INHIBITORY EFFECT OF *LAURUS NOBILIS* ON SPONTANEOUS AND AGONIST INDUCED UTERINE CONTRACTION IN NON-PREGNANT MICE

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A DISSERTATION SUBMITTED IN PARIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

TO

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research work titled "Inhibitory Effect of *Laurus nobilis* on spontaneous and agonist-induced uterine contraction" was carried out by Miss Nwachukwu Miracle Ugoma**,** with matriculation number PHA1908543 in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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DEDICATION

This work is dedicated to my loving family whose steadfast love and unwavering belief in me have been a constant source of strength and inspiration.

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I would like to begin by expressing my heartfelt gratitude to God for the strength, grace, and provision He has blessed me with throughout this research journey. Without His guidance, this endeavor would not have been possible.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATION

ATP	Adenosine triphosphate
AUC	Area under the Curve
CaM	Calcium calmodulin
Ca^{2+}	Calcium ions
$[Ca^{2+}]i$	Intacellular calcium ions
cAMP	Cyclic
cGMP	Cyclic guanosine monophosphate
COX	Cyclooxygenase
Cx	Connexin
DAG	Diacyl glycerol
ECC	Excitation-contraction coupling
EDTA	Ethylenediaminetetraacetic acid
EP1/EP2/EP3/EP4 receptors	Prostaglandin E2 receptor 1/Prostaglandin E2 receptor 2/Prostaglandin E2 receptor 3/Prostaglandin E2 receptor 4
FSH	Follicle Hormone
Gaq	G-protein activated alpha subunit
GC-MS	Gas chromatography-Mass spectrometry
GCPR	G-protein coupled receptor
GnRH	Gonadotrophin releasing hormone
HPLC	High performance liquid chromatography
HPO	Hypothalamic-pituitary-ovarian
IP3	Inositol 1,4,5 triphosphate
K^+	Potassium ions
KCl	Potassium chloride
kDa	Kilo Dalton
LH	Luteinizing hormone
LNE	*Laurus nobilis* extract
MAPK	Mitogen-activated protein kinase
MLCK	Myosin light chain kinase
MLC	Myosin light chain
MLCP	Myosin light chain phosphatase
NCX	Sodium/Calcium exchanger
NSAIDS	Non-steroidal anti-inflammatory drugs
OT	Oxytocin
OTR	Oxytocin receptor
PCOS	Polycystic ovarian syndrome
PG	Prostaglandin

PGE2	Prostaglandin E2
PGF2α	Prostaglandin F2 alpha
PIP2	Phosphatidylinositol 4,5-bisphosphate
PKA	Phosphokinase A
PKC	Protein kinase C
PKG	Protein kinase G
PLC	Phospholipase C
PMCA	Plasma membrane Ca^{2+}-ATPase
PSS	Physiological saline solution
RMP	Resting membrane potential
ROCK	Rho kinase
RyRs	Ryanodine receptors
SEM	Standard error of mean
SERCA	Smooth endoplasmic reticulum Ca^{2+}-ATPase
SR	Sarcoplasmic reticulum
UPS	Uninterrupted Power Supply
UV	Ultraviolet
VGCCS	L-type Voltage gated calcium channel

ABSTRACT

Laurus nobilis (Bay leaf) is traditionally used for culinary and medicinal purposes, including the management of pain, inflammation, and menstrual disorders. Its leaves and essential oil contain bioactive compounds such as 1, 8-cineole and eugenol with known smooth muscle effects, anti-inflammatory, and antioxidant properties. Some reports suggest possible reproductive and abortifacient effects of *L. nobilis*. However, its direct effect on uterine contractility is not well established.

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 swiss albino mice were used, and those in the estrous 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 equilibrium period with 0.5 g resting tension. Changes in isometric contractions were recorded using LabChart Software. The *L. nobilis* leaf extract (0.00625 - 0.4mg/ml) was added cumulatively to assess its effects on spontaneous, oxytocin-induced (14 nM), and high potassium-induced (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 ($p<0.05$).

L. nobilis leaf extract significantly and concentration-dependently reduced the amplitude and frequency of spontaneous uterine contractions and contraction induced by high KCl. It produced a slight, non-significant inhibition of oxytocin-induced contractions. In calcium-free medium, the extract markedly suppressed the frequency but only slightly reduced the amplitude of oxytocin-induced contractions.

These findings indicate that *L. nobilis* leaf extract exerts an inhibitory effect on spontaneous and agonist-induced uterine contractions, probably through mechanisms involving blockade of extracellular calcium influx and intracellular calcium release.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Uterine contractions are a basic physiological phenomenon that are important to female reproductive health because it is a necessary process in menstruation, parturition, and postpartum involution of the uterus. The mechanism that regulates this is a complicated interplay of hormonal, neural, and paracrine signals that regulate the excitability of myometrial smooth muscle cells (Aguilar and Mitchell, 2010). Despite being a well-tuned mechanism, dysregulated, abnormal or excessive uterine contractility may cause a range of gynecological and obstetric disorders, such as dysmenorrhea and preterm birth (Bano *et al*., 2023). All these conditions together pose a significant burden on female morbidity, reduced quality of life and in worst-case scenarios, there are severe adverse pregnancy outcomes, which have a health-related global impact.

Preterm labor, or the regular contraction of the uterine muscles along with the change of the cervix prior to 37 weeks of gestation, is still a tough challenge in the obstetric field. It is the leading cause of preterm birth, which has impacted around 15 million babies per year across the globe (Walani, 2020). These outcomes are severe, and complications of preterm birth are the most common causes of death in children and those below 5 years of age, causing more than 1 million deaths in children annually (Saalu *et al*., 2020; WHO, 2023). The financial cost is also quite overwhelming, as the expenses connected with intensive neonatal care and permanent disability impose enormous burden on the healthcare systems and families (Behrman *et al*., 2007).

Out of the framework of pregnancy, the pathophysiology of primary dysmenorrhea, one of the most frequent gynecological complaints is dysregulated myometrial activity translating as painful menstrual cramps, which is caused by uterine hypercontractility and ischemia caused by high levels of prostaglandins (Iacovides *et al*., 2015). It is extraordinarily common, with most populations

having up to 90 percent of adolescent girls and young women at risk. The adverse effects go beyond physical discomfort, often causing much disturbance of the normal life, such as school and work absenteeism, and being a key health concern about the well-being of women (Bano *et al*., 2023; De Sanctis *et al*., 2015).

The existing pharmacological therapies for excessive uterine contractions is mainly made up of tocolytic agents and analgesics. Examples of such tocolytic include calcium channel blocker (e.g., nifedipine), beta-adrenergic agonist (e.g., salbutamol), and oxytocin receptor antagonist (e.g., atosiban). In dysmenorrhea, the initial treatment is non-steroid anti-inflammatory drugs (NSAIDs) which suppress the production of prostaglandins (Proctor and Farquhar, 2006). The usefulness of these agents are affected by various restrictions. They are often linked to serious maternal and fetal side effects (e.g., cardiovascular effects of beta-agonists, hypotension by calcium channel blockers), contraindications, high cost, and reduced accessibility, especially in settings with limited resources (Haas *et al*., 2018; Bano *et al*., 2023). Moreover, most of them have a low potency of extending pregnancy and most of the synthetic medicines are not applicable in chronic diseases management when the long-term duration is needed.

The urgent demand to find safer, more efficacious, and more accessible uterine relaxants has led to an increased interest in the therapeutic benefits of natural products. An enormous and potentially abundant source of bioactive compounds are medicinal plants, many of which have a long history of application in traditional and ethnobotanical systems of managing reproductive health problems (Gruber and O'Brien, 2011). These phytocompounds can provide new modes of activity, which can even have better safety profiles.

Laurus nobilis is an attractive candidate to be explored with documented anti-inflammatory, antioxidant, and smooth muscle modulating activity in numerous experimental systems (Bano *et al*., 2023). A scientific assessment of whether it can inhibit uterine contractions could lead to production

of safer, more convenient, and culturally acceptable therapeutic options, thus filling a huge gap in the health of the women.

1.1 Uterine Physiology and Contraction

The uterus is a dynamic organ whose capacity to control and adjust its contractile activities lies at the center of almost all aspects of female reproduction such as transporting sperm, implantation, menstruation, gestation, and parturition (Aguilar and Mitchell, 2010). The functional muscle layer, the myometrium, has the extraordinary ability to exist in a state of relative quiescence through out most period of pregnancy only to change into a highly coordinated and contractile organ at the time of labor. The interaction of electrical activity, intracellular signaling dynamics, deep hormonal modulation, and intercellular communication is a complex interaction that controls this switch. Uterine contraction is a complicated process of which involves multiple stages and multidisciplinary team of contraction-controlling cells.

1.1.1 Myometrial Contraction Mechanisms.

The initiation of contraction in the smooth muscle cells (myocytes) of the uterus involves a process called excitation-contraction coupling, and this process solely relies on the increase in intracellular calcium concentration ($[Ca^{2+}]i$). Myocytes can generate spontaneous or stimulus-induced action potential that depolarize the cell membrane. This depolarization triggers the opening of L-type voltage gated calcium channels (VGCCs) that results in a rapid influx of extra-cellular Ca^{2+} (Wray *et al*., 2021). This influx is the major cause of contraction. Intracellularly, Ca^{2+} attaches itself to the calmodulin (CaM) calcium-binding protein. The resulting Ca^{2+}-CaM complex triggers myosin light chain kinase (MLCK) which in turn phosphorylates the 20 kDa regulatory light chain of myosin II (MLC20). This is the key molecular switch of the phosphorylation that allows the myosin head to engage with actin filaments, initiating the process of cross-bridge cycling and the production of

contractile force (Arrowsmith and Wray, 2014).

Besides the Ca^{2+} influx from the extracellular space, intracellular stores may be mobilized. Strong uterotonic agonists, including oxytocin and prostaglandin F2α (PGF2α) interact with their respective receptor Gq/11-protein coupled receptors on the surface of the myocytes. This stimulates the enzyme phospholipase C (PLC) that breaks down membrane phospholipids to inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 spreads to its receptors in the sarcoplasmic reticulum (SR), activating the release of stored calcium and amplifying the rise in cytosolic Ca^{2+}, which is called calcium-induced calcium release (Aguilar and Mitchell, 2010; Taggart and Morgan, 2007).

Absolute concentration of $[Ca^{2+}]i$ is not the ultimate determinant of the force of myometrial contraction. The contractile apparatus may be sensitized to Ca^{2+} allowing for a more sustained contraction at a given level of Ca^{2+}. This modulation mainly occurs by the inhibition of myosin light chain phosphatase (MLCP) which is the enzyme that inhibits the action of MLCK by dephosphorylating MLC20 and inducing relaxation. RhoA/Rho-kinase (ROCK) signaling cascade is the crucial calcium sensitization pathway. Some agonists such as oxytocin are able to stimulate this pathway, which causes ROCK-mediated phosphorylation and inhibition of MLCP maintaining the phosphorylated state of the myosin thus increasing the contractile force (Aguilar and Mitchell, 2010; Somlyo and Somlyo, 2003). Also, the phosphorylation of CPI-17, which is a potent endogenous inhibitor of MLCP phosphorylated by DAG-activated Protein Kinase C (PKC) may also play a role in sensitization (Wray *et al*., 2001).

1.1.2 Relaxation Mechanisms

Relaxation is an active process, which involves cytosolic Ca^{2+} regulation by decreasing it to basal levels and myosin dephosphorylation. A number of ATP-dependent pumps take Ca^{2+} out of the cytosol: sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps Ca^{2+} into the SR, and the

plasma membrane Ca^{2+}-ATPase (PMCA) and the Na^{+}/Ca^{2+} exchanger (NCX) pump it out of the cell (Tribe *et al*., 2000).

Moreover, the relaxation is actively facilitated through signaling pathways that enhance cyclic nucleotides. The agonists stimulate the adenylyl cyclase by activating β_2-adrenergic receptors (e.g., terbutaline) and increase the levels of intracellular cyclic AMP (cAMP). Others activate soluble guanylate cyclase to form cyclic Guano sine monophosphate (cGMP) with the help of nitric oxide (NO). Both cAMP and cGMP activate their corresponding protein kinases, PKA and PKG. These kinases enhance relaxation via various processes which include dephosphorylating and inhibiting MLCK, activating MLCP and stimulating Ca^{2+} reuptake into the SR thereby desensitizing the contractile machine to calcium (Word *et al*., 2007; Aguilar and Mitchell, 2010).

1.2 The Uterus: Human and Mice.

1.2.1 Human Uterus

The human uterus can be described as a hollow, pear shaped muscular organ found in the central part of the female pelvis located posterior to the bladder and anterior to the rectum. It plays a crucial role in the menstrual cycle, implantation, conception of fetus and paturition. The mean length, width and thickness of a non-gravid uterus is about 7-8 cm, 4-5 cm, and 2-3 cm, respectively, varying significantly with age, parity and hormonal status (Karunaharamoorthy and Mytilinaios, 2023). The human uterus is structurally divided to form four major parts. The upper dome-shaped superior part of the uterine tubes (tubal ostia) is called the fundus and its thick muscular wall provides the primary force of contraction in the later stages of labor. The uterine cavity is situated in the central, triangular part known as the corpus where the implantation of the blastocyst and further development of the fetus takes place. The corpus becomes narrower from the fundus to the isthmus which is a constrictive part that is about 1 cm long and interposes the corpus and the cervix. This area becomes

thin and elongated during late pregnancy to create the lower segment of the uterus, which is majorly a physiologic sphincter during childbirth (Thompson, 2025). This lower part or the cylindrical part that sticks out in the superior part of the vagina consists of the supravaginal cervix and vaginal part (portio vaginalis). The cervical canal connects the uterine cavity to the vaginal lumen via the internal and external os, serving as an entry point for sperm and an infection barrier (Hoagland and Kapoor, 2025).

Several supportive ligaments provide positional stability for the uterus. The broad ligament is a double layer of peritoneum that covering the uterus and encloses the uterine vessels. The round ligaments run through the inguinal canals through the uterine horns to the labia majora, maintaining an anteverted position. The uterosacral ligaments hold the cervix back to the sacrum, and the cardinal (or transverse cervical) ligaments which are extremely strong, support the uterine arteries and veins at the base of the broad ligament (Thompson, 2025).

Vascular supply is mainly from the two paired uterine arteries which are branches of the internal iliac arteries. These arteries ascend along the lateral wall of the uterus in the broad ligament and branch out into arcuate and radial arteries which branch into the myometrium. They end up becoming important spiral arteries that supply the functional layer of the endometrium and are important for both menstruation and the development of the maternal side of the placenta (Karunaharamoorthy and Mytilinaios, 2023; Pijnenborg *et al*., 1997).

Furthermore, the uterine wall is a trilaminar structure that consists of three concentric layers. These layers, arranged from the innermost to the outermost, are the endometrium, myometrium, and perimetrium. The perimetrium (Serosa) is the outermost thin layer of mesothelium (visceral peritoneum) which envelops the fundus, the majority of the body and provides a smooth slippery lining minimizing friction with other body organs in the pelvis. The myometrium comprises of the bulk of the uterine mass. It is the thickest layer and It primarily consists of complicated, interweaving

groups of smooth muscle fibers (myocytes) that form a functional syncytium. Although they are usually described as having an inner circular and outer longitudinal orientation, the fibers are organized as a mesh, which enables the uterus to contract in a uniformly in all the dimensions to push out the fetus at the term. These cells undergo hypertrophy (increase in size) and hyperplasia (increase in numbers) during pregnancy (Ferenczy, 2005). The endometrium serves as the inner mucosal lining that is further divided into two layers with the stratum functionalis being the most superficial layer which is highly secretory in response to progesterone influence to support implantation and shed during menstruation should fertilization fail to take place. The stratum basalis is the deeper regenerative layer that is not lost and contains the stem cells which form a new functionalis after every menses (Ferenczy, 2005; Brosens *et al*., 2011). The human uterus is designed to accommodate a single fetus during gestation, with a gestational period of approximately 40 weeks. Its contractile function is tightly regulated to ensure proper timing of menstruation, implantation, and delivery.

1.2.2 The Mouse Uterus

The mouse uterus is a bicornuate reproductive organ that is specialized for multiparous (litter bearing) gestation. It consists of a very short uterine body which then splits cranially to two elongated uterine horns. Towards the back, the body of the uterus narrows into one cervix, linking to the vagina, which means both horns have the same cervical canal (NIH, 2024). This anatomical arrangement makes it useful as a preclinical model.

The uterine horns are two elongated, thin-walled tubular cornua on which blastocyst implantation, placentation and fetal development take place, measuring about 15-20 mm in an adult making it is possible to give birth to a litter which usually includes 6-12 pups. The horns are attached to the abdominal cavity via the broad ligament (mesometrium) (NIH, 2024). The uterine body called the corpus refers to the short central segment (2-4 mm) where the two horns meet. (Elmore *et al*., 2020).

Between the corpus and the vagina is a rigid, fibro-muscular junction referred to as the cervix. In mice, a single cervical canal opens into the vaginal lumen, its dense stroma and smooth muscle enables it to function as a barrier to ascending infection (NIH, 2024). The vagina itself is a fibro-muscular tube lined by stratified squamous epithelium and its morphology changes between the various phases of the estrous cycle. This allows vaginal cytology (based on presence of cornified vs nucleated epithelial cells vs leukocytes) to be used as a dependable method for staging the cycle and matching it with the state of uterine function (Caligioni, 2009).

Histologically, the mouse uterus is composed of several layers, the endometrium, myometrium, and perimetrium, each of which has a specific role in its function and are consistent with mammalian uterine structure. The endometrium is undergoes rapid and hormonally driven proliferation and regression during the 4-5 day estrous cycle. It is made up of the luminal epithelium comprising of simple columnar cells lining the uterine lumen and regenerating after each estrous cycle, a glandular epithelium made of simple tubular glands that extend from the luminal surface into the stroma of luminal epithelium, and a highly cellular connective tissue stroma. (NIH, 2024). Stromal cells in reaction to implantation undergo a significant differentiation termed as decidualization which has been well examined in the mouse model (Huet-Hudson *et al*., 1990). Unlike the human uterus, the myometrium is visibly divided in two layers; an inner round smooth muscle layer which is a circular layer of inner smooth muscle, and a thinner outer longitudinal smooth muscle layer. This structure is comparable to the gastrointestinal tract and produces coordinated peristaltic and segmental contractions required to separate embryos along the horns and for their expulsion during birth (Nakajima *et al*., 2020). The perimetrium refers to a serosal lining that adorns the outer surface of the uterine horns and body.

1.3 Uterine Cycle: Humans and Mice

The female reproductive system is characterized by its remarkable cyclical nature, in preparation of

the uterus for potential pregnancy in a rhythmic and predictable manner. This process of regeneration is referred to as menstrual cycle in human beings. The similar process in rodents is referred to as the estrous cycle. Although both the cycles follow the same neuroendocrine axis, they vary greatly in terms of length, endometrial fate, and external expression, in accordance with their different reproductive strategies.

1.3.1 The Human Menstrual Cycle

The human menstrual cycle is a complex and recurring process of coordinated endocrine and endometrial processes events that are meant to prepare the uterus to blastocyst implantation. Its duration is usually 21 to 35 days with an average length of 28 days. The hypothalamic-pituitary-ovarian (HPO) axis is a complex system of feedback that controls the cycle and elicits the coordinated release of the hormones gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone (Jabbour *et al*., 2006; Kumar and Singh, 2025).

The hypothalamus at the summit of this axis secretes GnRH in a pulsatile manner, which inhibits the receptor desensitization of the anterior pituitary. This GnRH signal causes the pituitary to secrete the gonadotropins, FSH and LH. FSH starts to proliferate a group of ovarian follicles and induce the granulosa cells to produce estradiol. When the dominant follicle is formed, a combination of negative and positive feedback on the pituitary and hypothalamus arise due to the increase in estradiol levels, and the mid-cycle LH storm becomes the end result leading to ovulation (Reed and Carr, 2018). The positive or negative feedback mechanism that is formed between the ovarian steroids (estradiol and progesterone) and GnRH, FSH, and LH secretion makes the cycle self-regulating and cyclical (Lutsenko, 2019; Jabbour *et al*., 2006).

The cycle phases include the following:

I. **Menstrual Phase (Day 1-5)**: The menstrual phase is triggered by the dramatic drop in the levels of progesterone and estrogen which occur after degeneration of corpus luteum during a non-conception cycle. The hormonal withdrawal causes the manifestation of strong vasoconstriction of endometrial spiral arteries, ischemia of the tissues, hypoxia, and the formation of inflammatory mediators. The following loss of the necrotic stratum functionalis leads to menstrual bleeding (Critchley *et al*., 2020). The expulsion of this endometrial tissue is achieved by the contractions of the uterus under the influence of the synthesis of the local prostaglandins (especially PGF2α), and it is the main reason for menstrual pain, or dysmenorrhea (Iacovides *et al*., 2015; Rezaeizadeh *et al*., 2016).

II. **Follicular (Proliferative) Phase** (Days 6-13): After menstruation, ovarian follicle growth and maturation (folliculogenesis) is triggered by increased FSH levels. The chosen dominant follicle secretes rising levels of estradiol, which is the major mitogen of the endometrium. The endometrial stromal and epithelial cells of the remaining stratum basalis proliferate rapidly, under the impact of estradiol, to restore the lost functional layer, and make it thicker. Estradiol also increases the progesterone receptors in the endometrium, which consequently prepares the endometrial tissue to the next secretory phase (Lutsenko, 2019; Giudice, 2004). At the same time, cervical mucus get thinner, more alkaline and has a spinnbarkeit (stretchability), forming a more receptive environment for sperm transportation.

III. **Ovulation (Day -14):** A prolonged rise in circulating estradiol of about 48 hrs alters the negative feedback of the HPO axis to positive feedback, and a tremendous burst of LH secretion of the pituitary follows. This LH surge is the ultimate stimulus that causes the development of the prevailing follicle, its rupture, and the detachment of the mature oocyte (Jabbour *et al*., 2006; Kumar and Singh, 2025).

IV. **Luteal (Secretory) Phase** (Days 15-28): Ruptured follicle develops into the transient endocrine

gland known as the corpus luteum secreting large amounts of progesterone and moderate amounts of estrogen. The hormone prevalent in this phase is progesterone and it acts upon the estrogen-primed endometrium inhibiting proliferation and triggers extreme differentiation. It transforms the lining to the richly, vascularized receptive tissue, characterized by tortuous, glycogen-secreting glands and edematous stroma. This create the window of implantation, a phase during which the endometrium is open to an implanting blastocyst. In the absence of fertilization and implantation, the corpus luteum degenerates, the levels of progesterone and estrogen fall and the cycle restarts with menstruation (Critchley *et al*., 2020).

Throughout this entire cycle, the myometrial contractility varies in a predictable manner. It is characterized by high-frequency, low-amplitude contractions during the proliferative phase, which change to more powerful, fundus-to-cervix directed contractions around ovulation to help in sperm transport. During the luteal phase, which is dominated by progesterone, the contractile process is inhibited to ensure that the uterus becomes quiescent so as to provide an environment conducive to possible implantation. It is then followed by another peak of this contractile activity during menstruation to help exfoliate endometrium (Lyons *et al*., 2020).

1.3.2 The Mouse Estrous Cycle

The mouse estrous cycle is a rapid process typically lasting only 4-5 days, making it an efficient model for reproductive research. Like the human cycle,it is governed by the HPO axis and pulsatile GnRH from the hypothalamus drives pituitary secretion of LH and FSH. It is divided into four distinct stages- proestrus, estrus, metestrus and diestrus each marked by hormonal fluctuations and cytological changes in the vaginal epithelium(Biology Insights, 2025).

The cycle phases include the following:

I. **Proestrus** (<24 hours): This is equivalent to the follicular phase. Rising FSH promotes antral follicle growth and increases estradiol production by granulosa cells. This stimulates endometrial

proliferation, follicular development and uterine contractility begins to increase in preparation for mating. Vaginal smears shows predominance of nucleated epithelial cells.

II. Estrus (12-48 hours): This phase is defined by peak estrogen levels which induce sexual receptivity and ovulation in which oocytes are released into the oviduct. The vaginal epithelium cells become fully cornified and smears show a predominance of anucleated, keratinized squamous cells. Uterine contractions are heightened and coordinated, facilitating sperm transport.(NIH, 2024).

III. **Metestrus** (~24 hours):This transitional phase immediately follows ovulation. The corpora lutea starts to form, and progesterone levels begins to rise while estrogen levels falls sharply. The vaginal smear is mixed, characterized by the presence of both cornified cells and leukocyte. Uterine activity begins to decline as the influence of progesterone increases.

IV. Diestrus (48-72 hours): Equivalent to the luteal phase, it is dominated by high progesterone levels from the mature corpora lutea, maintaining a quiescent uterine state.The vaginal epithelium is thin, and leukocytes predominate in vaginal smears. The uterus remains in a resting phase until the corpora lutea regress, at which point the cycle returns to proestrus. (Ajayi and Akhigbe, 2020).

Mice do not menstruate. Instead, the endometrial lining is rapidly reorganized and reabsorbed if pregnancy does not occur. The distinct changes in vaginal epithelium provides a tool for researchers. Simple vaginal cytology is routinely used to stage the estrous cycle, allowing researchers to correlate uterine contractility with the animal's hormonal status(Elvis-Offiah *et al*., 2022).

Figure 1.1: Appearance of the vagina in different phases of the estrous cycle of a swiss albino strain mouse. (A)- Proestrus, (B)- Estrus, (C)- Metestrus, (D)- Diestrus (Ekambaram *et al*., 2017).

1.4 Comparison Of The Human and Mice Uterus

Although the human and mouse uteri both have a common embryologic development and share the same trilaminar structure, it demonstrates differences in gross morphology, physiology, and reproductive strategy. The greatest anatomical difference is the simplex human uterus, a single pear-shaped organ, which is fitted to gestate a single pregnancy, while the bicornuate mouse uterus, with two long horns is adapted to carry multiple pregnancies of litter.

Their cyclical also differs significantly. Human females experience an average menstrual cycle of 28 days with a resultant shedding of endometrial stratum functionalis (menses) in case of implantation failure. By comparison, menstruation-free 4-5 day estrous cycle occurs in mice; the endometrial tissue is quickly resorbed and restructured to start a new cycle (Ferenczy, 2005; NIH, 2024).

A major divergence at the histological level is observed at the myometrial architecture. Human myometrium is made up of intricate and crossing bundles of smooth muscle fibers that create a functioning mesh, which is perfect in creating powerful and uniform contractions that are used to push out one fetus. However the mouse myometrium is well differentiated into very fine inner circle and outer longitudinal smooth muscle layer. This bi-laminated structure enables the peristaltic-like motions needed to evenly separate embryos along the uterine horns and to force multiple pups out sequentially during birth (Nakajima *et al*., 2020). There are additional differences in mode of implantation that is deeply interstitial in humans and eccentric in mice.

Nevertheless, in spite of these important anatomical and physiological anomalies, underlying cellular and molecular processes that mediate smooth muscle contraction are very similar in the two species. Key processes such as excitation-contraction coupling, central role played by calcium signaling, and

function of major receptors, such as oxytocin receptor are shared. This is what makes the isolated mouse uterine horn an invaluable ex vivo tool. It enables the direct explanation of pharmacological actions of new molecules on myometrical contractility and offers a basis of information that can be used in the future in further translational experiments in human uterine physiology.

1.5 Pharmacological Agents Affecting Uterine Contraction

Pharmacological agents have the ability to manipulate the contractile state of the uterus in a very precise manner. The contraction-stimulating drugs are called uterotonics, and those ones that inhibit or suppress contractions are referred to as tocolytics (Fouche-Camargo, 2022; Ernst, 2021). These agents act on the various molecular pathways that regulate the functioning of the myometrium.

1.5.1 Uterotonics

Modern obstetrics cannot do without uterotonics, which play a leading role in induction or augmentation of labor and prevention and control of postpartum hemorrhage (PPH) by maintaining sufficient uterine tone after birth (Mousa *et al*., 2014).

1.5.1.1 Oxytocin

This is a nonapeptide hormone, which is the first major endogenous and therapeutic uterotonic. It is binds to Gq/11-linked oxytocin receptors that are highly increased in the myometrium towards term by estrogen. Its stimulation triggers the PLC/IP_3/DAG pathway that causes strong Ca^{2+} release out of the SR and depolarization of the contractile apparatus (Arrowsmith and Wray, 2014). This causes rhythmic, synchronized uterine contractions that are characteristic of labor.

1.5.1.2 Prostaglandins (e.g., PGE_2, PGF2α)

These are lipid autacids that play an important role in uterine activity. They are synthesized locally using arachidonic acid, by cyclooxygenase (COX) enzyme, especially COX-2 which is upregulated during labor and they activate certain G-protein coupled receptors (EP and FP receptors). PGF2α is a strong activator of myometrial contraction through Gq/PLC /IP_3 pathway, but PGE_2 has a significant role in both contraction and cervical ripening required to advance labor (Riciotti and FitzGerald, 2011).

1.5.1.3 Ergot Alkaloids (e.g., Ergometrine)

These are potent uterotonics derived from the fungus *Claviceps purpurea.* They are partial agonists of a-adrenergic and serotonergic receptors and lead to powerful and sustained (tetanic) uterine contraction. This non-physiological contractions makes them not suitable for inducing labor but very useful in the treatment of post partum haemorrhage as a result of uterine atony (de Groot, 1996).

1.5.2 Tocolytics

Tocolytics have a clinical application in preventing preterm labor and extending pregnancy to the level of fetal maturation or administering of antenatal corticosteroids to enhance neonatal outcomes (Haas *et al*., 2012).

1.5.2.1 β2-Adrenergic Agonists (e.g., Terbutaline, Ritodrine)

These are agonists that bind to myometrial cells to stimulate the cAMP/PKA signaling pathway at Gs-coupled β_2-adrenoceptors. As mentioned above PKA facilitates relaxation by inhibiting MLCK, facilitating the Ca^{2+} uptake process and hyperpolarizing the cell membrane to decrease Ca^{2+} influx. Their application can be limited by side effects of the medication on maternal cardiovascular and in the long term receptor desensitization (Aguilar and Mitchell, 2010).

1.5.2.2 **Calcium Channel Blockers** (e.g., Nifedipine)

Since the contraction of myocardial muscle is very much influenced by the influx of extracellular Ca^{2+}, it is a effective tocolytic strategy to block L-type voltage-gated calcium channels. Nifedipine inhibits these channels directly making it impossible to increase intracellular calcium concentration necessary to depolarize the cell and stimulate contraction. In most areas, it is a primary-line tocolytic because it is effective and has a good side-effect profile (Lee, 2025; King *et al*., 2003).

1.5.2.3 **Oxytocin Receptor Antagonists** (e.g., Atosiban)

These are structural analogues of oxytocin, which block the receptor of oxytocin. Their inhibition of the downstream signaling cascade by blocking oxytocin binding reduces the frequency and strength of uterine contractions. They are highly specific to oxytocin receptor hence less maternal side effects than other tocolytics (Papatsonis *et al*., 2005).

1.5.2.4 Nitric Oxide (NO) Donors (e.g., Glyceryl Trinitrate): These substances release NO that stimulates the cGMP /PKG pathway to cause radical relaxation of smooth muscles. Clinically, they are not intended for chronic use but are perfect for acute uterine relaxation (e.g., in complicated deliveries) since their vasodilatory effects on the body system may entail severe headaches and hypotension (Luo *et al*., 2021).

1.5.2.5 **Magnesium Sulfate**

Although this has been used as a tocolytic historically it has been found to be weak. It is believed to be a physiological calcium antagonist, competing with Ca^{2+} at VGCCs as well as at intracellular binding locations. Its major contemporary application in obstetrics is in fetal neuroprotection during imminent preterm delivery and seizure prevention during pre-eclampsia (Tonick and

Muneyyirci-Delale, 2016).

1.5.2.6 Prostaglandin Synthesis Inhibitors (NSAIDs, e.g., Indomethacin): These medicines suppress the COX enzymes, hence preventing the synthesis of contractile prostaglandins. Although useful as tocolytics, they should not be used after 32 weeks of gestation because of the risk of untimely closure of the fetal ductus arteriosus and oligohydramnios (abnormally low amniotic fluid) (Koren *et al*., 2006).

1.6 Ethnomedicine

Ethnomedicine is a wide term which involves the culturally specific knowledge, beliefs, theories and practices employed by indigenous and local people to keep themselves healthy, prevent disease as well as curing disease (Acharya and Shrivastava, 2008). It is a holistic healing system that tends to incorporate physical, spiritual, and social welfare in contrast to the typically purely biomedical orientation of Western medicine. It is an interdisciplinary field in nature. An example is medical anthropology which offers the means to record the rich sociocultural context of healing, how the illness is defined and viewed, who and who are the recognized healers (e.g., the shaman, the herbalist, the midwife), and how the complex therapeutic knowledge is passed down through the generations, usually by oral tradition (Heinrich and Gibbons, 2001).

Ethnomedicine is not only valuable as a source of cultural documentation, but also forms an important critical beginning point of contemporary scientific research. The ethnomedical knowledge has given historically invaluable leads, as far as the discovery of major pharmaceuticals is concerned. Such an ethno-directed approach to drug discovery has been much more effective than random screening of natural products. Famous ones have been discovery of Aspirin (Acetylsalicylic Acid), a

derivative of salicin, which is present in willow bark (Salix spp.) present in many cultures around the world, including ancient Egyptians and Greeks, used to relieve pain and fever since the ancient times.

Artemisinin is sweet wormwood (Artemisia annua) which is a common ingredient in Chinese traditional medicine on the treatment of fevers. It has now become a pillar of contemporary malaria treatment, an invention that won the 2015 Nobel Prize in Physiology or Medicine (Tu, 2011). Galantamine is an alkaloid derived by using snowdrops (Galanthus spp.), which was initially used in folk medicine in Europe against neurological conditions but is currently an approved drug to manage the symptoms of Alzheimer's disease (Fabricant and Farnsworth, 2001; Gurib-Fakim, 2006).

The scientific field which comes as a result of the investigation of ethnomedicine is known as ethnopharmacology. It refers to the transdisciplinary research of the physiological activity of plant, animal and other natural substances, which are the subjects of traditional medicines (Elisabetsky and Etkin, 2003). It tries to systemically report and confirm the usage, the effectiveness, and the safety of these remedies using strict scientific approaches, effectively establishing a link between anthropological understanding and contemporary pharmacology (Yeung *et al*., 2020).

Ethnopharmacological approach has four major objectives:

I. **Ethnobotanical And Anthropological Documentation**: This is observed fieldwork to document the traditional healing with specific emphasis on the identification of the exact species, the specific part of the plant (leaf, root, bark) used, the preparation (decoction, infusion, tincture), dosage and the cultural context of its usage.

II. **Phytochemical Characterization:** After a plant has been identified, its crude extracts are then

subjected to phytochemical analysis to identify the chemical profiles as well as isolate the bioactive compounds (e.g., alkaloids, flavonoids, terpenoids, saponins) that explain the noted therapeutic activities.

III. **Pharmacological Assessment:** The extracts and isolated compounds are assessed using established *in vitro* bioassays (e.g. organ bath tests of smooth muscle, cell culture tests) and *in vivo* animal models, to confirm their traditional applications and clarify their mechanisms of action (e.g., antispasmodic, anti-inflammatory, antimicrobial).

IV. **Safety and Toxicological Assessment:** A critical assessment is to analyze the possible toxicity of the remedies using acute and chronic toxicity tests to determine safe dosage limits, contraindications and record possible adverse effects.

Ethnopharmacology offers a highly effective model to help inform traditional knowledge into an evidence-based treatment alternative by combining ethnobotany, medical anthropology, pharmacognosy, and pharmacology (Heinrich and Gibbons, 2001).

1.7 Medicinal Plants in Reproductive Health.

Medicinal plants have been used in the women's reproductive healthcare since time immemorial, across all cultures. It is especially true in low- and middle-income nations, where traditional medicine often becomes the leading type of medical care since the cost or accessibility of the conventional pharmaceuticals is extremely high (WHO, 2019). Plants are used to treat a large range of conditions, such as dysmenorrhea, premenstrual syndrome, infertility, abnormal uterine bleeding, and pregnancy and childbirth complications (Brahmi *et al*., 2025; Osawaru and Ogwu, 2024).

Dysmenorrhea and preterm birth are both essentially associated with the inability to regulate uterine contractility. One of the primary health problems of the whole world is preterm labour due to premature uterine contractions, whereas dysmenorrhea, the resultant effect of painful uterine contractions, is a primary cause of recurrent short-term school and work absenteeism in young women (Iacovides *et al*., 2015). Surveys on ethnobotanical have shown that the world is rich in pharmacopeia of plants that are used to regulate these processes. An example is Vitex agnus-castus (Chasteberry) which is used to treat premenstrual symptoms, and Zingiber officinale (Ginger) which is well used in dysmenorrhea wherein studies indicate that the plant inhibits the synthesis of prostaglandins (Ozgoli *et al*., 2009).

Investigating these traditional remedies, scientists can discover the new bioactive compounds and mechanisms to create effective interventions to treat the conditions associated with uterine contractility (Bafor *et al*., 2019). In other places, such as southern Nigeria, ethnobotanical surveys have documented dozens of such species used by the traditional healers in caring about female reproductive health, such as Newbouldia laevis and Phyllanthus amarus (Osawaru and Ogwu, 2024). Although these remedies are low-cost and culturally viable, the absence of standard dosing, quality guarantees and strict demonstration of efficacy usually slows down the use of such remedies, and this makes ethnopharmacological study one of the most important in this field (Gurib-Fakim, 2006).

1.8 *Laurus nobilis*: An Overview

Laurus nobilis L., commonly known as bay leaf or sweet laurel, is an evergreen shrub native to the Mediterranean region and belongs to the Lauraceae family (Khodja *et al*., 2023). The binomial name *Laurus nobilis* L. was first validly published by Linnaeus in 1753. The species is dioecious, with

male and female flowers borne on separate individuals (Swamy, 2013).

Laurus nobilis is an evergreen aromatic tree or large shrub, typically growing 7-18m. It is native to the Mediterranean basin- southern Europe, North Africa, and western Asia. The plant thrives in maquis and garigue scrublands on calcareous well-drained soils. It tolerates drought, mild frost (down to -10C), and shade, and has been widely cultivated in temperate regions worldwide for culinary, ornamental, and medicinal use.

Its leaves are simple, entire, glossy dark green, and glabrous. They measure 6-12cm in length and 2-4cm in width, with an undulating margin. Petioles are short (5-10mm) and leave a characteristic scar when detached. The foliage emits a spicy fragrance when crushed, due to essential oils concentrated in leaf glands (Swamy, 2013; Khodja *et al*., 2023).

Cultivation is by seed or semi-hardwood cuttings. Growth is slow, with commercial leaf harvest beginning 3 to 5 years after planting. Leaves are harvested semi-annually and dried for storage. In addition to culinary seasoning, bay leaves are used in perfumery, cosmetics, and traditional medicine for their anti-spasmodic, anti-inflammatory, and antimicrobial properties.

Table 1: Taxonomic classification of *Laurus nobilis*

Sub-kingdom	Viridiplantae
Infra-kingdom	Streptophyta
Super-division	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Super-order	Magnolianae
Order	Laurales
Family	Lauraceae

Genus	Laurus
Species	*Laurus nobilis* L.

Source: Integrated Taxonomic Information System (ITIS, 2025)

Figure 1.2: Laurus nobilis plant is shown (International journal of secondary metabolite).

1.8.1 Phytochemical Composition of *Laurus nobilis.*

Laurus nobilis has a complex and diverse number of volatile and non-volatile secondary metabolites in its leaves which is responsible for its well-described organoleptic and pharmacological characteristics. The presence of modern methods of analysis, such as gas chromatography-mass spectrometry (GC-MS) of volatile compounds and high-performance liquid chromatography (HPLC) and a variety of detectors of non-volatile components allowed profiling these compounds in a holistic manner (Alejo-Armijo *et al*., 2017; Mrabet *et al*., 2024).

Essential oil is the most characteristic chemical component of bay leaf and is generally considered to be 1-4% of the dry mass of the leaf and comprises of a complex mixture of more than 150 individual substances. The composition of this oil is dominated by oxygenated monoterpenes, with 1,8-cineole (eucalyptol), being the most abundant component of the oil composition, most of the time it accounts to 25-50% of the oil composition (Carcel *et al*., 2013). Other significant constituents which characterize the oil aromatic and bioactive profile are the monoterpene hydrocarbon sabinene (5-15%), the ester a-terpinyl acetate (3-10%), and the alcohols linalool (2-6%) and terpinen-4-ol. It also contains the phenylpropanoid eugenol (1-5%) that is an established smooth muscle relaxant and analgesic. Other minor yet synergistically significant components, including methyl eugenol, a-pinene, b-pinene, and different sesquiterpenes have also been well-documented (Khodja *et al*., 2023; Alejo-Armijo, Altarejos and Salido, 2017).

In addition to its essential oil, *L. nobilis* is also a good source of non-volatile phenolic compounds, potent antioxidants. HPLC-UV-MS analyses have determined various hydroxycinnamic acids such as ferulic, caffeic and p-coumaric acid and simple phenolics such as protocatechuic acid. Depending on the geographical location, the time of harvest, and the mode of extraction, the total phenolic content may vary widely (between 120 and 260 mg of gallic acid equivalents per gram of dry leaf) (Mrabet *et al*., 2024). Moreover, the leaves have a large concentration of flavonoids, including flavonols and quercetin and kaempferol, and flavone apigenin, commonly in the form of glycoside. They are famous in terms of their strong antioxidant and anti-inflammatory properties as well as smooth muscle-modulating properties, with some of them, such as quercetin, having been demonstrated to suppress smooth muscle contraction via the calcium channel opening and potassium efflux (Capasso *et al*., 2010; Khodja *et al*., 2023).

Other types of bioactive substances are also found in smaller amounts, but they play a role in overall pharmacology of the plant. Minor alkaloid constituents, such as noraporphine, aporphine derivatives, have been reported, but generally only in concentrations less than 0.1 percent of the dry leaf weight. More importantly, the non volatile terpenoids, including sesquiterpene lactones (e.g. costunolide) were detected. The use of this group of compounds is also famed to possess a strong anti-inflammatory property, which is frequently achieved by blocking the NF-kB signaling pathway (Chadwick *et al*., 2013). There is also the presence of proanthocyanidins (condensed tannins) that are characterized by powerful antioxidant and free-radical scavenging effects (Alejo-Armijo, Altarejos and Salido, 2017). Although the major pharmacology impacts are caused by the secondary metabolites, proximate analyses have validated the nutritional content of bay leaf. It has a high level of dietary fiber (10-35%), protein (2-4%), lipids (7-10%), and a lot of mineral ash content (6-8%)

(Adoloju *et al*., 2019). High levels of potassium, calcium and magnesium, which are essential electrolytes of normal muscle activity, including smooth muscle quiescence, and trace minerals, such as iron make its mineral profile interesting.

1.8.2 *Laurus nobilis* in Reproductive Health

Based on the ethnobotanical record, *L. nobilis* has a long history of use in traditional medicine systems in treating conditions associated with pain and inflammation, some of them related to the health of women. Nevertheless, its impact on the reproductive system is not scientifically justified at the initial stage.

Some initial studies can be found regarding the context of male reproductive health. Animal models studies indicate that it has a protective effect against testicular damage. On the same note, Akunna *et al*. (2013) have depicted that an extract of bay leaf enhanced the sperm quality in rats whose varicocele had been induced experimentally, which the authors attribute to the high antioxidant phytochemical content of the extract.

On the other hand, the studies of its direct effect on the female reproductive health is limited and presents a more complicated picture. Bay leaf infusions have also been mentioned in folk medicine to treat menstrual cramps (dysmenorrhea). One way that this classical use is mechanistically possible is due to the known anti-inflammatory and antispasmodic constituents of the plant. The anti-inflammatory action of flavonoids and sesquiterpene lactones may regulate the production of prostaglandins, which are one of the major causes of uterine hypercontractility in dysmenorrhea,

while antispasmodic action on myometrial smooth muscle of compounds such as 1,8-cineole and eugenol may be due to direct relaxation (Times of India, 2025; Carcel *et al*., 2013). But it is very important to add that no rigorous and peer reviewed clinical trials have been done on *L. nobilis* with this particular indication.

Moreover, research points to the necessity of precautions especially in pregnancy. A study carried on pregnant rats *in vivo* revealed that high doses of bay leaf extract had negative dose-dependent impacts on maternal reproductive health, fetus development, and hormonal levels (Akunna *et al*., 2025). This highlights the extreme significance of dose and safety testing of any phytotherapeutic agent. Antioxidant and anti-inflammatory activity of flavonoids and polyphenols has the potential to positively impact female reproductive health by alleviating the disorders related to oxidative stress, including endometriosis or polycystic ovary syndrome (PCOS) (Al-Safi, 2021), although there are no direct studies on how *L. nobilis* can make an impact on these conditions.

1.9 Research Questions, Aims and Objectives

1.9.1 Research Question

The following question will guide our research:

1. Does ethanol extract of *L. nobilis* affect the contraction of isolated non-pregnant mice uterus in a dose-dependent manner?

2. What is the possible mechanism of action of the ethanol extract of *L. nobilis* on uterine contractility?

1.9.2 Study Hypothesis

Based on the existing literature and preliminary observations as studied above, we propose the

following hypothesis; Ethanol extract of *L. nobilis* will significantly induce a dose-dependent inhibition of spontaneous, oxytocin-induced,

high KCL-induced and oxytocin induced contractions in zero calcium by modulating calcium activity in myometrium.

1.9.3 Aim and Objectives of the Study

The aim of the study was to investigate the inhibitory effect of *L. nobilis* on spontaneous and agonist-induced contractions in isolated uterus of non-pregnant mice.

The objectives of this study were to;

1. determine the effect of ethanol extract of *L. nobilis* on spontaneous uterine contraction in non-pregnant mice'

2. determine the effect of ethanol extract of *L. nobilis* on oxytocin- induced uterine contraction in non-pregnant mice.

3. determine the effect of ethanol extract of *L. nobilis* on high KCL- induced uterine contraction in non-pregnant mice.

4. determine the effect of ethanol extract of *L. nobilis* on oxytocin- induced uterine contraction in a zero-calcium medium in non-pregnant mice.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1.1 Laboratory Materials

Materials used for the study include: micropipette (Microflux) 0 – 1000 uL, sample bottles, beakers (50 mL, 250 mL and 500 mL), Pasteur pipettes, syringes (1 ml, 2 ml, 5 ml, 10 ml, 20 ml), needles, white thread, masking tape, permanent markers (red, blue, green and black), dissecting instruments, glass stirrer, brushes, disposable gloves, cotton wool, plastic cages and aerated lids, spatula, measuring cylinders (100 ml, 250 ml and 500ml), microscope (Visiscope® VWR, UK), glass slides, distilled water, porcelain dish, pestle, hot plate/oven, organ and water bath, digital weighing balance, Refrigerator, Steel observation table, Uninterrupted Power Supply (UPS), 7003E-isometric force

transducer (PanLab AD Instruments, Australia), laptop with the Lab-chart software (AD Instruments) and connecting cables, PowerLab 2/26 Model ML826 data acquisition/recording unit (AD Instruments, Australia) and the GraphPad Prism v. 8.1 (San Diego, CA, USA) for analysis.

2.1.2 Chemicals and Reagents

Physiological saline solution (PSS), Ringer's Locke was prepared with the following composition (mM/L): Sodium Chloride-NaCl=154.00 (Guangdong Guanghua Sci-Tech Co. Ltd. China), Potassium Chloride - KCl = 5.63 (Guangdong Guanajua Sci-Tech Co. Ltd. China), D-Glucose - $C_6H_{12}O_6.H_20$=2.78 (Guangdong Guanghua Sci-Tech Co. Ltd. China), Sodium bicarbonate-$NaHCO_3$=5.95 (Sigma Aldrich, UK), Calcium Chloride-$CaCl_2H_20$=2.05 (Guangdong Guanghua Sci-Tech Co. Ltd. China).

Others include; Ethanol (Pharmatrends, Nigeria), Normal Saline (Bioflex; Biomedical Nigeria Ltd), Oxytocin (Roche pharmaceutical Ltd), Methylene Blue (Tianjin Kermel Chemical Reagent Co., Ltd), Distilled Water, Ethylenediaminetetraacetic acid (EDTA) (Molychem, Mumbai, India). All reagents used were of high analytical grade.

2.1.3 Collection and Extraction of the Plant Material

Dried leaves of *L. nobilis* were bought from Uselu market, Egor local government area, Edo State, Nigeria in March 2025. The plant material was identified by Professor Henry Akinnibosun Adewale from Department of Plant biology and Biotechnology (PBB), Herbarium Unit, Faculty of Life Sciences, University Of Benin, Benin City, Edo State as *Laurus nobilis* Linn. The plant material was given a voucher number of UBH-L300, few samples of the plant material were then deposited in the department's (PBB) Herbarium Unit for reference purpose.

The dried leaf were ground to a fine powder with the aid of a grinding machine. The powdered leaves(543.5g) were extracted with hydro-ethanol solvent (1:1) using a Soxhlet apparatus. The extract obtained was then concentrated by evaporation using a rotary evaporator at a controlled temperature of 60°c and a rotation speed of 90 revolutions per minute. The dried material yielded 19.94% by weight and was stored in a sealed container under refrigeration at 4°c.

2.1.4 Animal

The animals used for the study were healthy non-pregnant female Swiss albino mice weighing between 20 to 28 grams. The mice were purchased from the university of Benin, Nigeria in the Department of Pharmacology and Toxicology and they were kept and cared for in the same facility by the Faculty of Pharmacy animal housing unit. Subsequently, the mice were allowed an acclimatization of period of two weeks before their use for the experiment and maintained under standard conditions. They were housed at room temperature, roughly 27 ± 5°C, with a natural cycle of light and darkness. They were fed with standard rodent pellets (Chikun grower pellets Feeds, Crown flour mill Limited, Lagos, Nigeria), along with uninterrupted access to fresh tap water. All handling, care and treatment procedures for the mice strictly followed the established principles contained within the 'Guide for the Care and Use of Laboratory Animals' and 'Public Health Service Policy on Humane Care and Use of Laboratory Animals' according to the standard established by the (National Research Council 2010, Public Health Service Policy on Human Care and Use of Laboratory Animals, 2015.)

2.2 Methods

2.2.1 Uterine Tissue Preparation

The healthy non-gravid (not pregnant) mice were screened to identify those in estrus phase. Screening was done by evaluating vaginal cells under the microscope (cytology)., the estrus phase was identified using the technique highlighted by Bafor *et al*, (2019), where a Pasteur Pipette with a diameter of 0.1mm was used to flush the squamous epithelia of the vagina with approximately 0.1mL of normal saline. After careful transfer to a clean glass slide, the contents were dried on a hot plate, and fixed with cold Methanol. Methylene blue (0.1%) was used to stain the vaginal smear, and an

X10 objective lens (Visiscope® VWR, UK) was used for microscope viewing. The presence of cornified epithelial cells confirmed estrus phase. The abdomen was opened, the uterine horns were immediately removed and cleaned of connective tissues and adhering fats, and placed in a petri dish with warm, aerated PSS.

The uterine tissues were then divided into segments that were 1 to 2 mm long. These segments were then placed in an aerated organ bath with 10 ml of Ringers Locke solution, which was kept at 37ºc. The physiological salt solution's composition per mm/l as follows; $CaCl_2.H_2O$ = 2.05, D-Glucose = 2.78, KCl = 5.63, $NaHCO_3$ = 5.95, and NaCl = 154.00, as previously described (Bafor *et al*., 2019).

All of the uterine segments were vertically mounted by using a sterile needle to attach surgical threads at both ends, creating a loop on one end, and then submerged in the 10 mL organ bath. A 7003E-isometric force transducer (Pan-lab Instruments, Spain) was connected to the loop's long threaded opposite end, which was fastened to a tissue holder. Bridge amplifiers were

then connected to a power-lab data acquisition system, which included a recording unit for documenting and displaying variations in the force and frequency of the contraction (Power-lab 2/26 model ML826 Instruments, Australia). Lab-chart 7 reader software(v,8.0 AD Instruments North America, USA to write down the measurement. The uterine tissue was allowed to equilibrate under a suitable resting tension of 0.5 g in the PSS until regular rhythmic contractions were observed, ensuring proper conditions prior to the addition of drugs (Uchendu and Bafor, 2023).

2.2.2 Experiment on *L. nobilis* Leaf Extract in Spontaneous Uterine Contraction

To acquire concentration-response relationships, *L. nobilis* extract (LNE) was incrementally added (0.00625-0.4 mg/mL) to the isolated uterine tissue after acquiring regular spontaneous uterine contractions to function as a control. Each concentration was given a contact time of 5 minutes (Uchendu and Bafor, 2023).

2.2.3 Experiment on *L. nobilis* Leaf Extract in Oxytocin-Induced Uterine Contraction

The isolated uterine tissue of the non-pregnant mice was exposed to oxytocin at 14 nM for 10 minutes in order to increase uterine contraction. After 10 minutes, cumulative concentration of L. *nobilis* extract (LNE) in concentrations ranging from 0.00625-0.4 mg/mL were added to the oxytocin pre-contracted tissue, with a contact duration of 5 minutes. The tissue was then washed, and a recove ry phase followed. Differences in contraction amplitude and frequency were recorded for additional analysis (Bafor *et al* 2020; Uchendu and Bafor, 2023).

2.2.4 Experiment on *L. nobilis* Leaf Extract in High Potassium Chloride (KCl)-Induced Uterine Contraction

The study evaluated how Bayleaf ethanol extract affected the depolarization of the myometrial membrane brought on by high KCl levels. The uterine tissues of the isolated non-pregnant mice were treated with an 80 mM potassium chloride solution for five minutes. LNE was then added to the tissue pre-contracted by KCl in a series of increasing concentrations, ranging from 0.00625 to 0.4 mg/ml, without a washout step. Every addition was given five minutes to interact with the tissue. Afterwards, the tissues were washed and given time to recover. For analytical assessment, the resulting changes in the contraction frequency and amplitude were recorded. (Uchendu and Bafor, 2023; Bafor *et al*., 2020).

2.2.5 Experiment on *L. nobilis* Leaf Extract in Oxytocin-Induced Uterine Contraction in Ca^{2+}-Free Medium

The study examined the effects of LNE on intracellular calcium released from stores in a calcium-free physiological salt solution (PSS) containing 0.1 mM ethylene diamine tetraacetic acid (EDTA). First, in a standard PSS (Ringer's Locke solution), regular spontaneous contractions of uterine tissue were obtained for 10 minutes. A calcium-free PSS containing 0.1 mM EDTA was then used in place of this medium. After observing the effects caused by the calcium-free PSS for 2 minutes, oxytocin was added at a concentration of 14 nM in the absence of a washout of the calcium-free medium. Then, with oxytocin present, the maximal dose of 0.4mg/ml of LNE was added, and a 5 minute contact time was allowed (Bafor *et al*., 2019). The tissues were washed and given time to recover after the additions. Variations in contraction frequency and amplitude were noted recorded, measured and subjected to analysis. (Uchendu and Bafor, 2023; Bafor *et al*., 2020).

2.2.6 Data Analysis

The mean ± standard error of mean (SEM) is used to display all data. Dunnett's post hoc test was used after a one-way ANOVA for multiple comparisons and a value of $P<0.05$ was deemed statistically significant for all analyses. For this analyses, Graph-Pad Prism version 8.0 (Graph-Pad software, San Diego, CA, USA) was utilized. Lab-chart reader software version 8.0 was used to process all of the image data from the mouse uteri. The frequency and strength (amplitude) of the contractions were among the uterine contractility parameters that were assessed. Non-linear regression was used to fit the data to a four-parameter logistic model in order to examine the mean log concentration-response relationships. The following mathematical model was used: y = bottom + (top-bottom)/(1 + 10^((logec50-x) × hillslope)), where x is the log-transformed concentration and y is the measured response that starts at the lowest value (bottom) and increases sigmoidally to the maximum (top).

CHAPTER THREE

3.0 RESULTS

3.1 Effect of *L. nobilis* Extract (LNE) on Spontaneous Uterine Contraction

In a concentration-dependent manner (0.00625-0.4mg/mL), LNE gradually reduced spontaneous contractions of the non-pregnant uterus with each cumulative addition (Figure 3.1). The analysis of

the results revealed that there was a non-significant reduction in the frequency of spontaneous contractions of the non-pregnant uterus while the amplitude was inhibited significantly at a concentration of 0.2 (*$p<0.05$) and highly significantly at 0.4 mg/mL (***$p<0.001$). The area under the curve plot illustrating LNE's inhibitory effect is displayed in Figure 3.2, Following the washout of LNE with fresh PSS, there was immediate recovery spontaneous uterine contractions recovered.

3.2 Effect of *L. nobilis* Extract (LNE) on Oxytocin Induced Non-Pregnant Uterine Contractions

Oxytocin (OT) triggered an increased frequency and amplitude in uterine contraction (Figure 3.3). There was no effect on the amplitude and frequency of contractions on the addition of the vehicle. In the continued presence of (OT), cumulative additions of LNE led to concentration-dependent relaxation in non-pregnant mice uterine contractions (Figure 3.3). Analysis of the results revealed that there was no observed significant inhibition in the amplitude and frequency of the contractions (Figure 3.4). Following the washout of LNE with fresh PSS, spontaneous uterine contractions recovered immediately. (Figure 3.3).

A

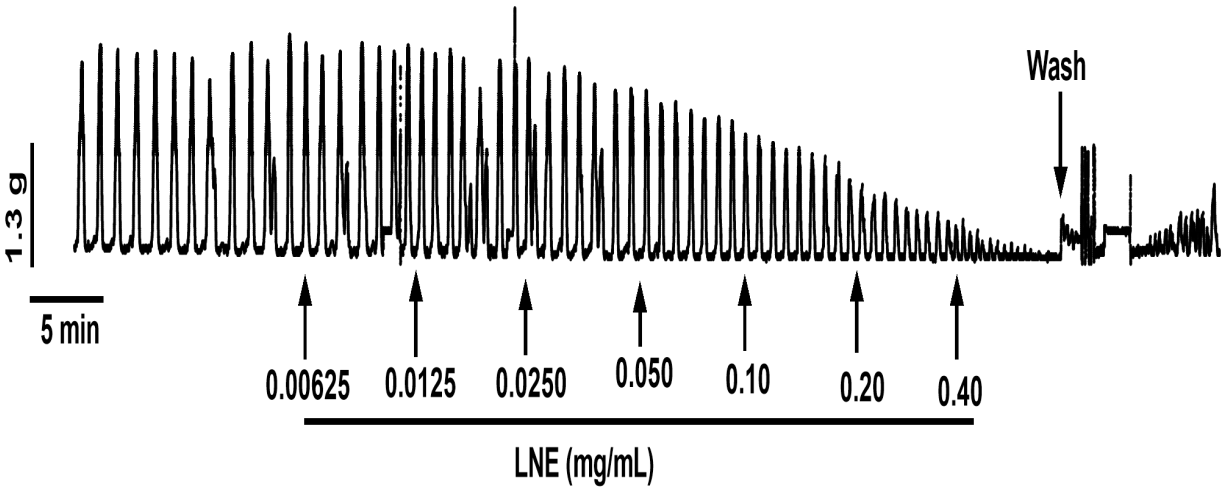
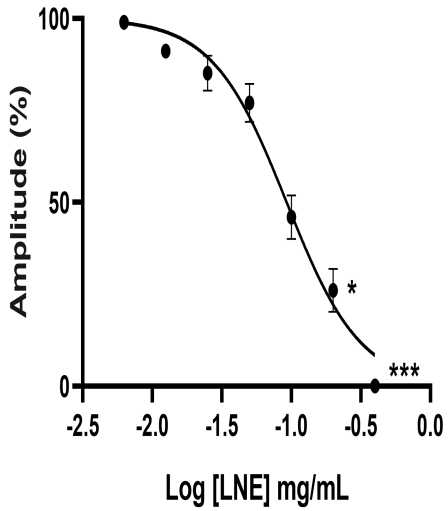
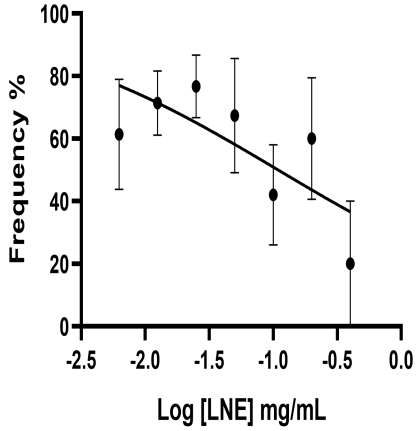


Figure 3.1: Original representative recording showing the effect of LNE on spontaneous uterine contractility

A



B



C

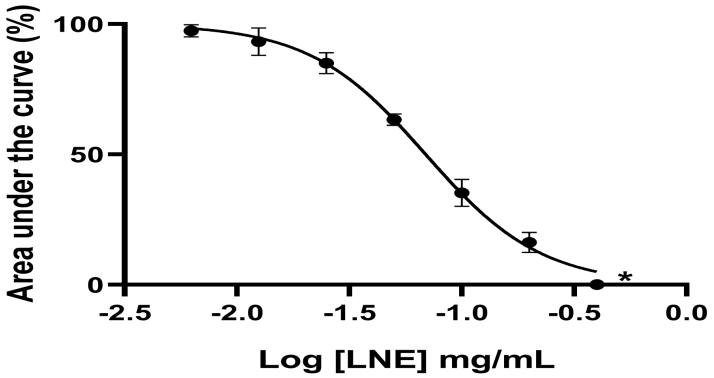
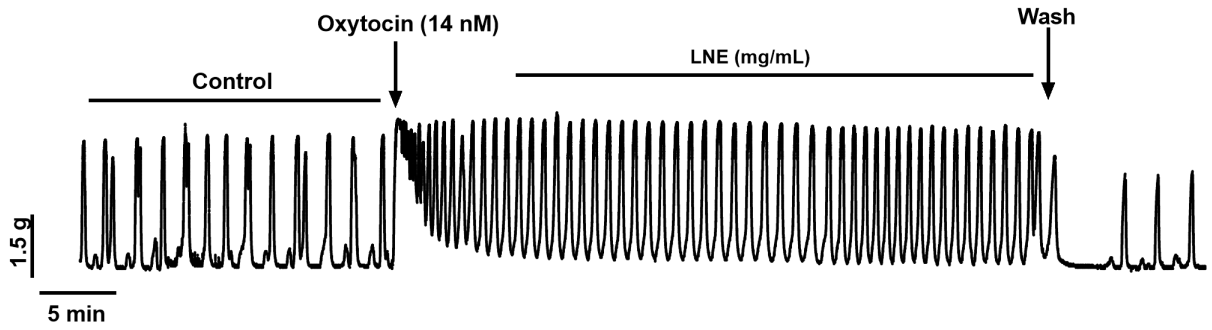


Figure 3.2: Effect of *L. nobilis* extract (LNE) on (A) amplitude, (B) frequency and (C) area under the curve of spontaneous uterine contractility of non-pregnant uterus. Values are expressed as mean ± SEM, *p<0.05, ***p<0.001 n=5 animals.

A



B

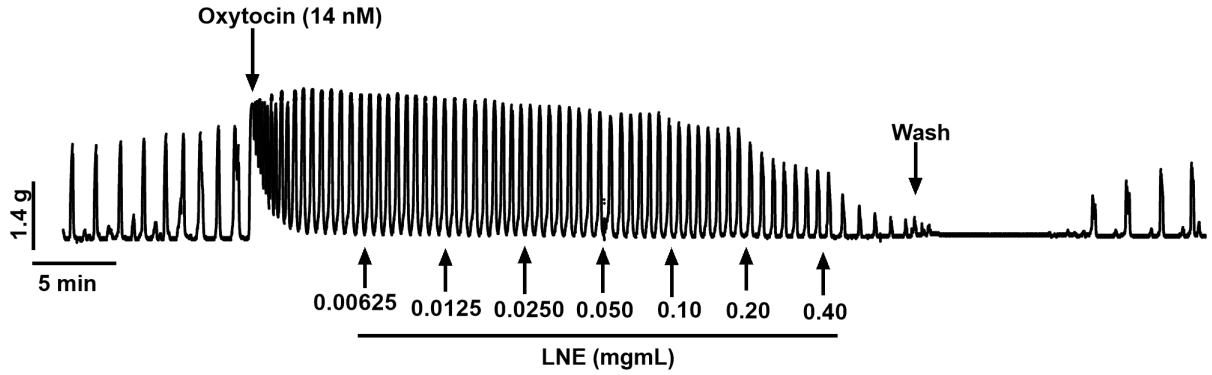
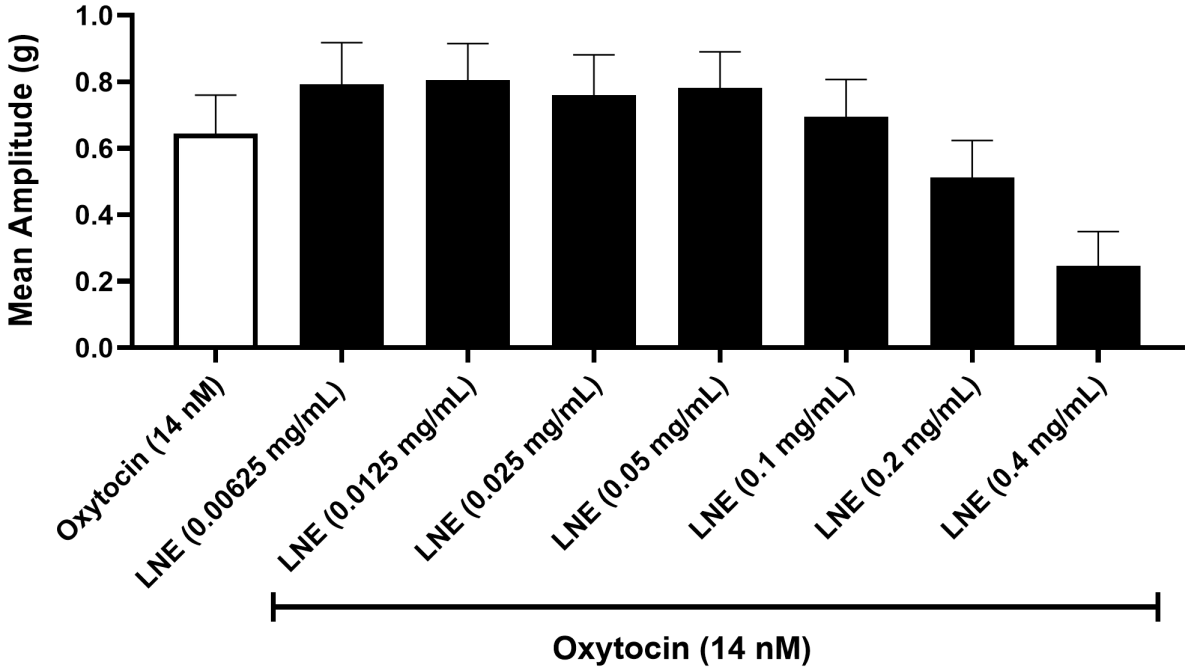


Figure 3.3: Effect of *Laurus nobilis* extract (LNE) on oxytocin (OT)-induced uterine contractions in non-pregnant mouse.

Original representative recordings showing **(A)** the effect of OT on non-pregnant uterine contractions as control; **(B)** the effect of LNE on OT-induced uterine contractions in non-pregnant mouse.

A


Mean Amplitude (g)
1.0
0.8
0.6
0.4
0.2
0.0
Oxytocin (14 nM)
LNE (0.00625 mg/mL)
LNE (0.0125 mg/mL)
LNE (0.025 mg/mL)
LNE (0.05 mg/mL)
LNE (0.1 mg/mL)
LNE (0.2 mg/mL)
LNE (0.4 mg/mL)
Oxytocin (14 nM)

B

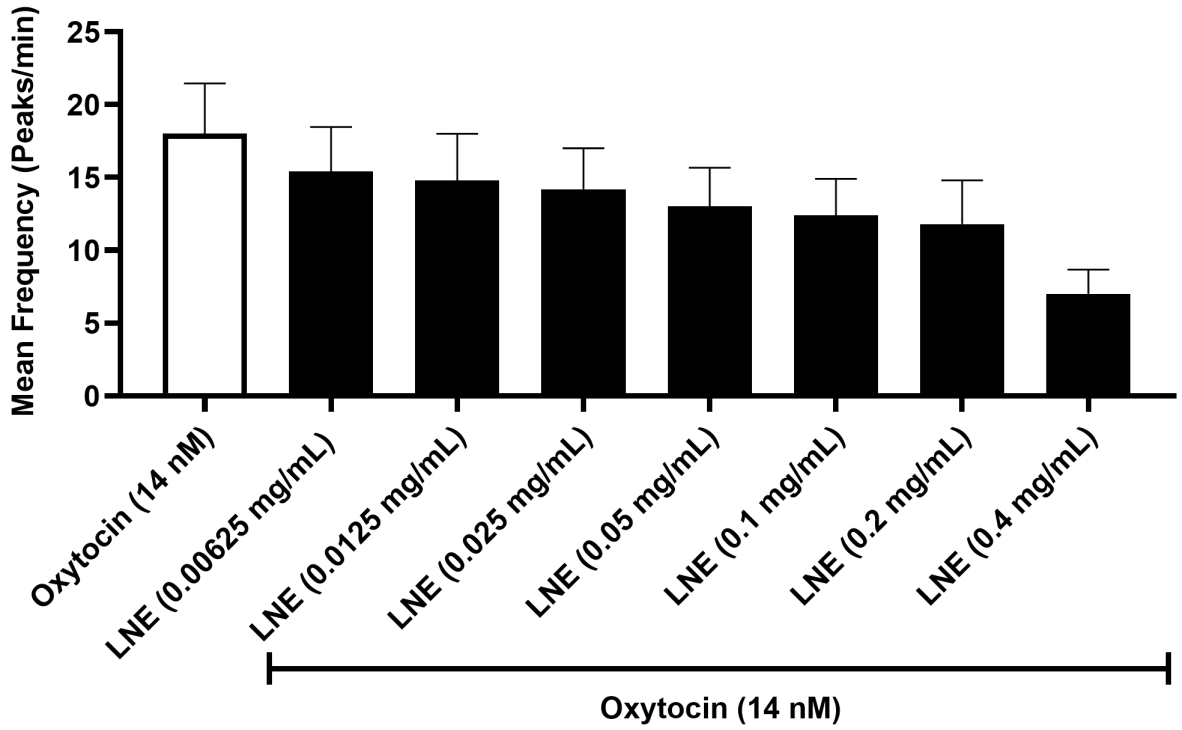


Figure 3.4: Bar graphs showing the effect of LNE on **(A)** amplitude and **(B)** Frequency of OT-induced contraction. Values expressed as mean ± SEM, n=5 animals

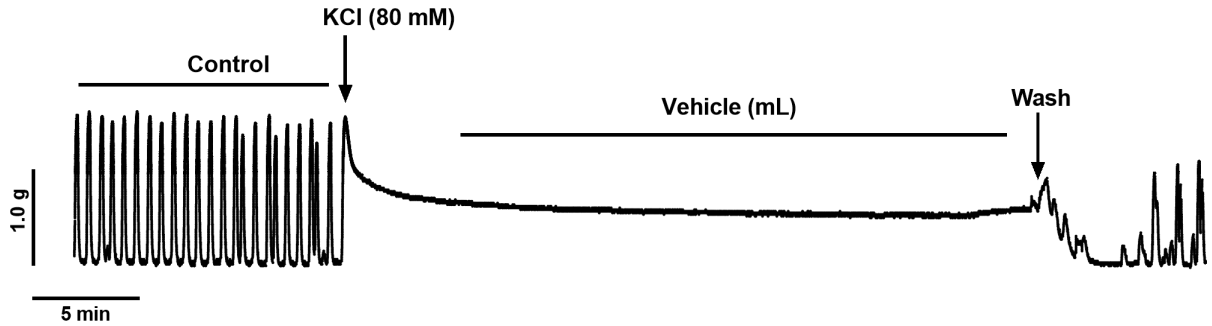
3.3 Effect of *L. nobilis* Extract (LNE) on High Kcl-Induced Non-Pregnant Uterine Contractions

The addition of high KCl results in sustained contractions, as seen in Figure 3.5. It also illustrates how LNE affects KCl induced contractions and that the vehicle has no effect on the contraction. There was a rapid and sustained increase in the force of contraction on addition of high KCl . When compared to KCl alone, the addition of LNE in the presence of KCl (80mM) significantly reduced the high KCl-induced contraction on the uterine tissues of non-pregnant mice. The inhibition of the contraction's amplitude was very highly significant (****$p<0.0001$) at a concentration of $0.4 mg/mL$ in the presence of LNE (Figure 3.6). Following the washout of LNE with fresh PSS, spontaneous uterine contractions recovered immediately.

3.4 Effect of *L. nobilis* Extract (LNE) on Oxytocin Induced Uterine Contractions in Calcium-Free Medium

Figure 3.7 illustrated how LNE affected the release of calcium from intracellular stores. The spontaneous contractions were increased slightly when OT was added to uterine tissue mounted in a zero-calcium PSS that contained the calcium chelating agent EDTA. However, the uterine contractions were totally inhibited when LNE was added. There was no significant inhibition of the amplitude of contraction but inhibition of the frequency was significant ($p<0.05$) at a concentration of 0.4mg/mL in frequency in the non-pregnant mice uterine tissues (Figures 3.7 and 3.8 respectively). Following the washout of LNE with fresh PSS, spontaneous uterine contractions recovered immediately.

A



B

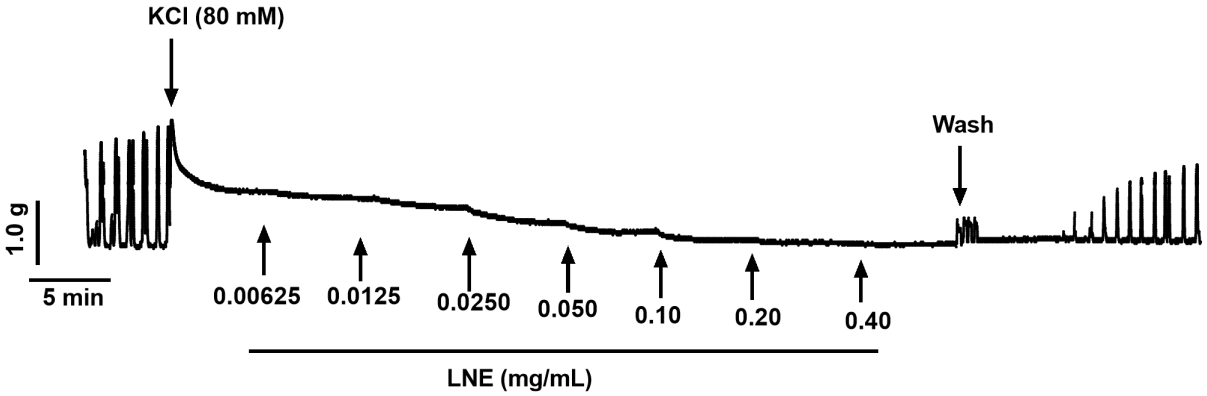


Figure 3.5: Effect of the *L. nobilis* extract (LNE) on high KCl-induced uterine contractions in non-pregnant mouse.

Original representative recordings showing **(A)** the effect of high KCl on non-pregnant uterine contractions as control **(B)** the effect of LNE on high KCl-induced contraction.

A

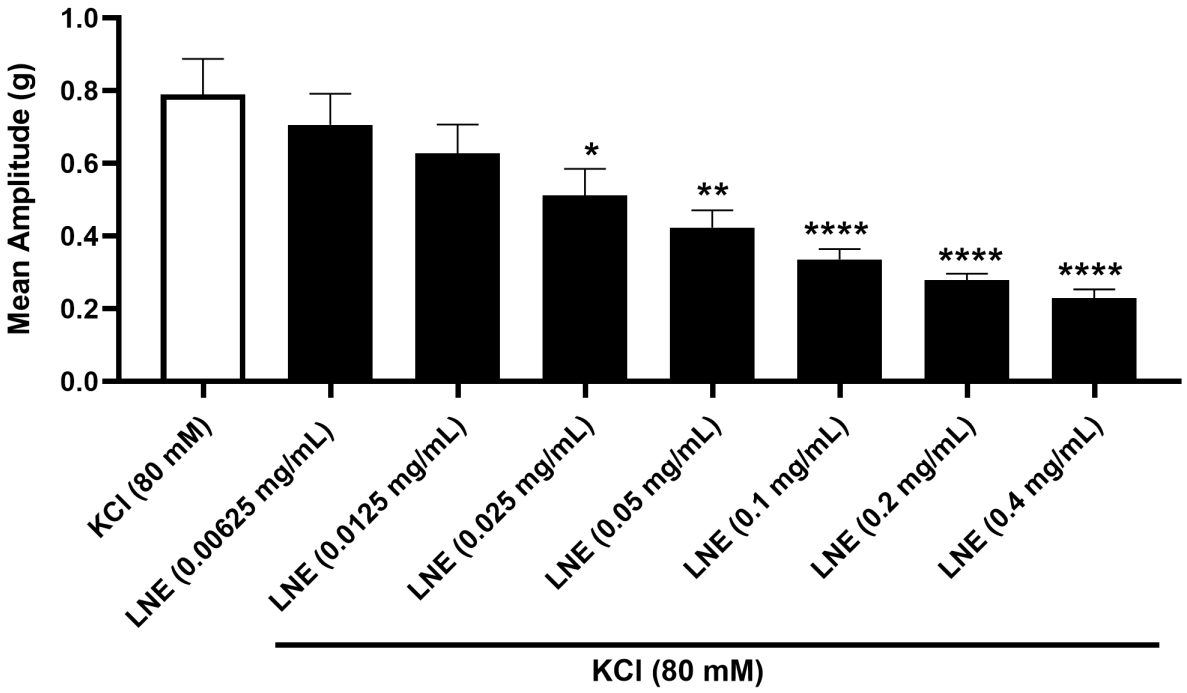
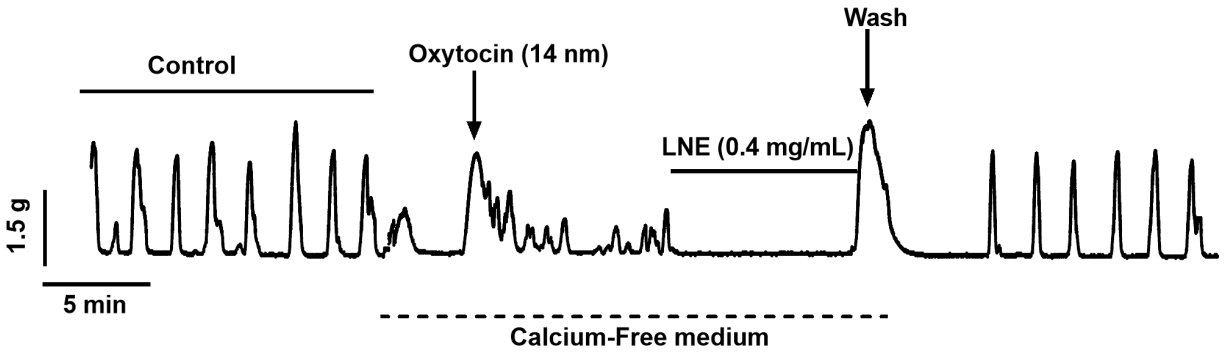


Figure3.6: Bar graph showing that *L. nobilis* extract **(**LNE) significantly ($p<0.0001$) inhibited the amplitude of High KCl-induced contraction. Values are expressed as mean ±SEM, n=5 animals

A


Wash
Control
Oxytocin (14 nm)
LNE (0.4 mg/mL)
1.5 g
5 min
Calcium-Free medium

B

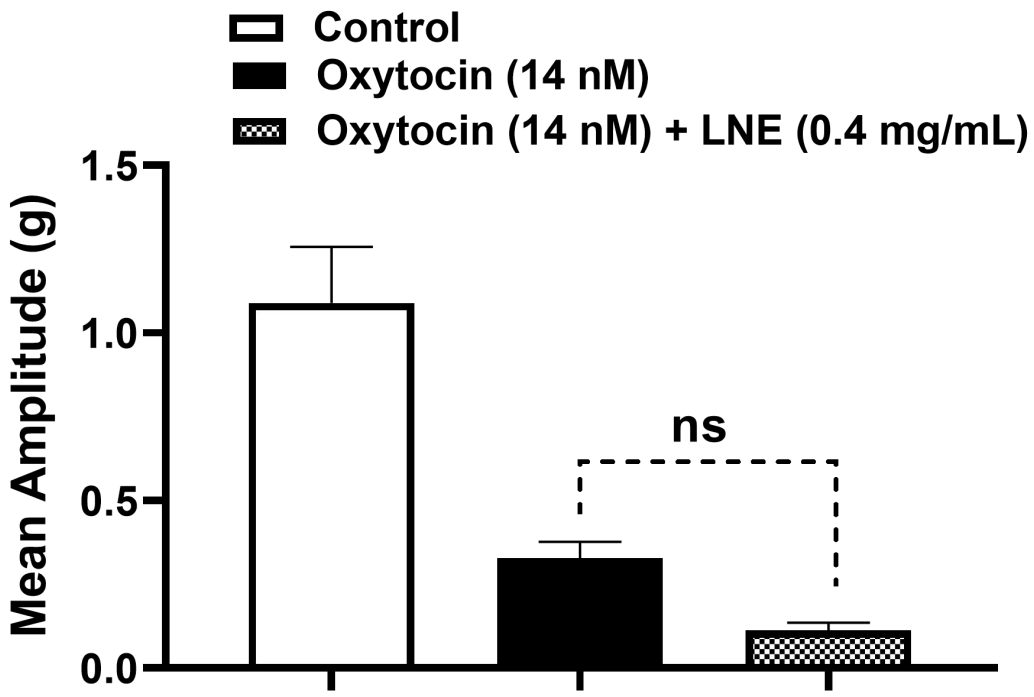
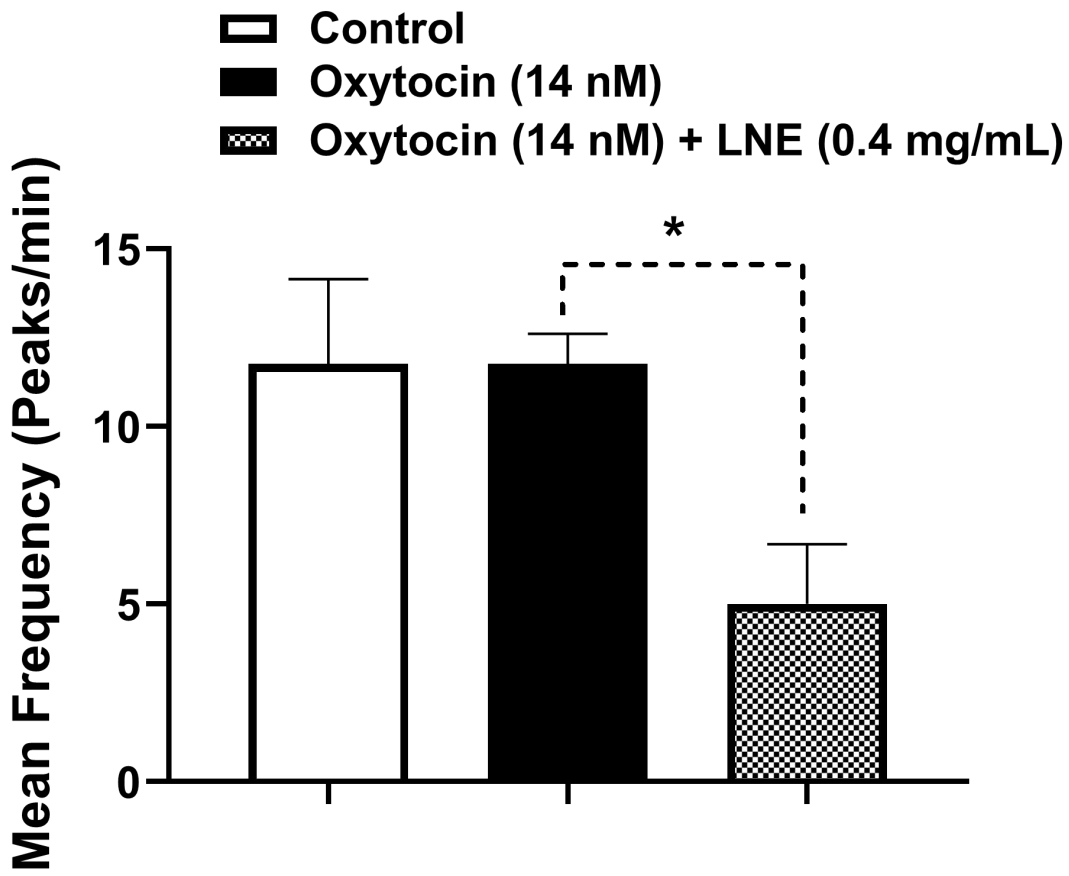


Figure 3.7: Effect of *L. nobilis* extract (LNE) on Oxyticin (OT)-induced uterine contractions in Ca^{2+}-free media.

(A) Representative recording of the effect of LNE on OT-induced contraction in Ca^{2+}- free medium.

(B) Bar graphs showing that LNE reduced the amplitude of OT-induced contraction in calcium free PSS.

A



C

Figure 3.8: Bar graphs showing that LNE significantly ($p<0.05$) inhibited the frequency of OT-induced contraction in Ca^{2+}- free PSS.

CHAPTER FOUR

DISCUSSION

Herbal medicines are rich sources for discovering new drugs, with major pharmaceuticals like aspirin and artemisinin originating from plants (Fabricant and Farnsworth, 2001: Gurib-Fakim, 2006). One of the most important areas of pharmacological research is the uterus due to significant global health issues associated with dysregulation of myometrial contractility including dysmenorrhea and preterm labour. *L. nobilis* was chosen due its traditionally use as an antispasmodic and its potent phytochemical profile. It contains well documented bioactive compounds like 1,8-cineole and the flavonoid quercetin, both of which are known to relax smooth muscle by interfereing with Ca^{2+} -dependent mechanisms of muscle contraction.

The aim of this study was to determine the uterorelaxant activity of the *Laurus nobilis* extract on the isolated myometrium of non-pregnant mice. The study employed four different pharmacological models of contraction in order to have a clear insight of the mechanism: Basal spontaneous rhythmic activity, Oxytocin (OT)-induced receptor-mediated contraction, High potassium chloride (KCl)-induced depolarization-mediated contraction, and OT-induced contraction in a calcium free environment.

The aim of the systematic comparison of the inhibitory strength of the extract in these models was to identify the main site(s) of action, that is, voltage-dependent Ca^{2+} channels, or intracellular Ca^{2+} processes. The study revealed that *L. nobilis* extract significantly and concentration-dependently reduced the amplitude and frequency of spontaneous uterine contractions and contractions induced by High KCl. It produced a slight, non-significant inhibition of the amplitude and frequency of

oxytocin-induced contractions. In calcium-free medium, the extract markedly suppressed the frequency but only slightly reduced the amplitude of oxytocin-induced contractions.

One significant finding was that, in every experimental setup, uterine contractions returned to their baseline levels following a washout period, during which the LNE was eliminated from the system. This reversible effect implies that its effect on uterine contractions is temporary and does not result in long-term harm to the uterine tissue. Rather, the extract appears to alter uterine activity momentarily without causing permanent changes, which is important for possible therapeutic applications. This result is consistent with earlier studies, which show that many substances that inhibit uterine contractions can also have reversible effects, making them safer for repeated or prolonged use (Loch-Caruso *et al*., 2003).

In order to truly understand how LNE inhibits spontaneous uterine contractions, it is important to examine the underlying mechanisms of contractions in uterine smooth muscle cells.

The myometrium, or the smooth muscle layer of the uterus, is a tissue that is able to produce spontaneous, rhythmic contractions. This innate electrical and mechanical action that does not need the stimulation of the neural or hormonal system is the basis of several physiological actions, such as menstruation, sperm transport, and, most importantly, the directional and powerful contractions that are needed during childbirth (Wray and Arrowsmith, 2012).

Spontaneous uterine contractions are not formed randomly but are caused by specialized pacemaker cells. During a long period of time, it was believed that every cell of the polyglandular myocardium could become a pacemaker. There is however, now widely evidence that there is a dedicated group of cells with special electrophysiological characteristics that dictate the rhythmicity of the whole organ.

They usually are called interstitial cells of Cajal-like cells (ICC-LCs) because they are similar to pacemaker cells of the gastrointestinal system (Duquette *et al*., 2005; Popescu *et al*., 2006). Verify this statement, myocardium is for heart and myometrium is for uterus

These pacing cells which are found all over the myometrium spontaneously produce slow-wave electrical potentials which are periodic oscillations of the membrane potential of the cell. Once a slow wave depolarizes the membrane to some threshold, it will cause a series of action potentials (sharp, rapid depolarization), which will spread to the surrounding smooth muscle cells and make them contract (Sanders *et al*., 2012). The rate of such slow waves eventually defines the primary rhythm of the uterine contractions. Previous research by Lammers (2013) has demonstrated that pacemaker activity tends to arise in a particular area i.e. the utero-tubal junction and propagate across the entire uterus ensuring a synchronous pattern of contraction.

The dynamics of the underlying electrical processes of spontaneous pacemaker activity and myometrial contraction are regulated by the movement of ions across the cell membrane by numerous ion channels. A balance between inward (depolarizing) and outward (hyperpolarizing) currents plays a vital role in determining the resting membrane potential and in determining the action potentials.

Calcium Channels (Ca^{2+}) channels that are arguably most important in the contraction of the uterus are Voltage-gated L-type calcium channels (VGCC). These channels open as a result of depolarization of cell membrane during an action potential, which leads to the influx of extracellular calcium ions (Ca^{2+}) into the cell (Arrowsmith and Wray, 2014). This Ca^{2+} influx has two functions; it further depolarizes the cell, which contributes to the upstroke of the action potential, but more to the

point, it directly activates the contractile machinery by activating calcium induced calcium release. There are also T-type calcium channels that are believed to play a part in the initial depolarization that causes the cell to reach the threshold to discharge an action potential (Shmigol *et al*., 2001).

Oscillations of $[Ca^{2+}]i$ are the basis of spontaneous contractions. This rise in calcium has two major sources namely influx across the extracellular space and release across the internal sarcoplasmic reticulum (SR) store (Wray *et al*., 2003). Calcium-induced calcium release (CICR) is a process which is triggered by the influx of Ca^{2+} through L-type channels. The calcium that comes in interacts with and opens ryanodine receptors (RyRs) on the SR membrane resulting in the release of a significantly greater amount of stored calcium into the cytosol (Burdyga *et al*., 2004).

This calcium wave in the cytoplasm binds to calmodulin protein. The resultant Ca^{2+}calmodulin complex triggers myosin light chain kinase (MLCK) that phosphorylates the regulatory light chain of myosin. Such a phosphorylation enables the interaction of myosin with actin, beginning the cross-bridge cycling and the formation of force, which leads to muscle contraction (Guerin *et al*., 2021). To relax, $[Ca^{2+}]i$ needs to be reduced through ATP-dependent pumps which either return Ca^{2+} into the SR (SERCA pumps) or into the cell (PMCA pumps).

To ensure that the uterus contracts as a single functional unit (a syncytium), the electrical impulses produced by pacemaker cells have to be disseminated to all other muscle cells as quickly and as efficiently as possible. This intercellular communication is known as gap junction- special protein channels that directly connect the cytoplasm of the neighboring cells (Garfield *et al*., 1977).

These crossings are mainly connexin - 43 (Cx43). They create low resistance channels through which the ions and small molecules pass which relays the action potentials between cells across the

myometrium. Cx43 is a highly regulated expression, mostly by steroid hormones. It is low at most times of the pregnancy, resulting in a low cell-to-cell coupling, and uncoordinated, localized contractions (Braxton Hicks). During parturition, a burst of estrogen levels increases the expression of Cx43 to create large amounts of gap junction plaques. Such increase in couplings is critical in developing the uterus to a highly efficient organ that is able to perform the strong, coordinated labour contractions (Hertelendy and Zakar, 2004; O'Brien, 2007).

The seemingly lack of significant inhibition on frequency of spontaneous contractions as seen in figure 3.1 might be because the extract had no direct effect on the endogenous pacemaker cells, which resides in uterine tissues. Thus, the extracts will have no effect on gap junction assembly and will not enhance or inhibit cellular communication, culminating in stable or unaffected frequency of uterine contraction.This observation suggests a potential inhibition of extracellular calcium influx

Oxytocin(OT) is a known endogenous utero-tonic agent and nonapeptide hormone produced in the hypothalamus and released by the posterior pituitary. It plays a central role of organizing the strong, rhythmic uterine contractions of labor and in the prevention of postpartum bleeding due to the occurrence of prolonged myometrial contraction after labor (Dale, 1906; Gimpl and Fahrenholz, 2001). Modern obstetrics is based on the clinical use of synthetic oxytocin (Pitocin) to induce or augment labor.

The activity of oxytocin is totally based on its interaction with a particular receptor the oxytocin receptor (OTR). The OTR is a typical Class I G-protein coupled receptor (GPCR) which is located on the membrane of the myometrial smooth muscle cells (Kimura *et al*., 1992). The sensitivity of the uterus to oxytocin depends on the expression of these receptors in the uterus.

During the majority of pregnancy, OTRs within the myometrium are comparatively minimal and make the uterus largely unresponsive to released oxytocin and thereby, cause uterine quiescence. Nevertheless, as the pregnancy progresses, the level of OTR gene expression is highly dramatic and profound that the receptor density increases 100-200 times (Fuchs *et al*., 1982; Soloff *et al*., 1979). This increased OTR expression is mainly precipitated by the increased concentration of estrogen that is a major transcriptional regulator of the OTR gene (Zingg *et al*., 1995). This upregulation is also caused by mechanical stretch of the uterine wall by the growing fetus. This timing of increase in OTRs is the molecular switch which primes the uterus and makes it highly sensitive to even minimal concentrations of oxytocin, and permits the labor contractions to become effectively active.

Once oxytocin binds to its receptor, it stimulates a known intracellular signaling cascade that leads to contraction of myometrial cells. The process is focused on mobilization of intracellular calcium ions ($[Ca^{2+}]i$), which is the final common smooth muscle contraction messenger. When oxytocin binds, the OTR undergoes conformational change leading to coupling and activatation of aparticular heterotrimeric G-protein, Gq/11. The G-protein's activated alpha subunit (Gaq) will in turn stimulate the membrane-bound effector enzyme, phospholipase C- beta (PLCβ) (Arrowsmith and Wray, 2014; Phaneuf *et al*., 1993). The activated PLCβ acts ona membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2), and cuts the compound into two important second messengers inositol 1,4,5-trisphosphate (IP^3) and diacylglycerol (DAG) (Aguilar and Mitchell, 2010). These two molecules trigger parallel sets of signals acting to trigger contraction.

IP_3, as a water-soluble molecule, diffuses along the cytosol and acts as a calcium releasing factor by binding to unique IP_3 receptors (IP_3Rs) on the myometrial cell membrane that are found on sarcoplasmic reticulum (SR). The result of this binding is the opening of the IP^3R channels and

a rapid and large discharge of the stored Ca^{2+} of the SR into the cytoplasm (Sanborn, 2001; Wray *et al*., 2003). It is this release of internal calcium that causes the initial sharp increase in the $[Ca^{2+}]i$ that initiates the onset of contraction. The depolarization with the release of the Ca^{2+}, among other receptor-mediated activities, stimulates the cell membrane voltage-gated L-type calcium channels. This enables the influx of extracellular Ca^{2+} to maintain the contraction (Young, 2007).

At the same time, the lipid soluble DAG is retained in the plasma membrane where it stimulates the Protein Kinase C (PKC) which contributes to the contractile response, in part by phosphorylating and inhibiting relaxation-promoting proteins. Moreover, the G-protein pathway is able to stimulate RhoA/Rho-kinase (ROCK) signaling cascade. ROCK phosphorylates and inhibits the enzyme called myosin light chain phosphatase (MLCP) which dephosphorylates myosin and leads to relaxation. This isa key mechanism of calcium sensitization, in which the contractile apparatus remains active for longer and thus maintains a strong contraction, without the need for further increase in $[Ca^{2+}]i$ (Word *et al*., 2007).

The elevated Ca^{2+} attaches to the calmodulin calcium-binding protein. This Ca^{2+}-calmodulin complex activates the enzyme myosin light chain kinase (MLCK). The 20-kDa regulatory light chain of myosin II is then phosphorylated by the activated MLCK. This phospohrylated enables the myosin heads to bind to actin filaments and begin the process of ATP-dependent cross-bridge cycling enabling the generation of mechanical force that forms muscle contraction (Guerin *et al*., 2021).

From the findings, LNE produced a modest, though not statistically significant, reduction in the ampitude and frequency of OT-induced uterine contractions. This trend towards inhibition may suggest that the extract possesses a weak antagonistic effect on the oxytocin pathway or may have

failed to block Ca^{2+} release from either extracellular or intracellular pathway. The lack of statistical significance could also be as a result of the inherent biological variability in uterine tissue preparations.

In order to isolate and study the particular part of the contractile pathway LNE has an effect on, further investigation on its effect on depolarized tissues was carried out. This was accomplished by application of high concentrations of KCl, which causes the influx of extracellular calcium through the openings in voltage-gated calcium channels resulting in a strong, prolonged (tonic) contraction (Karaki *et al*., 1997) leading to a sustained depolarization of the tissues.

The whole process starts with the resting membrane potential (RMP) of myometrial smooth muscle cell. In rest, the cell will have a negative electrical potentials across its membrane with an average of between -45 and -60 mV (Parkington and Coleman, 1990). This potential is mainly established and supported by the electrochemical gradient of potassium ions (K^+). The concentration of K^+ is high inside and low outside of the cell. At rest, the cell membrane is selectively permeable to K^+, and a small but consistent outward release of positive K^+ ions takes place, maintaining the negativity inside the cell compared to the outside (Lodge and Konje, 2006).

Exposing an organ bath preparation of uterine tissue with a solution of high concentration of KCl (usually 60-80 mM) disturbs the electrochemical equilibrium. The elevated K^+ extracellular level significantly reduces the concentration gradient which would have normally moved K^+ out of the cell. This decrease in the K^+ gradient, according to the equations of Nernst and Goldman-Hodgkin-Katz equations describing the dependence of ion gradients and membrane potential, shifts the RMP to a less negative value causing sustained depolarization of the cell

membrane (Bolton, 1979). The voltage-sensitive calcium channels are opened by this very strong depolarization. The results in this study indicated that LNE effectively inhibited the high KCl-induced depolarization, this provides evidence that LNE causes blockage of VGCC i.e it possesses activity on extracellular calcium channels.

It is important to note that the contribution of Ca^{2+} in uterine contractility also involves intracellular as well as extracellular stores. The activity of LNE was investigated in calcium free media to determine whether it affects the intracellular release of Ca^{2+}. This was done by the use of EDTA to chelate extracellular calcium, thus clarifying the mechanisms underlying LNE's action. In the absence of Ca^{2+} in the medium, OT still effected a slight increase in spontaneous contractions; the results revealed a significant inhibition of the frequency of contraction and a modest, non-significant reduction in the amplitude. This finding shows that the activity of LNE does not only rely on the influx of extracellular calcium but seems to suppress uterine contractions caused by intracellular calcium release.

CHAPTER FIVE

CONCLUSION

In summary, the *Laurus nobilis* extract (LNE) has been demonstrated to have significant inhibitory effects on spontaneous, high KCl-induced and oxytocin-induced uterine contractions in calcium free PSS in the isolated uterus of non-pregnant mice. The results indicate a mechanism of action that could include a blockage of extracellular influx of calcium via L-type voltage gated calcium channels and modulation of intracellular calcium release or sensitization pathways.

The findings of this paper indicate that LNE may be a promising source of new tocolytic (uterine relaxant) agents against those illnesses which are associated with excessive uterine contractility, Nevertheless, additional research should be done to isolate its specific bioactive compounds, to better understand the exact molecular mechanism of its effects, and to determine its safety and efficacy in the relevant preclinical models, and finally, in clinical models. The presented research provides a promising ground of the future of *Laurus nobilis* based therapies that will potentially enhance the reproductive well being of women and help to control health conditions linked to uterine hypercontractility.

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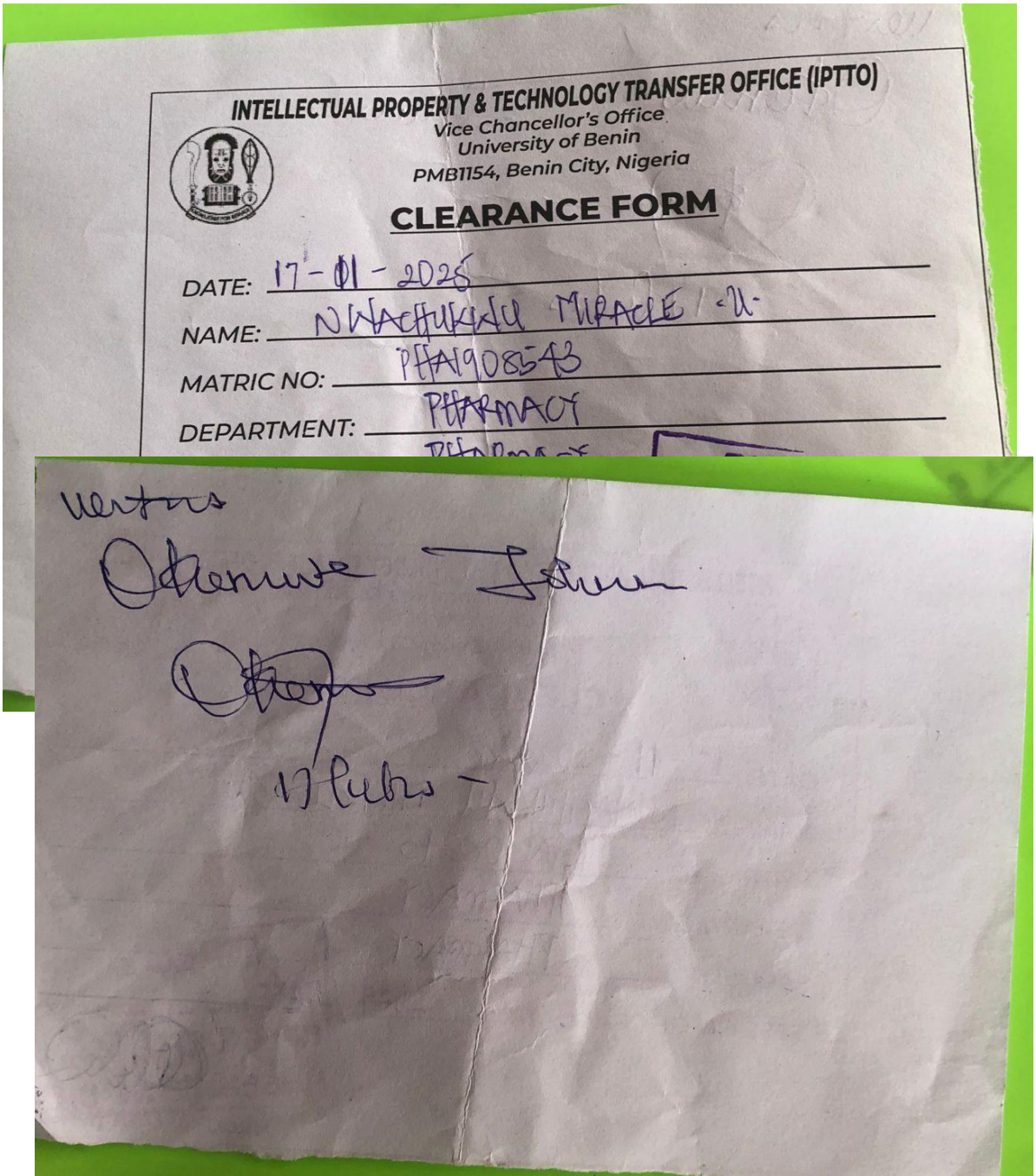
APPENDIX



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