EX VIVO STUDY ON THE TOCOLYTIC EFFECT OF *MORMODICA CHARANTIA* ON THE CONTRACTILITY OF ISOLATED MOUSE UTERUS.

BY

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CERTIFICATION

This is to certify that this research work titled "*Ex Vivo* study on the tocolytic effect of *Mormodica charantia* on the contractility of mouse isolated uterus." was carried out by Miss Annabella Chinyere Chukwuemeka, with the matriculation number PHA1910241 in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin City, Nigeria.

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DEDICATION

This work is dedicated to God Almighty, whose boundless grace, wisdom, and guidance have Helped me in every step of this journey. I also dedicate it to my late parents, late Mr and Mrs Emeka and Chinyere Sonwu, my sister Mirabella, my friend Femi and all my lecturers and newly found mummies and daddies whose unwavering support and love have been a constant source of strength and inspiration throughout my journey.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
ACOG	American College of Obstetrics and Gynecology
Ca2+	Calcium Ion
CAM	Complementary/alternative medicine
CaM	Calmodulin
cAMP	Cyclic AMP (Cyclic Adenosine Monophosphate)
cGMP	Cyclic GMP (Cyclic Guanosine Monophosphate)
COX	Cyclooxygenases
CYP	Cytochrome P450
DAG	Diacylglycerol
E2	Estradiol
ECC	Excitation-Contraction Coupling
FDA	United States Food & Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
FSH	Follicle-Stimulating Hormone
GBS	Group B Streptococcus
hCG	Human Chorionic Gonadotropin
IP3	Inositol-1, 4,5-triphosphate (or Inositol Triphosphate
KCl	Potassium Chloride
LH	Luteinizing Hormone
MCE	Momordica charantia Extract
MCP	Myosin Light Chain Phosphatase
Mg-ATP	Magnesium-Adenosine Triphosphate

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MLCK	Myosin Light Chain Kinase
MLCo	Myosin's Regulatory Light Chain
Na+Ca2+exchanger	Sodium Calcium Exchanger
NaCl	Sodium Chloride
NICE	National Institute for Health and Care Excellence
NSAIDs	Nonsteroidal Anti-inflammatory drugs
OT	Oxytocin
PBB	Plant Biology and Biotechnology
PMCA	Plasma Membrane Ca++-ATPase
PSS	Physiological Saline Solution
SAN	Senior Advocate of Nigeria
SERCA	Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase
SR	Sarcoplasmic Reticulum
TW-80	Polysorbate 80
UPS	Uninterruptible Power Supply
VOCCs	Voltage-Operated Calcium Channels (or Voltage-Gated Calcium Channels)
WHO	World Health Organization

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ABSTRACT

The traditional use of Momordica charantia (bitter melon) includes the treatment of various ailments, notably diabetes, and its application as an abortifacient and contraceptive. Reports that its seeds can induce uterine contractions raise concerns regarding its safety during pregnancy. This study was therefore conducted to investigate the effect of the M. charantia leaf extract on the isolated uterus of non-pregnant mice. Hydro-alcoholic extract was obtained from extracting the powdered leaf material with Hydro-ethanol (1:1) solvent using a Soxhlet apparatus. Twenty-five non-pregnant albino mice were used, and those in the estrus phase (identified by vaginal smears) were sacrificed by cervical dislocation. Uterine strips were isolated, cleaned, mounted in a 10 ml organ bath containing aerated physiological saline solution maintained at 37∘C, and subjected to a 40-minute equilibration period with 0.5 g resting tension. Changes in isometric contractions were recorded using LabChart Software. The *M. charantia* leaf extract (0.00625 – 0.4 mg/mL) was added cumulatively to assess its effects on spontaneous, oxytocin-induced (14 nM), and high potassium-induced contractions (80 mM) as well as oxytocin-induced contractions in a calcium-free medium. Data were analyzed using one-way ANOVA with Dunnett's post hoc test ($p<0.05$). The leaf extract of M. charantia inhibited spontaneous uterine contractions in a concentration-dependent manner, causing a significant decrease in the force of contraction(amplitude) but not the frequency. The extract also significantly and concentration-dependently decreased the force of contractions induced by both oxytocin and high potassium, again with no observable changes in contraction frequency. However, the extract did not significantly alter oxytocin-induced contractions when tested in a calcium-free medium. In conclusion the inhibitory effect of M. charantia leaf extract on both spontaneous and induced uterine contractions suggests a calcium-dependent mechanism. This mechanism likely involves the blockade of calcium influx from the extracellular compartment rather than the inhibition of intracellular calcium release, offering insight into the plant's traditional use and potential pharmacological targets.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1The female reproductive system

The female reproductive system is a highly organized set of organs that does more than just reproduction. It also controls the complex hormone levels that manage a woman's fertility and overall physical health throughout her life (Tortora and Derrickson, 2017*)*. This system includes parts both inside and outside the body. The main internal structures inside the pelvis are the ovaries, Fallopian tubes, uterus, and vagina.

The ovaries are two small organs that serve as the main reproductive glands. They have two key jobs: to create the female sex cells (eggs or oocytes) in a process called oogenesis and to act like hormone factories, making crucial sex hormones like estrogen and progesterone (Marieb & Hoehn, 2019). These hormones control monthly cycles and female body changes.

The Fallopian Tubes are narrow passages that act as a road for the egg to travel from the ovary to the uterus. Tiny hairs (cilia) inside the tubes help push the egg along (Tortora & Derrickson, 2017), and fertilization (where the sperm meets the egg) usually happens here.

The Vagina is a muscular tube connecting the cervix (bottom of the uterus) to the outside. It serves as the channel for sex, the exit point for the monthly period, and the birth canal (Thibodeau and Patton, 2019).

Amongst these organs, the uterus (womb) occupies a central role. It is a strong, muscular, pear-shaped organ whose main purpose is to shelter, protect, and feed a developing baby during the

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nine months of pregnancy (Marieb and Hoehn, 2019). Aside from serving as the site of implantation of a fertilized egg, the uterus also aids in the development of the embryo and fetus. It is also the site of menstruation and is made up of smooth muscles capable of finely regulated contractions which aid processes such as sperm transport and childbirth (parturition) (Gasner *et al.*,2023).

The middle muscular layer of the uterus, known as the myometrium, is responsible for these rhythmic contractions. Dysfunctional uterine activity may be implicated in various reproductive health problems such as severe period pain (dysmenorrhea), post-partum hemorrhage, endometriosis, spontaneous miscarriage, or preterm birth (Aguilar and Mitchell, 2010). Understanding precise mechanisms allows for the development of targeted pharmaceutical interventions, such as tocolytics (to inhibit contractions and prevent preterm labor) and uterotonics (to stimulate contractions to induce labor or control PPH).

1.2 The Physiology and Electrophysiology of Uterine Contraction and Relaxation

The contractility of the uterine wall, or the myometrium, is a pivotal physiological process necessary for reproductive functions including menstruation, gestation, and, critically, for childbirth, or parturition (Caldeyro-Barcia *et al*., 1960). The shift in the myometrium from a relaxed, quiescent state during pregnancy to a highly active, contractile state during labor is regulated by a complex interplay of hormonal, electrical, and ionic mechanisms (Wray *et al.,* 2003).Uterine contraction and relaxation are fundamentally controlled by the concentration of intracellular calcium ions (Ca2+) within the myometrial smooth muscle cells, a process known as excitation-contraction coupling (Word *et al*., 2007).Contraction begins when myometrial cells exhibit spontaneous electrical activity, generating intermittent bursts of spike action potentials

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that initiate the process (Luo *et al*., 2017). This electrical excitation leads to membrane depolarization, which opens voltage-gated L-type Ca2+ channels, allowing extracellular calcium to flow into the myocyte cytoplasm (Wray *et al*., 2003). This initial influx of calcium further triggers the release of more calcium from the internal storage compartment, the sarcoplasmic reticulum (Shmygol *et al*., 2007). The resulting elevated intracellular calcium level then binds to calmodulin, and this complex activates myosin light chain kinase (MLCK). MLCK then phosphorylates the myosin regulatory light chain, which initiates the interaction between the actin and myosin filaments, leading to muscle shortening and subsequent contraction (Roh and England 2021). Conversely, relaxation requires a sharp decrease in the intracellular calcium concentration (Shmygol *et al*., 2007). Calcium is actively removed from the cytoplasm by being pumped back into the sarcoplasmic reticulum and by being extruded from the cell by the sodium-calcium exchanger and the plasma membrane calcium-ATPase. The drop-in calcium inactivates the MLCK, allowing the enzyme myosin light chain phosphatase (MLCP) to dephosphorylate the myosin light chain (MacDonald & Word, 2002). The dephosphorylated myosin detaches from actin, resulting in muscle relaxation.

1.3 Pharmacological Modulation of Uterine Contractility

The clinical management of abnormal uterine contractility relies on drugs that either stimulate or inhibit myometrial activity, broadly categorized as uterotonics or tocolytics, respectively, representing a crucial pharmacological interface with the fundamental physiological mechanisms of smooth muscle (Wray *et al*., 2003).

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Uterotonics are agents used to initiate or augment labor, or more commonly, to control severe bleeding after childbirth, known as postpartum hemorrhage (Mousa *et al.*,2014). Common uterotonic agents include:

A. Oxytocin

Oxytocin is widely regarded as the first-line agent for the prevention and treatment of postpartum hemorrhage and for labor induction (WHO, 2019). This synthetic nonapeptide increases the excitability and strength of myometrial contraction (Rao *et al*., 1986). It achieves this effect by binding to specific G-protein coupled receptors on the myometrium, which triggers an intracellular signaling cascade that ultimately results in a rise in the concentration of cytoplasmic calcium within the uterine myofibrils (Taylor and Francis, 2025). This increase in intracellular calcium is the final common pathway for enhancing the frequency, intensity, and coordination of uterine contractions. Furthermore, oxytocin can stimulate the local production of prostaglandins, which contributes to the maintenance of efficient uterine contractions (Rao *et al*., 1986).

B. Ergot Alkaloids

Ergot alkaloids specifically methylergonovine (Methergine), are potent uterotonics often reserved as second-line agents due to a greater risk of adverse effects like hypertension (WHO, 2019). These semisynthetic derivatives cause a rapid and powerful uterine response. Their activity is accomplished through direct action on the uterine smooth muscle, where they interact as agonists at certain adrenergic, dopaminergic, and serotonergic receptors, which strongly

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increases the rate and amplitude of rhythmic contractions. At therapeutic doses, this results in a sustained, continuous, or tetanic uterotonic effect that is effective for controlling hemorrhage.

C. Prostaglandins

This class of drugs represent another critical class of uterotonics, including agents like misoprostol (PGE1 analogue), dinoprostone (PGE2), and carboprost (PGF2α analogue) (Taylor & Francis, 2025). These compounds enhance uterine contractility by directly engaging prostaglandin receptors on the myometrial cells (Sahmay *et al*., 1988). Misoprostol is particularly valuable in resource-limited settings due to its stability and ease of administration (WHO, 2019). The stimulation of these receptors' initiates signaling cascades that lead to increased calcium mobilization, thereby augmenting the frequency and force of contractions. Furthermore, dinoprostone also promotes changes in the cervical collagen structure, facilitating cervical ripening, which is an important prerequisite for labor induction. Carboprost is an injectable prostaglandin primarily utilized for treating severe postpartum hemorrhage that is refractory to first-line agents (Monga, 2021).

D. Carbetocin

This is a newer, long-acting synthetic analogue of oxytocin, possessing agonist properties at the oxytocin receptors (WHO, 2019). Its benefit lies in its extended duration of action compared to natural oxytocin, producing sustained uterine contractions within minutes of intravenous injection that can last for over an hour (WHO, 2019). The compound achieves its effect by selectively binding to the oxytocin receptors on the smooth uterine muscle, leading to the same downstream signaling of increased intracellular calcium as oxytocin, but with a significantly prolonged effect.

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Conversely, tocolytics (also referred to as anti-contraction medications or labor suppressants) are pharmaceuticals utilized to inhibit premature labor (Wray *et al*., 2003). These therapeutic agents are administered with the expectation of mitigating fetal morbidity and mortality. Tocolysis is designed to prolong gestation for a duration of two to seven days and functions by creating a serene environment within the uterus. This is crucial to facilitate transfer to a superior medical care establishment, to administer a regimen for fetal pulmonary maturation with prenatal corticosteroids, and the additional interval is also leveraged to ascertain the Group B streptococcus (GBS) status of the expectant mother and provide prophylactic treatment if she is either positive or the GBS culture status is indeterminate.

Tocolysis is not intended to extend the fetus's gestation to full term but is focused on furnishing a window of opportunity to support therapeutic interventions that have been demonstrated to enhance outcomes for delivery.

Tocolytic therapy is advantageous for pregnant women with a likelihood of premature labor or miscarriage.

Tocolysis is efficacious because it concentrates on both deferring and attenuating uterine contractions. The pharmacology targets the activity of the myometrium. The myometrium is a smooth muscle located in the uterus. The various pharmaceutical tocolytics currently in use are:

A. Beta-Adrenergic receptor agonists

This class of drugs specifically operate on the beta-2 receptor. Activation of the beta-2 receptors causes an augmentation in cyclic AMP (cAMP) which is associated with heightened smooth muscle relaxation. (Eric *et al* 2023). The most prevalent medication in this category is

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terbutaline. Hexoprenaline, a beta-2 receptor agonist, is used in numerous nations but has yet to be approved by the US Food and Drug Administration. Ritodrine is also another pharmaceutical in this class that is utilized internationally for tocolysis but is not approved in the United States. A black box warning has been issued by the US Food and Drug Administration against the use of injectable terbutaline in the context of protracted preterm labor management (over 72 hours) due to maternal cardiac complications. These complications are suggested from terbutaline's activity at beta-1 receptors, which are situated in cardiac muscle. Research has indicated that tocolytic agents in this family might pose a risk for the development of childhood asthma (Ogawa *et al.*, 2019). Maternal risks include but are not limited to cardiac arrhythmias, tachycardia, hypotension, nausea, and emesis. Fetal risks include but are not limited to tachycardia.

B. Calcium channel blockers

This drugs specifically act on T-type calcium channels by impeding the ingress of calcium into the uterine smooth muscle (Cretoiu *et al.*, 2015). The absence of free calcium directly impacts the capacity of the calcium-calmodulin activation of myosin light chain kinases. The most common pharmaceutical in this class is nifedipine. The two available routes for this medication are oral and sublingual. Recent literature has demonstrated that sublingual nifedipine achieved swifter tocolysis. Maternal risks include but are not limited to flushing, headache, lightheadedness, nausea, and hypotension (Leal-Júnior *et al*., 2015). Studies have shown no fetal risks with nifedipine usage for the management of preterm labor. Women who have documented hypotension and other cardiovascular ailments should employ nifedipine with extreme caution. Recent literature has posited that nifedipine is the superior tocolytic agent because of improved neonatal outcomes and fewer adverse effects (Hanley *et al.*, 2019).

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C. Magnesium sulfate

This drug has an unresolved mechanism of action concerning uterine contractions, but it has been described to inhibit the entry of calcium into the uterine smooth muscle (Renzo *et al*.,2017). It also possesses vasodilatory effects on uterine blood vessels. Magnesium sulfate is utilized for neuroprotection and studies have failed to demonstrate its efficacy in pregnancy prolongation in the setting of preterm labor. It is not recommended to use magnesium sulfate in tandem with calcium channel blockers, unless for neuroprotection, due to the hazard of maternal respiratory depression. Its sole route of administration is intravenous, and it is excreted by the kidneys. There are dangers of magnesium toxicity, and the adverse reactions include flushing, nausea, diminished deep tendon reflexes, blurred vision, and reduced cardiac contractility. Calcium gluconate and fluids are employed to manage the side effects of magnesium toxicity.

D. Nonsteroidal anti-inflammatory drugs (NSAIDS)

NSAIDS function by inhibiting cyclooxygenases (COX). These enzymes are responsible for the generation of prostaglandins from arachidonic acid (Vane 1971). The most prevalent medication in this class is a non-selective COX inhibitor, indomethacin. The method of administration for indomethacin is orally or rectally. This medication has renal consequences by causing vasoconstriction through the absence of prostaglandins being produced at the site of the afferent arteriole. Indomethacin also has gastrointestinal repercussions by augmenting the rate of ulcer formation. The lack of prostaglandin synthesis in the stomach increases the prevalence of mucosal injury and gastrointestinal hemorrhage. It is contraindicated in women who have a history of bleeding maladies, gastritis, aspirin hypersensitivity, and hepatic impairment. Indomethacin is also contraindicated after 32 weeks of gestational age because of premature

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closure of the ductus arteriosus. Other fetal effects from indomethacin include oligohydramnios, gastric perforation, and pulmonary hypertension.

E. Oxytocin inhibitors

These drugs **f**unction by competitively acting at the oxytocin receptor site. Oxytocin works to increase the intracellular levels of inositol triphosphate. The medications currently in this class are atosiban and retosiban. This group of tocolytics is currently not sanctioned in the United States but was approved in Europe in 200 (Saade *et al.,* 2021). The medication is delivered intravenously. Neither maternal nor fetal side effects have been documented for this tocolytic.

F. Nitroglycerine

In addition to the aforementioned tocolytic agents, nitroglycerine has also been evaluated for its effectiveness with preterm labor. Nitroglycerine operates by inducing myometrial guanylyl cyclase to generate cyclic GMP (cGMP). Increases in cGMP inhibit the capability of intracellular calcium levels to ascend. Furthermore, cGMP is also utilized to dephosphorylate myosin heads which arrests the capacity for contractions to proceed. The two routes for nitroglycerine are intravenous and transdermal. Although the side effect profile is superior when compared to beta-adrenergic receptor agonists, transdermal nitroglycerine is not advised for tocolysis (Conde *et al.* 2013). This contrasts with other literature which demonstrates that transdermal nitroglycerine is more effective at delaying birth compared with nifedipine.

When choosing a tocolytic, patient-specific attributes must be taken into consideration. In patients who necessitate magnesium sulfate for neuroprotection, it may be appropriate to employ this therapy at tocolytic dosages because it can serve a dual purpose. Indomethacin should be

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reserved for pregnancies that are less than 32 weeks' gestation to evade the risk of premature closure of the ductus arteriosus (Ohlsson *et al.,* 2020).

1.4. The Female Reproductive Cycle

The female reproductive cycle undergoes predictable, cyclical transformations, in contrast to the males. This regular process readies the body for ovulation and a potential pregnancy. The most evident part of this cycle is menstruation, or recurring vaginal bleeding, which unfolds alongside a series of coordinated hormonal shifts. Menstruation, known initially as menarche, typically commences around puberty with a median age of 12.4. (ACOG 2015). These cycles cease at menopause, which onsets, on average, at age 51.

For tracking purposes, the initial day of significant menstrual flow is considered day 1. According to the International Federation of Gynecology and Obstetrics (FIGO), normal menstrual cycles ought to possess consistent frequency, regularity, length, and amount of discharge. A normal menstrual frequency is defined as cycles occurring every 24 to 38 days. Cycles exceeding 38 days are termed infrequent, while those shorter than 24 days are considered frequent (Munro *et al.,* 2018). Amenorrhea denotes the complete absence of menstrual bleeding. Normal menstrual duration is characterized by bleeding lasting 8 days or less, whereas bleeding extending beyond that is classified as prolonged (Munro *et al.,*2018).

The volume of menstrual flow is categorized as light, normal, or heavy. Objective criteria to distinguish these classifications are often impractical in clinical settings. For research, heavy menstrual bleeding is defined as blood loss surpassing 80 mL per cycle, based on weighed menstrual products. The National Institute for Health and Care Excellence (NICE) defines it as excessive menstrual bleeding that disrupts a person's physical, social, emotional, and/or material

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quality of life. Conversely, light menstrual bleeding is rarely tied to an underlying medical issue, though it may occur in patients with uterine adhesions or cervical stenosis. In a research context, light menstrual bleeding is typically defined as less than 5 mL of blood loss per cycle.

Menstrual regularity is gauged by the variation in cycle lengths from one cycle to the next. Slight variations are normal. Cycles are deemed regular if the difference between the shortest and longest cycle lengths is 7 days or less for individuals aged 26 to 41, and 9 days or less for those aged 18 to 25 or 42 to 45. A cycle is considered irregular when its lengths vary by 8 or more days for individuals between 26 and 41, or by 10 or more days for those between 18 and 25 or 42 and 45. Intermenstrual bleeding is defined as any bleeding that occurs between regularly scheduled menstrual periods (Munro 2018).

1.4.1 The Ovarian and Endometrial Cycles

The menstrual cycle is comprised of two distinct cycles—one within the ovary and one within the endometrium. The ovarian cycle encompasses the follicular phase, ovulation, and the luteal phase. The endometrial cycle, on the other hand, consists of the proliferative phase, the secretory phase, and the menstrual phase. Generally, the ovarian follicular phase corresponds to the endometrial menstrual and proliferative phases, while the ovarian luteal phase corresponds to the endometrial secretory phase.

Phase 1: Follicular and Proliferative Phases

The initial stages of the menstrual cycle include the follicular and proliferative phases, which correlate with the maturation of ovarian follicles and the proliferation of the endometrium. The follicular phase, which varies in length, consistently begins on day 1 of the menstrual cycle and

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culminates in ovulation. Concurrently, the proliferative phase within the uterus starts after menstrual bleeding ends and continues until ovulation.

During the follicular phase, FSH stimulates a group of primordial follicles to develop into Graafian follicles and fosters the production of 17-β estradiol and inhibin B within the ovary. As 17-β estradiol and inhibin B provide negative feedback to lower FSH levels, the nondominant follicles begin to degenerate. The dominant follicle continues its maturation, and FSH prompts the development of LH receptors, preparing it for the next phase—ovulation (Pache *et al.,*1990*).*

The 17-β estradiol produced by the growing follicles stimulates the growth of the endometrial stroma and glands and increases the depth of the spiral arteries supplying the endometrium. By the conclusion of the proliferative phase, the endometrium reaches its maximum development, typically measuring between 8 and 12 mm. These changes ready the endometrium for a possible pregnancy after ovulation (Fleischer *et al.,* 1986).

Ovulation

Ovulation typically occurs 14 days before the start of menstruation. Throughout the follicular phase, estradiol levels climb, and at its conclusion, 17-β estradiol shifts from providing negative feedback to positive feedback at the anterior pituitary. This transition triggers a sudden surge of LH secretion. As a consequence of this hormonal milieu, the mature follicle releases plasminogen activator and other cytokines, prompting the rupture of the follicle and the expulsion of the oocyte. Ovulation generally happens approximately 36 to 44 hours after the onset of the LH surge (Messinis *et al.,* 2014).

Phase 2: Luteal and Secretory Phases

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The subsequent phase of the menstrual cycle includes the luteal and secretory phases. This stage begins with ovulation and ends when menstrual bleeding starts. The luteal or secretory phase is relatively consistent in length within an individual, typically lasting 14 days.

The key hormone during this phase is progesterone, which is stimulated by LH. Progesterone promotes the maturation of the endometrium in readiness for the potential implantation of a fertilized ovum. A fertilized ovum releases human chorionic gonadotropin (hCG), which stimulates the corpus luteum to maintain progesterone production. However, in the absence of a fertilized ovum, the natural rise in progesterone slows LH release through negative feedback, leading to a swift decrease in progesterone and estradiol levels at the phase's end.

Normal Menstruation

The abrupt decline in progesterone and estradiol levels at the end of the luteal phase compels the shedding of the endometrium, which can no longer be sustained without these hormones. This shedding is referred to as menses. The first day of menstrual bleeding is designated as day 1 of the menstrual cycle, meaning menstruation occurs during the initial days of the follicular phase. The duration of menses is variable, but normal menses lasts 8 days or less (Munro *et al.*, 2018). Menstrual fluid contains blood, endometrial cells, vaginal secretions, and various biochemical molecules (Yang *et al.*, 2012).

The menstrual cycle is a vital indicator of a female's reproductive health, and any irregularities demand a thorough evaluation. A deep understanding of menstrual physiology is crucial for clinicians, as it enables them to diagnose and manage various gynecological issues, including both hormonal and structural pathologies.

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1.4.2 Female Mice Reproductive Cycle

The human menstrual cycle lasts around 28 days, but in rodents, this cycle, known as the estrous cycle, is much shorter, lasting approximately 4-5 days. The mouse estrous cycle is divided into four stages:

1. **Proestrus**: Characterized by a high number of nucleated epithelial cells. This is the pre-ovulatory stage when estradiol (E2) levels rise (Walmer *et al.*, 1992), leading to a nighttime surge in LH and FSH that triggers ovulation (Parkening *et al*., 1982).
2. **Estrus**: Distinguished by cornified squamous epithelial cells that lack a nucleus. E2 remains high in the morning and then returns to basal levels in the afternoon (Walmer *et al.*, 1992).
3. **Metestrus**: A mix of cell types, primarily leukocytes, with some nucleated epithelial and cornified cells. During this stage, plasma E2 concentration is low (Walmer *et al*., 1992).
4. **Diestrus**: Consists mainly of leukocytes. E2 levels begin to increase during this stage (Walmer *et al.*,1992). Throughout estrus, metestrus, and diestrus, the circulating levels of LH and FSH are low.

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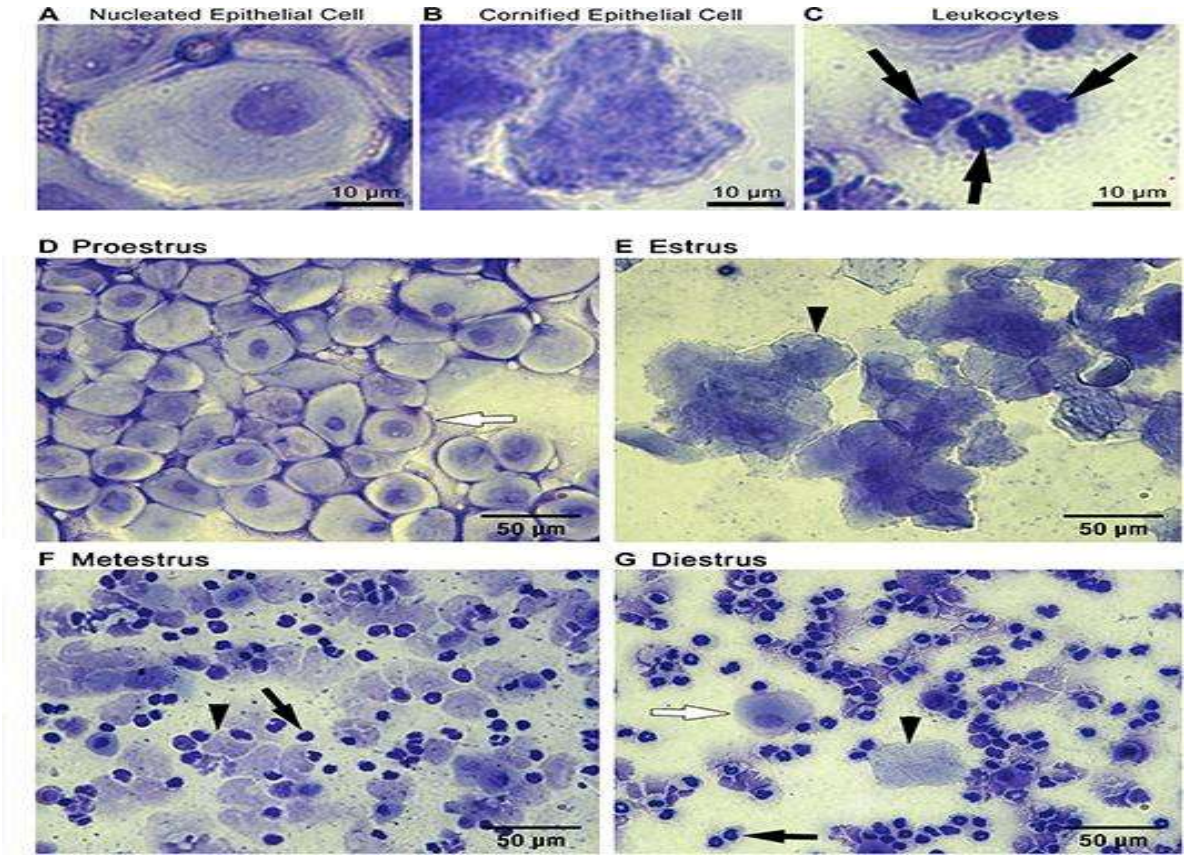

Figure 1.1 Diagrammatic representation of the female mice reproductive cycle

Journal of Experimental and Applied Animal Sciences.

1.5 Comparative Anatomy and Physiology of the Human and Mouse Uterus

The uterus is a fundamental organ in the female reproductive system, centrally involved in embryo implantation, nourishment, and ultimately, parturition. Structurally, both human and mouse uteri are built from the same three fundamental layers: the inner mucosal lining, which is called the endometrium or decidua; the muscular middle layer, the myometrium; and the outer protective serosal layer, the perimetrium. The myometrium is crucial for generating uterine contractions, and its function is tightly regulated by endocrine (hormonal), paracrine (local), and neurogenic (nerve-related) factors.

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Despite these basic structural similarities, important anatomical and physiological differences exist between the two species. For instance, the shape of the uterus differs significantly; mice possess a bicornuate (two-horned) uterus that accommodates multiple implantation sites within each horn, reflecting their poly-ovulatory reproductive process. In contrast, humans have a pyriform (lightbulb-shaped) uterus, which typically supports a single fetus per pregnancy. Furthermore, the muscular layers of the myometrium are organized differently: the mouse myometrium has clearly delineated inner circular and outer longitudinal layers, whereas in humans, these longitudinal and circular fibers are difficult to distinguish (Malik, and England, 2021).

At the cellular level, the mechanisms of contraction show remarkable conservation across species. Myocytes in both human and mouse uteri are capable of spontaneous depolarization, which generates action potential that trigger contractions via L-type calcium channels (Mali and England, 2021). Synchronizing these contractions across the entire myometrium is achieved through gap junctions, primarily composed of connexin-43, the expression of which increases during the period leading up to labor (peri-partum) in both species. Endocrine regulation also exhibits similarities: progesterone maintains uterine quiescence by inhibiting contractile-associated proteins, while estrogen prepares the uterus for labor by increasing the expression of oxytocin receptors and connexin in both models. While oxytocin and prostaglandins universally regulate contractility, their pharmacological sensitivity can differ; for instance, the mouse uterus often demonstrates greater responsiveness to oxytocin *in vitro* compared to the human uterus, possibly due to variations in receptor density or downstream signaling (Wang *et al*., 2022).

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These shared features and differences underscore the value of the mouse model for studying reproductive pharmacology. Mice offer an easily accessible and genetically tractable system that successfully replicates many aspects of human uterine physiology, particularly in hormone control and contractile process. Female mice are an excellent animal model for studying reproductive changes because of their short, regular cycles (4-5 days). They are also easy to handle, and their cycles are not easily disrupted by routine stress, making them ideal for research (Festing and Lovell 1981). However, when applying findings from mouse studies to humans, it remains crucial to account for species-specific differences, especially concerning the anatomical structure and overall morphology of the uterus and the process of pregnancy.

1.6. Medicinal Plants in Health and Gynecology and Obstetrics

1.6.1. Overview of Herbal Medicines in Health

Herbs, plants, and ethnobotanicals have been used for health promotion and disease treatment since ancient times and continue to be used globally. These natural sources form the foundation of modern medicine and are a significant part of commercial drug preparations today.

Herbal and traditional medicines are used to treat various chronic and acute conditions, such as cardiovascular disease, prostate problems, depression, inflammation, and to boost the immune system. According to the World Health Organization (WHO), traditional medicine is defined as "the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness."

Plants are rich in various compounds, many of which are secondary metabolites, including aromatic substances like phenols and tannins. Ethnobotanicals are crucial for pharmacological

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research and drug development, both as direct therapeutic agents and as starting materials or models for the synthesis of new drugs (Li and Vederas 2009).

Medicinal plants offer three main benefits: economic benefits to those who collect and sell them; health benefits for users; and societal benefits like employment and tax revenue (World Health Organization, 2005; Farnsworth *et al*, 1985). However, the advancement of plants with potential medical use is impeded by inadequate funding, substandard drug development procedures, and a lack of scientific proof (Rates, 2001; Gurib-Fakim, 2006).

Regulations for herbal medicines vary by country. Some countries regulate them as a stand-alone category, while others include them under a broader group of products like "natural health products." Some are classified as prescription or non-prescription medicines, or as various food categories. Regardless of the regulatory approach, the aim is to protect consumer health by ensuring herbal medicines are safe and of appropriate quality.

The international use of herbal and traditional medicines is substantial, though its prevalence varies by country due to differences in access, regulations, cultural aspects, historical influence, and the advancement of conventional healthcare systems. Use is generally higher in low-income countries where access to conventional healthcare is limited, and traditional medicine is prominent due to its cultural and historical importance.

The popularity of herbal medicine is driven by factors such as affordability and accessibility, as well as the consumer belief that these "natural products" are safe and free from adverse effects. This widespread use, given the current regulatory landscape, raises concerns about the sufficiency of public health protection from unsafe and poor-quality products.

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1.6.2. Medicinal Plants Used as Tocolytics

Tocolytic agents are medicines that inhibit uterine contractions and are used to prevent premature labor. Several medicinal plants have been studied for their potential tocolytic effects.

1. ***Bryophyllum pinnatum***: This plant has been used as a sedative since 1921. It was introduced in 1970 at a German complementary/alternative medicine (CAM) center to treat premature labor (Hassauer *et al.*, 1985).
2. ***Justicia flava***: The leaves of this plant are traditionally used in Southern Nigeria to prevent preterm births. Studies have shown that *Justicia flava* can potently inhibit uterine contractions in both non-pregnant and pregnant isolated mouse uteri.
3. ***Tectona grandis***: *Tectona grandis* is a well-known Indian herb. In Ayurveda, an extract from its stem has been noted for its tocolytic effect (Sharma *et al.*, 1978).
4. ***Alchornea laxiflora***: The leaves of *Alchornea laxiflora* are used in herbal medicine as a remedy for threatened abortion (Pierre *et al*., 2019).

1.7 Momordica Species (Bitter Melon)

1.7.1. Taxonomic Classification and Overview of *Momordica charantia*

Bitter gourd (*Momordica charantia* L.), also known as bitter melon, is a tropical vine belonging to the order Cucurbitales, family Cucurbitaceae, and genus *Momordica*.

Table 1.1: Taxonomic classification of *Mormodica charantia* (bitter melon).

Rank	Taxon

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Subkingdom	Embryophyta (or Bryobiotina)
Phylum	Magnoliophyta
Super division	Magnoliophyta
Division	Magnoliophyta (Angiosperms)
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Momordica
Species	*M. charantia*

International Journal of Pharmaceutical Sciences & Medicine.

Plant Description

Bitter gourd is a slender, tendril-climbing annual vine that grows to a height of 2 to 4 meters. The plant has distinct leaves with serrated edges, giving them a jagged, "bitten" appearance. Each plant produces separate yellow male and female flowers. The fruits vary in shape from discoid to ovoid or oblong, with pointed ends (Kole *et al*. 2020). They are typically 2 to 10 cm long, with a warty exterior and a hollow cross-section containing a thin layer of flesh, flattened seeds, and pith (Gupta *et al*. 2011). Immature fruits are whitish or pale green, while mature ones can be light green, green, or dark green, turning orange-yellow as they ripen. The fruit matures in 45 to 80 days (Sorifa 2018). The seeds are 8 to 15 mm long and straw-colored, covered by a fleshy layer that is white in unripe fruits and red in ripe ones (Poolperm & Jiraungkoorskul 2017).

1.7.2 Nutritional Profile

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Despite its bitter taste, bitter gourd is a highly nutritious vegetable, containing more vitamins and minerals than other cucurbits like squash, pumpkin, and cucumber (Krawinkel & Keding 2006). The fruit is rich in vitamins A, E, C, thiamine, riboflavin, niacin, and folate, as well as minerals such as potassium, iron, calcium, magnesium, phosphorus, and zinc. It also contains a good amount of dietary fiber. The calorific value per 100g is 213.26 kcal for the leaf, 241.66 kcal for the fruit, and 176.61 kcal for the seed (Joseph and Jini 2013). Vitamin C is one of the most abundant compounds, with an average of 205 mg/100g DW in the leaf and 2022 mg/100g DW in the fruit, with higher content in young fruits (Goo *et al*. 2016). Bitter gourd seeds are a rich source of quality proteins, meeting amino acid requirements for preschool children set by FAO/WHO/UNU. The seeds contain 35-40% oil, rich in polyunsaturated fatty acids (59.96%), and are one of the few edible fruits containing conjugated α-linolenic acid (Saeed *et al*., 2018; Yoshime *et al*., 2016). They are also one of the best natural sources of chromium (5.65 mg/100g) and zinc (45.45 mg/100g) (Saeed *et al.,* 2018).

1.7.3 Composition of *Momordica charantia*

Momordica charantia contains a diverse range of cucurbitacins, a class of compounds unique to the Cucurbitaceae family. The basic structure of a cucurbitacin resembles a lanostane ring but with a key difference in the position of the C-19 methyl group (Nadkarni, K.M., 2007; Pullaiah, T. and Naidu, 2003). Most cucurbitacins in *M. charantia* have an eight-membered branched side-chain at C-17, which can be saturated (Groover *et a*l., 2007) or unsaturated (Basch *et al.,* 2007). A double bond is typically present at C-5, which may shift to C-6 if an epoxy ring is formed. Additional double bonds can be found at C-23 (Chang *et al*., 2008) or C-24 (Jiang *et al.,* 2016). The C-19 methyl group can be oxidized to an aldehyde, ketone, or carboxylic function. Cucurbitacins can also have hydroxyl, keto, carboxyl, methoxy, or acetoxy substitutions, leading

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to great structural diversity (Lotliker *et al.*, 1966). Some have an extra epoxy ring, as seen in goyaglycosides, while momordicosides are a specific group of cucurbitacins with an oxidized C-19 methyl group, found only in *M. charantia*. They can exist in glycosidic (Singh 1989) or free form (Raish *et al.*, 1989). Polynorcucurbitacins with a five-membered, three-membered, or no side-chain have also been reported (Sreejayan,). Besides cucurbitacins, the plant also contains triterpenoids, sterols, vicine, and p-methoxy benzoic acid.

1.7.4 Ethnomedical Uses of *Momordica charantia*

The pantropical vine *Momordica charantia* is widely used in traditional medicine to treat various conditions. It is frequently used to manage diabetes in many cultures (Halberstein and Saunders, 1978; Mossa, 1985; Zhang, 1992; Arvigo and Balick, 1993). Leaves, fruits, and roots are also used to treat fevers (Ayensu, 1978; Halberstein and Saunders, 1978; Singh, 1986; Girón *et al*., 1991). In reproductive health, it has been used as an abortifacient, a birth control agent, to treat painful menstruation, and to facilitate childbirth (Kerharo and Adam, 1974; West *et al*., 1981; Burkill, 1985; Mossa, 1985). In Togo, the most common uses are for gastrointestinal and viral infections in children.

1.7.4.1 Anti-Diabetic Activity

Diabetes mellitus is a metabolic disease characterized by high blood sugar levels. *Momordica charantia* is a traditional remedy for this condition, and extensive research has been conducted to identify the compounds and mechanisms responsible for its anti-diabetic effects. (Halberstein and Saunders, 1978; Mossa, 1985; Zhang, 1992; Arvigo and Balick, 1993).

1.7.4.2 Anti-Cancer Property

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Preliminary trials over the last few decades have explored the anti-cancer properties of *Momordica charantia*. Studies suggest that its bioactive compounds may help regulate various cancers, including cervical, breast, liver, nasopharyngeal, leukemia, and colon cancer. However, systematic clinical trials in cancer patients are needed to confirm these effects.

- *In vitro* studies have shown that an ethanolic extract of the whole fruit has anti-cancer activity against breast and cervical cancer cell lines (Shobha *et al*., 2015). Gunes *et al*. (2019) also reported that ethanolic fruit extract showed the highest anti-tumor activity against lung cancer, breast cancer, and leukemia cell lines.
- *Momordica charantia* lectin, a protein from the seeds, has been shown to have potent cytotoxicity against nasopharyngeal carcinoma cell lines (Fang *et al*., 2012). A similar protein, MAP30, isolated from the seeds, promoted apoptosis in liver cancer cells *in vitro* and *in vivo* (Fang *et al*., 2012).
- A novel anti-cancer peptide, BG-4, isolated from the seeds, showed cytotoxicity to human colon cancer cells *in vitro* (Dia and Krishnan 2016).
- A **triterpenoid** from the whole plant inhibited the growth of breast cancer cells *in vitro* (Bai *et al*., 2016).
- Crude bitter melon extract has been suggested as a supplement to improve the efficacy of cisplatin-based chemotherapy for ovarian cancer (Yung *et al*., 2016).

1.7.4.3 Anti-Oxidative Property

Oxidative stress is a major contributor to various lifestyle diseases. Numerous studies suggest that *Momordica charantia* has significant antioxidant properties, with good antioxidant capacity

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compared to other vegetables like colocasia (Gayathri 2014) and pumpkin (Hamissou *et al*., 2013).

- *In vitro* studies have established the anti-oxidative activity of the whole fruit pulp, extracts, seed powder, leaves, and stem (Kubola & Siriamornpun 2008; Padmashree *et al*. 2011; Leelaprakash *et al*., 2011).
- An *in vivo* study in mice showed that bitter gourd polysaccharides scavenge free radicals, increase antioxidant enzyme activity (SOD and CAT), and reduce oxidative stress (Tsai *et al*., 2011).
- Zinc nanoparticles synthesized from bitter gourd extract also exhibited potent antioxidant activity (Ekezie *et al*., 2016).
- One study found no significant difference in the antioxidant potential of bitter melon fruits at different ripening stages (Aminah and Anna 2011).

1.7.4.4 Anti-Dementia Activity

Neurodegenerative diseases, which affect brain cells and cause irreversible effects on memory and cognition, are often linked to metabolic or toxic stress. Various preclinical trials are exploring the neuroprotective effects of compounds from *M. charantia*, particularly their anti-dementia activity.

- Charantin, a steroidal glycoside isolated from the plant, showed neuroprotective effects in *in vitro* studies on neuroblastoma cell lines by scavenging free radicals and inhibiting butyrylcholineesterase (Tamilanban 2018; Kuanhuta *et al*., 2014).

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- Polysaccharides from *M. charantia* have been shown to protect against cerebral ischemia/reperfusion injury due to their antioxidant activities (Gong *et al*., 2015).
- An ethanol extract protected against oxidative stress-induced neuronal cell death in human neuroblastoma cells (Ju and Kim 2018).
- *In vivo* studies in mice have demonstrated that a hydroalcoholic extract of *M. charantia* can restore memory (Miri *et al*., 2019) and inhibit lipid peroxidation and acetylcholinesterase activity in the brain (Pathakota *et al*., 2017). A similar effect was also reported in scopolamine-induced rats (Joshi *et al*., 2017).

1.7.4.5 Hypolipidemic and Hypotensive Activity

Hyperlipidemia, a condition with abnormally high levels of cholesterol and triglycerides, is a risk factor for cardiovascular diseases. Hypotension is low blood pressure. Research explores the role of *M. charantia* in managing these conditions.

- An *in vivo* study showed that bitter gourd juice had a hypolipidemic effect in rats similar to that of the drug atorvastatin, reducing serum total cholesterol, LDL-cholesterol, and triglycerides (Sharmin *et al*., 2017).
- Other studies found that bitter gourd seeds were more effective than other parts in controlling hyperlipidemia (Arshad *et al*. 2018), and that whole fruit powder had the highest hypolipidemic activity (Mahwish *et al*., 2018).
- An extract of bitter melon leaves showed high anti-hypertensive activity by inhibiting angiotensin-converting enzyme (ACE), with the ethyl acetate fraction showing the highest activity (Lestari and Mahayasih 2017).

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- A polysaccharide from the fruit also showed high ACE-inhibitory activity (*in vitro*) (Tan and Gan 2016), and two novel ACE-inhibitory peptides were identified in the seeds, which showed a significant anti-hypertensive effect in rats (*in vivo*) (Priyanto *et al*., 2015).

1.7.4.6 Anti-Microbial and Anthelmintic Activity

Bitter gourd is a folk remedy for various skin and stomach ailments. Its potential as an antimicrobial and anthelmintic agent has been proven.

- An ethanol extract of the leaves has been found to be effective against a wide range of bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Leelaprakash *et al*., 2011; Ingle & Kapgatte 2018), and the fungus *C. albicans* (Jagessar *et al*. 2008). The antimicrobial activity is solvent-dependent, with ethanol extract being more potent than aqueous (Ingle & Kapgatte 2018).
- Methanolic extracts of the fruit and seed pulp also have antimicrobial activity against various bacteria and fungi (Mahmood *et al*., 2012).
- Plumericin, an iridoid lactone isolated from the vine, showed better inhibition against certain bacteria than cloxacillin (Saengsai *et al*., 2015).
- Extracts of the leaf, fruit, and seeds also have anthelmintic activity against various parasites in birds and mammals (Poolperm & Jiraungkoorskul 2017).

1.8 RATIONALE FOR THIS STUDY

Preterm labor, defined as uterine contractions leading to cervical changes before 37 weeks of gestation, is a significant contributor to neonatal morbidity and mortality. Current management

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often involves the use of tocolytic agents to suppress uterine contractions and prolong pregnancy. However, these conventional drugs, such as beta-agonists and calcium channel blockers, can be associated with significant maternal and fetal side effects, including pulmonary edema, tachycardia, and fetal distress. This has led to a growing interest in exploring alternative, natural therapies that may be effective and have a more favorable side-effect profile.

Momordica charantia, commonly known as bitter melon, is a plant with a long history of use in traditional medicine. It is known for its purported anti-inflammatory, antioxidant, and smooth-muscle relaxing properties. These qualities make it a promising candidate for investigation as a natural tocolytic agent. This study seeks to investigate whether Momordica charantia extract can serve as a safe and potentially effective alternative or adjunctive therapy for the management of preterm labor. The findings could provide evidence supporting its use and offer an additional treatment option for individuals experiencing preterm labor.

1.9 RESEARCH QUESTIONS

Does the *Momordica charantia* ethanol extract exhibit a tocolytic effect on spontaneous uterine contractions in non-pregnant mice?

1.10 STUDY HYPOTHESIS

Momordica charantia extract, when administered in appropriate doses, will significantly reduce or inhibit spontaneous uterine contractions, oxytocin-induced uterine contractions, high KCl-induced uterine contractions and in a calcium-free medium of non-pregnant mice.

1.11 AIM AND OBJECTIVE OF THE STUDY

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The aim of the study is to investigate the tocolytic properties of the *M. charantia* extract on the uterus of non-pregnant mice.

The objectives of the study were to:

1. determine the effect of *M. charantia* extract on spontaneous uterine contraction.
2. determine the effect of *M. charantia* extract on oxytocin-induced uterine contraction in non-pregnant mice.
3. determine the effect of *M. charantia* extract on high KCl-induced uterine contraction in non-pregnant mice.
4. determine the effect of *M. charantia extract* on oxytocin- induced uterine contraction in a calcium free medium in non-pregnant mice.

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CHAPTER TWO

MATERIALS AND METHOD

2.1 Materials

The study utilized a range of equipment, including a micropipette (Microflux, 0–1000 µL), various sizes of sample vials, beakers (50 mL, 250 mL, and 500 mL), Pasteur pipettes, syringes (1 mL, 2 mL, 5 mL, 10 mL, 20 mL) with accompanying needles, white thread, and masking tape. Other instruments included permanent markers (red, blue, green, and black), a dissecting kit, a glass stirrer, brushes, disposable gloves, plastic cages, a spatula, and graduated cylinders (100 mL, 250 mL, and 500 mL).

For microscopic and preparatory work, a microscope, glass slides, a porcelain dish, a pestle, a hot plate or oven, and an organ and water bath were used. Data was collected with a digital weighing balance, a refrigerator, a steel observation table, and an Uninterruptible Power Supply (UPS). The main data acquisition system consisted of a 7003E-isometric force transducer (PanLab ADInstruments, Australia), a laptop running Lab-chart software (ADInstruments), and a PowerLab 2/26 Model ML826 data acquisition unit (ADInstruments, Australia). Statistical analysis was performed using GraphPad Prism v. 8.1 (San Diego, CA, USA).

2.1.1 Chemicals and Reagents

The physiological saline solution (PSS), a modified Ringer's Locke, was prepared with the following components in mM/L concentrations:

- Sodium Chloride (NaCl): 154.00 (Guangdong Guanghua Sci-Tech Co. Ltd. China)
- Potassium Chloride (KCl): 5.63 (Guangdong Guanajua Sci-Tech Co. Ltd. China)

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- D-Glucose (C6H12O6.H2O): 2.78 (Guangdong Guanghua Sci-Tech Co. Ltd. China)
- Sodium Bicarbonate (NaHCO3): 5.95 (Sigma Aldrich, UK)
- Calcium Chloride (CaCl2H2O): 2.05 (Guangdong Guanghua Sci-Tech Co. Ltd. China)

Additional chemicals and reagents included: Ethanol (Pharma trends, Nigeria), Normal Saline (Bioflex; Biomedical Nigeria Ltd), Oxytocin (Roche pharmaceutical Ltd), Methylene Blue (Tianjin Kermel Chemical Reagent Co., Ltd), Distilled water, Tween 80 (Tianjin Kermel Chemical Reagent Co., Ltd), and EDTA (Guangdong Guanghua Sci-Tech Co. Ltd. China).

2.1.2. Collection and Extraction of Plant Materials

In May 2025, a plant specialist obtained leaves of *Momordica charantia* in capito around Ugbowo Benin city, Edo State, Nigeria. The plant was formally identified as *Momordica charantia.* by Professor Henry Akinnibosun Adewale of the Department of Plant Biology and Biotechnology (PBB), Herbarium Unit, at the University of Benin, Benin City, Edo State. A specimen was given the voucher number UBH-M617 and was subsequently stored in the department's herbarium for future reference.

The leaves underwent a cleaning process to eliminate foreign matter before being thoroughly dried and ground into a fine powder using an electric milling machine. The powdered leaves (441.9g) were then subjected to extraction with Hydro-ethanol (1:1) solvent using a Soxhlet apparatus. The resulting Hydro-alcoholic extract was concentrated by removing the solvent with a rotary evaporator, with the temperature maintained at 60°C and a rotation speed of 90 rpm. The final dried extract accounted for 31.46% of the original weight and was kept in a sealed container under refrigeration at 4°C.

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2.1.3 Animals

The experiment utilized twenty-five healthy non-pregnant female albino mice, each weighing between 20 and 30 grams. The mice were acquired from the animal division of the Faculty of Pharmacy and were housed and cared for in the animal facility of the Department of Pharmacology and Toxicology at the University of Benin, Nigeria. The mice were given a two-week acclimatization period before the study began. They were maintained under a controlled environment with a natural light-dark cycle and regulated temperature. All animals had constant access to a standard rodent pellet diet (Chikun grower pellets Feeds, Crown flour mill Limited, Lagos, Nigeria) and fresh tap water.

All animal care and handling procedures were conducted in strict accordance with the guidelines outlined in the 'Guide for the Care and Use of Laboratory Animals' and the 'Public Health Service Policy on Humane Care and Use of Laboratory Animals,' as established by the National Research Council (2010) and the Public Health Service (2015).

2.1.4 Uterine Tissue Preparation

Non-pregnant mice were screened to select those in the estrus phase which was confirmed by microscopic observation of vaginal smears and macroscopic observation of the vulva. Their abdomens were opened, and the uterine horns were swiftly removed. The horns were then cleaned of any connective tissues or fat and placed in a Petri dish containing warm, aerated physiological saline solution (PSS). The uterine tissue was then cut into segments of approximately 1–2 mm in length.

Each segment was vertically mounted in an aerated 10 mL organ bath containing Ringer's Locke solution, which was kept at 37°C. The solution had the following composition in mM/L: CaCl2

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.2H2O = 2.05, D-Glucose = 2.78, KCl = 5.63, NaHCO3 = 5.95, and NaCl = 154.00, as previously described (Bafor *et al*., 2019). The segments were secured at both ends with surgical thread, with one end attached to a tissue holder and the other to a 7003E-isometric force transducer (Pan-lab ADInstruments, Spain). This transducer was connected to bridge amplifiers and a PowerLab 2/26 Model ML826 data acquisition system (ADInstruments, Australia), which recorded changes in contraction force and frequency. The data were further analyzed using Lab-chart 7 reader software (v.8.0, ADInstruments, North America, USA). The tissue was allowed to acclimate for a minimum of 30 minutes at a resting tension of 0.5 g until stable, rhythmic contractions were observed before any drugs were introduced (Uchendu and Bafor, *2023*).

2.2 Methods

2.2.1 Effect of *M. charantia* Extract on Spontaneous Uterine Contraction.

To evaluate the effect on spontaneous contractions, *M. charantia* extract (MCE) was administered to the isolated non-pregnant mouse uterine tissues at increasing concentrations (0.00625 to 0.4 mg/ml), each addition was allowed a contact time of 5 minutes to interact with the tissue. This was done after establishing a baseline of regular spontaneous contractions, which served as the control. Each concentration was allowed to act for 5 minutes before the next dose was added (Uchendu and Bafor, 2023).

2.2.2 Effect of *M. charantia* Extract on Oxytocin-Induced Uterine Contraction

To induce contractions, 14 nM of oxytocin was added to the isolated tissue and allowed to take effect for 5 minutes. Without a subsequent washout, MCE was administered to the oxytocin-pre-contracted tissue at increasing concentrations (0.00625 to 0.4 mg/ml). Each concentration was given a contact time of 5 minutes. Following the additions, the tissue was thoroughly washed and

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allowed to recover before any new analysis. The resulting changes in contraction strength and rate were documented (Bafor *et al., 2020;* Uchendu and Bafor, *2023*).

2.2.3 Effect of *M. charantia* Extract on High KCl-Induced Uterine Contraction

This part of the study assessed the extract's influence on myometrial membrane depolarization induced by high concentrations of potassium chloride. An 80 mM KCl solution was applied to the isolated tissue for 5 minutes. Without subsequent washout, MCE was administered to the tissue at increasing concentrations (0.00625 to 0.4 mg/ml). Each concentration was given a contact time of 5 minutes. The tissue was then washed and allowed to recover, and the resulting changes in contraction amplitude was recorded for analysis (Bafor *et al., 2020;* Uchendu & Bafor, *2023*).

2.2.4 Effect of *M. charantia* Extract on Oxytocin-Induced Contraction in a Calcium-Free Medium

This investigation focused on how the extract might affect the release of intracellular calcium from its stores. The experiment was conducted in a calcium-free PSS supplemented with 0.1 mM EDTA. The uterine tissue's spontaneous contractions were first stabilized in a standard PSS. This solution was then replaced with the calcium-free PSS. Without a washout, 14 nM of oxytocin was added, followed by an optimal dose of the ethanol extract (0.4 mg/ml), which was allowed to act for 5 minutes (Bafor *et al., 2019*). The tissues were then washed and permitted to recover. The subsequent alterations in the amplitude and frequency of contractions were recorded for analysis (Bafor *et al*., 2020; Uchendu and Bafor., 2023).

2.3 Data Analysis

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All data are presented as the mean ± standard error of the mean (SEM). For comparing multiple groups, a one-way ANOVA was used, followed by Dunnett's post hoc test. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. These statistical analyses were carried out using GraphPad Prism version 8.0. Uterine contractility data, including contraction frequency and strength (amplitude), were processed using Lab-chart reader software version 8.0.

The mean log concentration-response relationships were analyzed by fitting the data to a four-parameter logistic model using non-linear regression. The mathematical model employed was $y=bottom+(top-bottom)/(1+10((logEC50-x) \times hillslope))$. Here, *y* represents the measured response that transitions from a minimum value (*bottom*) to a maximum (*top*) in a sigmoidal pattern, while *x* is the log-transformed concentration.

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CHAPTER 3

RESULTS

3.1. Effect of *M. charantia* extract on Spontaneous Uterine Contractions.

Cumulative additions of *M. charantia* extract (MCE) slowly decreased spontaneous contractions of the non-pregnant uterus (0.00625 – 0.4 mg/mL) in a concentration-dependent manner (Figure 3.1). The result analysis showed that the amplitude (Figure 3.2 (A)) of spontaneous contractions on the non-pregnant uterus progressively reduced with a significant reduction at 0.1 and 0.2 mg/mL (*p <0.05) and a highly significant reduction at 0.4 mg/mL (**P<0.01) while the frequency (Figure 3.2 (B)) of spontaneous contraction showed no significant change. Figure 3.2 (C) shows the area under the curve plot depicting the inhibitory effect of MCE. There was immediate recovery of spontaneous uterine contractions after the washout of MCE with fresh PSS.

3.2. Effect of *M. charantia* extract *on* Oxytocin-Induced Non-Pregnant Uterine Contractions

Oxytocin (OT) (14 nM) stimulated an increased amplitude and frequency of uterine contractions (Figure 3.3 (A)). There was no effect on contractions in the presence of the vehicle, TW-80 (Polysorbate 80). In the continued presence of OT, MCE cumulative additions led to a dose-dependent inhibition in non-pregnant mice uterine contractions (Figure 3.4 (B)). In the presence of MCE, the amplitude of the contractions progressively reduced with a highly significant reduction at 0.1 and 0.2 mg/mL (**p <0.01) and a very highly significant reduction at 0.4 mg/mL (**P<0.001). There was no significant inhibition in the frequency (Figure 3.3 (A) & 3.3 (B)).

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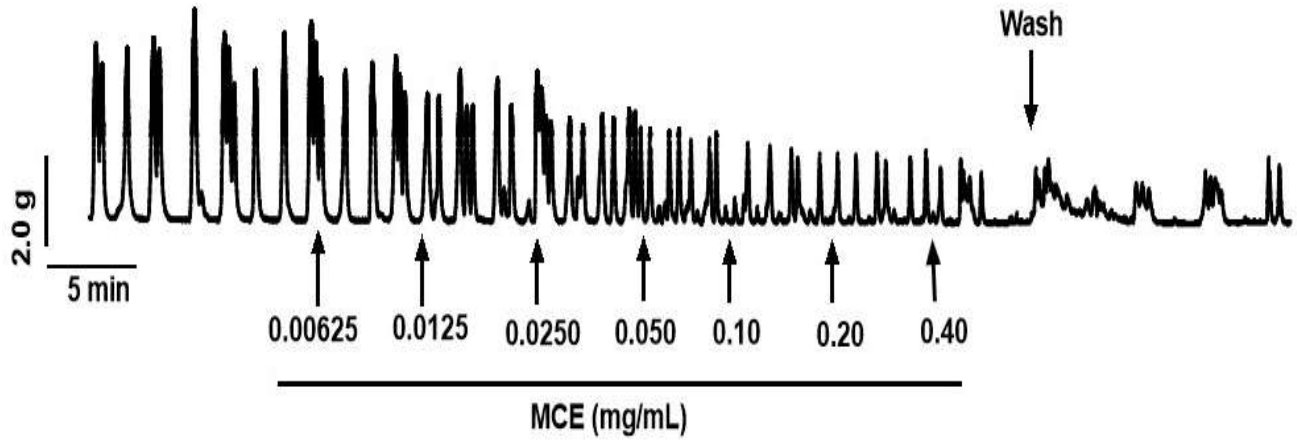

Figure 3.1. Representative recording showing the effect of cumulative concentration (0.0625 - 0.4 mg/mL) of MCE on spontaneous uterine contractility of the non-pregnant mouse uterus.

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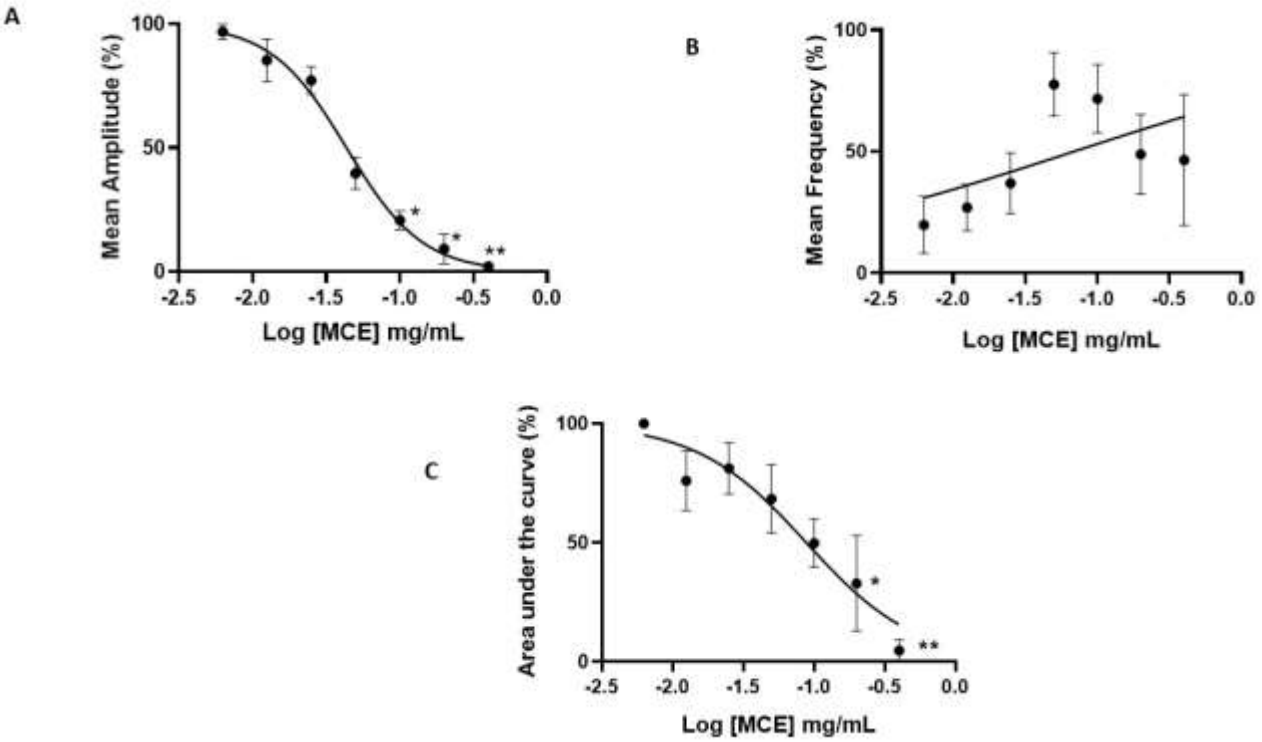

Figure 3.2 Effect of *M. charantia* extract (MCE) on (A) Amplitude, (B) frequency and (C) The area under the curve (AUC) on spontaneous uterine contractility of the non-pregnant uterus. Values are mean ±SEM, n=5 animals, *P< 0.05, **P<0.01, n=5 animals.

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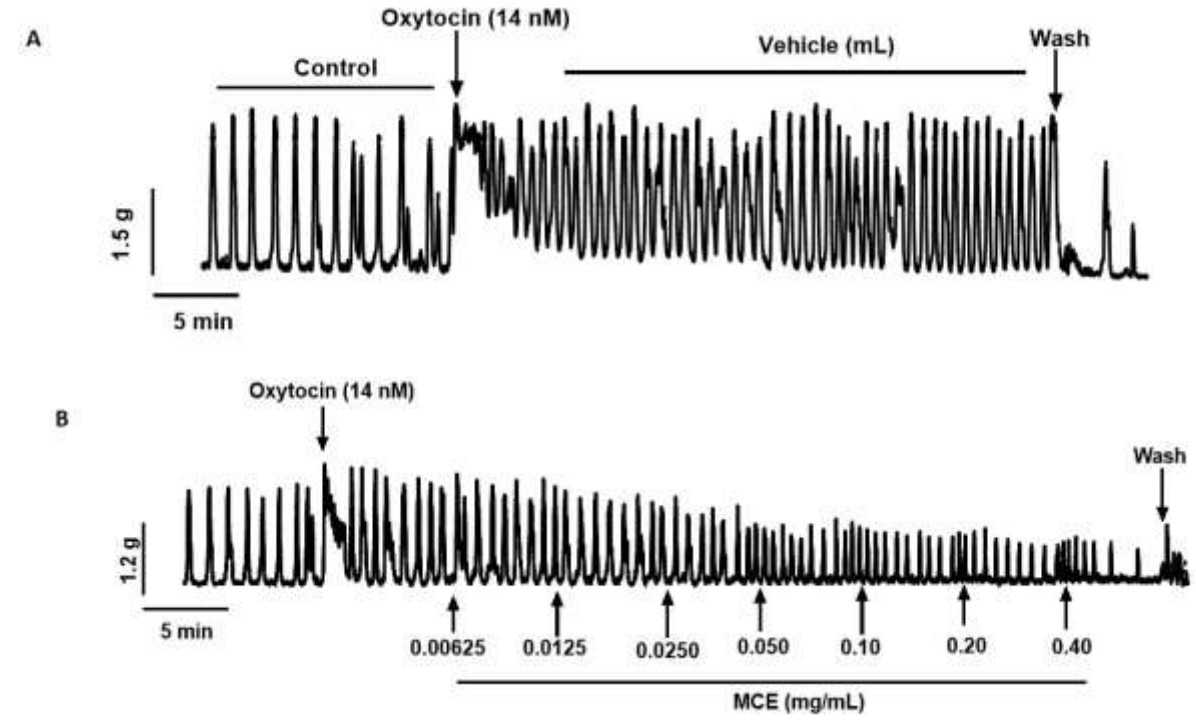

Figure 3.3 Effect of MCE on oxytocin-induced uterine contraction of a non-pregnant mouse

Representative recordings showing (A) The effect of oxytocin on non-pregnant uterine contraction as control; (B) The effect MCE (0.00625-0.4 mg/mL) on Oxytocin-induced contraction of the non-pregnant uterus.

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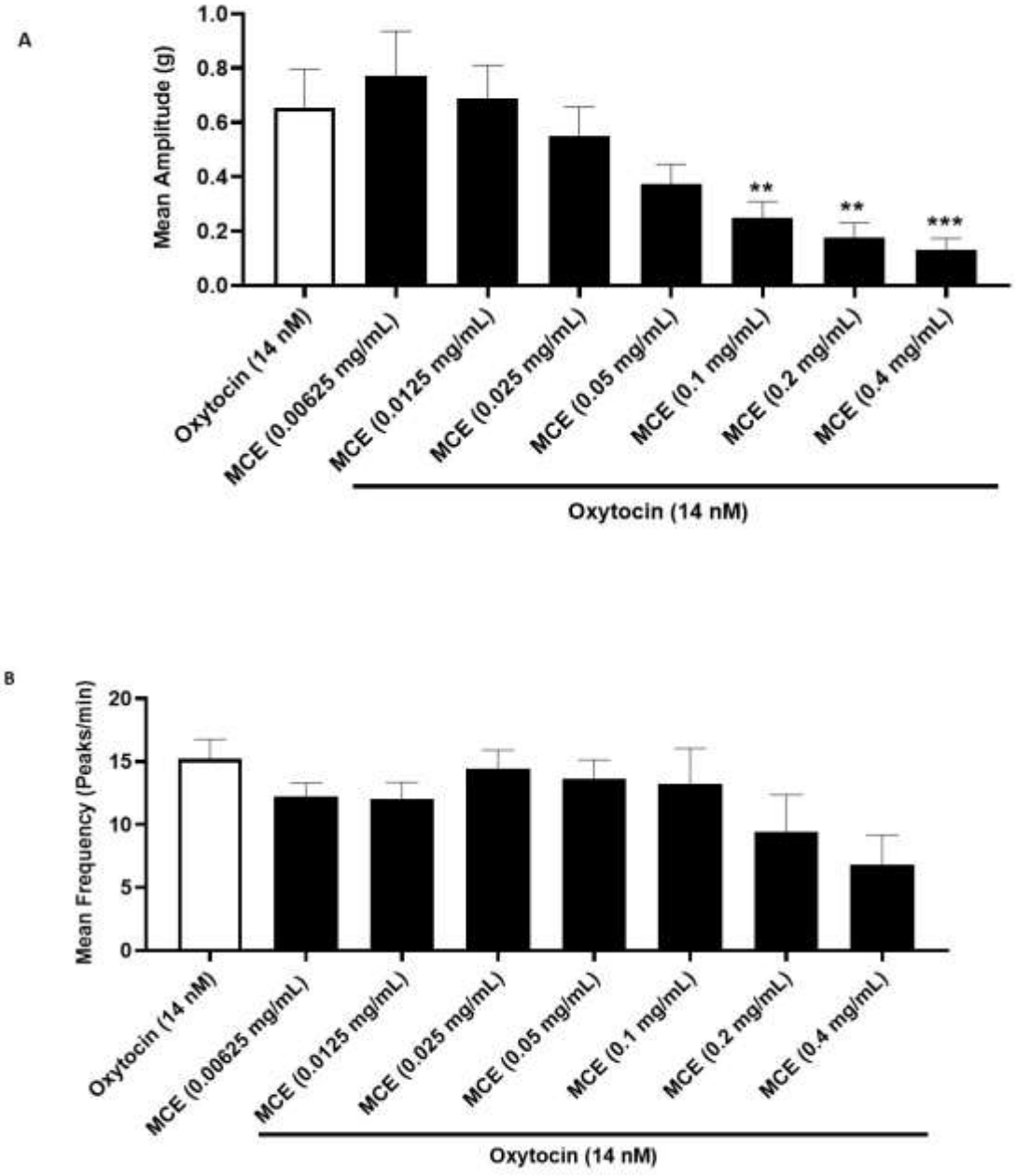

Figure 3.4. Bar graph showing effect of MCE on (A) amplitude and (B) frequency on oxytocin-induced uterine contraction. values are mean ±SEM, n=5 animals. HValues are mean ±SEM, **P< 0.01, ***P<0.001, n=5 animals.

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3.3. Effect of *M. charantia* Extract on High KCl-Induced Non-Pregnant Uterine Contractions.

Figure 3.5 (A) shows sustained contractions caused by the addition of high KCl. It also shows that there was no effect of the vehicle on its contraction. The effect of MCE on KCl-induced contractions is shown in Figure 3.5. High KCl produced a rapid and sustained increase in force of contraction (Figure 3.5(A)). The introduction of MCE in the presence of KCl (80 mM) significantly diminished the high KCl-induced contraction on non-pregnant mice uterine tissues (Figure 3.5(B), compared to KCl alone. In the presence of MCE, the amplitude of the contractions progressively reduced with a significant reduction at 0.1 and 0.2 mg/mL (*p <0.05) and a highly significant reduction at 0.4 mg/ml (*P<0.01), figure 3.6.

3.4. Effect of *M. charantia* Extract on Oxytocin-Induced Uterine Contractions in Calcium Free Medium.

The effects of MCE on the release of calcium from intracellular stores were shown in Figure 3.7(A). The addition of OT to the uterine tissue mounted in a zero-calcium PSS, containing the calcium chelating agent, EDTA, slightly increased the spontaneous contractions. However, upon addition of MCE, there was no significant reduction in the uterine contractions. The frequency and amplitude showed no significant reduction in the non-pregnant mice uterine tissues. (Figures 3.8). There was immediate recovery of spontaneous uterine contractions after the washout of MCE with fresh PSS (Figure 3.8(A)).

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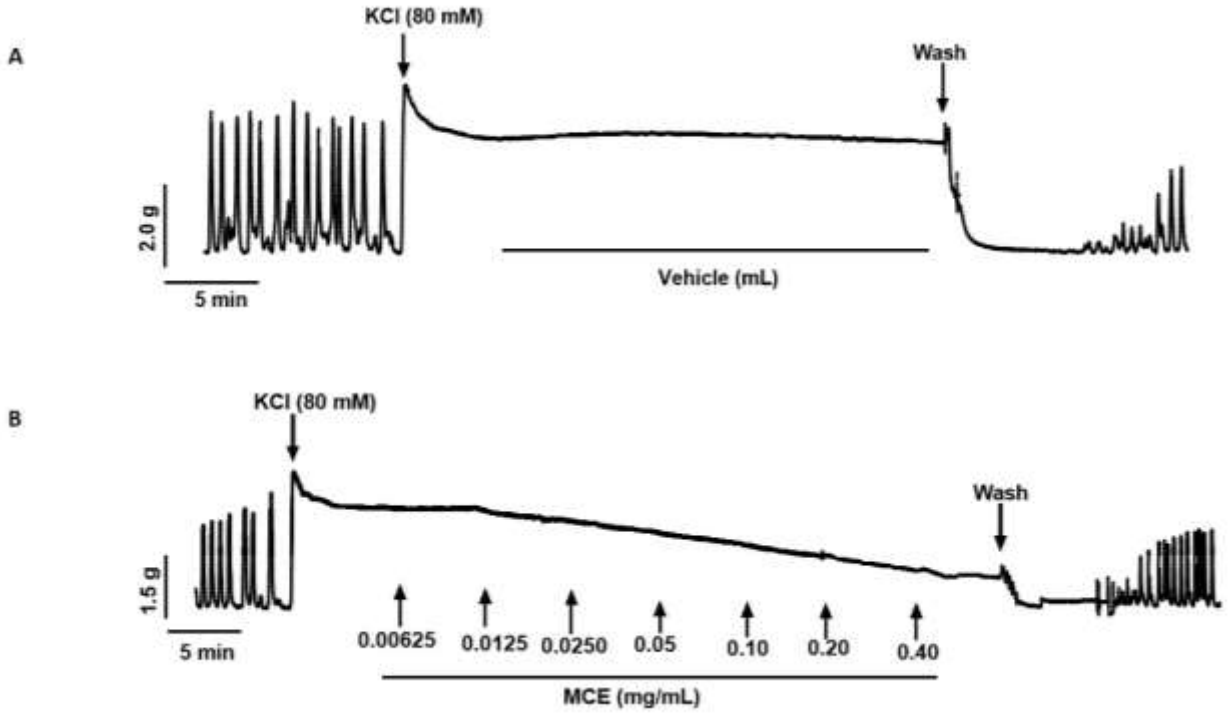

Figure 3.5. Effect of MCE on oxytocin-induced uterine contraction of a non-pregnant mouse

Representative recordings showing (A) The effect of high KCL-induced on non-pregnant uterine contraction as control; (B) The effect of MCE (0.00625-0.4 mg/mL) on high KCL-induced contraction of the non-pregnant uterus.

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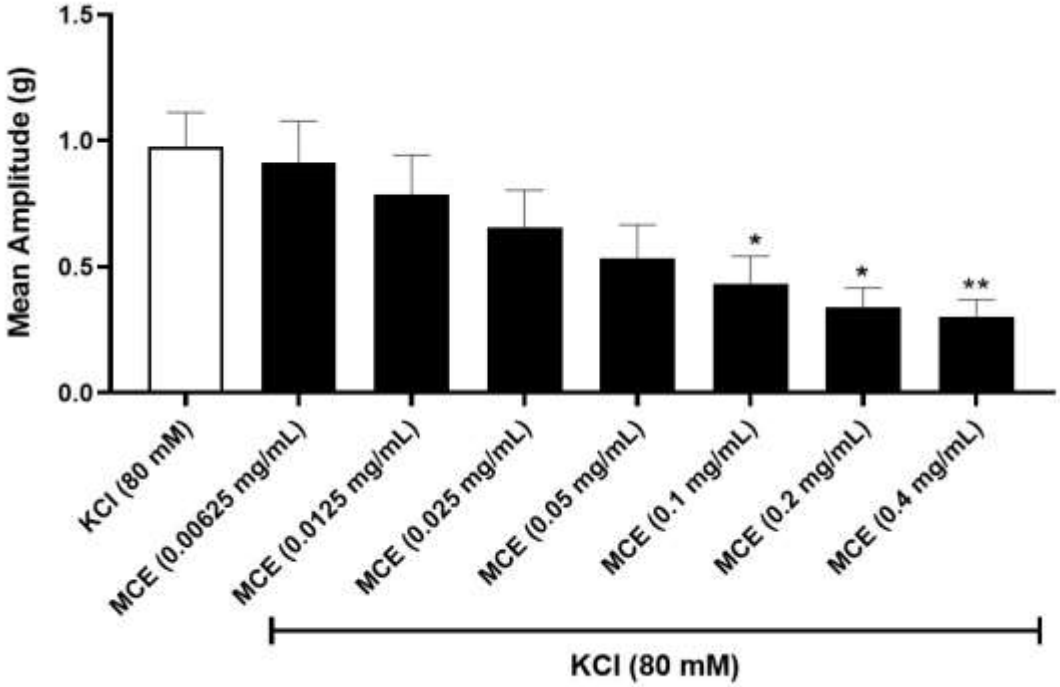

Figure 3.6 Bar graph showing effect of MCE on amplitude on high KCL-induced contraction of the non-pregnant uterus. Values are mean ±SEM, n=5 animals, *P< 0.05, **P<0.01, n=5 animal.

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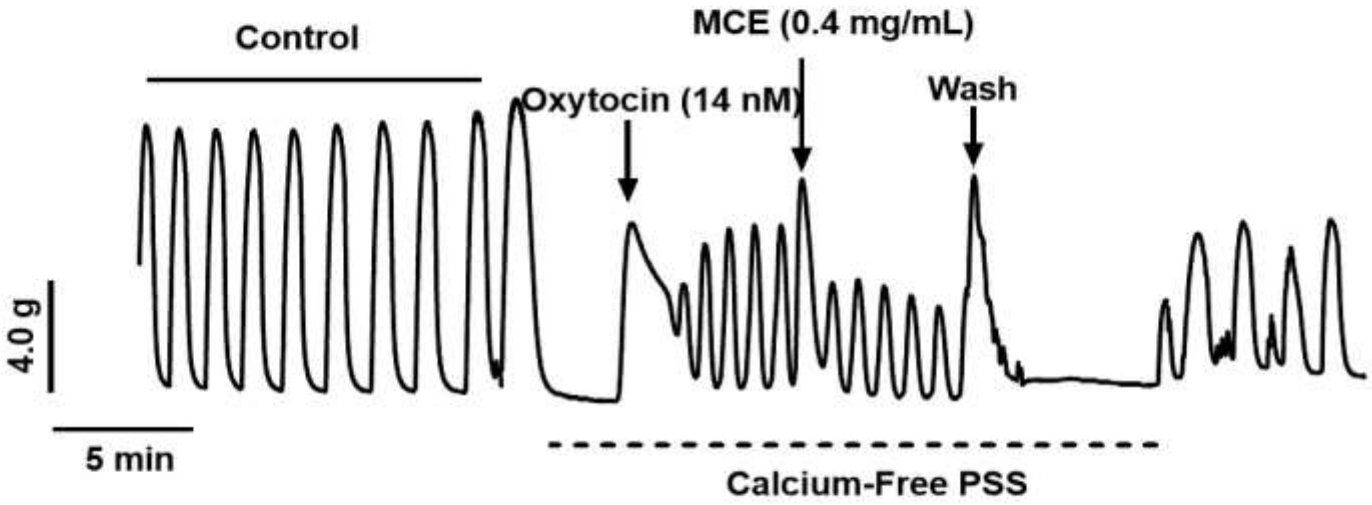

Figure 3.7. Representative recording showing the effect of cumulative concentration (0.0625 - 0.4 mg/mL) of MCE on Oxytocin-induced contraction in a Ca2+-free medium of the non-pregnant uterus.

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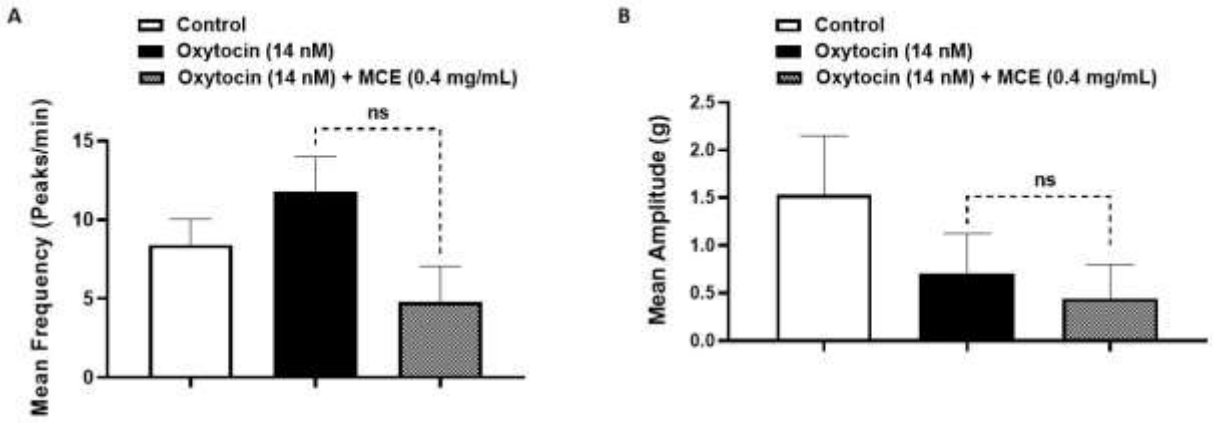

Figure 3.8. Bar graph showing effect of MCE on (A) amplitude and (B) frequency on Oxytocin-induced contraction in a Ca2+-free medium of the non-pregnant uterus. Values are mean ±SEM, n=5 animals. Values are mean ±SEM, n=5 animals.

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CHAPTER 4

DISCUSSION

The enduring significance of traditional medicine in modern healthcare is evident in the global reliance on plant-based remedies for conditions like reproductive disorders and pregnancy complications (Goldenberg *et al.,* 2008). *Momordica charantia* (bitter melon) is a prominent traditional medicine in Asia, Africa, and the Caribbean, valued for its purported anti-diabetic and anti-malarial properties (Kandikattu *et al.,* 2021). Its ethnopharmacological use as an abortifacient and tonic provides the rationale for investigating its potential as a natural therapeutic agent in pregnancy management (Sharanabasapa *et al.,* 2002).

This study was designed to investigate the tocolytic effect of *Momordica charantia* extract (MCE) on isolated mouse uterine segments, using oxytocin or KCl as positive contractile agents. The results strongly indicate that the extract possesses significant dose-dependent effects on uterine contractility parameters, suggesting its potential as a natural agent for treating preterm labor.

The evaluation of the tocolytic effect of MCE was based on some parameter's indicative of smooth muscle relaxation. These included its effect on spontaneous contraction, oxytocin induced contraction, high KCl induced contraction and oxytocin induced contraction in a calcium free medium all carried out on non-pregnant mouse uterus.

Another key finding was the reversibility of the extract's effect. Following a washout period (removal of MCE from the system), uterine contractions fully returned to their starting levels across all tests. This demonstrates that MCE's action is temporary and does not result in irreversible damage to uterine tissue. Instead, the compound appears to modulate activity

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temporarily, which is a highly desirable quality for any potential therapeutic drug. This finding is consistent with earlier research (Loch-Caruso *et al*., 2003) showing that many uterine relaxants operate reversibly, enhancing their safety profile for potential long-term or repeated use.

MCE demonstrated a dose-dependent inhibitory effect on spontaneous uterine contractility, marked by a significant reduction in the amplitude of contraction. However, MCE did not produce any significant difference in the frequency of spontaneous contractions across the tested dose range.

In isolated mouse uteri, spontaneous contractions are driven by intrinsic pacemaker currents and are an exhibition of the tissue's intrinsic myogenic activity (Shynlova *et al*., 2024).

The spontaneous contractions process begins with Myometrial smooth muscle cells exhibiting rhythmic fluctuations in membrane potential known as slow waves, which are controlled by complex K^+ and Na^+ ion channel activity. When the depolarization of a slow wave reaches a critical threshold, it triggers a rapid sequence of action potentials (APs), or spike bursts. These APs are the electrical events that open the dominant L-type Ca^{2+} channels on the plasma membrane. The opening of these Voltage-Operated Calcium Channels (VOCCs) permits a sustained influx of extracellular Ca^{2+}, resulting in an increased cytosolic Ca^{2+} concentration. This Ca^{2+} then binds to Calmodulin (CaM). The Ca^{2+}-CaM complex then activates myosin light chain kinase (MLCK), which adds a phosphate group to myosin's regulatory light chain (MLCo). This phosphorylation is crucial for muscle contraction, driving the acto-myosin cross-bridge cycle and using Mg-ATP (Taggart, 2001). For the uterus to relax, myosin light chain phosphatase (MCP) must remove the phosphate group from myosin, and calcium must be actively expelled from the cell via mechanisms like the plasma membrane Ca^{2+}-ATPase (PMCA), stored in the

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sarcoplasmic reticulum (SR) by SERCA pumps, or exchanged via the $Na^{+}Ca^{2+}$exchanger. Spontaneous contractions primarily depend on Ca^{2+} entry, which starts the contraction by using internal calcium stores and maintains or increases it using external sources (Shmigol, Eisner and Wray 1998; Wray *et al*. 2003; Floyd & Wray 2007).

The observation that the extract suppressed amplitude while leaving frequency unchanged suggests that MCE does not significantly influence the intrinsic electrical properties or the primary pacemaker activity that sets the rate of contraction initiation. Instead, MCE functions as a myorelaxant or an inhibitor of force generation (Hector *et al*., 2010). This means the electrical firing (action potentials) may continue at the same rate, but the mechanical power (the resulting force) produced by each electrical event is reduced. The finding in this study agrees with previous study conducted on the vaso-relaxant of *M. charantia* on rat aorta (Zainol *et al.*,2024).

MCE significantly inhibited the amplitude of Oxytocin (OT)-induced contractions but, mirroring the spontaneous contraction data, showed no significant difference in the frequency of contraction.

Oxytocin is a procreative chemical messenger involved in the act of giving birth and widely administered during labor. Oxytocin is created within the supraoptic core and paraventricular core of the hypothalamic region and discharged from the posterior pituitary section into the bloodstream. In addition, oxytocin is synthesized in the peripheral organs, such as the cardiovascular system; in the alimentary canal, ovaries, maternal lining of the uterus, and chorioamniotic membranes; and in cells, such as lining cells and skin cells. (Miller and Mitchell 1993). Locally oxytocin mainly exerts regional, regulating roles via paracrine mechanisms. (Burbach, *et al.*, 2006)

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When oxytocin binds to its receptors, it triggers womb-muscle spasms. Elevated quantities of circulating estrogen at full term make the receptors more responsive. Furthermore, oxytocin spurs prostaglandin creation and discharge in the maternal lining and chorioamniotic membranes by activating a particular type of oxytocin receptor which further boosts uterine contractions.

Oxytocin plays parts in labor and breastfeeding. It promotes the maturing of eggs in the follicles, tube movement of the eggs, fusion of eggs, nesting of the early embryo, and encouragement of growth of the unborn baby. (Mitchell *et al.*, 1980).

Synthetic oxytocin has been employed for more than 60 years to initiate and intensify labor, to lessen the incidence of excessive bleeding after delivery and sometimes to provoke milk letdown, such as when an infant is prematurely born. Currently, many women in labor get intravenous drips of synthetic oxytocin to start or increase their labor. Additionally, hospitals often administer mothers a single, concentrated dose of oxytocin after delivery.

The oxytocin receptor, such as the closely related vasopressin receptor, is categorized as the G-protein–linked receptor, which relays data from the cell membrane into the cell via G proteins. Only one oxytocin receptor has been identified, whereas three different vasopressin receptors exist. The oxytocin receptor is composed of 389 amino acids and is a 7-span transmembrane spiral receptor with 3 external and 3 internal loops. (G. Gimpl, Fahrenholz 2001)

The oxytocin receptor is connected to a three-part assemblage of G proteins, made up of one G alpha unit and one beta or gamma unit. Several different forms of the proteins are present, which contribute to various compositions of the G-protein assemblage.

By binding to its receptor, the beta or gamma unit is separated from the G-alpha protein. This, in turn, causes the activation of phospholipase-C. This biological catalyst breaks down

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phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol 1,4,5, triphosphate and initiates calcium ($Ca2^{+}$) release from the smooth internal network. This Ca^{2+} release activates calmodulin, culminating in the addition of a phosphate group to myosin light-chain kinase that adds a phosphate group to myosin light chains, which, in turn, assists actin-myosin interplay and tightening, Simultaneously, DAG activates protein kinase C. This succession of events is known as the standard pathway through which oxytocin provokes uterine contractility (Rao *et al*., 1986).

MCE had the ability to significantly inhibit the force of contraction and had no significant effect on the frequency of oxytocin induced (OT) contraction and as stated earlier implies that MCE does not significantly influence the intrinsic electrical properties or the primary pacemaker activity that sets the rate of contraction initiation (Hector *et al*., 2010).

To investigate the interaction of MCE with extracellular calcium influx, MCE activity on high KCI was investigated. This was accomplished by applying high concentrations of KCl, which activates L-type VOCCs (Granger, Hollingsworth and Weston 1986; Niedergerke 1956), leading to a sustained depolarization of the tissues (Little, Teaf and Hurwitz 1985). This depolarization forces the VOCC channels into their open state, inducing a substantial and sustained influx of extracellular Ca2+ that is independent of any electrical rhythm or OT binding. This large increase in Ca2+ leads to a strong, sustained tonic contraction (Word and Shaul, 2020).

The ability of the MCE to inhibit this KCl-induced contraction is the definitive pharmacological evidence that suggests that MCE acts by interfering with VOCCs (Shynlova *et al*., 2024).

It is also important to know that the isolated uterine smooth muscle cell (myometrium) has two primary mechanisms for increasing intracellular Ca^{2+} the essential signal for contraction and they are: the Inflow from outside the cell (extracellular compartment) where Ca^{2+} enters the cell from

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the PSS via voltage-gated Ca^{2+} channels (the main source for sustained contraction, especially in labor) and the Release from internal stores (intracellular compartment) where calcium Ca^{2+} is released from the Sarcoplasmic Reticulum (SR), often triggered by agonists like oxytocin (via the IP3 pathway). (De Heus R *et al.,* 2009). To investigate whether MCE affects the intracellular release of Ca^{2+}, its activity was evaluated in a Ca^{2+}-free medium. This involved the use of EDTA to chelate extracellular calcium, thereby elucidating the mechanisms underlying MCE's action. In the absence of Ca^{2+} in the medium, OT still elicited a minor increase in spontaneous contractions.

If a contraction occurs at all in Ca^{2+}-free PSS, it suggests that the extract's most significant mechanism of action (its "potency") is directed towards extracellular Ca^{2+} influx rather than the intracellular calcium release. (Wray *et al* 2003). This is because the primary purpose of the Ca^{2+} free PSS test is to isolate the intracellular pathway. And if the extract is a strong inhibitor, it would completely block this isolated pathway. If the Contraction Was Completely Abolished in Ca^{2+}-Free PSS; It suggests that the extract prevents the agonist from working, i.e., the extract blocks the release of Ca^{2+} from the intracellular stores. This is because the only source of Ca^{2+} available to the cell in this medium is the internal store.

From the observation since MCE did not inhibit oxytocin- induced smooth muscle contraction in a Ca^{2+}-free medium it suggest MCE does not affect intracellular release of calcium. This finding in this study agrees with previous study conducted on the inhibitory effect of rutin on uterine contraction in non-pregnant mice (Uchendu *et al.,* 2025).

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CHAPTER 5

CONCLUSION

This study demonstrates that *Momordica charantia* extract (MCE) possesses significant tocolytic properties, evidenced by its ability to inhibit both spontaneous and agonist-induced uterine contractions in non-pregnant mice. The extract reduced the force of contractions in a dose-dependent manner without altering contraction frequency, indicating that its primary action lies in suppressing contractile strength rather than pacemaker activity.

Furthermore, the inhibitory effect of M. charantia leaf extract on both spontaneous and induced uterine contractions suggests a calcium-dependent mechanism. This mechanism likely involves the blockade of calcium influx from the extracellular compartment rather than the inhibition of intracellular calcium release, offering insight into the plant's traditional use and potential pharmacological targets. The

results of this study suggest that MCE could serve as a novel therapeutic agent for conditions characterized by excessive uterine contractions, preterm labor, or other spasm-related disorders.

5.1 Recommendations

Further studies are needed to fully elucidate the molecular mechanisms underlying its effects and to assess its safety and efficacy in clinical settings. This study lays a promising foundation for the development of MCE-based treatments aimed at improving uterine health.

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