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Effects of *Syzygium aromaticum* (L.) Merr. & LM Perry (Cloves) Extract on Uterine Contractions and its Underlying Mechanisms: An *Ex vivo* Study

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Abstract

Abnormal uterine contractility contributes significantly to gynaecological and obstetric disorders such as dysmenorrhea and preterm labour, and current tocolytic therapies are often limited by adverse effects, cost, and inconsistent efficacy. These limitations have stimulated interest in plant-derived agents with smooth muscle relaxant properties. *Syzygium aromaticum* (clove) is traditionally used for pain and spasms, but its effects on uterine smooth muscle and underlying mechanisms have not been previously reported. In this study, the effects of a hydroethanol extract of *Syzygium aromaticum* (SAE) was investigated on uterine contractility using isolated uterine strips from non-pregnant Swiss albino mice. Uterine tissues were mounted in organ baths containing aerated physiological solution, and isometric contractions were recorded after equilibration. Cumulative concentrations of SAE (6.25-200 µg/mL) were assessed on spontaneous uterine contractions as well as contractions induced by oxytocin, prostaglandin $F_{2\alpha}$, potassium chloride, and methacholine. To elucidate the mechanism of action, experiments were conducted in the presence of propranolol and tetraethylammonium, and in the calcium-free medium containing EDTA. Contractile responses were quantified by measuring amplitude, frequency, and area under the curve. SAE produced a concentration-dependent and reversible inhibition on spontaneous uterine contractions, significantly reducing contractile amplitude, frequency, and overall activity. The extract markedly suppressed oxytocin-, prostaglandin $F_{2\alpha}$-, and methacholine-induced contractions but showed no significant effect on high potassium-induced tonic contractions. In calcium-free conditions, SAE completely abolished oxytocin-induced contractions. The inhibitory effect persisted despite β-adrenergic blockade and potassium channel inhibition. In conclusion, SAE exhibits potent uterine relaxant activity mediated primarily via suppression of receptor-operated signaling and intracellular calcium release, providing pharmacological support for its traditional use in uterine spasmodic disorders.

Keywords: *Syzygium aromaticum,* clove, uterine smooth muscle, uterine contractility, oxytocin, mice.

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Introduction

Myometrial contractions are rhythmic and tightly regulated processes that facilitate several reproductive functions, including menstrual shedding of the endometrium, and expulsion of the fetus during labour (Huang *et al*., 2017; Sajadi *et al*., 2018). However, altered uterine contractile activity is implicated in several gynaecological and obstetric disorders, such as dysmenorrhea, preterm labour, endometriosis, postpartum haemorrhage, and spontaneous miscarriage (Aguilar and Mitchell, 2010; Sajadi *et al*., 2018).

Uterine contractility plays a central role in conditions such as primary dysmenorrhea and both term and preterm labour (Bafor *et al*., 2019). Primary dysmenorrhea affects a large proportion of women of reproductive age and is associated with significant pain, discomfort, and reduced quality of life (Bernadi *et al*., 2017). Preterm labour which results in preterm birth, remains a major global health concern due to its contribution to neonatal and maternal morbidity and mortality. Approximately 15 million babies are born preterm each year, and complications of preterm birth are responsible for nearly one million child deaths annually (Kolan and Hall, 2023). The prevalence of primary dysmenorrhea has been reported to range from 41.7% to 94% worldwide (Hu *et al.,* 2020), while in Nigeria it ranges from 42.5% to 71.8% (Onu *et al*., 2020). Preterm labour is characterized by premature uterine contractions, whereas dysmenorrhea in non-pregnant women presents as painful uterine cramps due to increased uterine contractions (Tonini, 2002; Bafor *et al*., 2019).

Clinically, excessive or premature uterine contractions are managed using tocolytic drugs including calcium channel blockers (e.g., nifedipine), β_2-adrenergic agonists (e.g., salbutamol and ritodrine), magnesium sulphate, and oxytocin receptor antagonists such as atosiban (Munjal and Allam, 2024; Gopy *et al.*, 2024). Despite their effectiveness, these drugs are associated with significant maternal and fetal adverse effects, limited efficacy, high cost and potential drug interactions, which restrict their widespread use and long-term safety (Lamont, 2016). These limitations have stimulated interest in alternative therapies, particularly plant-derived constituents with established ethnomedicinal use and potentially safer pharmacological profiles.

Syzygium aromaticum (clove) is an aromatic spice widely used in traditional medicine across Asia and African for the management of pain, inflammation, infections, and gastrointestinal disturbance. Phytochemical analyses have shown that clove is rich in phenolic compounds, including eugenol, eugenyl acetate, β-caryophyllene, flavonoids, tannins, and phenolic acids, which collectively contribute to its wide range of biological activities (Batiha *et al*., 2020; Abdul Aziz *et al*., 2023). Pharmacological studies have demonstrated that clove and its major bioactive constituent, eugenol, possess antioxidant, anti-inflammatory, analgesic, antimicrobial, antispasmodic, and smooth muscle-relaxant properties (Hussain *et al*., 2017; Yadav *et al*., 2020). Previous experimental studies have reported that clove extract induces relaxation of gastrointestinal and airway smooth muscles, suggesting a potential modulatory effect on smooth muscle contractility (Lima *et al*., 2011; Oyinloye *et al*., 2025). Additionally, eugenol has been shown to inhibit high voltage-activated calcium channel currents and suppress intracellular calcium signaling in various smooth muscle tissues (Pramod, 2010). Despite these findings and the traditional use of clove in the management of menstrual pain, its direct effects on uterine smooth muscle contractility and the mechanism underlying such effects have not been systematically investigated. Therefore, this study aimed to evaluate the effects of a hydroethanol extract of *Syzygium aromaticum* on spontaneous and agonist-induced uterine contractions in isolated myometrial strips from non-pregnant mice.

Materials and Methods

Drugs, chemicals and reagents

Oxytocin (Anhui Hongye Pharmaceutical Co., Ltd., Anhui Province, China, Propranolol hydrochloride (Sigma Aldrich, UK), Methacholine chloride (Sigma Aldrich, UK), Prostaglandin $F_{2\alpha}$ (Bioveta, a.s. Czech Republic.), and Tetraethylammonium (Sigma Aldrich, UK), Ethanol (Pharmatrends Nigeria), Methylene Blue (Tianjin Kermel Chemical Reagent Co., Ltd), ethylenediaminetetraacetic acid (EDTA) (Guangdong Guanghua Sci-Tech Co., Ltd. China). Potassium chloride (KCl), sodium chloride (NaCl), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), and D-glucose ($C_6H_{12}O_6.H_2O$) were of analytical grade from Sigma Aldrich, UK and Guangdong Guanghua Sci-Tech Co. Ltd China. All drugs were freshly prepared in distilled water on the day of experiment. Ringer's Locke physiological saline solution (PSS) was prepared with the following composition (mM/L): NaCl 154.0, KCl 5.63, $C_6H_{12}O_6.H_2O$ 2.78, $NaHCO_3$ 5.95, $CaCl_2H_2O$ 2.05. For calcium-free PSS, $CaCl_2$ was omitted and EDTA (0.1 mM) was added. Distilled water was used as the solvent for all drugs.

Experimental animals.

Thirty Swiss albino non-pregnant female mice (20-28 g, 8-10 weeks old) were used in the study. Animals were procured from the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. Animals were maintained under standard laboratory conditions with a 12 h light/dark cycle, ambient temperature of 24 ±2ºC, and *ad libitum* access to standard rodent chow and water. All experimental procedures were approved by the Faculty of Pharmacy Ethics Committee, University of Benin, Nigeria.

Plant material collection and extraction.

Dried flower buds of *Syzygium aromaticum* were purchased from Uselu Market, Benin City, Edo state, Nigeria. The plant was authenticated at the Herbarium Unit, Department of Plant Biology and Biotechnology, University of Benin, (voucher number; UBH-S385). The buds were cleaned, and pulverized into a fine powder. A total of 525 g of the powdered material was extracted using a hydroethanol solvent (ethanol: water, 1:1 v/v) in a Soxhlet apparatus. The *S. aromaticum* extract (SAE) was concentrated under reduced pressure at low temperature in a rotary evaporator, and dried in an oven at a temperature of 40ºC, yielding 42.31% (w/w), and stored at 4°C until use.

Isolation of uterine tissue and measurement of uterine contractility.

Only mice in estrus phase were used for uterine contractility studies. Estrus was determined using vaginal cytology. Vaginal smears were collected using a sterile pipette (approximately 0.1 mm in diameter) containing 0.1 mL of normal saline, air-dried, fixed in cold methanol, and stained with 0.1% methylene blue. Slides were examined under a light microscope (VWR microscope VisiScope series 200), and animals displaying predominantly cornified epithelial cells were identified as being in the estrus phase (Uchendu et al., 2025).

The mice were euthanized humanely by cervical dislocation. The abdomen was opened, and both uterine horns were excised, cleaned of adhering fats and connective tissues, and placed in aerated warm PSS. Each uterine horn was cut into longitudinal strips measuring approximately 1 - 2 mm in length. Uterine strips were mounted vertically in a 10 mL organ bath containing Locke's solution maintained at a temperature of 36.5 ± 0.5ºC and continuously aerated with 100% oxygen. One end of the tissue was fixed to a tissue holder, while the other was connected to an isometric force transducer (MLT0210/A Pan Lab AD Instruments, Spain). Contractile activities were recorded using a Power-lab data acquisition system (ML826, Power lab 2/26, AD Instruments, Spain) with Lab-chart software (MLS060/8 version 8.0, AD Instruments, Spain).

Tissues were allowed to equilibrate under a resting tension of 0.5 g for 35 to 40 min, or until stable spontaneous rhythmic contractions were obtained.

Effect of SAE on spontaneous uterine contractions.

After spontaneous contractions were allowed to equilibrate, the rhythmic uterine contractions were recorded for 10 min, and taken as control (100% contraction). Increasing concentrations of SAE (6.25 – 200 µg/mL) was added cumulatively at 5 min intervals. Changes in contraction amplitude and frequency were recorded, and after the final concentration, the tissues were washed and allowed to recover.

Effect of SAE on agonist-induced contractions

To assess the effect of SAE extract on agonist-induced contractions, uterine tissues were precontracted with the following agents: oxytocin (OT, 14 nM), potassium chloride (KCl, 80 mM), or prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$, 10^{-6} M). Each agonist was allowed a contact time of 5 min to stimulate contractions. Without washing, cumulative concentrations of SAE (6.25 – 200 µg/mL) were added at 5 min intervals. Control experiments were conducted using the vehicle (distilled water).

Investigation of mechanism(s) of action

To investigate the possible mechanisms underlying the observed effects, increasing concentrations of SAE were tested in the presence of methacholine (MCh, 10 µM), tetraethylammonium (TEA, 5 mM), and propranolol (PRO, 20 µM). Each drug was left in contact with the tissue for 5 min before cumulative additions of SAE (6.25 – 200 µg/mL) at 5 min interval in the presence of the drugs. Control experiments were conducted using the vehicle (distilled water).

Effect of SAE on oxytocin-induced contractions in calcium-free medium

The effect of SAE on intracellular calcium release was evaluated. The rhythmic contractions of uterine tissues were recorded in normal Locke's solution for 5 min. The PSS was then replaced with calcium-free Locke's solution containing EDTA (0.1 mM) to chelate residual extracellular calcium. In the absence of spontaneous contractions, oxytocin (14 nM) was added to the organ bath and allowed a contact time of 5 min to induce contractions mediated by intracellular calcium release. In the presence of oxytocin, 200 µg/mL of SAE was added and allowed a contact time of 5 min. Changes in uterine contractile activity were recorded.

Data analysis

Contractile activity was quantified by measuring contraction amplitude, frequency, and area under the curve (AUC). Data are expressed as mean ± standard error of mean (SEM), with n = 5 animals. Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed Dunnett's post hoc test. A p value < 0.05 was considered statistically significant.

Concentration-response curves were fitted using nonlinear regression based on a four-parameter logistic model: Y = bottom + (top-bottom)/(1+10^((logIC_{50}-X) × hillslope), where y represents the response, X is the logarithm of SAE concentration, IC_{50} is the concentration producing 50% inhibition. Graph-pad prism version 8.0 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses.

Results

Effect of SAE on spontaneous uterine contractions

Cumulative additions of SAE (6.25 – 200 µg/mL) produced a concentration-dependent inhibition of spontaneous uterine contractions in isolated non-pregnant uterus (Figure 1A). SAE (6.25 – 200 µg/mL) significantly reduced contraction amplitude (Figure 1B), and contraction frequency (Figure 1C). The half maximal inhibitory concentration (IC_{50}) values for the contraction amplitude and frequency were 38.47 µg/mL and 48.07 µg/mL respectively. For the area under the curve, IC_{50} value was 25.25 µg/mL. Analysis of area under the curve confirmed a marked suppression of overall uterine contractile activity (Figure 1D). Contractile activity recovered fully following washout.

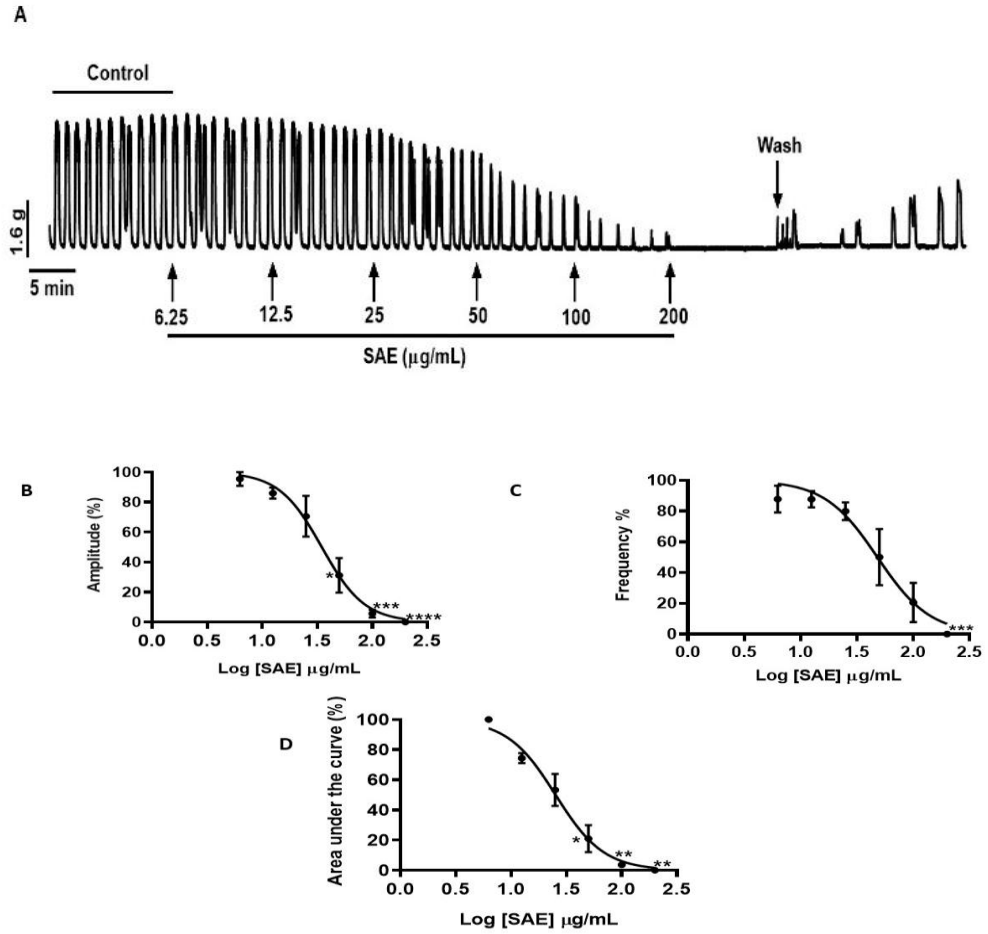
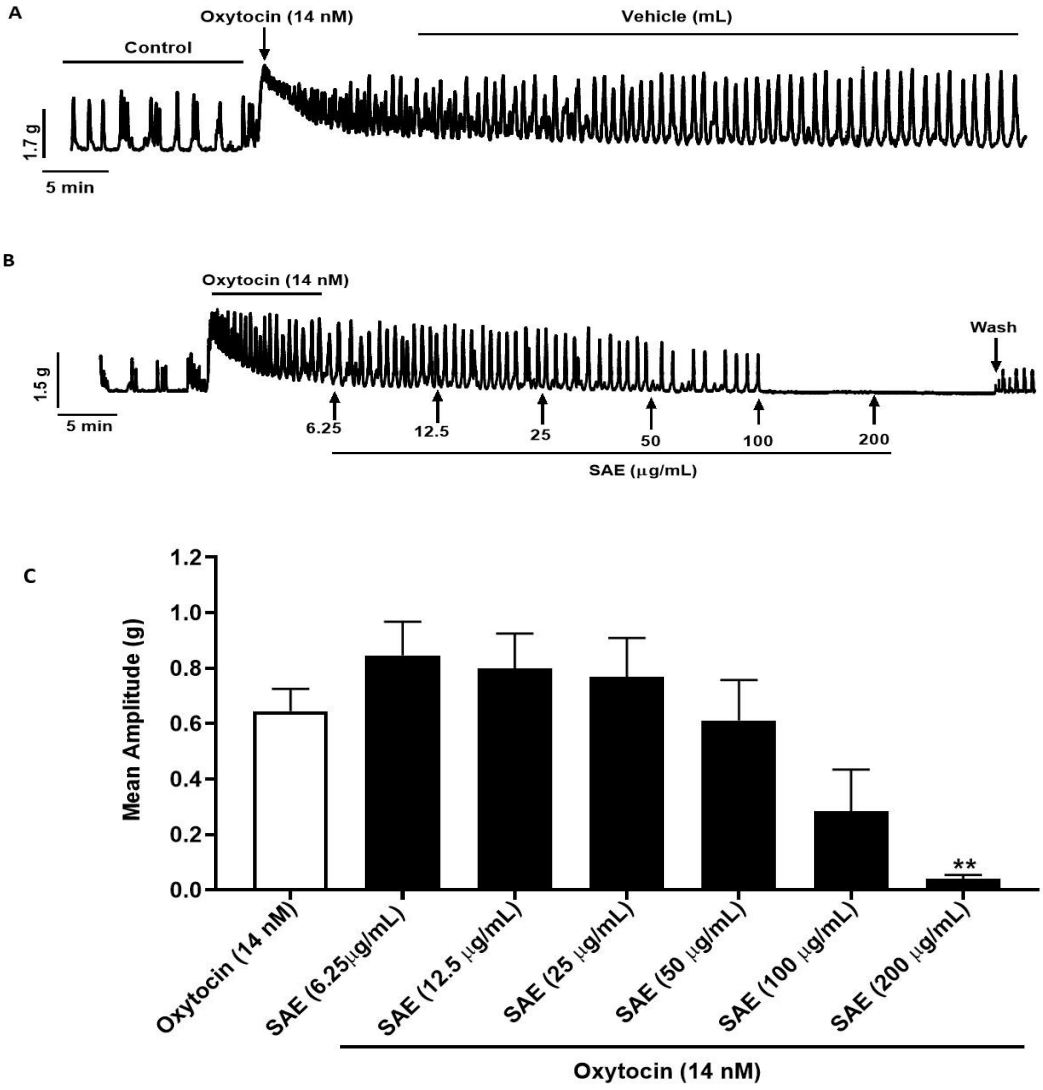


Figure 1. Effect of SAE on spontaneous uterine contractions in non-pregnant mice. (A) the effect of cumulative concentrations of SAE (6.25 – 200 µg/mL) on spontaneous uterine contractions in non-pregnant mice. Concentration-response curves showing the effects of SAE on the spontaneous contraction amplitude (B), frequency (C) and area under the curve (D). n = 5 animals. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 compared to control

Effect of SAE on oxytocin-induced uterine contraction

Oxytocin (14 nM) significantly increased uterine contraction amplitude and frequency (Figure 2A). In the presence of oxytocin, cumulative addition of SAE (6.25 – 200 µg/mL) caused a concentration-dependent inhibition of uterine contractions (Figure 2B). A significant reduction (P < 0.01) in contraction amplitude was observed at 200 µg/mL only (Figure 2C), while contraction frequency was significantly reduced at 50 - 200 µg/mL (Figures 2D).


A
Oxytocin (14 nM)
Vehicle (mL)
Control
1.7 g
5 min
B
Oxytocin (14 nM)
Wash
1.5 g
5 min
6.25
12.5
25
50
100
200
SAE (μg/mL)
C
Mean Amplitude (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
Oxytocin (14 nM)
SAE (6.25μg/mL)
SAE (12.5 μg/mL)
SAE (25 μg/mL)
SAE (50 μg/mL)
SAE (100 μg/mL)
SAE (200 μg/mL)
Oxytocin (14 nM)

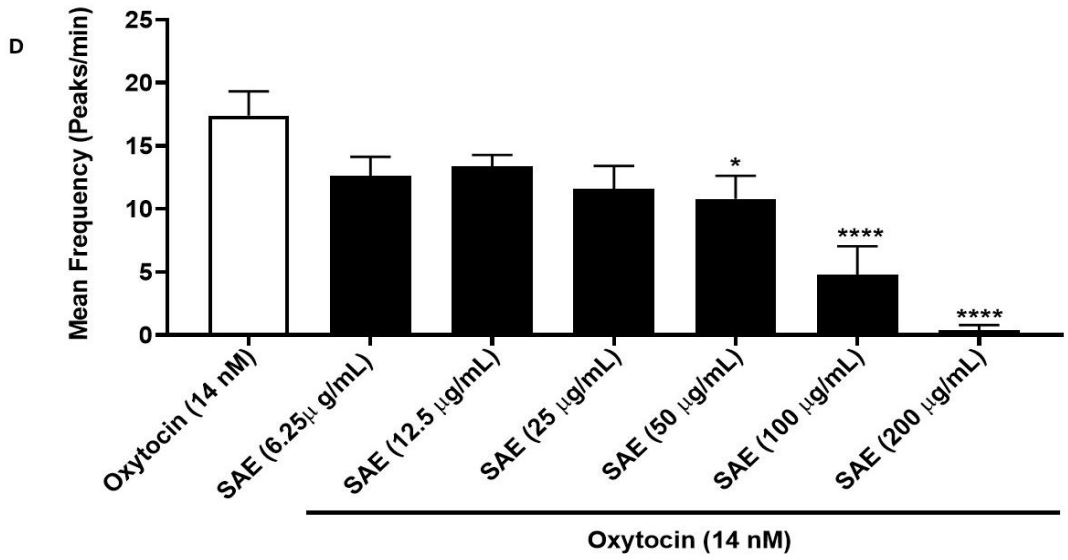
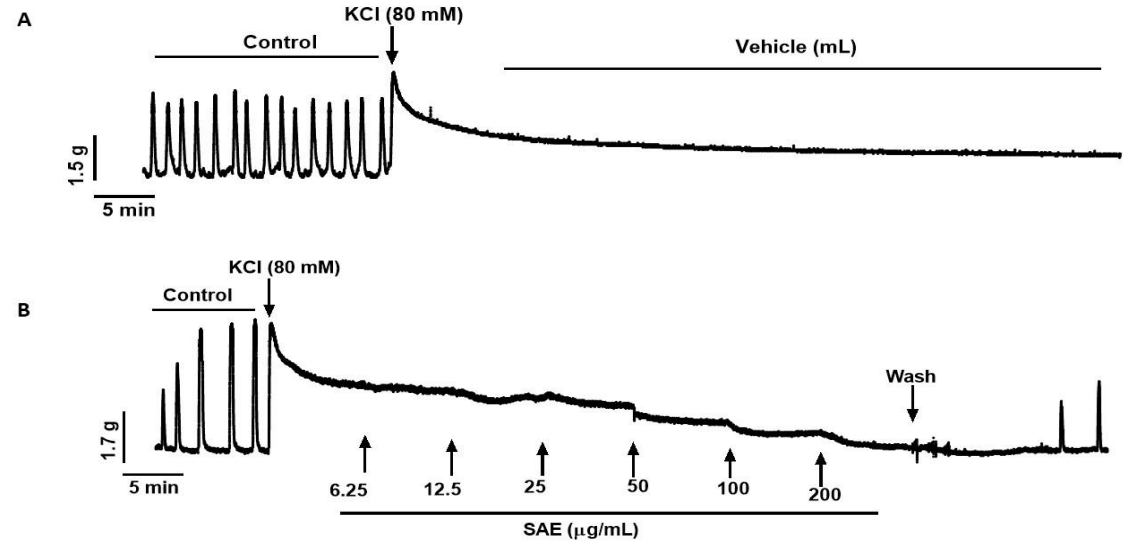


Figure 2. Effect of SAE on uterine contractions induced by oxytocin (14 nM) in non-pregnant mice. (A) the response of uterine tissue to oxytocin (B) the effect of SAE on uterine contractions induced with oxytocin in non-pregnant mice, (C and D). the inhibitory effect of SAE on contractions amplitude, and frequency induced by oxytocin. Values are mean ± SEM, n = 5 animals. *P < 0.05, **P < 0.01, and ****P < 0.0001 compared to oxytocin effect.

Effect of SAE on high KCL-induced uterine contractions

High potassium chloride (80 mM) induced a sustained tonic contraction of uterine tissues (Figure 3A). Subsequently, cumulative additions of SAE (6.25 – 200 µg/mL) produced a mild reduction in contractile force (P > 0.05), across all tested concentrations (Figure 3B and 3C).



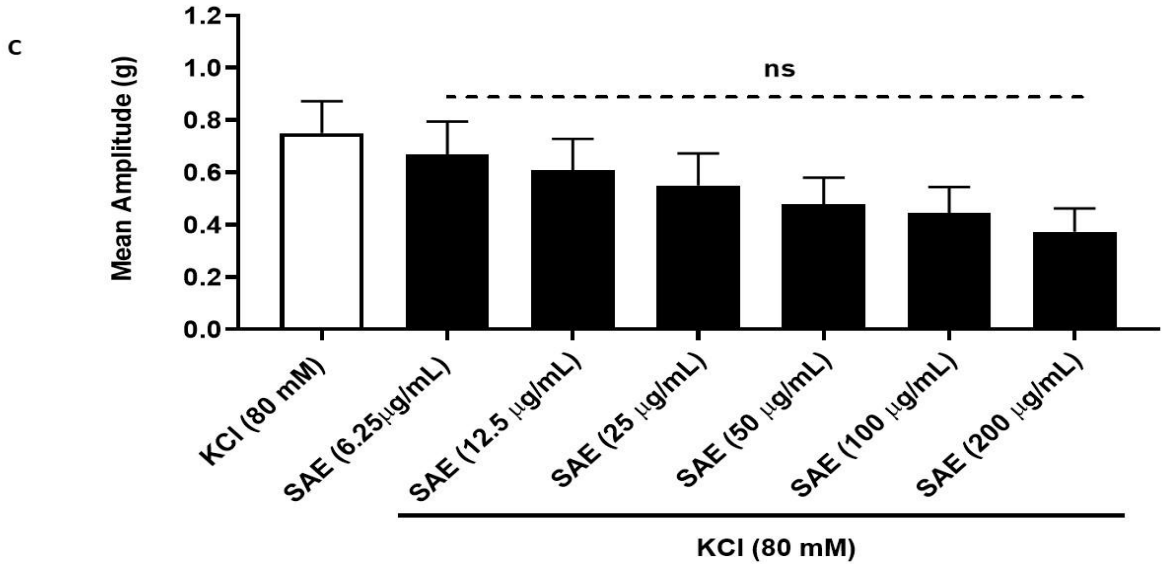
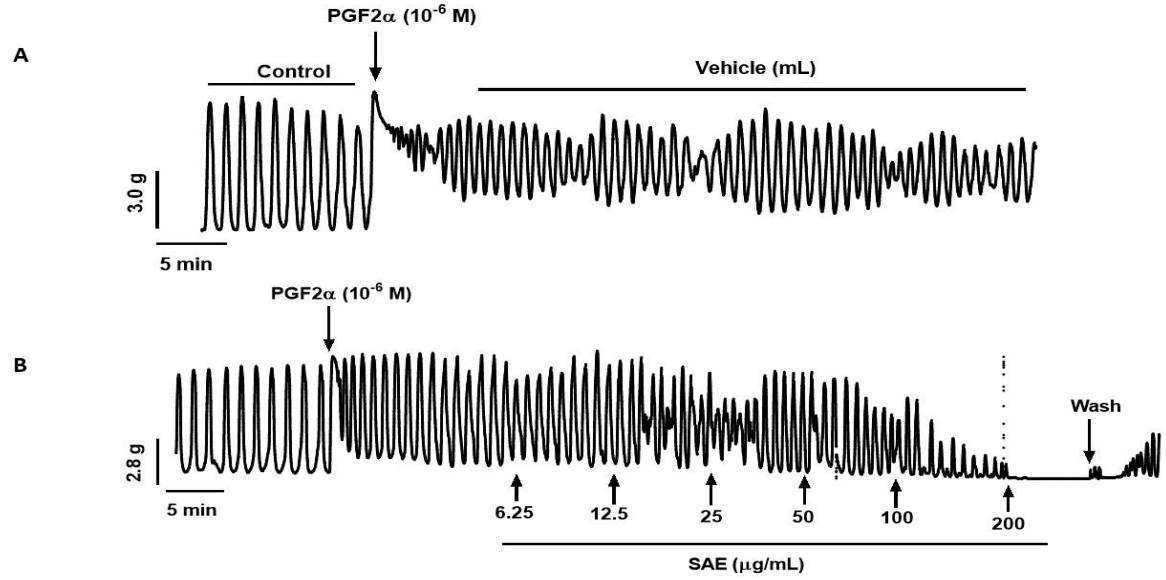


Figure 3. Effect of SAE on uterine contractions induced by high KCl (80 mM)) in non-pregnant mice, (A) the response of uterine tissue to high KCl (B) the effect of SAE on uterine contractions induced with high KCl in non-pregnant mice, (C). the effect of SAE on contractions amplitude induced by high KCl. Values are mean ± SEM, n = 5 animals; ns (not significant).

Effect of SAE on prostaglandin $F_{2\alpha}$-induced uterine contractions

$PGF_{2\alpha}$ (10^{-6} M) significantly increased uterine contractile activity (Figure 4A). Cumulative additions of SAE (6.25 – 200 µg/mL) caused a concentration-dependent inhibition of $PGF_{2\alpha}$-induced contractions, with complete abolition at 200 µg/ml (Figure 4B). A high significant reduction in contraction amplitude was evident (Figures 4C and 4D).



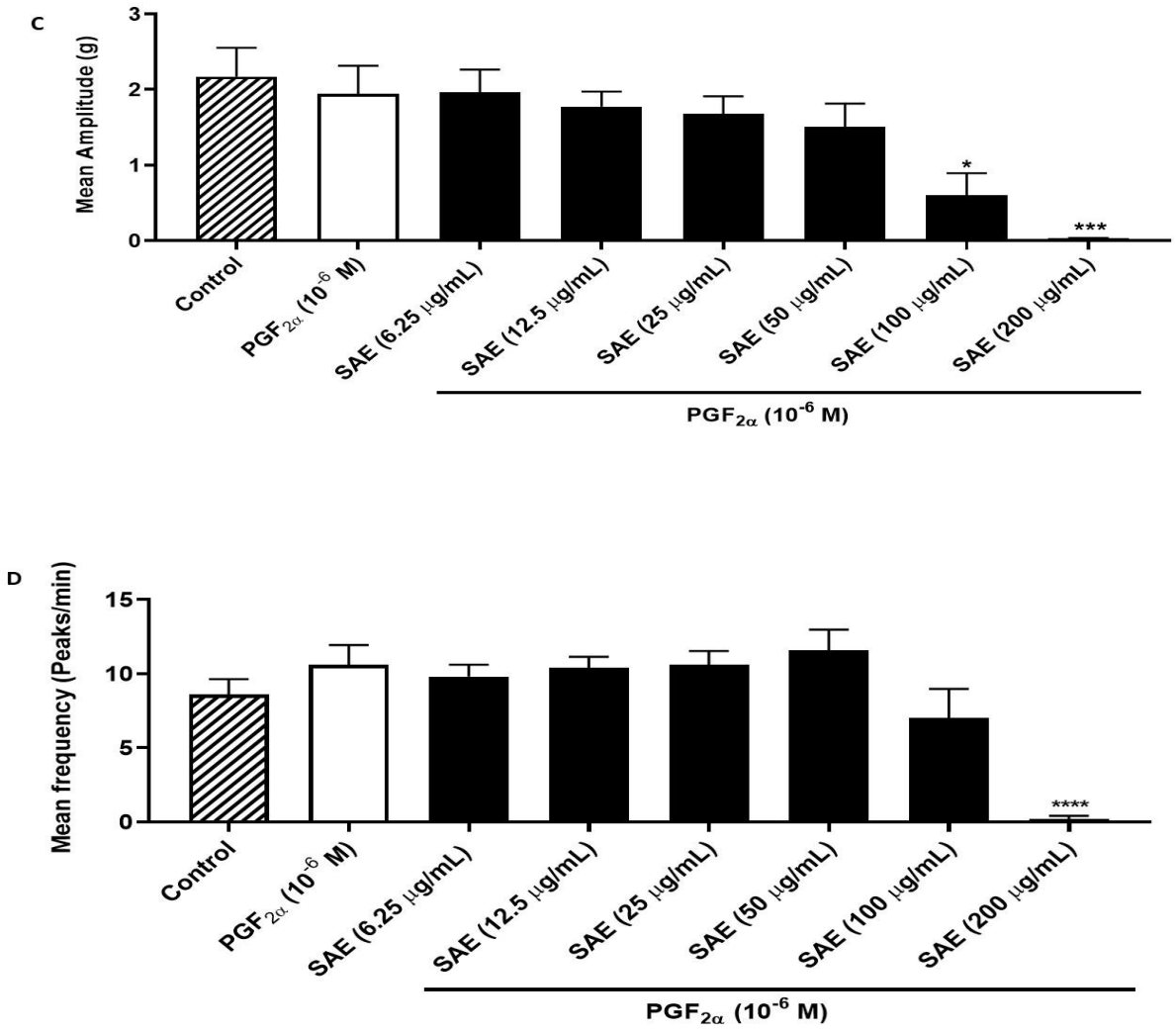


Figure 4. Effect of SAE on uterine contractions induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10^{-6} M) in non-pregnant mice. (A) the response of uterine tissue to $PGF_{2\alpha}$, (B) the effect of SAE on uterine contractions induced with $PGF_{2\alpha}$ in non-pregnant mice, C and D show the inhibitory effect of SAE on contractions amplitude, and frequency induced by $PGF_{2\alpha}$. Values are mean ± SEM, n = 5 animals. *P < 0.05, ***P < 0.001, and ****P < 0.0001 compared to $PGF_{2\alpha}$ effect.

Effect of SAE on oxytocin-induced contractions in calcium-free medium

In calcium-free medium containing EDTA, spontaneous uterine contractions were completely eliminated, as the extracellular calcium entry via L-type calcium channel is absent (Figure 5A). Oxytocin induced transient uterine contractions, indicating the release of intracellular calcium from the stores. SAE (200 µg/mL) completely suppressed these contractions (Figure 5A). Result analysis showed significant inhibition of contraction amplitude and frequency of oxytocin-induced contractions in calcium-free media (Figure 5B and 5C)

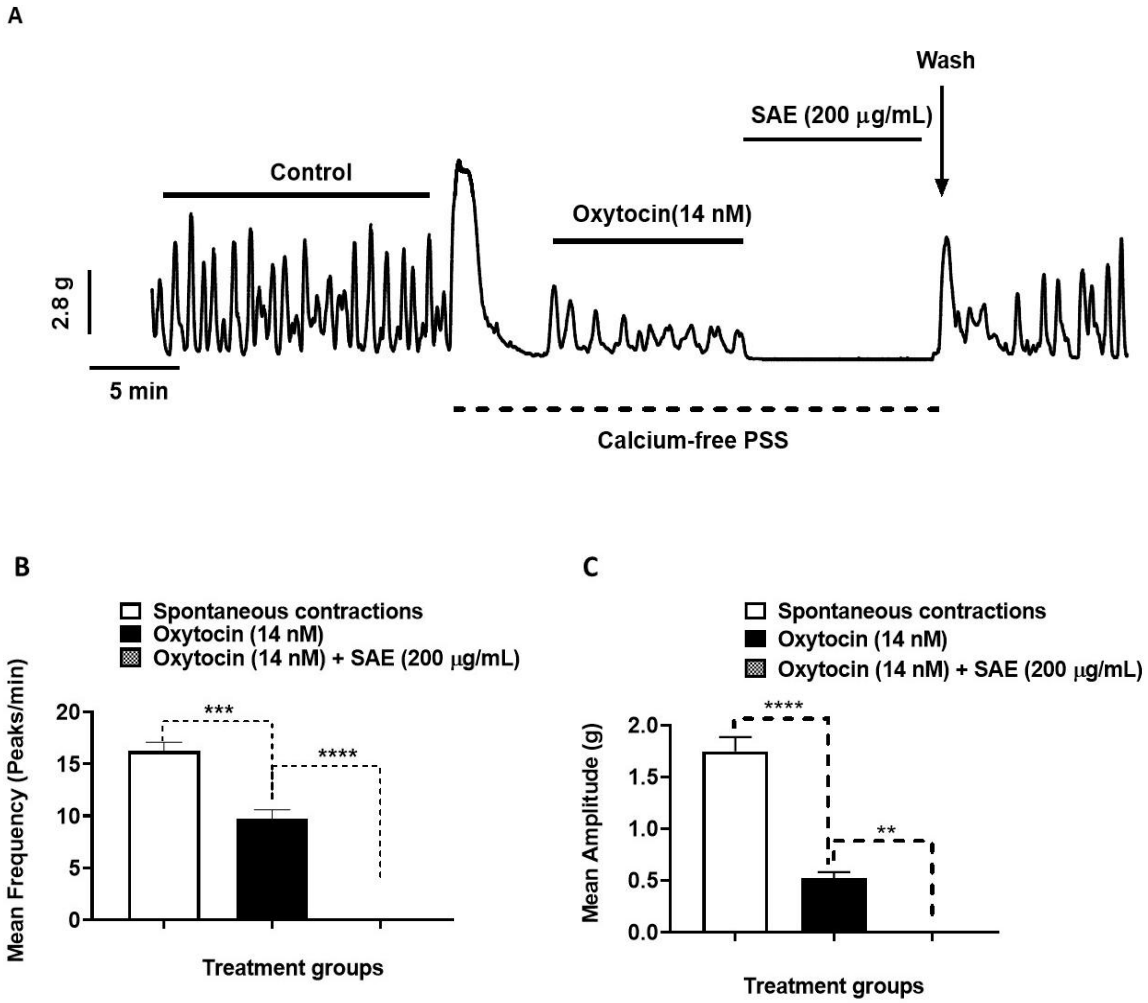
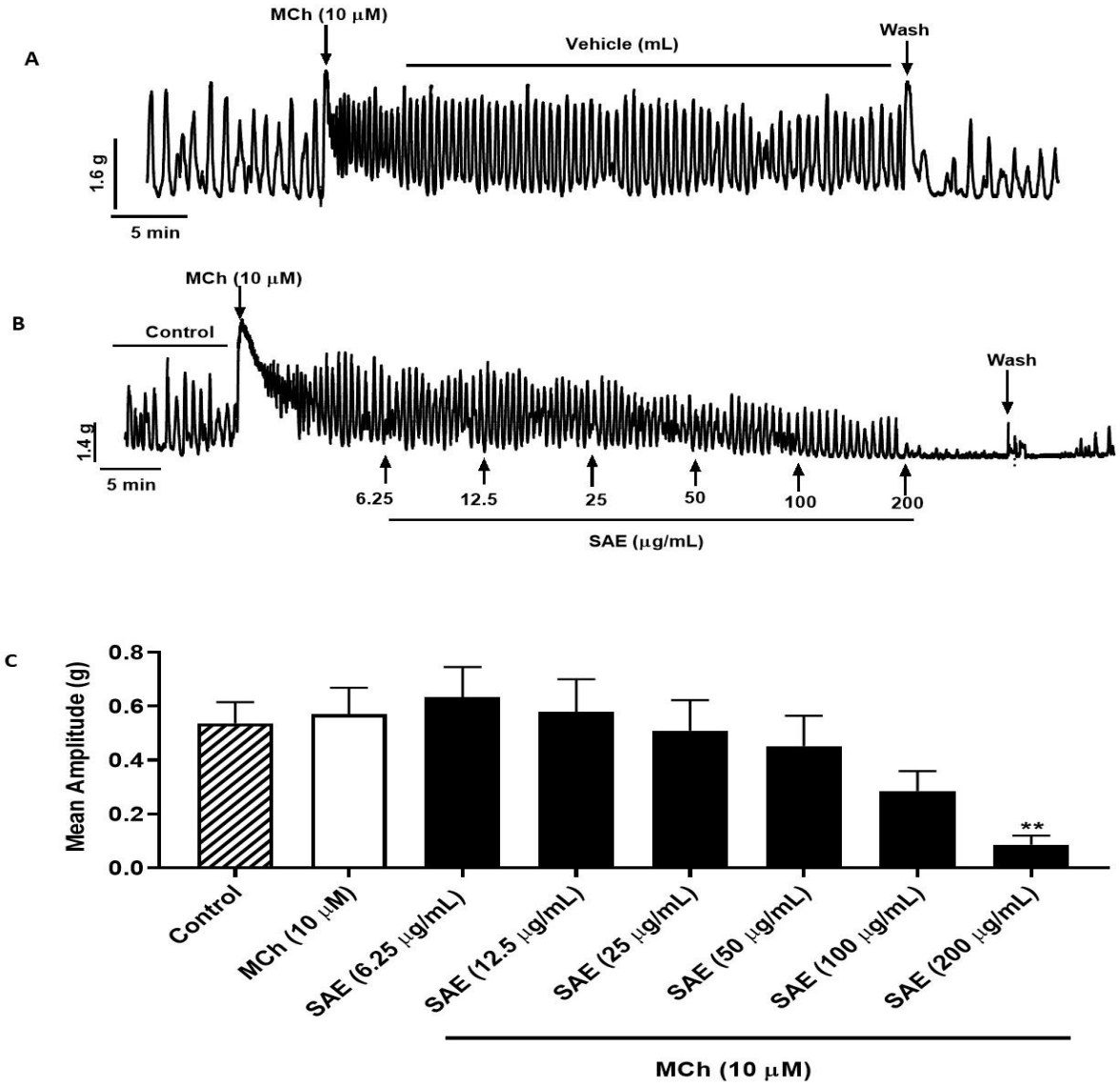


Figure 5. Effect of SAE on uterine contractions induced by oxytocin (14 nM) in calcium-free medium in non-pregnant mice. (A) the response of uterine tissue to oxytocin in calcium-free medium and effect of SAE on oxytocin-induced contractions in non-pregnant mice, (B) and (C). the inhibitory effect of SAE on contractions frequency, and amplitude induced by oxytocin. Values are mean ± SEM, n = 5 animals. **P < 0.01, ***P < 0.001, and ****P < 0.0001 compared to oxytocin effect.

Effect of SAE on methacholine (MCh)-induced uterine contractility.

Methacholine (10 μM) significantly increased uterine contraction amplitude and frequency (Figure 6). In the presence of MCh, SAE (6.25 – 200 μg/mL) produced a concentration-dependent reduction in uterine contractility. A significant decrease in contraction frequency ($P < 0.05$) and amplitude ($P < 0.01$) were observed at 200 μg/ml. Normal contractile activity was restored following washout.


A
MCh (10 μM)
Vehicle (mL)
Wash
1.6 g
5 min
B
MCh (10 μM)
Control
Wash
1.4 g
5 min
6.25
12.5
25
50
100
200
SAE (μg/mL)
C
Mean Amplitude (g)
0.8
0.6
0.4
0.2
0.0
**
Control
MCh (10 μM)
SAE (6.25 μg/mL)
SAE (12.5 μg/mL)
SAE (25 μg/mL)
SAE (50 μg/mL)
SAE (100 μg/mL)
SAE (200 μg/mL)
MCh (10 μM)

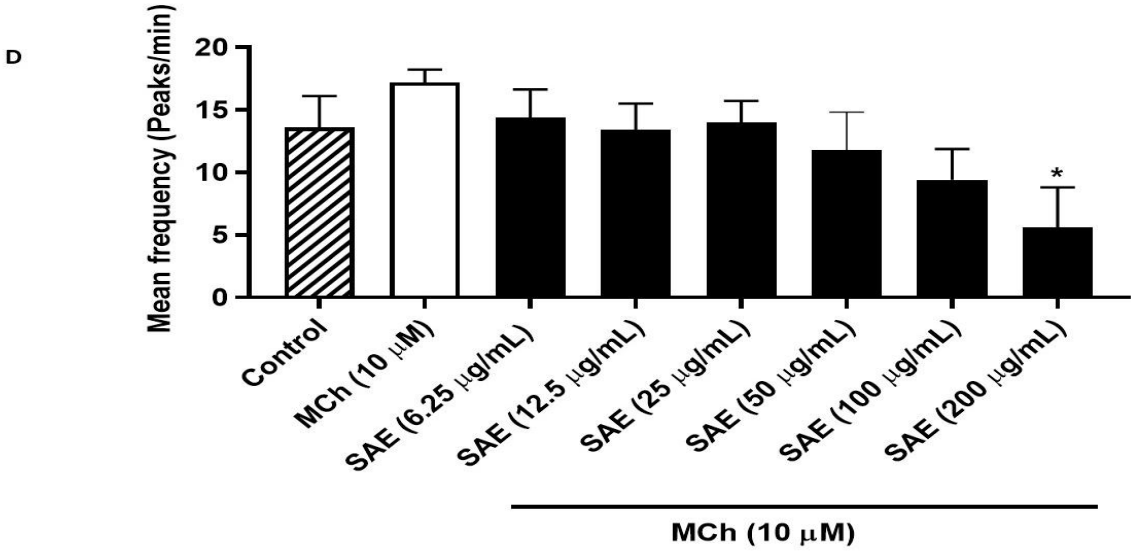
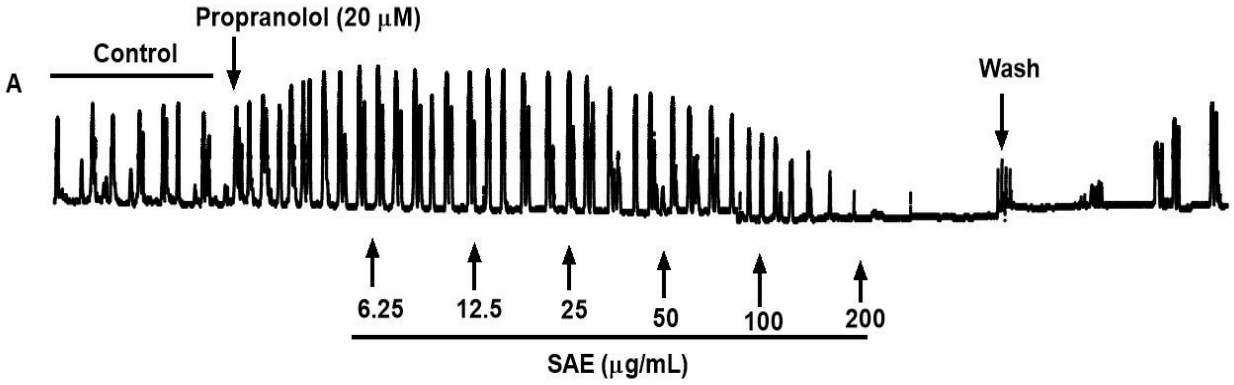


Figure 6. Effect of SAE on uterine contractions induced by methacholine (MCh, 10 μM) in non-pregnant mice. (A) the response of uterine tissue to MCh, (B) the effect of SAE on uterine contractions induced with MCh in non-pregnant mice, (C) and (D). inhibitory effect of SAE on contractions amplitude, and frequency induced by MCh. Values are mean ± SEM, n = 5 animals. *P < 0.05, and **P < 0.01 compared to methacholine effect.

Effect of SAE in the presence of propranolol

Pretreatment with propranolol (20 μM) did not abolish the inhibitory effect of SAE on both uterine contractility, frequency and amplitude. (Figure 7). Normal contractile activity was restored after washout.



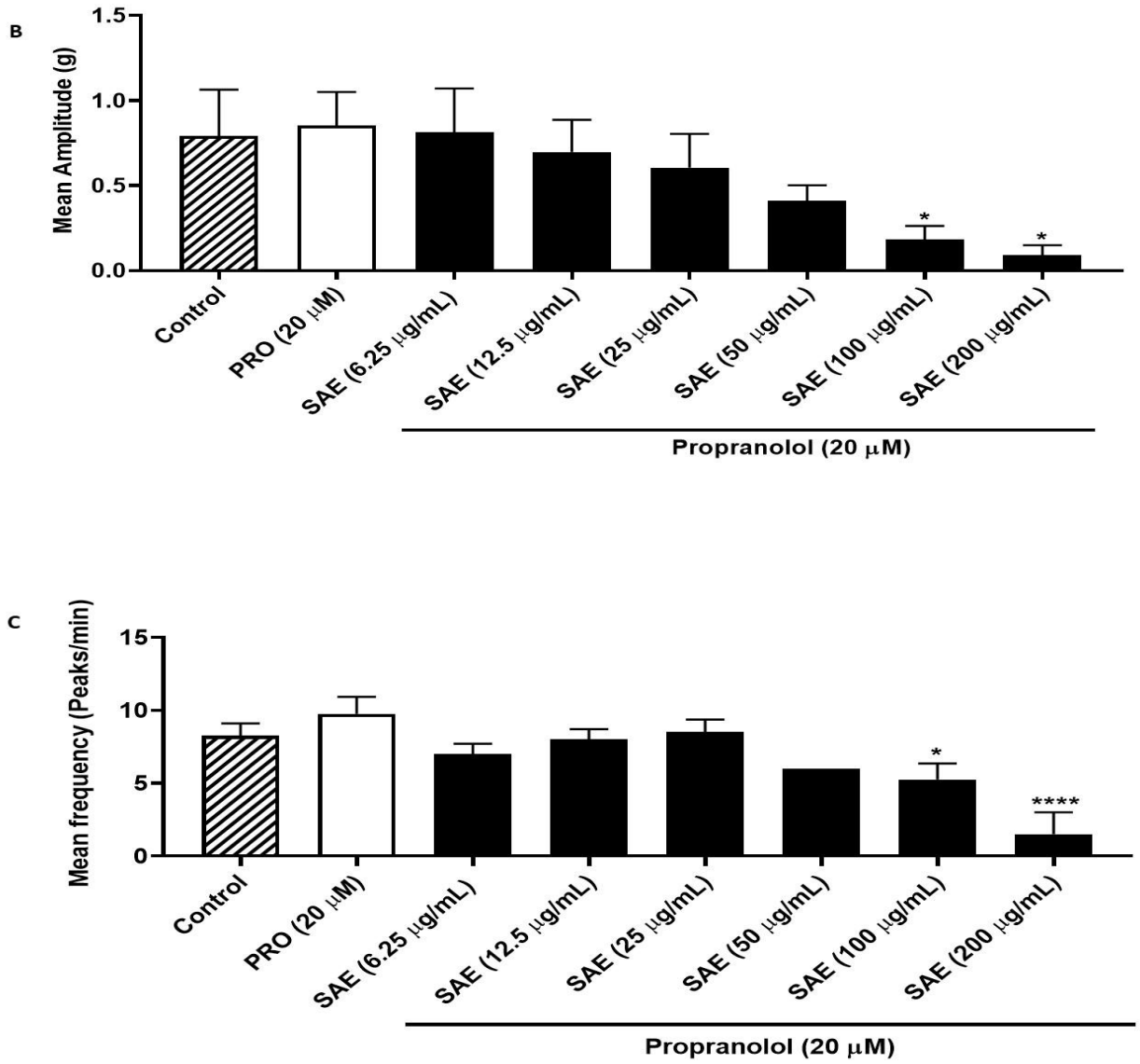
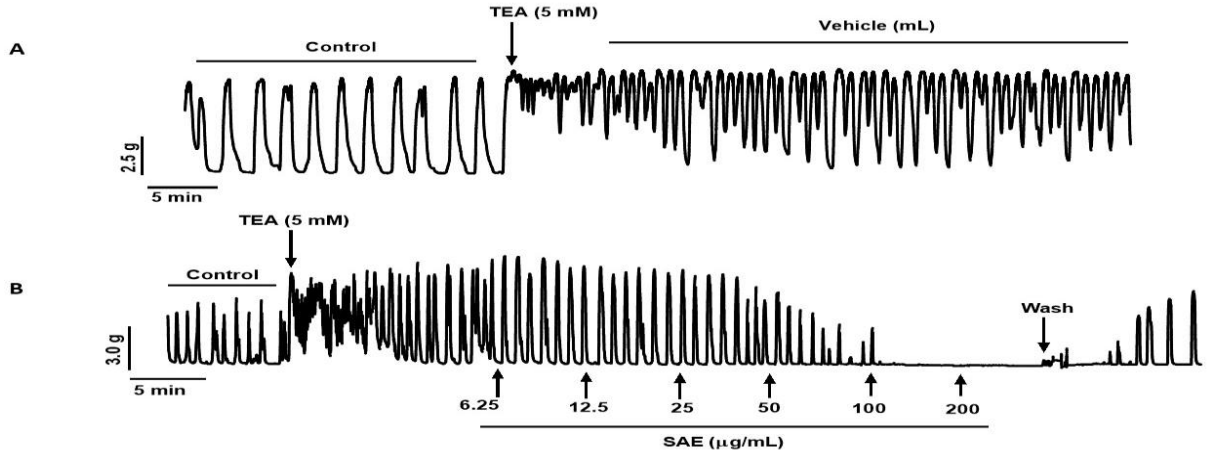
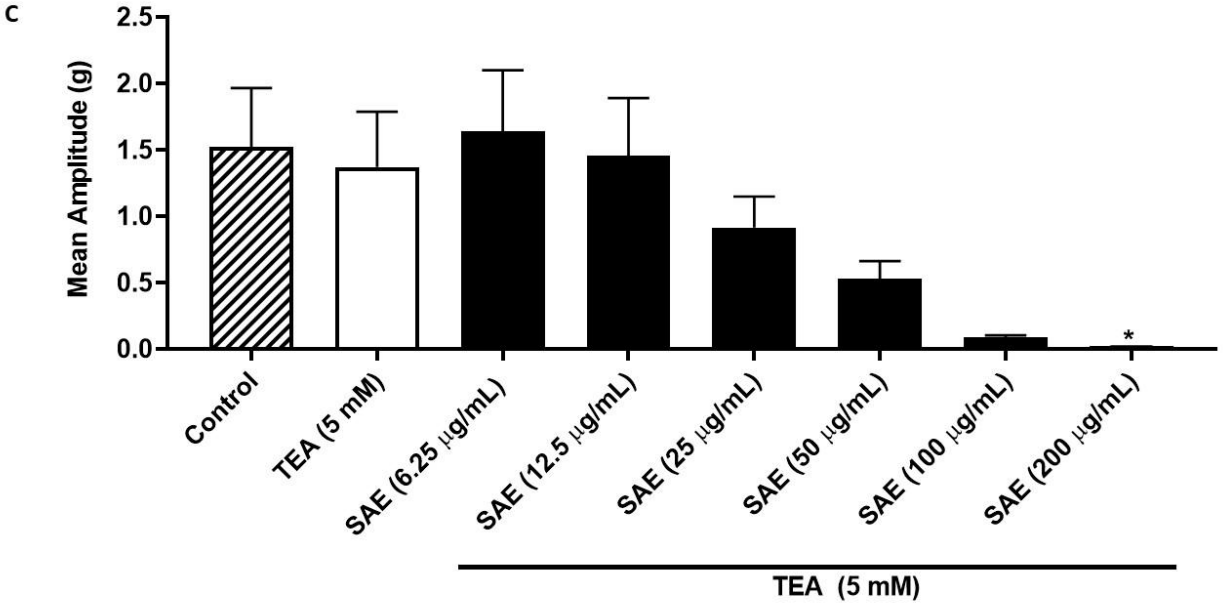


Figure 7. Effect of SAE on uterine contractions in the presence of propranolol (PRO, 20 μM) in non-pregnant mice. (A) the effect of SAE on uterine contractions in the presence of propranolol, (B) and (C). the effect of SAE on amplitude and frequency of uterine contractions in non-pregnant mice in the presence of propranolol. Values are expressed as mean ± SEM; *P < 0.05, ****P < 0.0001 compared to propranolol effect, n = 5 animals.

Effect of SAE on tetraethyl ammonium-induced uterine contractions

Tetraethylammonium (TEA,5 mM) increased uterine contraction amplitude and frequency. In the presence of TEA, SAE (6.25 – 200 μg/mL) progressively inhibited uterine contractions, with complete suppression at 200 μg/mL. Significant reductions in contraction frequency and amplitude were observed at concentrations ≥ 100 μg/mL (P < 0.05) (Figure 8).


A
TEA (5 mM)
Control
Vehicle (mL)
2.5 g
5 min
B
TEA (5 mM)
Control
Wash
3.0 g
5 min
6.25
12.5
25
50
100
200
SAE (µg/mL)


C
Mean Amplitude (g)
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
TEA (5 mM)
SAE (6.25 µg/mL)
SAE (12.5 µg/mL)
SAE (25 µg/mL)
SAE (50 µg/mL)
SAE (100 µg/mL)
SAE (200 µg/mL)
TEA (5 mM)

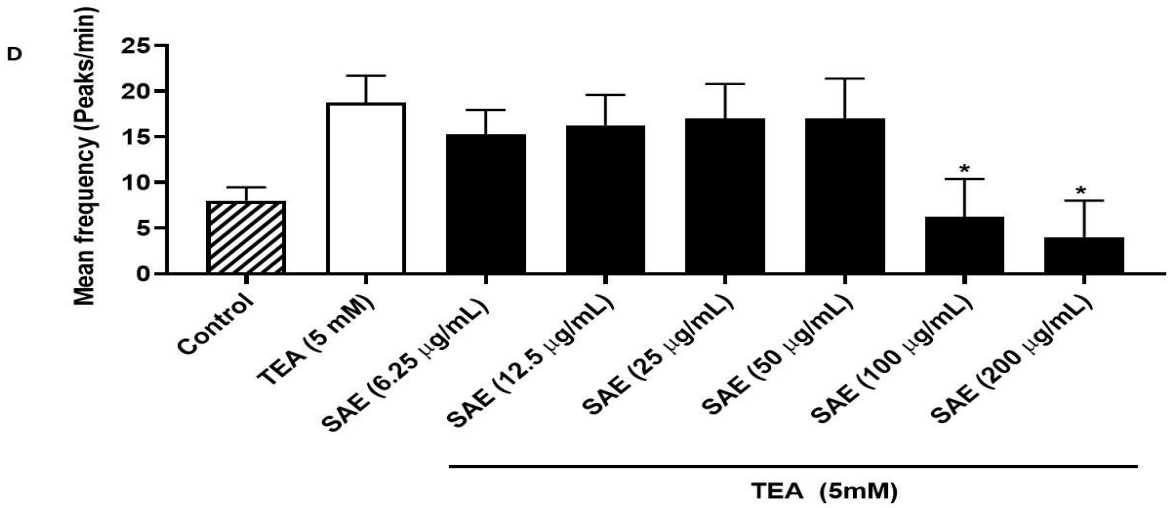


Figure 8. Effect of SAE on uterine contractions in the presence of tetraethylammonium (TEA, 5 mM) in non-pregnant mice. (A) the response of uterine strip to TEA, (B) the effect of SAE on uterine contractions in the presence of TEA, (C) and (D). the effect of SAE on amplitude and frequency of uterine contractions in non-pregnant mice in the presence of TEA. Values are expressed as mean ± SEM; *P<0.05 compared to TEA effect, n = 5 animals.

Discussion

The present study provides experimental evidence that the SAE exerts a potent inhibitory effect on uterine smooth muscle contractility in non-pregnant mice. The findings suggest that SAE exerts uterine relaxant effects primarily through modulation of receptor-mediated and intracellular calcium-dependent signaling pathways rather than direct blockade of voltage-gated calcium entry.

Controlled uterine smooth muscle activity is essential for female reproductive health. Changes in calcium signaling within the myometrium have important functional consequences, as they determine contractility (Wray *et al*., 2003; Burdyga *et al*., 2007). In uterine smooth muscle, phasic contraction is driven primarily by calcium influx through voltage-gated L-type calcium channels (VGCCs) following membrane depolarization, while the sarcoplasmic reticulum (SR) plays a secondary role, mainly regulating excitability via negative feedback (Wray *et al*., 2003; Burdyga *et al.,* 2007). In this study, SAE significantly inhibited the amplitude and frequency of spontaneous uterine contraction in a concentration-dependent manner, suggesting a reduction in myometrial excitability. This effect is likely mediated by inhibition of Ca^{2+} entry through L-type channels and/or enhanced SR Ca^{2+} sequestration, resulting in decreased intracellular Ca^{2+} availability and reduced contractile activity.

To further assess the influence of SAE on calcium influx, uterine strips were exposed to a high potassium solution to selectively activate calcium entry through L-type VGCCs. Elevated extracellular potassium induces sustained, tonic uterine contractions by depolarizing the myometrial membrane, thereby opening L-type VGCCs and increasing intracellular Ca^{2+}levels (Alotaibi, 2020; Malik *et al.,* 2021). In this study, SAE did not significantly attenuate high KCl-induced contractions, suggesting that its relaxant effect is unlikely to involve direct inhibition of voltage-dependent calcium entry via L-type channels. This finding distinguishes SAE from classical calcium channel-blocking tocolytics such as nifedipine and supports the involvement of alternative pathways, including receptor-operated signaling and intracellular calcium mobilization.

Oxytocin increases uterine contractility by increasing both the amplitude and frequency of myometrial contractions relative to spontaneous activity through activation of oxytocin receptor (a G-protein coupled receptor) and facilitation of L-type calcium channel activity (Sukwan *et al.,* 2014; Alotaibi, 2020). Oxytocin-induced contractions are primarily mediated via oxytocin receptor coupling to phospholipase C, resulting in inositol 1,4,5-triphosphate (IP_3)-dependent release of $Ca^{2+}$from intracellular stores (Vrachnis *et al.,* 2011; Sukwan *et al*., 2014). The pronounced inhibition of oxytocin-induced contractions by SAE, both in normal calcium and calcium-free media, provides strong evidence that SAE interferes with intracellular Ca^{2+} mobilization rather than extracellular Ca^{2+} influx. This interpretation is further supported by the ability of SAE to suppress oxytocin-induced contractions in a calcium-free medium, where contractile responses depend almost entirely on Ca^{2+} release from intracellular stores.

The inhibitory effect of SAE on MCh-induced contractions further supports receptor-mediated mechanisms. MCh (a

non-selective muscarinic receptor agonist) activates muscarinic M_3 receptors, which are Gq-coupled and stimulate uterine through phospholipase C (PLC) activation and inositol 1,4,5-triphosphate (IP_3)-dependent calcium release (Kudlak and Tadi, 2023). The significant reduction in MCh-induced uterine contractility indicates that SAE may exert antagonistic effects on muscarinic receptors or inhibit downstream signaling pathways common to both muscarinic and oxytocin receptor activation.

The involvement of β-adrenergic pathways in SAE-induced uterine relaxation was evaluated using propranolol, a non-selective β-adrenergic receptor antagonist. β_2-adrenergic receptor activation normally promotes uterine relaxation via cyclic adenosine monophosphate (cAMP)-dependent pathways that reduce intracellular calcium levels and myosin light chain kinase activity (Balki *et al.*, 2025). In the present study, SAE retained its inhibitory effect on uterine contractions in the presence of propranolol, indicating that its relaxant action is independent of β-adrenergic receptor activation.

Potassium channels are known to play critical role in regulating myometrial membrane potential and excitability. Blockade of these channels with TEA, a non-selective blocker of potassium (K^+) channels, leads to membrane depolarization and enhanced uterine contractility (Hu *et al*., 2011; Xu *et al.*,2011; Zak *et al.,* 2021). Furthermore, the results of this study provide evidence that SAE effectively inhibited TEA-induced contractions, suggesting that its uterine relaxant effect does not depend on the activation of potassium channels. TEA is a non-selective blocker of potassium channels

Prostaglandin $F_{2\alpha}$ is a powerful uterotonic agent that stimulates myometrial contraction via prostaglandin F receptor activation, leading to increased intracellular calcium release and enhanced calcium sensitization through Rho-kinase and protein kinase C pathways (Riaposova *et al.*, 2023). The significant and concentration-dependent inhibition of $PGF_{2\alpha}$-induced contractions by SAE indicates interference with prostaglandin receptor-mediated signaling or downstream excitation-contraction mechanisms. This finding is particularly relevant clinically, as prostaglandins plays a central role in dysmenorrhea and preterm uterine activity.

Conclusion

In conclusion, the findings of this study suggest that SAE produces uterine relaxant effects through multiple, overlapping mechanisms. The underlying mechanisms appear to be primarily associated with suppression of intracellular calcium release, inhibition of receptor-operated signaling pathways, including oxytocin, muscarinic, and prostaglandin receptors, and attenuation of calcium sensitization processes. However, these effects clearly do not involve direct blockade of voltage-gated calcium channels, activation of potassium channel, or stimulation of β-adrenergic receptors.

These findings position clove as a promising natural candidate for the management of uterine hypercontractile conditions such as dysmenorrhea and preterm labour.

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Conflict of interest

None declared

References

Abdul Aziz AH, Rizkiyah DN, Qomariyah L et al (2023). Unlocking the full potential of clove (*Syzygium aromaticum*) spice: an overview of extraction techniques, bioactivity, and future opportunities in the food and beverage industry. *Processes* 11(8): 2453.

Aguilar HN, Mitchell BF (2010). Physiological pathways and molecular mechanisms regulating uterine contractility. *Human reproduction update* 16(6): 725-744.

Alotaibi MF (2020). *Pimpinella anisum* extract attenuates spontaneous and agonist-induced uterine contraction in term-pregnant rats. *Journal of Ethnopharmacology* 254: 112730.

Arman BM, Binder NK, de Alwis N et al (2023). Repurposing existing drugs as a therapeutic approach for the prevention of preterm birth. *Reproduction* 165(1): R9-23.

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

Bafor EE, Ukpebor F, Omoruyi O et al (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.

Balki M, Miller LM, Caliaperumal J et al (2025). Propranolol and Oxytocin-Induced Contractility in Gravid Human Myometrium: An Ex Vivo Laboratory Study. BJOG: *International Journal of Obstetrics and Gynaecology* 132(9): 1228-1237.

Batiha GE, Alkazmi LM, Wasef LG et al (2020). *Syzygium aromaticum* L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules* 10(2): 202.

Bernardi M, Lazzeri L, Perelli F et al (2017). Dysmenorrhea and related disorders. *F1000Research 6*.

Burdyga T, Wray S, Noble K (2007). In situ calcium signaling: no calcium sparks detected in rat myometrium. *Annals of the New York Academy of Sciences* 1101(1): 85-96.

Gopy M, Prameeda PR, Radhakrishnan A, et al (2024). Efficacy and safety of tocolytic therapy with oral nifedipine for the management of preterm labor: Prospective Observational Study. *International Journal of Life Sciences, Biotechnology and Pharma Research* 13(9): 75-87.

Hu XQ, Xiao D, Zhu R et al (2011). Pregnancy Upregulates Large-Conductance Ca $^{2+}$-Activated K^+ Channel Activity and Attenuates Myogenic Tone in Uterine Arteries. *Hypertension* 58(6): 1132–1139

Hu Z, Tang L, Chen L, et al (2020). Prevalence and risk factors associated with primary dysmenorrhea among Chinese female university students: a cross-sectional study. *Journal of Pediatric and Adolescent Gynecology 33*(1): 15-22

Huang M, Li X, Guo P et al (2017). The abnormal expression of oxytocin receptors in the uterine junctional zone in women with endometriosis. *Reproductive Biology and Endocrinology* 15(1): 1.

Hussain S, Rahman R, Mushtaq A, Zerey-Belaskri AE (2017). Clove: A review of a precious species with multiple uses. *International Journal of Chemical and Biochemical Sciences* 11: 129-133.

Kolan AS, Hall JM (2023). Association of preterm birth and exposure to endocrine disrupting chemicals. *International Journal of Molecular Sciences* 24(3): 1952.

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

Lamont CD, Jørgensen JS, Lamont RF (2016). The safety of tocolytics used for the inhibition of preterm labour. *Expert Opinion on Drug Safety* 15(9): 1163-1173.

Lima FC, Peixoto-Neves D, Gomes MD et al (2011). Antispasmodic effects of eugenol on rat airway smooth muscle. *Fundamental and Clinical Pharmacology* 25(6): 690-699.

Malik M, Roh M, England SK (2021). Uterine contractions in rodent models and humans. *Acta Physiologica* 231(4): e13607.

Munjal A, Allam AE (2024). Indomethacin. In: *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing.

Onu A, Aluh D Ikehi M (2020). Prevalence and Management of Dysmenorrhea among Secondary School Adolescents in Enugu State, Nigeria. *Research Square.*

Oyinloye EO, Abdullahi AM, Farouk AO et al (2025). In vitro Antioxidant and effects of ethanol extract of *Syzygium aromaticum* on Gastrointestinal smooth muscle. *The Nigerian Journal of Pharmacy* 59(1): 154-164.

Pramod K, Ansari SH, Ali J (2010). Eugenol: a natural compound with versatile pharmacological actions. *Natural Product Communications* 5(12): 1934578X1000501236.

Riaposova L, Kim SH, Hanyaloglu AC et al (2023). Prostaglandin F2α requires activation of calcium-dependent signalling to trigger inflammation in human myometrium. *Frontiers in Endocrinology* 14: 1150125.

Sajadi M, Noroozzadeh M, Bagheripour F, Tehrani FR (2018). Contractions in the isolated uterus of a rat model of polycystic ovary syndrome compared to controls in adulthood. *International Journal of Endocrinology and Metabolism* 16(2): e63135.

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.

Tonini G (2002). Dysmenorrhea, endometriosis and premenstrual syndrome. *Minerva Pediatrica* 54(6): 525-538.

Uchendu AP, Amaechina FO, Okonmah EC et al (2025). Inhibitory effects of rutin on uterine contractions in nonpregnant and pregnant mice *ex vivo*. *Journal of Pharmacy and Bioresources* 22(2): 107-118.

Vrachnis N, Malamas FM, Sifakis S et al (2011). The oxytocin-oxytocin receptor system and its antagonists as tocolytic agents. *International Journal of Endocrinology* 2011(1): 350546.

Wray S, Jones K, Kupittayanant S et al (2003). Calcium signaling and uterine contractility. *Journal of the Society for Gynecological Investigation* 10(5): 252-264.

Xu C, You X, Gao L et al (2011). Expression of ATP-sensitive potassium channels in human pregnant myometrium. *Reproductive Biology and Endocrinology* 9(1): 35.

Yadav S, Gupta SK, Bharti D, Yogi B (2020). *Syzygium aromaticum* (clove): a review on various phytochemicals and pharmacological activities in medicinal plant. *World Journal of Pharmaceutical Research* 9: 349-363.

Zak M, Kestler B, Cornwell T, Taylor MS (2021). Augmented KCa2. 3 channel feedback regulation of

oxytocin stimulated uterine strips from nonpregnant mice.
International Journal of Molecular Sciences 22(24): 13585.

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