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Tocolytic Activity of Hydroethanol Leaf Extract of *Momordica Charantia* Linn. (Cucurbitaceae) in Isolated Non-Pregnant Mouse Uterus: Involvement of Extracellular Calcium Influx Inhibition

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ABSTRACT

Momordica charantia L. (Cucurbitaceae) is widely used in traditional medicine for the management of various ailments, including reproductive disorders. However, its direct effects on uterine contractility remain poorly understood. This study investigated the tocolytic activity of the hydroethanol leaf extract of *M. charantia* (MCE) and its possible mechanisms of action in isolated non-pregnant mouse uterus. Uterine tissues obtained from estrus-phase Swiss albino mice were mounted in organ baths, and contractile responses were recorded using an isometric force transducer. The effects of cumulative concentrations of MCE (6.25–400 µg/mL) were evaluated on spontaneous contractions and contractions induced by oxytocin (14 nM), potassium chloride (KCl, 80 mM), prostaglandin $F_2\alpha$ ($PGF_2\alpha$, 10^{-6} M), and methacholine (MCh, 10 µM). Mechanistic studies were conducted using calcium-free physiological salt solution containing EDTA, propranolol (20 µM), and tetraethylammonium (5 mM). MCE produced concentration-dependent inhibition of spontaneous uterine contractions, significantly reducing contraction amplitude and overall contractile activity ($n = 5$; $p < 0.05$, $p < 0.01$). Similar inhibitory effects were observed against oxytocin-, KCl-, $PGF_2\alpha$-, and MCh-induced contractions. In calcium-free medium, MCE did not significantly inhibit oxytocin-induced transient contractions. Pretreatment with propranolol or tetraethylammonium did not abolish the inhibitory activity of MCE, although slight rightward shifts in concentration–response curves were observed. These findings provide evidence that MCE exerts potent uterine relaxant effects predominantly through inhibition of extracellular calcium influx, with a possible secondary contribution from potassium channel activation, supporting its potential as a source of novel tocolytic agents.

Keywords: *Momordica Charantia*, Uterine Contractility, Tocolytic Activity, Calcium Channel Blockade, Oxytocin-Induced Contractions, Smooth Muscle Relaxation

INTRODUCTION

The female reproductive system is a highly organized network of organs that performs functions beyond reproduction, including the regulation of complex hormonal processes that govern female fertility and overall health throughout life (Pan *et al.*, 2025). These functions are facilitated by the coordinated contractile activity of the myometrium, the smooth muscle layer of the uterine wall (Berridge *et al.*, 2003).

Myometrial contractility is a fundamental physiological process required for menstruation, maintenance of pregnancy, and childbirth (Aguilar & Mitchell, 2010). The regulation of uterine contractions is primarily dependent on intracellular calcium (Ca^{2+}) homeostasis and it is initiated when cytosolic Ca^{2+} concentrations increase from resting levels of approximately 100 nM to values exceeding 500 nM (Shmygol *et al.*, 2007; Pehlivanoğlu *et al.*, 2013; Bootman & Bultynck, 2020). This rise in intracellular Ca^{2+} occurs through two principal mechanisms: influx of extracellular Ca^{2+} via voltage-operated calcium channels (VOCCs) and receptor-operated calcium channels (ROCCs), and release of Ca^{2+} from intracellular stores within the sarcoplasmic reticulum (SR) through inositol 1,4,5-trisphosphate (IP_3)-sensitive channels (Wray, 2007; Pehlivanoğlu *et al.*, 2013). Elevated intracellular Ca^{2+} binds to calmodulin, forming a Ca^{2+}–calmodulin complex that activates myosin light-chain kinase (MLCK). Activated MLCK phosphorylates the 20-kDa regulatory light chain of myosin, facilitating actin–myosin cross-bridge formation and subsequent muscle contraction (Wray, 2007; Pehlivanoğlu *et al.*, 2013). Consequently, modulation of intracellular Ca^{2+} availability represents a key mechanism through which uterine contractility can be regulated.

Abnormal uterine contractility is associated with several reproductive disorders, including dysmenorrhea, endometriosis, spontaneous miscarriage, postpartum hemorrhage, and preterm labor (Aguilar & Mitchell, 2010). Understanding the molecular mechanisms underlying uterine contractility is therefore essential for the development of therapeutic agents capable of either suppressing or stimulating uterine contractions. Tocolytic agents inhibit uterine contractions and are primarily used in the management of preterm labor, whereas uterotonic agents promote contractions and are employed to induce labor or control postpartum haemorrhage.

Current tocolytic agents include β_2-adrenergic agonists (e.g., salbutamol), calcium channel blockers (e.g., nifedipine), oxytocin receptor antagonists (e.g., atosiban), and non-steroidal anti-inflammatory drugs (NSAIDs) used in the management of dysmenorrhea (WHO, 2005). However, their clinical use is often limited by adverse maternal and fetal effects, including tachycardia, pulmonary edema, and hypotension (WHO, 2005). Consequently, increasing attention has been directed toward medicinal plants as potential sources of safer and more affordable uterine relaxants (Rabizadeh, 2025).

Momordica charantia L. (Cucurbitaceae), commonly known as bitter melon, bitter gourd, or balsam pear, is an annual climbing vine widely distributed throughout tropical and subtropical regions. Traditionally, it has been used in the treatment of diabetes mellitus and various reproductive disorders. The plant contains numerous bioactive

constituents, including triterpene glycosides (momordicosides), alkaloids (momordicine), and cucurbitacins (Kole *et al*., 2020). These compounds have demonstrated diverse pharmacological activities, including smooth muscle relaxation, antidiabetic, anticancer, antimicrobial, hypolipidemic, and antioxidant effects in experimental models (Shobha *et al*., 2015; Sharmin *et al*., 2017; Tamilanban, 2018;].

Although previous studies have reported antifertility and abortifacient effects of *M. charantia* seeds in female rats (Yakubu *et al.,* 2022; Mili *et al.,* 2025), traditional practitioners in Asian regions like India employ leaf preparations for the management of painful menstruation and uterine hypercontractility (Kumar *et al.,* 2010). Therefore, the present study investigated the tocolytic activity of the hydroethanol leaf extract of *M. charantia* by evaluating its effects on spontaneous uterine contractions and contractions induced by oxytocin, potassium chloride, prostaglandins $F_{2\alpha}$, and methacholine. Furthermore, the study explored the involvement of extracellular calcium influx and intracellular calcium release pathways using calcium-free physiological salt solution (PSS), propranolol, and tetraethylammonium to elucidate the possible mechanisms underlying its inhibitory effects on uterine contractility.

MATERIALS AND METHODS

Drugs, Chemicals, and Reagents

Oxytocin (Anhui Hongye Pharmaceutical Co., Ltd. (China). Methacholine chloride, Tetraethylammonium, and propranolol (Sigma-Aldrich, UK). Prostaglandin $F_{2\alpha}$ (Bioveta, Czech Republic). Normal saline (Bioflex®, Biomedical Nigeria Ltd.), Physiological saline solution (PSS) components including Sodium Chloride (NaCl), Potassium Chloride (KCl), D-Glucose, Sodium Bicarbonate ($NaHCO_3$), and Calcium Chloride ($CaCl_2$) (Guangdong Guanghua Sci-Tech Co. Ltd, China) and (Sigma-Aldrich, UK). Other reagents included ethanol (Pharmatrends, Nigeria), methylene blue (Tianjin Kermel Chemical Reagent Co., Ltd., China), and ethylenediaminetetraacetic acid (EDTA) (Guangdong Guanghua Sci-Tech Co., Ltd., China).

Collection and Extraction of Plant Material

Fresh leaves of *M. charantia* were collected in July 2025 from the University of Benin campus, Ugbowo, Ovia North-East Local Government Area, Edo State, and authenticated at the University of Benin Herbarium, Benin City, Nigeria (Voucher No. UBH-M617). The leaves were cleaned, air-dried at room temperature, and pulverized into a fine powder using an electric grinder. A total of 441.9 g of powdered leaf material was extracted with 2 L of hydroethanol (1:1, v/v) using a Soxhlet extraction apparatus. The extract was concentrated under reduced pressure using a rotary evaporator at 60°C and 90 rpm. The resulting dried extract represented 31.46% w/w of the initial plant material. The extract was stored in airtight containers at 4°C until use.

Experimental Animals

Thirty adult non-pregnant female Swiss albino mice weighing 20 – 30 g were used for the study. The animals were obtained from the Animal House, Faculty of Pharmacy, University of Benin, Nigeria. They were housed under standard laboratory conditions with free access to commercial pellet feed and water and maintained under a 12-hour light/dark cycle. Experimental procedures were approved by the Faculty of Pharmacy Ethics Committee, University of Benin (Approval No. EC/FP/026/035) and conducted in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (National Research Council, 2010).

Isolation of Uterine Tissue and Measurement of Contractility

Prior to experimentation, the estrous cycle stage of each animal was determined by vaginal cytology, and only mice in the estrus phase were included in the study. Vaginal smears were obtained by gently flushing the vaginal canal with 0.1 mL physiological saline using a sterile narrow-bore pipette. The lavage fluid was transferred onto clean glass slides, air-dried, fixed in chilled methanol, and stained with 0.01% methylene blue. Slides were examined under a digital light microscope (Visiscope®, VWR, UK), and the estrus stage was confirmed by the predominance of cornified epithelial cells. Animals were euthanized by cervical dislocation, and the uterine horns were immediately excised and placed in warm aerated PSS. Connective tissue and fat were carefully removed. Uterine segments (1 – 2 mm) were mounted vertically in a 10-mL organ bath containing aerated PSS (composition of PSS (mM/L) used: NaCl 154.00, D-glucose 2.78, KCl 5.63, NaHCO3 5.95, and CaCl2 · 2H2O 2.05) maintained at 37°C (Bafor *et al.*, 2019). One end of the tissue was attached to the tissue holder, while the other was connected to an isometric force transducer (PanLab, ADInstruments, Spain). A resting tension of 0.5 g was applied, and tissues were allowed to equilibrate for 35 – 40 min before experimentation.

Effect of MCE on Spontaneous Uterine Contractions

Tissues exhibiting regular, rhythmic spontaneous contractions were used. After a 10-minute control period, MCE was added cumulatively (6.25 – 400 µg/mL) to the bath. Each concentration remained in contact with the tissue for 5 minutes. This allowed the establishment of the concentration-response relationship. The tissue was washed and allowed to recover after the addition of the last MCE concentration.

Effect of MCE on Agonist-Induced Contractions

Following stable spontaneous activity, various agonists including oxytocin (14 nM), high potassium chloride concentration (KCl, 80 mM), prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$, 10^{-6} M) and methacholine (MCh, 10 µM) were used to stimulate the uterine tissues. Each agonist was allowed to act on the tissue for 5 min before cumulative concentrations of MCE (6.25 – 400 µg/mL) were added at 5-min intervals. The tissue was then thoroughly washed and allowed to recover. Control experiments were conducted using the vehicle and changes in contractile force and frequency documented accordingly.

Investigation of the Mechanism of Action of MCE

To elucidate the possible mechanisms underlying the inhibitory effect of MCE, cumulative concentrations of MCE (6.25 – 400 µg/mL) were administered in the presence of propranolol (PRO, 20 µM), and tetraethylammonium (TEA, 5mM). The tissues were pre-incubated with each agent for 5 minutes prior to the addition of MCE, which was introduced cumulatively at 5-minute intervals while the agents remained present. Vehicle-treated tissues served as controls.

Effect of MCE on Oxytocin-Induced Contractions in Calcium-Free Medium

The effect of MCE on intracellular calcium release was investigated using oxytocin-induced uterine contractions in a calcium-free medium. Uterine strips were initially equilibrated in normal Locke's PSS, and spontaneous contractions were recorded for 5 min. The bathing solution

was subsequently replaced with calcium-free Locke's PSS containing EDTA (0.1 mM) to remove extracellular Ca^{2+}. After spontaneous contractions had ceased, oxytocin (14 nM) was added and left in contact with the tissue for 5 min to evoke contractions through the release of intracellular Ca^{2+} stores. Thereafter, MCE (400 µg/mL) was added in the continued presence of oxytocin for an additional 5 min. Changes in uterine contractile activity were then recorded and compared with vehicle-treated controls.

Data Analysis

Data are expressed as mean ± SEM, and "n" represents the number of uterine strips, with each strip obtained from a different mouse. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison post hoc test. Differences were considered statistically significant when $p < 0.05$. All analyses were conducted using GraphPad Prism version 8.0.

Uterine contractility was quantified by measuring contraction amplitude, frequency, and area under the curve (AUC) using LabChart Reader software version 8.0. Concentration–response curves were analyzed by nonlinear regression using a four-parameter logistic model:

$$y = \text{Bottom} + (\text{Top} - \text{Bottom}) / [1 + 10^{((\text{LogIC50} - x) \times \text{HillSlope})}]$$

Where y represents the observed response, x is the logarithm of the extract concentration, Bottom and Top correspond to the minimum and maximum responses, respectively, HillSlope describes the steepness of the curve, and IC_{50} represents the concentration required to produce 50% inhibition of the measured response.

RESULTS AND DISCUSSION

Effect of MCE on Spontaneous Uterine Contractions

Cumulative applications of MCE (6.25 – 400 µg/mL) progressively inhibited, in a concentration-dependent manner, the spontaneous uterine contractions of the non-pregnant mouse uterus (Figure 1A). MCE significantly ($p<0.05$, $p<0.001$) reduced contraction amplitude and overall uterine activity despite a concomitant increase in contraction frequency (Figure 1B - D). The estimated IC_{50} values for amplitude, frequency and AUC were 0.044, 0.069 and 0.088 µg/mL respectively. Instant recovery of spontaneous contractions was observed after washing out the MCE with fresh PSS.

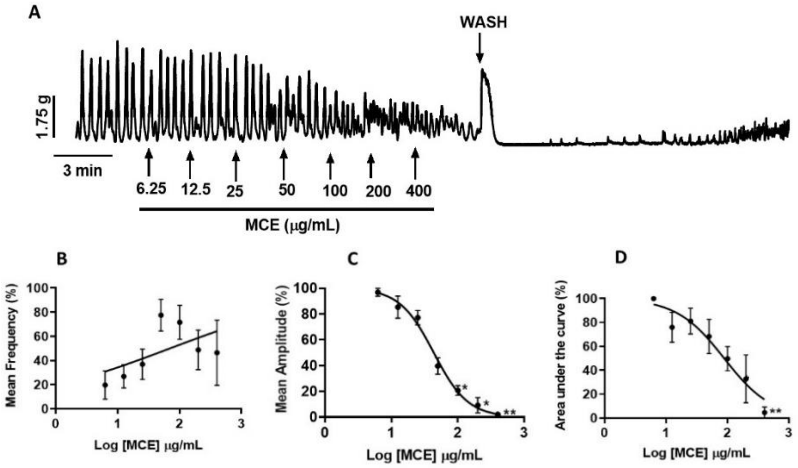

Figure 1: Effect of MCE on spontaneous uterine contractions in non-pregnant mice. (A) Representative recording showing the effect of MCE (6.25 – 400 µg/mL) on spontaneous contractions. (B - D) Concentration-response curves illustrating the effects of MCE on contraction frequency, amplitude, and overall activity (AUC). Values are expressed as mean ± SEM; n = 5 animals. *P < 0.05, **P < 0.01

Effect of MCE on Oxytocin-Induced Contractions

Application of oxytocin (14 nM) resulted in a marked increase in the amplitude and frequency of contractile responses in the non-pregnant uterus (Figure 2A). In the continued presence of oxytocin, cumulative application of MCE (6.25 – 400 µg/mL) produced a dose-dependent inhibition of the contractions (Figure 2B). MCE significantly reduced the amplitude of these contractions, showing significant reductions at 200 µg/mL ($p<0.05$) and 400 µg/mL ($p<0.01$) (Figure 2D). Similar to its effect on spontaneous activity, MCE produced no significant inhibition of the frequency of oxytocin-induced contractions (Figure 2C).

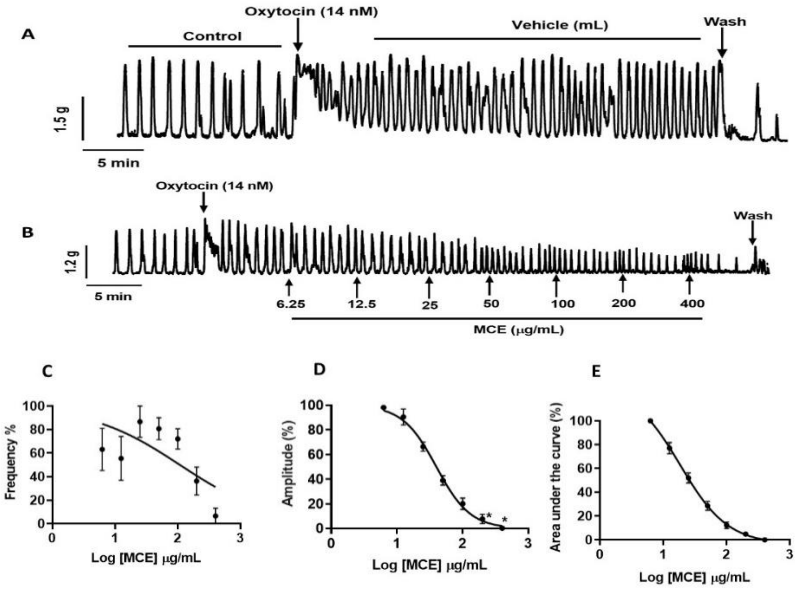

Figure 2: Effect of MCE on oxytocin (14 nM)-induced uterine contractions in non-pregnant mice. (A) Representative recording of the uterine response to oxytocin. (B) Representative recording showing the effect of MCE (6.25 – 400 µg/mL) on oxytocin-induced contractions. (C - E) Concentration-response curves illustrating the effects of MCE on contraction frequency, amplitude, and overall activity (AUC). Values are expressed as mean ± SEM; n = 5 animals. *P < 0.05

Effect of MCE on High KCL-Induced Contractions

Application of high KCl (80 mM) solution induced depolarization, producing a rapid and sustained increase in the force of contraction (Figure 3A). MCE (6.25 – 400 µg/mL) significantly diminished these high KCl-induced tonic contractions (Figure 3B). In the presence of MCE, the amplitude of contractions progressively reduced, with significant inhibition at 100 and 200 µg/mL ($p<0.05$) and highly significant inhibition at 400 µg/mL ($p<0.01$) (Figure 3C).

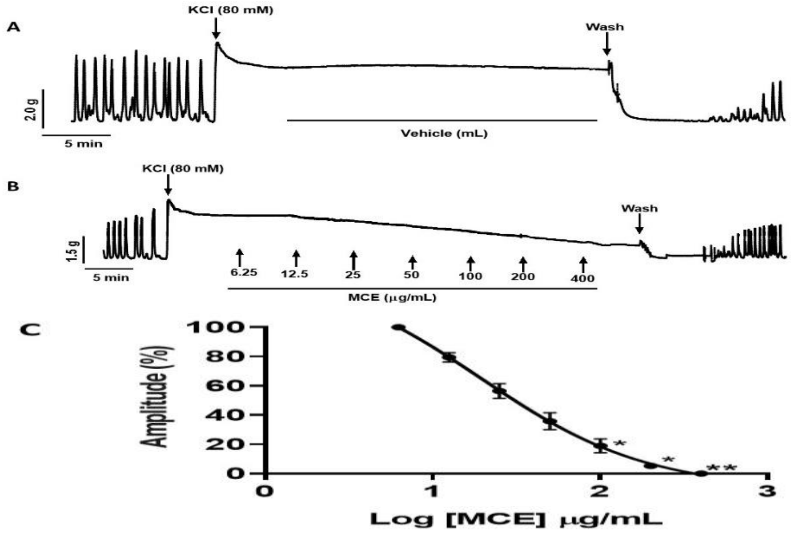

Figure 3: Effect of MCE on high KCl-induced uterine contractions in non-pregnant mice. (A) Representative recording of the uterine response to high KCl. (B) Representative recording showing the effect of MCE (6.25 – 400 µg/mL) on KCl-induced contractions. (C) Concentration-response curves illustrating the effects of MCE on contraction amplitude. Values are expressed as mean ± SEM; n = 5 animals. *P < 0.05, **p<0.01

Effect of MCE on Uterine Contractions Induced by $PGF_{2\alpha}$

$PGF_{2\alpha}$ induced strong uterine contractions, evidenced by increased contractile force (Figure 4A). Treatment with MCE (6.25 – 400 µg/mL) produced a concentration-dependent inhibition of $PGF_{2\alpha}$-induced contractions (Figure 4B). As shown in Figure 4C – E, the analysis of the contractile parameters revealed a concentration-dependent reduction in contraction amplitude, accompanied by an increase in contraction frequency. Despite the increase in frequency, the overall uterine activity was significantly suppressed by MCE, with marked inhibition of uterine contractions observed from 50 µg/ml onward.

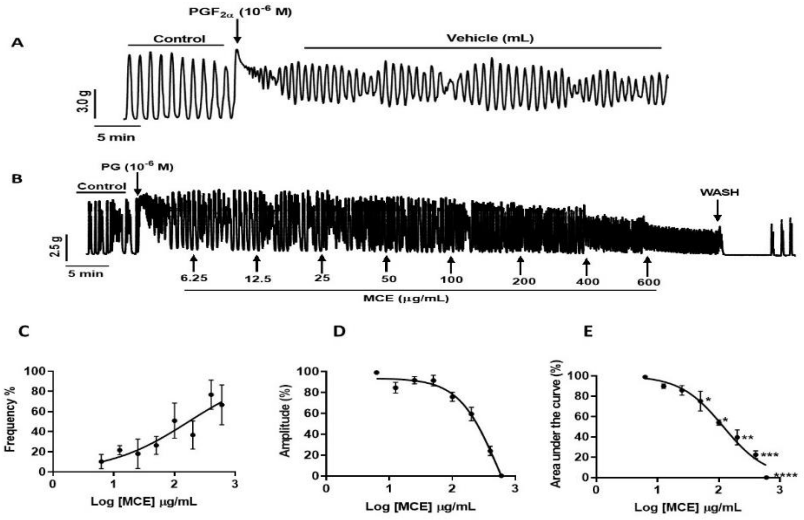

Figure 4: Effect of MCE on prostaglandin $F_{2\alpha}$ -induced uterine contractions in non-pregnant mice. (A) Representative recording of the uterine response to $PGF_{2\alpha}$. (B) Representative recording showing the effect of MCE (6.25 – 400 µg/mL) on $PGF_{2\alpha}$-induced contractions. (C - E) Concentration-response curves illustrating the effects of MCE on contraction frequency, amplitude, and overall activity (AUC). Values are expressed as mean ± SEM; n = 4 animals. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001

Effect of MCE on Oxytocin-Induced Contractions in Calcium-Free Medium

In calcium-free medium containing EDTA, a small transient contractile response attributable to intracellular calcium release was observed upon the addition of oxytocin (Figure 5A). The application of MCE in the continued presence of oxytocin in this calcium-free medium did not result in a significant reduction of these contractions. No evident effects on either the amplitude or frequency were observed (Figure 5B, 5C).

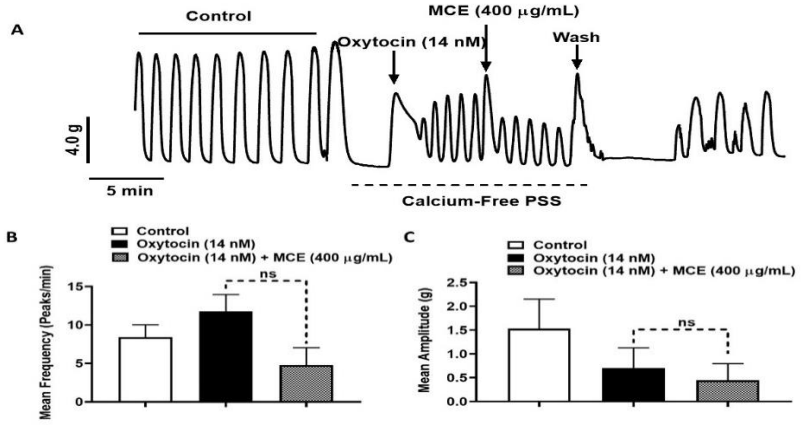

Figure 5: Effect of MCE on oxytocin-induced uterine contractions in calcium-free medium in non-pregnant mice. (A) Representative recording of the uterine response to oxytocin in calcium-free PSS and effect of MCE (400 µg/mL) on oxytocin-induced contractions. (B, C) Bar charts illustrating the effects of MCE on contraction frequency, and amplitude. Values are expressed as mean ± SEM; n = 5 animals. ns – Not significant

Effect of MCE on Methacholine-Induced Uterine Contractions

Compared with spontaneous contractions, MCh (10 µM) markedly increased both the amplitude and frequency of uterine contractions (Figure 6A). Cumulative administration of MCE (6.25 – 400 µg/mL) in the presence of MCh resulted in a concentration-dependent suppression of uterine contractility (Figure 6B). This inhibitory effect was characterized by a gradual decline in contractile activity, culminating in a significant reduction in overall uterine activity at concentrations of 200 and 400 µg/mL (Figure 6E).

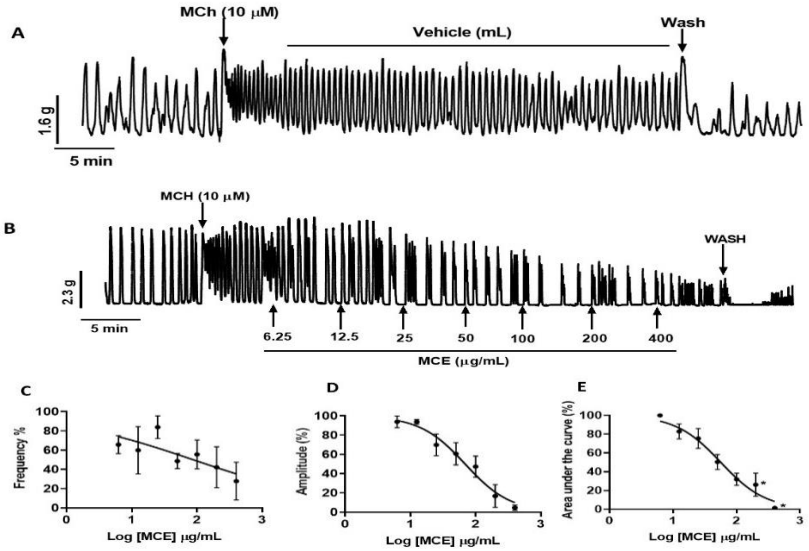

Figure 6: Effect of MCE on methacholine-induced uterine contractions in non-pregnant mice. (A) Representative recording of the uterine response to MCh. (B) Representative recording showing the effect of MCE (6.25 – 400 μg/mL) on MCh-induced contractions. (C - E) Concentration-response curves illustrating the effects of MCE on contraction frequency, amplitude, and overall activity (AUC). Values are expressed as mean ± SEM; n = 4 animals. *P < 0.05

Influence of Propranolol on the Uterine Relaxant Effect of MCE

Propranolol (20 μM) pretreatment did not prevent the inhibitory effect of MCE on uterine contractility (Figure 7A). MCE (6.25 – 400 μg/mL) produced concentration-dependent reductions in contraction amplitude and overall uterine activity (Figure 7C & D), while contraction frequency increased with increasing concentrations (Figure 7B). Relative to MCE alone, propranolol caused a rightward shift of the concentration-response curves for frequency and amplitude. Accordingly, the IC_{50} values for frequency and amplitude increased from 69.33 to 164.60 μg/mL and from 43.45 to 73.17 μg/mL, respectively. In contrast, the IC_{50} values for AUC decreased from 88.06 to 45.38 μg/mL.

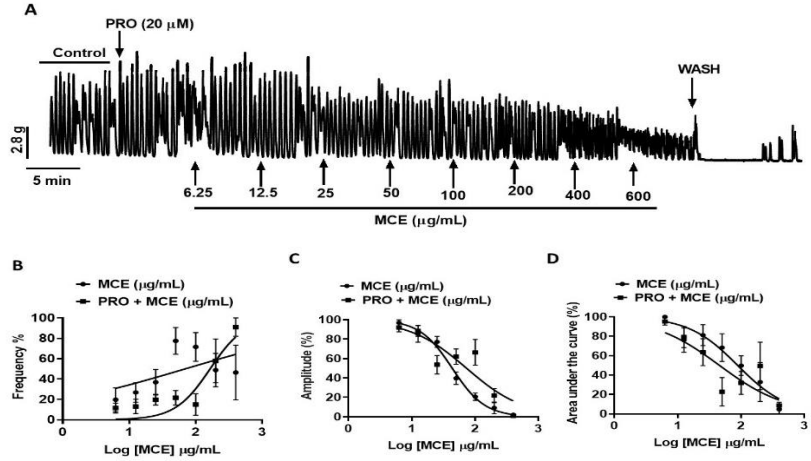

Figure 7: Effect of MCE on uterine contractions in the presence of propranolol (PRO, 20 μM) in non-pregnant mice. (A) Representative recording showing the effect of MCE (6.25 – 400 μg/mL) on uterine contractions in the presence of propranolol. (B - D) Concentration-response curves for contraction frequency, amplitude, and AUC (i.e. overall activity) in the presence and absence of propranolol. Values are expressed as mean ± SEM; n = 4 animals

Influence of Tetraethylammonium on the Uterine Relaxant Effect of MCE

TEA (5 mM) enhanced uterine contractility (Figure 8A); however, MCE retained its concentration-dependent inhibitory effect (Figure 8B). As shown in Figures 8C and 8D, TEA pretreatment shifted the concentration-response curves to the right, increasing the IC_{50} values for frequency (69.33 to 144.00 μg/mL) and amplitude (43.45 to 81.50 μg/mL). Conversely, the IC_{50} value for AUC decreased from 88.06 to 42.44 μg/mL (Figure 8E).

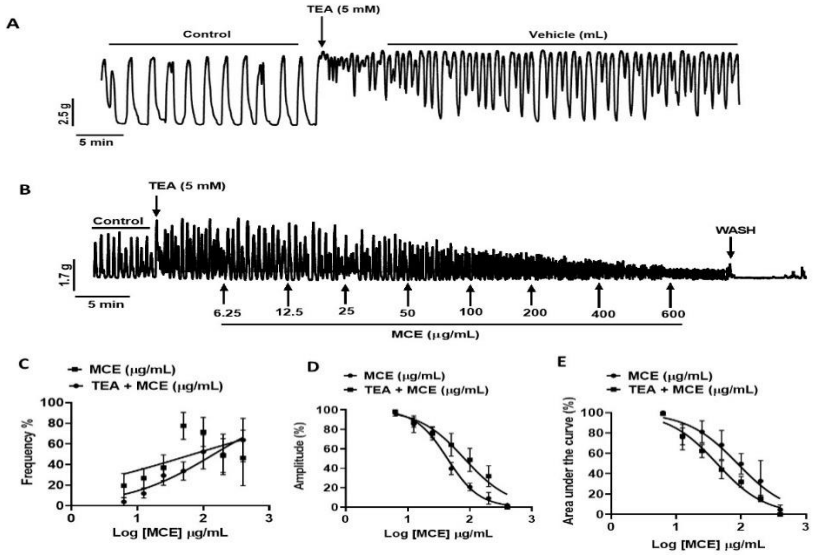

Figure 8: Effect of MCE on uterine contractions in the presence of tetraethylammonium (TEA, 5 mM) in non-pregnant mice. (A) Representative recording of the uterine response to TEA. (B) Representative recording showing the effect of MCE (6.25 – 400 µg/mL) in the presence of TEA. (C - E) Concentration-response curves for contraction frequency, amplitude, and AUC (i.e. overall activity) in the presence and absence of TEA. Values are expressed as mean ± SEM; n = 4 animals

Discussion

The continued reliance on medicinal plants for the management of reproductive disorders has stimulated interest in identifying novel uterine relaxants from natural sources (Kole *et al.*, 2020). *M. charantia* (bitter melon) is widely used in traditional medicine for the treatment of various ailments and has been reported to possess anti-inflammatory, antioxidant, hypotensive, and vasorelaxant properties (Li *et al*., 2020; Zainol *et al*., 2024). These diverse pharmacological activities, together with its traditional use in reproductive disorders (Grover & Yadav, 2004), underscore the need to investigate the direct effects of MCE on uterine contractility.

The present study demonstrates that the hydroethanol leaf extract of *M. charantia* produces a reversible, concentration-dependent inhibition of spontaneous and agonist-induced contractions in the isolated non-pregnant mouse uterus.This inhibitory effect was characterized primarily by a marked reduction in contraction amplitude and overall contractile activity, as measured by the area under the curve (AUC), whereas contraction frequency was either unaffected or slightly increased. The rapid restoration of spontaneous contractile activity following washout of the extract indicates that the inhibitory effect is reversible and is unlikely to result from tissue toxicity or irreversible impairment of the contractile apparatus. Such reversibility is a desirable characteristic of potential tocolytic agents, suggesting a pharmacological rather than a cytotoxic mechanism of action (Bafor *et al*., 2019).

Spontaneous uterine contractions depend largely on periodic membrane depolarization and calcium influx through voltage-operated calcium channels (VOCCs) (Burdyga *et al.*, 2007; Wray, 2007). The preferential reduction in contraction amplitude, suggests that MCE primarily interferes with excitation–contraction coupling rather than pacemaker activity. Similar findings including those from our previous studies, have been reported for several plant-derived uterine relaxants that exert their effects by reducing intracellular calcium availability (Uchendu & Nwachukwu, 2026).

The inhibitory activity of MCE was further confirmed against oxytocin-induced contractions. Oxytocin is a potent endogenous uterotonic agent that stimulates uterine contraction through receptor-mediated calcium mobilization (Vrachnis *et al.*, 2011; Sukwan *et al.*, 2014). MCE significantly attenuated oxytocin-induced contractions, particularly contraction amplitude and overall contractile activity, indicating that the extract effectively counteracts receptor-mediated uterine stimulation and may interfere with calcium-dependent signaling pathways downstream of oxytocin receptor activation.

To further investigate the role of extracellular calcium influx in the observed relaxant effect, MCE was evaluated against contractions induced by high potassium chloride (KCl). High extracellular potassium concentrations depolarize the myometrial membrane, leading to sustained activation of L-type VOCCs and a substantial influx of extracellular calcium (Alotaibi, 2020; Malik *et al*., 2021). Consequently, inhibition of KCl-induced contractions is widely accepted as evidence of calcium channel antagonism. The significant suppression of KCl-induced tonic contractions by MCE strongly suggests that the extract interferes with calcium entry through voltage-operated calcium channels.

The inhibitory effects of MCE on $PGF_2\alpha$-induced contractions provided additional insight into its uterine relaxant activity. $PGF_2\alpha$ is an important endogenous uterotonic mediator that increases myometrial contraction through FP receptor activation and intracellular calcium mobilization (Riaposova *et al*., 2023). MCE produced a concentration-dependent inhibition of $PGF_2\alpha$-induced contractions. The ability of the extract to inhibit contractions induced by both oxytocin and $PGF_2\alpha$ indicates that its relaxant effect is not receptor-specific but rather acts on a common downstream pathway shared by these agonists.

To distinguish between effects on extracellular calcium influx and intracellular calcium release, experiments were conducted in calcium-free physiological salt solution containing EDTA. Under these conditions, extracellular calcium was unavailable, and oxytocin-induced contractions

depended primarily on calcium released from intracellular stores. Interestingly, MCE failed to significantly inhibit these residual contractions. This finding suggests that the extract does not substantially interfere with IP_3-mediated calcium release from the sarcoplasmic reticulum. Therefore, the inhibitory activity of MCE appears to be directed mainly toward extracellular calcium entry rather than intracellular calcium mobilization. Collectively, the findings from the KCl and calcium-free experiments strongly indicate that the uterine relaxant effect of MCE is mediated predominantly through inhibition of extracellular calcium influx rather than suppression of intracellular calcium mobilization.

The involvement of muscarinic receptor-mediated contractile pathways was examined using MCh, a stable muscarinic receptor agonist. MCh markedly enhanced uterine contractility, consistent with activation of M_3 muscarinic receptors and subsequent stimulation of phospholipase C-mediated calcium signaling (Kudlak & Tadi, 2023). MCE significantly inhibited MCh-induced contractions, indicating that its relaxant effect extends to cholinergic stimulation of the uterus. Because muscarinic receptor activation ultimately increases intracellular calcium concentrations through both calcium release and calcium influx pathways, the inhibitory effect of MCE is again consistent with suppression of calcium-dependent contractile mechanisms.

To investigate the possible contribution of β-adrenergic receptors, uterine tissues were pretreated with propranolol, a non-selective β-adrenoceptor antagonist. Pretreatment with propranolol did not abolish the inhibitory effect of MCE, although a modest rightward shift in the concentration–response curves were observed. The persistence of uterine relaxation despite β-adrenoceptor blockade indicates that activation of β-adrenergic receptors is unlikely to be the primary mechanism responsible for the extract's activity. The slight reduction in potency observed following propranolol pretreatment may reflect a minor modulatory influence of endogenous adrenergic pathways but does not support direct β-adrenoceptor agonism as the principal mechanism of action.

The role of potassium channels was examined using TEA, a non-selective potassium channel blocker. Although TEA pretreatment enhanced uterine contractility and partially attenuated the inhibitory effects of MCE, substantial relaxation was preserved. These findings suggest that potassium channels activation may contribute to the uterine relaxant effect of MCE but unlikely to represent its principal mechanism. Instead, potassium channel activation appears to play a supportive role that complements the predominant inhibition of extracellular calcium influx.

Nevertheless, this study has some limitations that should be considered when interpreting the findings. The experiments were conducted *ex vivo* using isolated uterine tissues from non-pregnant mice, which do not fully replicate the physiological, hormonal environment of pregnancy. Furthermore, the study evaluated a crude hydroethanol extract without identifying the active constituents. Therefore, further studies involving pregnant animal models, bioassay-guided fractionation, pharmacokinetic studies, and comprehensive safety evaluation are needed to confirm its therapeutic potential

CONCLUSION

The present findings demonstrate that MCE exerts potent uterine relaxant activity against both spontaneous and receptor-mediated contractions in the isolated non-pregnant mouse uterus. The consistent inhibition of contractions induced by oxytocin, KCl, $PGF_{2}\alpha$, and MCh, together with the absence of a significant effect in calcium-free medium, provides strong pharmacological evidence that inhibition of extracellular calcium influx is the predominant mechanism underlying its tocolytic activity, with a possible secondary contribution from potassium channel activation. These findings provide mechanistic support for the traditional use of *M. charantia* in conditions associated with uterine hypercontractility

REFERENCES

Aguilar, H.N., Mitchell, B.F. (2010). Physiological pathways and molecular mechanisms regulating uterine contractility. *Human reproduction update*, 16(6): 725-744. DOI: 10.1093/humupd/dmq016

Alotaibi, M.F. (2020). *Pimpinella anisum* extract attenuates spontaneous and agonist-induced uterine contraction in term-pregnant rats. *Journal of Ethnopharmacology* 254: 112730. https://doi.org/10.1016/j.jep.2020.112730

Bafor, E.E., Ukpebor, F., Omoruyi, O., Ochoyama, E., Omogiade, G., Ekufu, J. and Edrada-Ebel, R. (2019). Tocolytic activity assessment of the methanol leaf extract of *Justicia flava* Vahl (Acanthaceae) on mouse myometrial contractility and preliminary mass spectrometric determination of secondary metabolites. *Journal of ethnopharmacology*, *243*: 112087. DOI: 10.1016/j.jep.2019.112087

Berridge, M.J., Bootman, M.D. and Roderick, H.L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nature reviews Molecular cell biology*, *4*(7): 517-529. https://doi.org/10.1038/nrm1155

Bootman, M.D., Bultynck, G. (2020). Fundamentals of cellular calcium signaling: a primer. *Cold Spring Harbor perspectives in biology*, *12*(1), p.a038802. Http://doi.org/10.1101/cshperspect.a03880

Grover, J.K., Yadav, S.P. (2004). Pharmacological actions and potential uses of *Momordica charantia*: a review. *Journal of ethnopharmacology*, 93(1):123-132. Http://doi.org/10.1016/j.jep.2004.03.035

Kole, C., Matsumura, H., Behera, T.K. eds. (2020). The bitter gourd genome, 10: 978-3. Cham Switzerland: Springer.

Kudlak, M., Tadi, P. (2023). Physiology, muscarinic receptor. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Kumar, D.S., Sharathnath, K.V., Yogeswaran, P., Harani, A., Sudhakar, K., Sudha, P., Banji, D. (2010). A medicinal potency of *Momordica charantia*. *International Journal of Pharmaceutical Sciences Review and Research*, *1*(2), pp.95-100.

Li, Z., Xia, A., Li, S., Yang, G., Jin, W., Zhang, M., Wang, S. (2020). The pharmacological properties and therapeutic use of bitter melon (*Momordica charantia* L.). *Current Pharmacology Reports*, *6*(3):103-109. https://doi.org/10.1007/s40495-020-00219-4

Malik, M., Roh, M., England, S.K. (2021). Uterine contractions in rodent models and humans. *Acta Physiologica* 231(4): e13607. Http://doi.org/10.1111/apha.13607

Mili, S., Konwar, A., Singh, Y.R. and Kalita, J.C. (2025). Comprehensive Phytochemical Analysis and Antifertility Assessment of Medicinal Plants Traditionally Used in Contraception across Different Cultures. *Pharmacological Research-Natural Products*, p.100403. https://doi.org/10.1016/j.prenap.2025.100403

National Research Council. Guide for the care and use of laboratory animals. In: Guide for the Care and Use of Laboratory Animals, eighth ed. National Academies Press 2010; 118.

Pan, M., Chen, H., Deng, K. and Xiao, K. (2025). Organ-on-a-Chip Models of the Female Reproductive System: Current Progress and Future Perspectives. *Micromachines*, *16*(10), p.1125. https://doi.org/10.3390/mi16101125.

Pehlivanoğlu, B., Bayrak, S., Doğan, M. (2013). A close look at the contraction and relaxation of the myometrium; the role of calcium. *Journal of the Turkish German Gynecological Association*, *14*(4): 230-234. https://doi.org/10.5152/jtgga.2013.67763

Rabizadeh, F. (2025). A review of medicinal plants used for uterine disorders in Makhzan al-Adwiyyah: Insights from Persian Medicine. *Iranian Journal of Pharmaceutical Research*, *24*(1): e160193. Http://doi.org/10.5812/ijpr-160193

Riaposova. L., Kim, S.H., Hanyaloglu, A.C., Sykes, L., MacIntyre, D.A., Bennett, P.R., Terzidou, V. (2023). Prostaglandin $F_{2\alpha}$ requires activation of calcium-dependent signalling to trigger inflammation in human myometrium. *Frontiers in Endocrinology* 14: 1150125. Http://doi.org/10.3389/fendo.2023.1150125. Http://doi.org/10.3389/fendo.2023.1150125

Sharmin, Z.R., Akter, M.T., Rahman, R., Hoque, M., Khan, I., Mosaddek, A.S.M. (2017). Comparative study of hypolipidemic effects of Momordica charantia (karela) with atorvastatin in fat fed rats. *Journal of Human Health Research*, 1(1):11. https://doi.org/10.14302/issn.2576-9383.jhhr-17-1816

Shmygol, A., Blanks, A.M., Bru-Mercier G., Gullam, J.E., Thornton, S. (2007). Control of uterine Ca^{2+} by membrane voltage: toward understanding the excitation–contraction coupling in human myometrium. *Annals of the New York Academy of Sciences*, *1101*(1): 97-109. Http://doi.org/10.1196/annals.1389.031

Shobha, C.R., Vishwanath, P., Suma, M.N., Prashant, A., Rangaswamy, C., Gowdappa, B.H. (2015). *In vitro* anti-cancer activity of ethanolic extract of *Momordica charantia* on cervical and breast cancer cell lines. *International Journal of Health Allied Science*, *4*(4): 210-210. https://doi.org/10.4103/2278-344X.167649

Sukwan, C., Wray, S., Kupittayanant, S. (2014). The effects of Ginseng Java root extract on uterine contractility in nonpregnant rats. *Physiological Reports*, 2(12): e12230. Http://doi.org/10.14814/phy2.12230

Tamilanban, T. (2018). *In vitro* neuroprotective effect of charantin from *Momordica charantia* against neurotoxin and endoplasmic reticulum stress-induced cell death in SH-SY5Y cells. *International Journal of Green Pharmacy*, 12(03): S555–S560.

Uchendu, A., & Nwachukwu, M. U. (2026). Uterorelaxant Activity of *Laurus nobilis* L. Leaf Extract on Mouse Uterine Contractility. *Nigerian Journal of Pharmaceutical and Applied Science Research*, *15*(1): 36–46. https://doi.org/10.60787/nijophasr-v15-i1-640

Vrachnis, N., Malamas, F.M., Sifakis, S., Deligeoroglou, E. and Iliodromiti, Z. (2011). The oxytocin-oxytocin receptor system and its antagonists as tocolytic agents. *International Journal of Endocrinology*, (1), p.350546. https://doi.org/10.1155/2011/350546

World Health Organization, (2005). National policy on traditional medicine and regulation of herbal medicines: Report of a WHO global survey. *World Health Organization*.

Wray, S., (2007). Insights into the uterus. *Experimental physiology*, 92(4): 621-631. https://doi.org/10.1113/expphysiol.2007.038125.

Yakubu, J., Sodipo, O.A. and Umarfarouk, S.A. (2022). Phytochemical profiling, toxicity study and abortifacient activity of seed and whole plant of *Momordica charantia* Linn. (Cucurbitaceae). *Chemical Review and Letters*, 5(3), pp.200-206.

Zainol, S., Dewa, A., Mohamed, N., Razali, N., Manshor, N.M., Jusoh, R.R. (2024). Vasorelaxant Activity and Its Mechanisms of *Momordica charantia* Fruit Extract in Diabetic Rat Aorta. *Malaysian Journal of Medicine & Health Sciences*, *20* (SUPP1): 93-104.