium chloride induced contractions)

To investigate the potential calcium channel blockade by *Phyllanthus amarus,* uterine tissues were depolarized using 80 mMol K, inducing sustained contractions. Following this, 25µL, 62.5µL, 125 µL and 250µL of *Phyllanthus amarus* extract at concentrations of 0.1mg/mL, 1mg/ mL, 10 mg/mL, 100 mg/mL, and 200 mg/mL were administered cumulatively to generate a concentration-response curve for Potassium chloride induced contractions. A contact time of 5 minutes was maintained for each administration. This procedure was repeated two times, ensuring a final n =6 for all conditions.

2.5 STATISTICAL DATA ANALYSIS AND PRESENTATION

All data were expressed as mean ± Standard deviation (S.D) using tables, continuous line graphs and pictorial charts for the in-vivo and in-vitro results. Dose response curves were generated and data analysis as well as data presentation were carried out using Graphpad

prism. Statistical evaluation for the in-vivo results was performed using one way analysis of variance (ANOVA), with a significant level of $p \leq 0.05$ and a 95% confidence interval.

CHAPTER THREE

RESULTS

3.1 IN-VIVO STUDY

3.1.1 The effect of *Phyllanthus amarus* on Conception and Litter Weights in Female Wistar Rats

Table 1: Summary of conception outcomes and mean litter weights in rats treated with varying doses of Ph*yllanthus amarus* extract.

Treatment Group (mg/kg)	No. of Rats	No. of Litters Produced	Mean Litter Weight (g ± SD)	Observation
Control(Distilled water)	5	12	15.33 ± 2.14	Normal conception
6.25	5	0	—	No litters observed
12.5	5	8	12.18 ± 1.76	Normal conception
25	5	0	—	No litters observed
50	5	8	13.69 ± 2.03	Normal conception

NOTES:

- Values are expressed as mean ± standard deviation (SD) for groups with litters.
- "—" indicates no litters were produced.

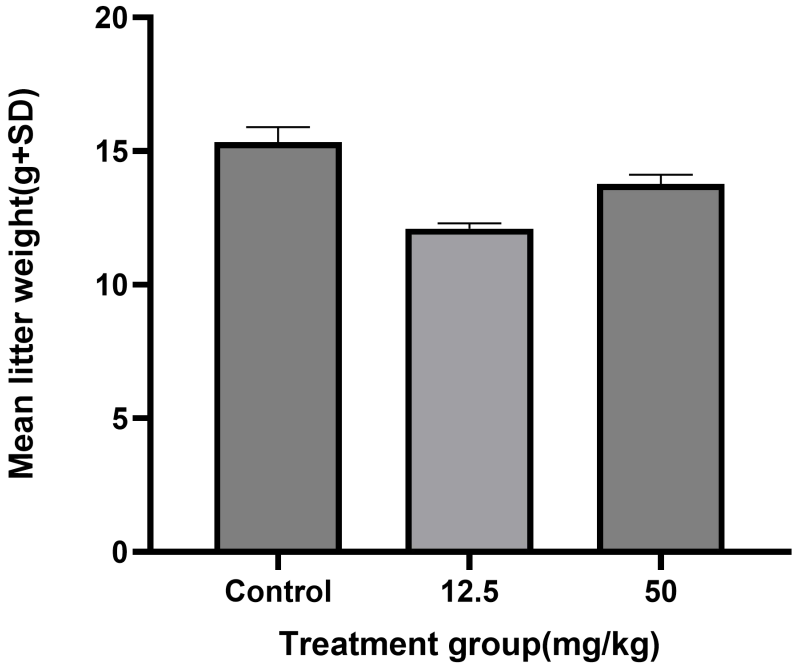

Fig 2.1: Effect of Phyllanthus amarus on Mean Litter Weight in Female Wistar Rats

3.2 IN-VITRO STUDY

The findings from the various experiment carried out on isolated uterine smooth muscle tissue of non-gravid female Wister rats are presented below ;

3.2.1 The effects of varying concentrations of *Phyllantus amarus* extract on the spontaneous contractions of isolated rat uterus are presented below

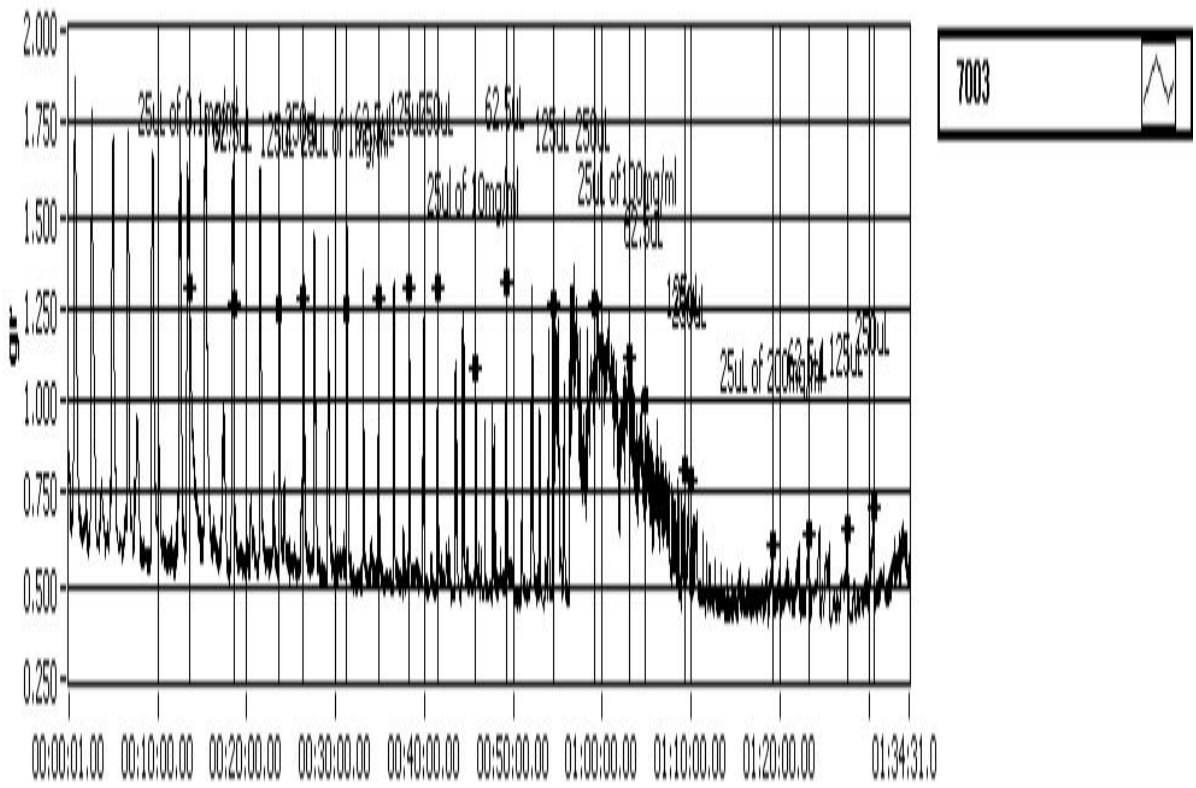

Fig.2.2: Representative chart of the effect of *Phyllantus amarus* extract on spontaneous contraction of an isolated female uterine tissue

b)

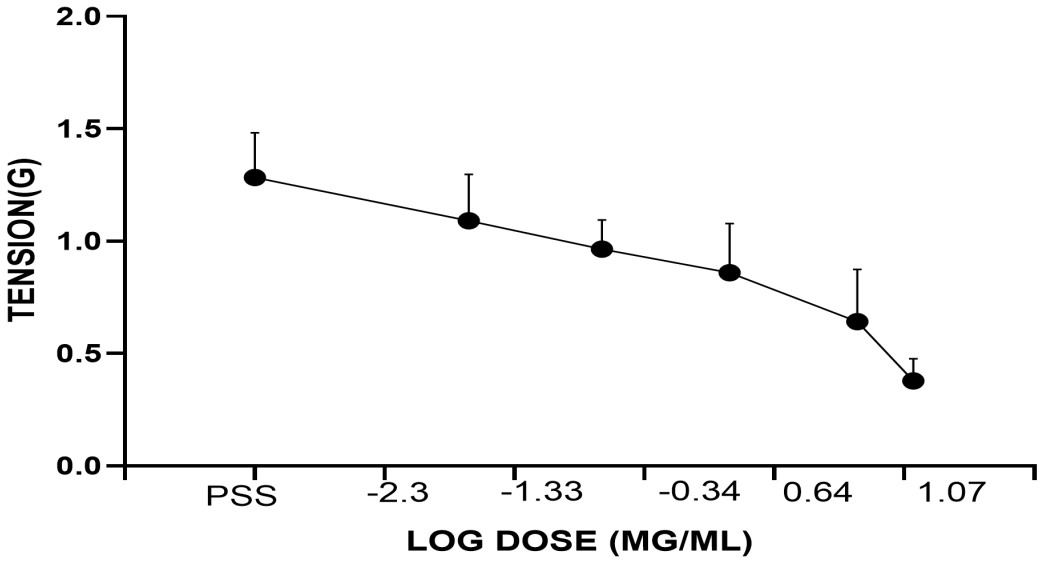

Fig. 2.3: A graphical representation of the mean values of uterine response to the mean values of the different concentrations of Ethanolic Extract of *Phyllantus amarus*.

Where n = 6

3.2.2 The effects of varying concentrations of *Phyllantus amarus* on the isolated rat uterus with Oxytocin induced contractions are presented below;

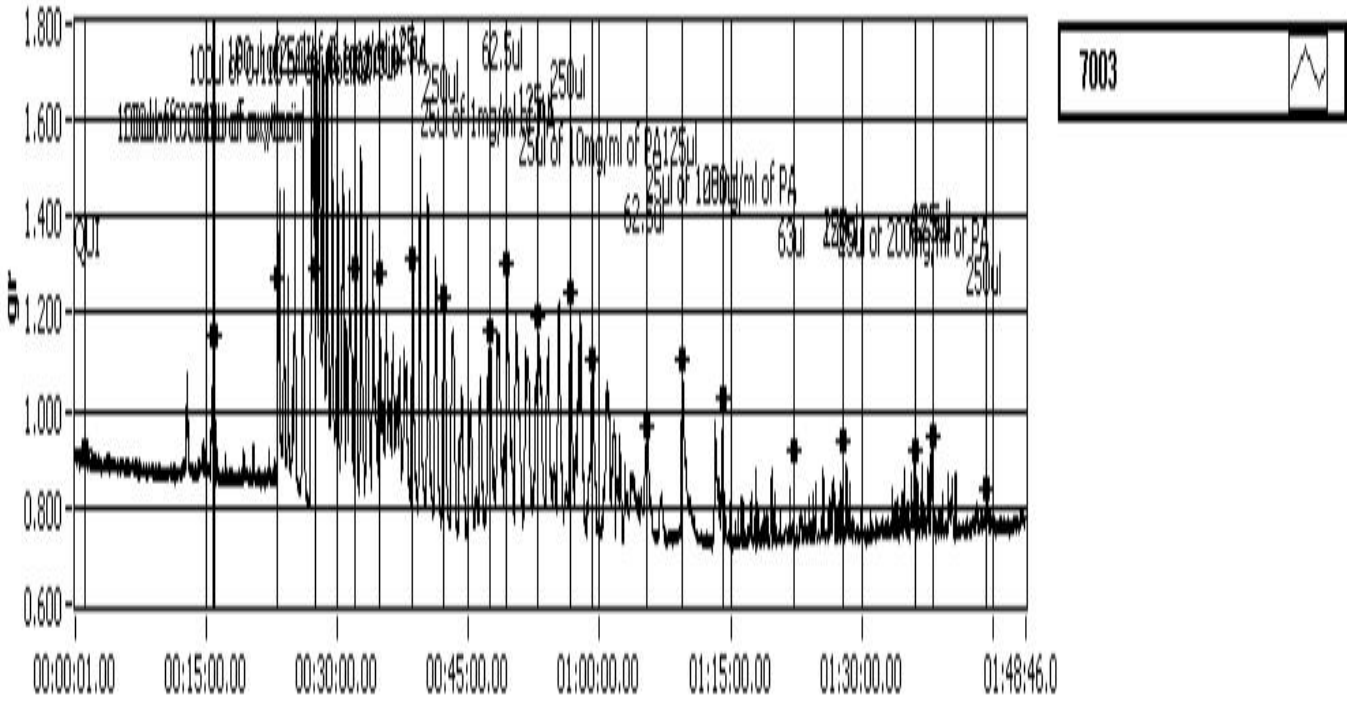

Fig.2.4: A representative chart of the uterine response to different concentrations of *Phyllantus amarus* extract

b)

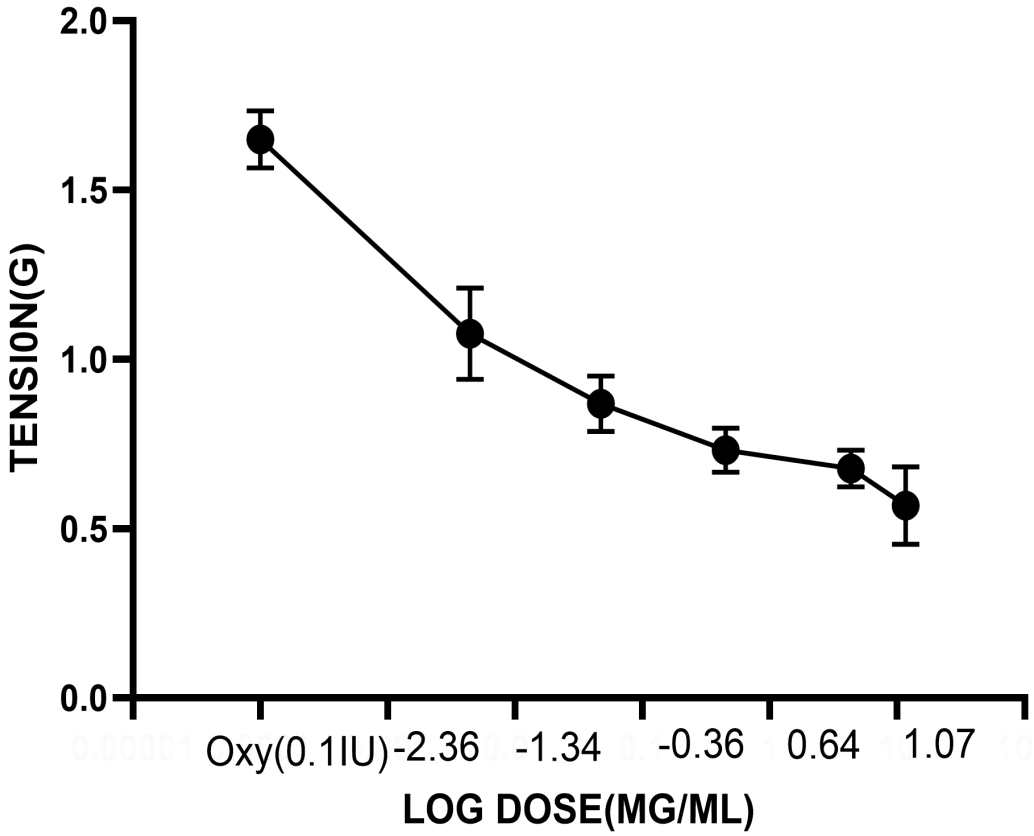

Fig. 2.5: A graphical representation of the mean values of uterine response to the mean values of the different concentrations of Ethanolic Extract of *Phyllantus amarus*.

Where n = 6

3.2.3 The effects of varying concentrations of *Phyllantus amarus* on the isolated rat uterus with KCL induced contractions are presented below;

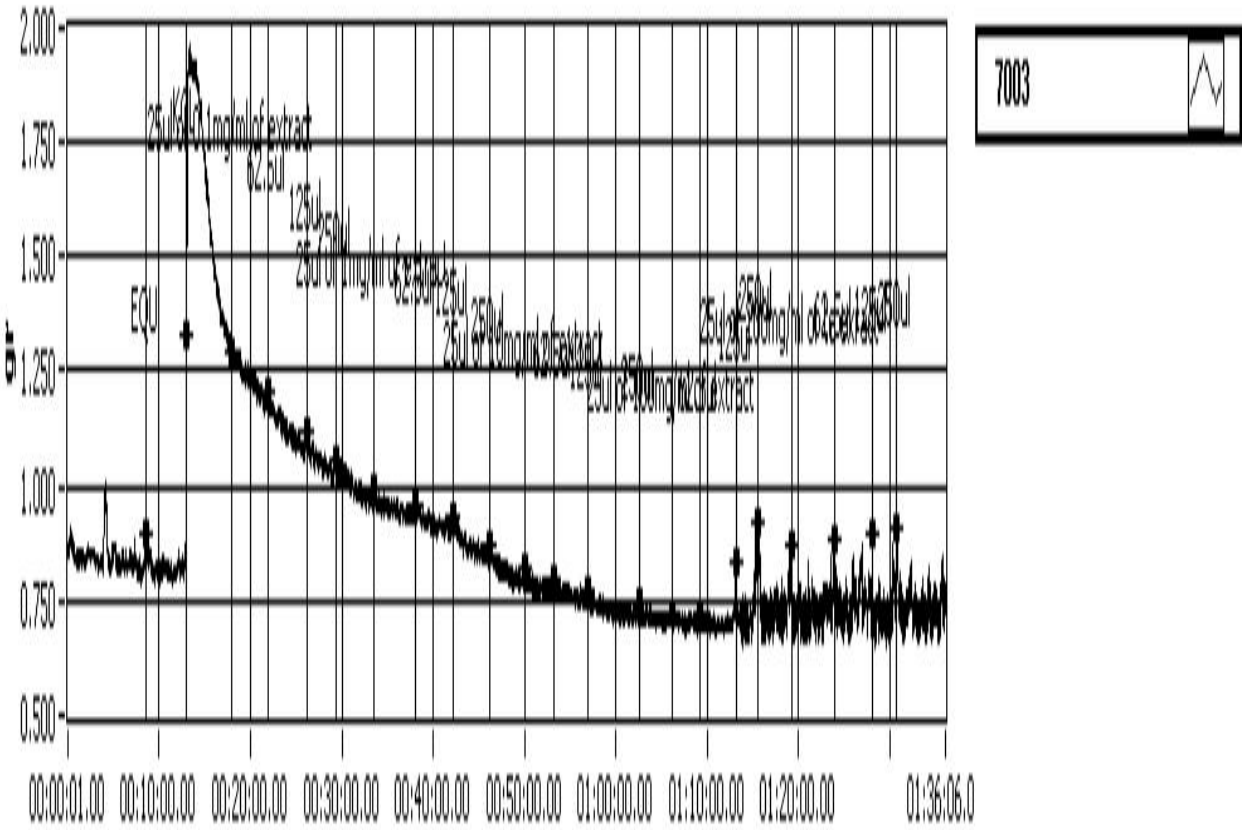

Fig. 2.6: A representative chart of the uterine response to different concentrations of *Phyllantus amarus* extract

b)

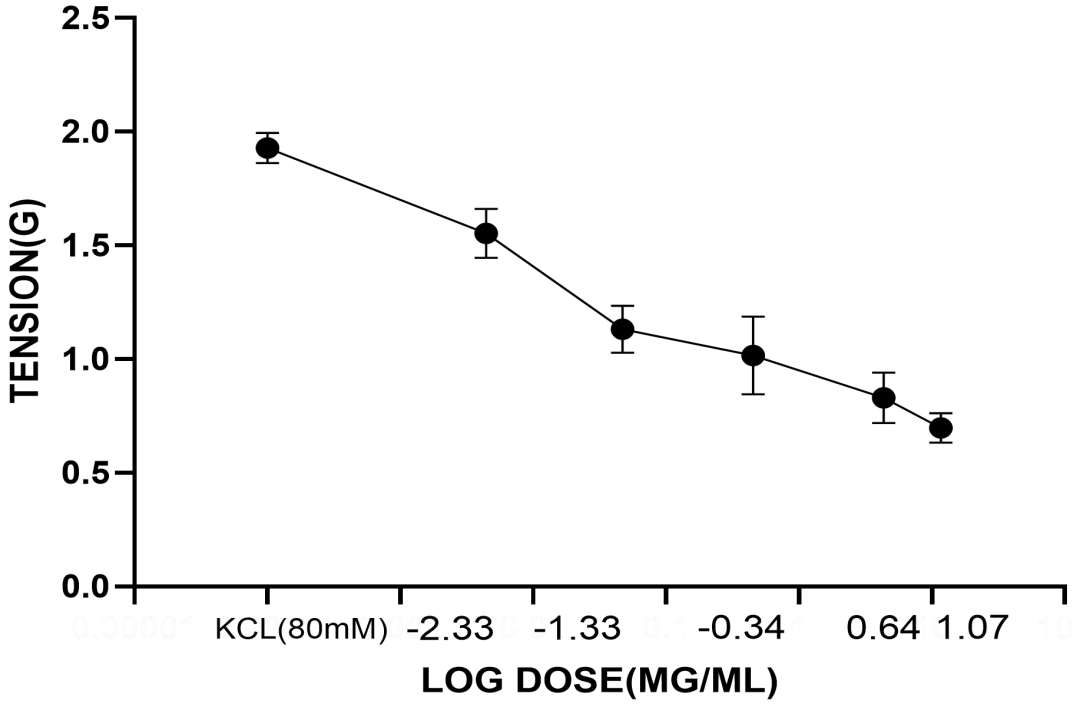

Fig. 2.7: A representative graph of the mean values of uterine response to the mean values of the different concentrations of *Phyllantus amarus* extract

Where n = 6

CHAPTER FOUR

DISCUSSION

Uterine activity is carefully controlled by a complex interaction of hormones, brain inputs, and local signalling molecules. The structural and functional adaptability of the uterus under hormonal influence ensures the success of reproduction (Marieb and Hoehn, 2019).

The cyclical changes in the uterine endometrium are largely driven by estrogen and progesterone, controlled by the hypothalamic-pituitary-ovarian (HPO) axis. (Thiyagarajan *et al.,* 2024). During the follicular phase, oestrogen increases the endometrial lining's growth and vascularization, whilst progesterone induces glandular production and preserves endometrial receptivity. (Guyton & Hall, 2021). In the absence of fertilization, progesterone withdrawal leads to endometrial shedding menstruation or estrous sloughing, depending on the species.

Myometrial contractility is another key physiological process influenced by calcium ion influx through voltage-gated channels and receptor-operated pathways. Oxytocin, one of the most potent uterotonic agents, activates phospholipase C and increases intracellular calcium concentration, leading to powerful rhythmic contractions necessary for both menstruation and labor (Arrowsmith and Wray, 2014). Prostaglandins, especially $PGF_2\alpha$, also enhance uterine motility and contribute to luteolysis (Stocco *et al*., 2007).

Overall, the uterus operates as a hormonally responsive and dynamically adaptable organ. Its cyclical transformation, muscular activity, and biochemical environment collectively ensure reproductive competence. The coordination of hormonal and cellular events underscores the uterus’s central role in fertility, implantation, and successful gestation.

4.1 EFFECTS OF PLANT EXTRACT

4.1.1 Effect of *Phyllanthus amarus* extract on conception

The result from the experiment showed that *Phyllanthus amarus* extract influenced conception outcomes in female Wistar rats. Conception occurred in the control, 12.5 mg/kg, and 50 mg/kg groups, while no litters were recorded in the 6.25 mg/kg and 25 mg/kg groups. This indicates that the extract supported reproductive performance at specific doses, the median and the highest doses used in the experiment.

Previous studies that investigated the effects of high doses of *Phyllanthus amarus* on the morphology and reproductive function, particularly at very high doses around 250mg/kg and for prolonged periods, were largely aimed at assessing its potential toxicological or antifertility profile. (Ataman and Sakpa, 2017). In contrast, the present study employed much lower doses (6.25–50 mg/kg) administered for 14 days and observed conception at specific dose ranges. This difference suggests that the reproductive effects of *P. amarus* may be dose- and duration-dependent, with lower doses potentially supporting conception while higher or extended treatments may inhibit fertility.

The mean litter weights of the treated groups (12.5 and 50mg/kg) were slightly lower compared to the control; however the difference was not statistically significant at a significant level of $p \leq 0.05$.

Careful adjustment of a mean dose of 12.5mg/kg and 50mg/kg of the extract may be beneficial in female reproductive health and hence modulate conception and pregnancy oucomes positively. Overall, these results suggest that *Phyllanthus amarus* extract exerts a positive effect on reproduction and hence supports conception outcomes depending on the dose and timing of exposure.

4.1.2 Effect of *Phyllanthus amarus* extract on Spontaneous Contraction Of An Isolated Non Gravid Rat Uterus

The ethanol extract of *Phyllanthus amarus* leaves has been studied for its potential to modulate uterine myometrial contractions. From the results gotten in the first protocol, the extract of *P. amarus* leaves was observed to relax the normal spontaneous contractions of the uterus in a dose dependent manner.

The extract exhibited a cumulative dose-dependent relaxation effect on spontaneous uterine contractions, as evidenced by a gradual decline in contraction frequency with increasing doses. A significant reduction in contractile amplitude was observed at 250 µL of 200 mg/mL concentration, indicating a strong relaxant effect. The relaxation mechanism is likely attributed to the inhibition of voltage-gated calcium channels, which restricts calcium ion (Ca^{2+}) influx into myometrial cells.

This blocks Ca^{+} binding to calmodulin, which inhibits the activation of myosin light chain kinase (MLCK). Because MLCK phosphorylation of the myosin light chain is required for actin-myosin cross-bridge synthesis, its blockage causes muscle relaxation rather than contraction. (Wray *et al*., 2021; Burdyga *et al*., 2022; Grajales *et al.,* 2005). Some studies suggest that the lignans, hypophyllanthin and phyllantin, found in *Phyllanthus amarus* may have smooth muscle relaxant effects, which could contribute to reducing uterine contractions (Olajide *et al.,*2000).

4.1.3 Effect of *P . amarus* extract on Oxytocin Induced Contraction On An Isolated Non Gravid Rat Uterus

Oxytocin (OT) has a stronger effect on the pregnant uterus than on the non-gravid uterus, but its influence remains observable. It regulates uterine contractions, the menstrual cycle, and

sensory perception, primarily facilitating labor contractions and childbirth (Wray *et al.*, 2014). In this protocol, oxytocin was administered cumulatively in a dose-dependent manner to induce uterine contractions. There was a progressive increase in both the amplitude and frequency of uterine contractions which was observed with increasing concentrations of oxytocin, confirming its stimulatory action on uterine smooth muscle.

The plant extract was found to relax oxytocin induced uterine contraction significantly upon cumulative dose administration. Oxytocin is a nonapeptide hormone that acts by increasing intracellular calcium level by facilitating the activation of voltage gated calcium ion channels during the process of excitation (S. Wray *et al,* 2014). Oxytocin induces contractions by increasing intracellular calcium (Ca^{2+}) levels, activating voltage-gated calcium channels, and inhibiting Ca^{2+} efflux via Ca^{2+}/ATPase (Reiner *et al.*, 1986). It also stimulates IP_3-mediated Ca^{2+} release from the sarcoplasmic reticulum, contributing to muscle contraction (Mazukka *et al.*, 2011). The observed relaxation suggests that *P. amarus* may directly or indirectly inhibit oxytocin receptors (OTR), block Ca^{2+} influx, and prevent voltage-gated calcium channel activation. Since relaxation increased with higher doses, the extract appears to act in a dose-dependent manner.

4.1.4 Effect of *Phyllanthus amarus* extract on KCl Induced Contraction

From the result of this protocol, the plant extract was observed to relax the potassium chloride (KCL) induced uterine contraction significantly upon cumulative dose administration. The exposure to high K+ in the intracellular induced a more sustained contraction compared to the normal resting spontaneous contractions.

The introduction of potassium chloride (KCl) increases the concentration of extracellular K^+ ions, leading to depolarization of uterine cell membranes. This depolarization causes the

opening of voltage-gated calcium channels, hence allowing an influx of extracellular Ca^{2+} into the cytoplasm (Perez and Sanderson, 2005).

4.2. LIMITATIONS OF THE STUDY

- This study was restricted by a small sample size and the absence of hormonal assays, which restricted understanding of the endocrine influence of *Phyllanthus amarus* in the in-vivo model.
- Dose response and toxicity analyses were not established, and the short experimental duration limited assessment of long-term effects on conception.
- Concentration differences, as the concentrations of *Phyllanthus amarus* used in vitro may not match those in a living organism due to differences in absorption and distribution.
- In vitro environments lack the interaction between different cell types (e.g., smooth muscle, endothelial cells) present in vivo, potentially affecting outcomes.

CHAPTER FIVE

CONCLUSION, CONTRIBUTION AND RECOMMENDATION

5.1 CONCLUSION

From the results of the various experiments, it can be concluded that the extract has relaxant effect on the isolated uterus and has positive outcomes on the pregnant female wistar rats.

5.2 CONTRIBUTIONS TO KNOWLEDGE

This study has contributed to knowledge through

- It has reported the uterine smooth muscle relaxant effect of the *Phyllantus amarus* extract.
- It also reported the positive outcome on pregnancy of the extract.

5.3 RECOMMENDATION

Further studies should be carried out to isolate the active constituents of *Phyllanthus amarus* and determine their hormonal and biochemical mechanisms on uterine function and conception. Long-term safety and histological studies are also recommended to assess possible reproductive effects, while clinical research is needed to confirm its potential use in managing female reproductive disorders.

REFERENCES

Ajayi, A.F. & Akhigbe, R.E., 2020. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertility Research and Practice*, 6(1), p.5.

Ajayi, G.O., Olorunrinu, T.J. & Shittu, M.A., 2020. Elucidation of bioactive compounds in hydroalcohol extract of *Phyllanthus amarus* Schum. and Thonn. leaf using GC-MS analysis. *Journal of Scientific and Innovative Research*, 9, pp.40–47.

Ameer, M.A., Fagan, S.E., Sosa-Stanley, J.N. & Peterson, D.C., 2022. Anatomy, abdomen and pelvis: uterus. *StatPearls Publishing*.

Ameen, O.A., Hamid, A.A., Yusuf, Q., Njoku, O.G., Oseni, T.O. & Jamiu, W., 2020. Quantitative and qualitative assessment of phytochemicals in methanolic extracts of hurricane weed (*Phyllanthus amarus* Schumach. & Thonn) Plant. *Journal of Applied Sciences and Environmental Management*.

Amaechina, F. & Omogbai, E.K.I., 2007. Hypotensive effect of aqueous extract of the leaves of *Phyllanthus amarus* Schum. and Thonn (Euphorbiaceae). *Nigerian Journal of Pharmacy Acta Pol*, 64(6), pp.547–552.

Amaechina, F.C. & Omogbai, E.K.I., 2013. Some central nervous system effects of the aqueous extract of the leaves of *Phyllanthus amarus* Schum, (Euphorbiaceae). *Nigerian Journal of Natural Products and Medicine*, 17, pp.61–65.

Aplin, J.D. & Ruane, P.T., 2017. Embryo–epithelium interactions during implantation at a glance. *Journal of Cell Science*, 130(1), pp.15–22.

Andina, I., 2023. An overview of the female reproductive system: A narrative literature review. *Sriwijaya Journal of Obstetrics and Gynecology*, 1(2), pp.73–80.

Arrowsmith, S. & Wray, S., 2014. Oxytocin: its mechanism of action and receptor signalling in the myometrium. *Journal of Neuroendocrinology*, 26(6), pp.356–369.

Ataman, J.E. & Sakpa, C.L., 2017. Effects of ethanolic leaf extract of *Phyllanthus amarus* on ovarian morphology and reproductive parameters in Wistar rats. *Nigerian Journal of Physiological Sciences*, 32(2), pp.171–176.

Bose Mazumdar Ghosh, A., Banerjee, A. & Chattopadhyay, S., 2022. An insight into the potent medicinal plant *Phyllanthus amarus* Schum. and Thonn. *The Nucleus*, 65(3), pp.437–472.

Casteel, C.O., 2023. Physiology, Gonadotropin-Releasing Hormone. *StatPearls Publishing*.

Coan, P.M., Angiolini, E., Sandovici, I., Burton, G.J., Constância, M. & Fowden, A.L., 2008. Adaptations in placental nutrient transfer capacity to meet fetal growth demands depend on placental size in mice. *The Journal of Physiology*, 586(Pt 18), pp.4567–4576.

Cortes, D.R.E. et al., 2024. Modeling normal mouse uterine contraction and placental perfusion with non-invasive longitudinal dynamic contrast enhancement MRI. *PLoS One*, 19(7), e0303957.

Drake, R.L., Vogl, A.W., Mitchell, A.W., Tibbitts, R. & Richardson, P., 2014. *Gray's Atlas of Anatomy* (E-book). Elsevier Health Sciences.

Gibson, E. & Mahdy, H., 2019. Anatomy, abdomen and pelvis, ovary. *StatPearls Publishing*.

Gold, J.M. & Shrimanker, I., 2023. Physiology, vaginal. *StatPearls Publishing*.

Hall, J.E. & Hall, M.E., 2020. *Guyton and Hall Textbook of Medical Physiology*. 13th ed. Elsevier Health Sciences.

Han, J. & Sadiq, N.M., 2023. Anatomy, abdomen and pelvis: Fallopian tube. *StatPearls Publishing*.

Hou, X., Cheng, Z. & Wang, J., 2020. Preparative purification of corilagin from *Phyllanthus* by combining ionic liquid extraction, prep-HPLC, and precipitation. *Analytical Methods*.

Ismail, C.A.M., Deris, Z.Z., Bakar, R.A. & Ismail, N., 2021. In vitro anti-leptospiral activity of *Phyllanthus amarus* extracts and their combinations with antibiotics. *International Journal of Environmental Research and Public Health*, 18(6), p.2834.

Iwanaga, R. et al., 2016. Comparative histology of mouse, rat, and human pelvic ligaments. *International Urogynecology Journal*.

Joshi, B.S. et al., 1986. Isolation and structure (X-ray analysis) of ent-norsecurinine, an alkaloid from *Phyllanthus niruri*. *Journal of Natural Products*.

Joshi, H. & Parle, M., 2007. Pharmacological evidences for antiamnesic potentials of *Phyllanthus amarus*.

Kiemer, K.A., Hartung, T., Huber, C. & Vollmar, A.M., 2003. *Phyllanthus amarus* has anti-inflammatory potential by inhibition of iNOS, COX-2, and cytokines via the NF-κB pathway. *Journal of Hepatology*, 38(3), pp.289–297.

Longo, M., Liuzzi, F., De Carlini, S. & La Marca, A., 2025. The role of LH in follicle development: from physiology to new clinical implications. *Reproductive Biology and Endocrinology*, 23(Suppl. 1), Article 22.

Manikkoth, S., Deepa, B., Joy, A.E. & Rao, S.N., 2011. Anticonvulsant activity of *Phyllanthus amarus* in experimental animal models.

Marieb, E.N. & Hoehn, K., 2019. *Human Anatomy & Physiology*. 11th ed. Pearson Education.

Mascarenhas, M.N., Flaxman, S.R., Boerma, T., Vanderpoel, S. & Stevens, G.A., 2012. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Medicine*, 9(12), e1001356.

Mazukka, R. et al., 2011. Oxytocin-mediated calcium responses in neurons and myometrial cells. *Neuroscience Letters*, 498(2), pp.102–108.

Mazumdar, U.K., Islam, A., Selvan, T., Gupta, M. & Ghosal, S., 2008. Antitumour effect of phyllanthin and hypophyllanthin from *Phyllanthus amarus* against Ehrlich ascites carcinoma in mice. *Pharmacologyonline*, 2(2), pp.796–807.

Moshi, J.M., Lutale, J.J.K., Rimoy, G.H., Abbas, Z.G., Josiah, R.M. & Swai, A.B.M., 2001. The effect of *Phyllanthus amarus* aqueous extract on blood glucose in non-insulin dependent diabetic patients. *Phytotherapy Research*, 15(7), pp.577–580.

Murtijaya, J. & Lim, Y.Y., 2007. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *LWT–Food Science and Technology*, 40(9), pp.1664–1669.

Nguyen, J.D. & Duong, H., 2023. Anatomy, abdomen and pelvis: female external genitalia. *StatPearls Publishing*.

Nowak, R.A., 2018. Estrous and menstrual cycles. In: *Encyclopedia of Reproduction*. 2nd ed. Vols. 1–6. Elsevier, pp.114–120.

Olajide OA, Awe SO, Makinde JM. *Effects of Phyllanthus amarus extracts on smooth muscle activity.* Phytotherapy Research. 2000;14(5):356–360.

Oluwafemi, F. & Debiri, F., 2008. Antimicrobial effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi*. *African Journal of Biomedical Research*, 11(2).

Orlowski, M., 2023. Physiology, Follicle Stimulating Hormone. *StatPearls Publishing*.

Oshomoh, E.O. & Uzama-Avenbuan, O., 2020. Quantitative phytochemical composition and bioactive constituents of ethanolic extract of *Phyllanthus amarus* (Schum. et Thonn) leaves. *European Journal of Engineering Research and Science (EJERS)*.

Patel, J. et al., 2021. Comparative profiling of four lignans (phyllanthin, hypophyllanthin, nirtetralin, and niranthin) in nine *Phyllanthus* species from India using a validated reversed phase HPLC-PDA method. *Journal of AOAC International*, 104(3), pp.833–842.

Perez, J.F. & Sanderson, M.J., 2005. The contraction of smooth muscle cells of intrapulmonary arterioles is determined by the frequency of Ca^{2+} oscillations induced by 5-HT and KCl. *Journal of General Physiology*, 125(6), pp.555–567.

Reed, B.G. & Carr, B.R., 2018. The normal menstrual cycle and the control of ovulation. *Endocrine Reviews*.

Reiner, A. et al., 1986. Oxytocin signaling and calcium regulation in myometrium. *Endocrinology*, 119(3), pp.789–795.

Ribeiro, A.M.B. et al., 2019. Antimicrobial activity of *Phyllanthus amarus* and inhibition of the NorA efflux pump of *Staphylococcus aureus* by phyllanthin. *Microbial Pathogenesis*.

Saranraj, P. & Sivasakthivelan, P., 2012. Screening of antibacterial activity of the medicinal plant *Phyllanthus amarus* against urinary tract infection-causing bacterial pathogens. *Applied Journal of Hygiene*, 1(3), pp.19–24.

Sato, J., Nasu, M. & Tsuchitani, M., 2016. Comparative histopathology of the estrous or menstrual cycle in laboratory animals. *Journal of Toxicologic Pathology*, 29(3), pp.155–162.

Sharma, A., Singh, R.T. & Anand, S., 1993. Estimation of phyllanthin and hypophyllanthin by high performance liquid chromatography in *Phyllanthus amarus*. *Phytochemical Analysis*, 4, pp.226–229.

Singh, B., Makkar, H.P.S. & Negi, S.S., 1989. Rate and extent of digestion and potentially digestible dry matter and cell wall of various tree leaves. *Journal of Dairy Science*, 72(12), pp.3233–3239.

Stocco, C., Telleria, C. & Gibori, G., 2007. The molecular control of corpus luteum formation, function, and regression. *Endocrine Reviews*, 28(1), pp.117–149.

Thiyagarajan, D.K., Basit, H. & Jeanmonod, R., 2024. Physiology, menstrual cycle. *StatPearls Publishing*.

Ueno, H., Horie, S., Nishi, Y., Kawasaki, M. & Suzuki, S., 1988. Chemical and pharmaceutical studies on medicinal plants in Paraguay, Geraniin, an angiotensin-converting enzyme inhibitor from "Paraparai Mi" (*Phyllanthus niruri*). *Journal of Natural Products*, 51, pp.1.

World Health Organization, 2022. *Critical considerations and actions for achieving universal access to sexual and reproductive health in the context of universal health coverage through a primary health care approach*. World Health Organization.

Wray, S. et al., 2014. Calcium signaling and uterine contractility. *Journal of the Society for Reproduction and Fertility*, 27(1), pp.183–195.

Yousaf, O., Grunfeld, E.A. & Hunter, M.S., 2015. A systematic review of the factors associated with delays in medical and psychological help-seeking among men. *Health Psychology Review*, 9(2), pp.264–276.