© 2020 Journal of Pharmacy & Pharmacognosy Research, 8 (2), 92-104, 2020
ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

Ex-vivo inhibition of uterine contractility by the stem bark extracts of *Omphalocarpum procerum* P.Beauv. (*Sapotaceae*) in mouse models

[Inhibición *ex vivo* de la contractilidad uterina por los extractos de corteza de tallo de *Omphalocarpum procerum* P. Beauv. (*Sapotaceae*) en modelos de ratón]

Enitome Bafor[1]*, Tosan Eyegbagharen[2]*, Ejiro Ochoyama[1], MacDonald Idu[2]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.
[2]Department of Plant Biology and Biotechnology, Faculty of Science, University of Benin, Benin City, Nigeria.
*E-mail: enitome.bafor@uniben.edu; toseyes@gmail.com

Abstract

Context: The plant *Omphalocarpum procerum* is used traditionally in Africa to manage female reproductive health issues such as infertility and menstrual cycle regulation. In the search for new therapies for the management of female reproductive issues, the plant *O. procerum* was investigated in this study.

Aims: To determine the activity of the methanol extract of *O. procerum* stem bark (MOPS) on uterine contractility, as a measure to determine its usefulness in uterine contractility associated disorders.

Methods: MOPS' activity on spontaneous, oxytocin, KCl-induced contractions and in calcium-free solution was determined on the isolated uterus. Possible mechanisms of activity were determined using receptor and channel blockers.

Results: MOPS inhibited spontaneous and oxytocin-induced contractions. MOPS also inhibited oxytocin-induced contractions in calcium-free solution. MOPS was also found to exert its inhibitory effect through interaction with β-adrenoceptors in the uterus.

Conclusions: MOPS has been shown to inhibit uterine contractility in the non-pregnant and pregnant uterus primarily through the activation of β-adrenoceptors in the uterus.

Keywords: labor; oxytocin; plant extract; propranolol; uterus.

Resumen

Contexto: La planta *Omphalocarpum procerum* se usa tradicionalmente en África para controlar problemas de salud reproductiva femenina, como la infertilidad y la regulación del ciclo menstrual. En la búsqueda de nuevas terapias para el manejo de los problemas reproductivos femeninos, se investigó la planta *O. procerum* en este estudio.

Objetivos: Determinar la actividad del extracto de metanol de la corteza del tallo de *O. procerum* (MOPS) sobre la contractilidad uterina, como una medida para determinar su utilidad en los trastornos asociados a la contractilidad uterina.

Métodos: Se determinó la actividad de MOPS sobre las contracciones espontáneas y las inducidas por oxitocina, por KCl y en solución libre de calcio en el útero aislado. Los posibles mecanismos de actividad se determinaron utilizando receptores y bloqueadores de canales.

Resultados: MOPS inhibió las contracciones espontáneas e inducidas por oxitocina. MOPS también inhibió las contracciones inducidas por oxitocina en solución libre de calcio. También se descubrió que MOPS ejerce su efecto inhibitorio a través de la interacción con los adrenoceptores β en el útero.

Conclusiones: Se ha demostrado que MOPS inhibe la contractilidad uterina en el útero no embarazado y en gestante principalmente a través de la activación de los adrenoceptores β en el útero.

Palabras Clave: extracto de planta; oxitocina; parto; propranolol; útero.

ARTICLE INFO
Received: August 25, 2019.
Received in revised form: November 8, 2019.
Accepted: November 9, 2019.
Available Online: November 13, 2019.
Declaration of interests: The authors declare no conflict of interest.
Funding: This research was not funded and did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

INTRODUCTION

Dysmenorrhea is a condition that affects women of reproductive age who are not pregnant but are going through their menstrual cycles (Bulletti et al., 2000). It is a condition associated with increased uterine contractility, which is a necessity during the monthly cycles to assist in the evacuation of the endometrial contents (de Vries et al., 1990). Similarly, uterine contraction of the pregnant uterus is involved in labor where it functions to release the fetus from the uterus. However, should there be stimulation of uterine contractions too early during pregnancy, this result in a condition known as preterm labor (PTL) (Maltaris et al., 2006). PTL which occurs prematurely is a prevalent cause of maternal and neonatal mortality and morbidity in the world today (Liu et al., 2015). Targeting uterine contractility therefore is one of the therapeutic measures that can be employed in managing these conditions (Bafor et al., 2017). Therapy currently in use are not without their complications ranging from adverse effects to limited usefulness (Park et al., 2014). Natural products, such as plants, are reliable sources to search for new and efficient therapies for inhibition of uterine contractility (Chen et al., 2006; Bafor et al., 2019). Plants have shown usefulness in managing female reproductive health issues, which support the scientific validation of their traditional utilization in these conditions (Gruber and O'Brien, 2011).

O. procerum is popularly utilized traditionally in the management of infertility and other female reproductive issues including regulation of the menstrual cycle (Quiroz et al., 2016). *O. procerum* is a tree popularly found in tropical Africa. It grows to a height of about 30 m and is characterized by the presence of hard fruits (Ngamgwe et al., 2014). Phytochemical investigations had shown the presence of flavonoids, triterpenes, and alkaloids (Akihisa et al., 2010; 2011; Baliga et al., 2011). In addition the compounds, procerenone, betulin, β-amyrin, lupeol acetate, stigmasterol, and β-sitosterol have been isolated and reported from the fruits of *O. procerum* (Ngamgwe et al., 2014).

Due to the reported traditional effect of the plant on the female reproductive system, the plant was selected for investigation to determine its potential usefulness in the therapy of female reproductive health issues associated with uterine contractility disorders. The current study was therefore set to investigate the activity of the stem bark extract of *O. procerum* on uterine contractility utilizing the functional uterus assay, which involves use of the isolated uterine tissue.

MATERIAL AND METHODS

Chemicals and reagents

Tween 80 (Kermel- kn®, China), oxytocin (Roche pharmaceutical Ltd, UK). Some salts used included: sodium chloride, potassium chloride and D-glucose (Guangdong GuanghuaSci-Tech Co. Ltd. China), sodium bicarbonate (Sigma-Aldrich, UK), calcium chloride (XL® China), salbutamol, propranolol, and tetraethyl ammonium chloride (Sigma-Aldrich, UK). All solvents used were of analytical grade.

Plant material

O. procerum stem bark materials were collected from the Uroho village located at Ikpoba Okha Local Government Region in Edo State Nigeria (6°09'52.02" N 5°37'22.22" E). The stem bark was identified by Professor Macdonald Idu of Plant Biology and Biotechnology Department, Faculty of Life Sciences, University of Benin, Nigeria. The herbarium number of the plant is UBH0438 and a voucher specimen has been deposited for future reference at the Plant Biology and Biotechnology Department, University of Benin, Nigeria.

Preparation of plant extract

The stem bark of *O. procerum* was cut into bits and powdered using a mortar and pestle. From the resulting powder, 500 g was macerated in 1 L of methanol for 24 h with constant stirring. After 24 h, the macerate was filtered and concentrated over a water bath set at 60°C. The extract yield was calculated as 21.46% (w/w). The extract was pre-

served in a sealed container in the refrigerator until needed for experiments.

Animals

Non-pregnant and pregnant female albino mice (22-35 g) were used. The animals were maintained in the animal unit located at the Department of Pharmacology and Toxicology, University of Benin, Nigeria. Ethical approval (reference number: EC/FP/016/011) for the study was granted by the Faculty of Pharmacy Ethics Committee University of Benin Nigeria. Maintenance of animals was according to the guide for the care and use of laboratory animals (National Research Council, 2010). The animals were kept under an environmentally controlled room temperature of approximately 32 ± 5 °C and natural dark and light cycles. The mice were fed a diet of animal pellets and clean tap water was provided *ad libitum*.

Experimental protocols

Preparation of uterine tissue

Animals were euthanized humanely by cervical dislocation. The uterine horns were rapidly isolated, placed in a Petri dish containing aerated, warmed physiological solution and freed of connective tissues. The horns were dissected and uterine segments of about 1.5 – 2 mm in length were obtained and mounted in an aerated, organ bath (10 mL) kept at 37°C. The organ bath contained physiological salt solution of the following composition in mM/L: $CaCl_2 \cdot 2H_2O$ 2.05, D-glucose 2.78, KCl 5.63, $NaHCO_3$ 5.95 and NaCl 154.00 (Bafor et al., 2015; 2019). The pregnant animals utilized for this study were time-mated and were euthanized on day 18 by cervical dislocation. The uterine horns, freed of fetuses, were cut into segments and mounted in the organ bath as earlier described.

All uterine segments were mounted vertically in an organ bath (10 mL), which was connected to an isometric force transducer (7003E-Ugo Basile, Varise, Italy) attached to a 17400 data capsule digital recorder (Ugo Basile, Varese, Italy). Prior to the addition of extracts and drugs, the uterine tissue was equilibrated under resting tension of 4.90 mN for 30 min or until regular rhythmic contractions were observed (Bafor et al., 2019).

Assessment of extract on spontaneous uterine contractility

MOPS (0.001 - 0.440 mg/mL) was cumulatively added to the isolated non-pregnant and pregnant uterine tissues. The concentrations had been previously determined to represent the full spectrum of activity of the extract. On addition of each concentration, a contact time of 5 min was allowed (Bafor et al., 2019).

Assessment of extract on oxytocin-induced uterine contraction

Oxytocin (OT) (11.62 nM) was added to the non-pregnant uterine tissues for 5 min, and without washing out OT, MOPS (0.1 mg/mL) was added and left in contact for an additional 5 min. The changes in amplitude and frequency were measured and analyzed (Bafor et al., 2019).

Assessment of extract on high KCl-induced uterine contraction

KCl (80 mM) was added to the bath for 5 min and without washing out, MOPS (0.1 mg/mL) was added and left in contact for an additional 5 min. A time-matched control for KCl was also performed (Bafor et al., 2019).

Assessment of extract activity in Ca^{2+}-free media

The activity of MOPS on uterine contraction was also investigated in the presence of zero calcium physiological salt solution where calcium salt was withheld and ethylenediaminetetraacetic acid (EDTA) (0.1 mM) was included. After equilibration in the regular physiological salt solution, the non-pregnant uterine tissue was further equilibrated in the zero calcium solution for 5 min. OT (1.16 µM) was subsequently added to the bath for 5 min and without washing or changing the physiological salt solution, MOPS (0.1 mg/mL) was then added for a further 5 min. Time-matched controls were performed (Bafor et al., 2019).

Determination of possible mechanism of activity of MOPS

Other experiments where performed using receptor antagonists for determination of the mechanisms of MOPS' activity on uterine contraction (Bafor et al., 2019). The receptor antagonists used were tetraethyl ammonium chloride (TEA), a blocker of calcium-dependent potassium channels (1.03 mM), neomycin (NM), a phospholipase C inhibitor and ryanodine receptor blocker (4.39 μM) and propranolol (PR), a beta-adrenoceptor antagonist (0.02 mM). After equilibration of the non-pregnant uterine tissues, regular spontaneous contractions were recorded for 5 min. Thereafter, the receptor antagonists were added for a contact period of 5 min, and without washing out, MOPS (0.1 mg/mL) was added for an additional 5 min and washed out.

Statistical analysis

Data is presented as mean ± standard error of mean (SEM). The uterine contraction parameters, frequency and amplitude were measured in each experiment using the GraphPad Prism, (version 5.03; CA, USA). One-way analysis of variance (ANOVA) with Dunnett's post hoc test was performed and p values ≤ 0.05 was considered significant in all cases. For spontaneous contractility, datasets were computed as mean log concentration-response curves and fitted to a variable slope logistic equation, with the equation values [1].

$$Y = Bottom + (Top-Bottom)/(1+10^{((LogIC_{50}-X)*HillSlope)}) \quad [1]$$

In this case Y represents response from the bottom to the top while X represents the logarithm of concentration and IC_{50} represents the concentration producing responses half way between the top and bottom.

RESULTS

Effect of MOPS on spontaneous contractility in the non-pregnant uterus

MOPS (0.001 - 0.440 mg/mL) was observed to inhibit spontaneous uterine contractions, which was more evident at the higher concentrations (Fig. 1a). MOPS, concentration-dependently inhibited the amplitude of spontaneous contraction (Fig. 1b). For the frequency, MOPS stimulated an initial increase in the frequency of spontaneous contractions but at higher concentrations the increase was attenuated (Fig. 1c). The IC_{50} of MOPS on the amplitude of the non-pregnant uterus was 0.057 ± 0.127 (Table 1). The high SEM observed possibly indicates a wide spread of the extract's inhibition data (Cumming et al., 2007).

Table 1. Estimates of the methanolic extract of *O. procerum* stem bark on spontaneous contractility parameters of the uterus in mice.

Group	IC_{50} AMP (mg/mL)	IC_{50} Freq (mg/mL)
Non-pregnant	0.057 ± 0.104	0.045 ± 0.306
Pregnant	0.054 ± 0.067	0.109 ± 0.280

Values are mean ± SEM, n = 5 animals.

Effect of MOPS on spontaneous contractility in the pregnant uterus

MOPS (0.001 - 0.440 mg/mL) was observed to have an inhibitory effect on spontaneous contraction of the pregnant uterus (Fig. 2a). MOPS inhibited the amplitude of spontaneous contractions in the pregnant uterus in a concentration-dependent manner (Fig. 2b). Similarly, the frequency of uterine contractions in the pregnant uterus was inhibited (Fig. 2c). The IC_{50} of MOPS on the amplitude of the pregnant uterus was 0.054 ± 1.167 (Table 1). The high SEM observed again possibly indicates a wide spread of the extract's inhibition data (Cumming et al., 2007).

Effect of MOPS on oxytocin-induced uterine contractility in the non-pregnant uterus

MOPS (0.1 mg/mL) was observed to have variable effect on OT-induced uterine contractions (11.62 nM) with an increase in the baseline (Fig. 3a). A decrease ($p<0.05$) in amplitude was observed in the presence of MOPS (Fig. 3b) but a stimulatory effect was observed on the frequency (Fig. 3c).

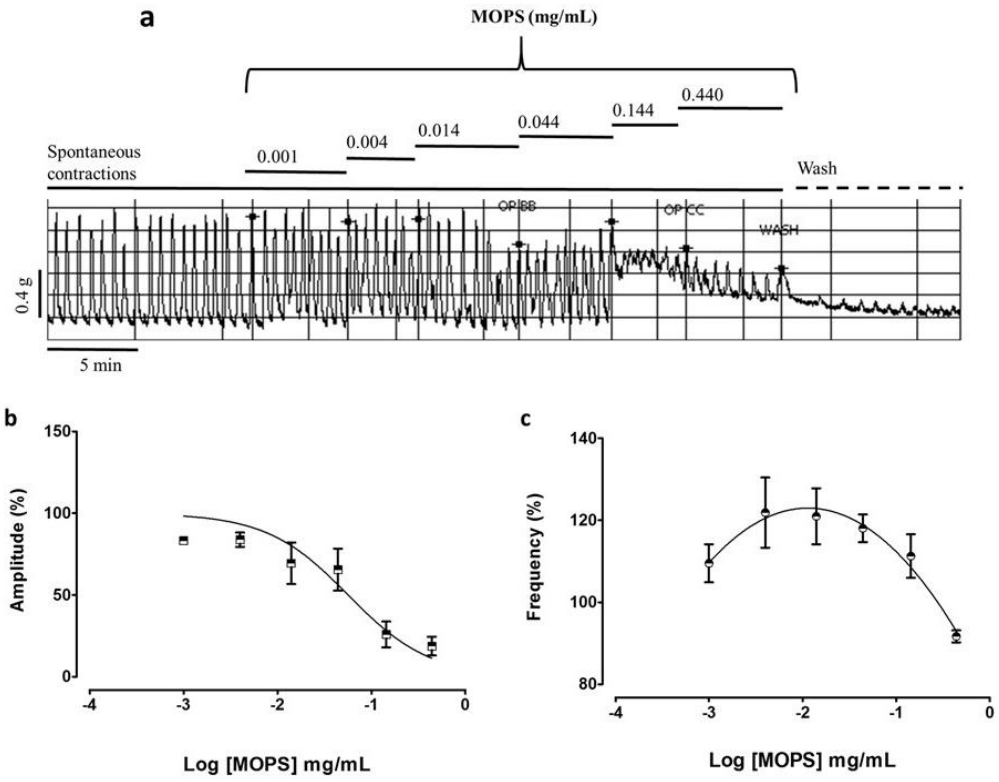

Figure 1. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on spontaneous uterine contractility of non-pregnant animals.

(a) Original representative recording showing the effect of MOPS on spontaneous uterine contractility; **(b)** MOPS inhibited the amplitude of spontaneous uterine contraction; **(c)** MOPS inhibited the frequency of spontaneous uterine contraction; Values are mean ± SEM, n = 5 animals.

Effect of MOPS on high KCl-induced uterine contraction in the non-pregnant uterus

MOPS was observed to have no effect on the tonic contractions induced by KCl (80 mM) (Fig. 4a). This was evident on analysis of the amplitude from five different experiments, which showed that MOPS had no effect on the amplitude of high KCl-induced uterine contractions (Fig. 4b).

Effect of MOPS on OT-induced contraction of the non-pregnant uterus in Ca^{2+}- free media

MOPS was observed to inhibit uterine responses to OT (1.16 µM) in Ca^{2+}- free PSS (Fig. 5a). This was clearly evident on analysis, which showed an inhibition of the amplitude of OT by MOPS and a significant inhibition ($p<0.01$) of the frequency of OT –induced uterine contraction (Fig. 5b-c).

Effect of receptor antagonist on MOPS activity on uterine contraction

MOPS (1.00 mg/mL) inhibited spontaneous uterine contractions (Fig. 6). The amplitude was significantly inhibited ($p<0.05$) while a non-significant increase in the frequency was observed (Fig. 6b-c). In the presence of TEA, MOPS appeared to still cause slight inhibition of spontaneous contractions (Fig. 7a). MOPS caused a non-significant decrease in the amplitude and frequency of spontaneous uterine contractions. (Fig. 7b-c).

MOPS inhibited spontaneous uterine contraction in the presence of neomycin (Fig. 8). MOPS was observed to produce a non-significant inhibition in the amplitude of spontaneous uterine contraction in the presence of NM, however a significant inhibition ($p<0.05$) of the frequency was observed (Fig. 8b-c). MOPS (1.00 mg/mL) had no effect on the amplitude and frequency of spontaneous uterine contractions in the presence of propranolol (Fig. 9).

In order to determine these receptor antagonists' effect in order of potency, a percentage computation was done for the activities of MOPS on spontaneous contractions in the presence of each antagonist. For the amplitude, it was observed that TEA and NM only slightly counteracted the inhibitory effect of MOPS; however, PR produced a sig-

nificant attenuation ($p<0.001$) of the inhibitory effect of MOPS on spontaneous uterine contraction (Fig. 10a). On the frequency, MOPS at 1.00 mg/mL was shown to stimulate an increase in frequency of spontaneous uterine contractions. In the presence of TEA and PR, MOPS stimulatory effect was significantly attenuated ($p<0.05$). However, NM produced a greater significant attenuation ($p<0.001$) of the stimulatory effect of MOPS on spontaneous contractions (Fig. 10b).

The pharmacological agents used in determining the activity of MOPS in this study as well as the proposed mechanism of activity are summarized in Table 2.

DISCUSSION

MOPS was shown in this study to inhibit spontaneous uterine contraction in the pregnant and non-pregnant uterus. The myometrial layer of the uterus composed of smooth muscle fibers is primarily responsible for contraction of the uterus (Kelly, 1962; Wray and Arrowsmith, 2012). In the non-pregnant uterus, contraction serves to evacuate the sloughed endometrial layer, which occurs in the follicular phase (de Vries et al., 1990). In some females, this may cause mild to severe pain known clinically as dysmenorrhea (Bulletti et al., 2000). In the pregnant uterus at term, contractions serve to expel the fetus but in the early pregnant uterus this may lead to PTL (Floyd and Wray, 2007; Wray et al., 2003). MOPS was shown to inhibit these uterine contractions in both pregnant and non-pregnant uterus. Spontaneous contractions are regulated in part by calcium influx into the myometrial cells (Wray and Arrowsmith, 2012), this suggests that MOPS may interact with calcium influx.

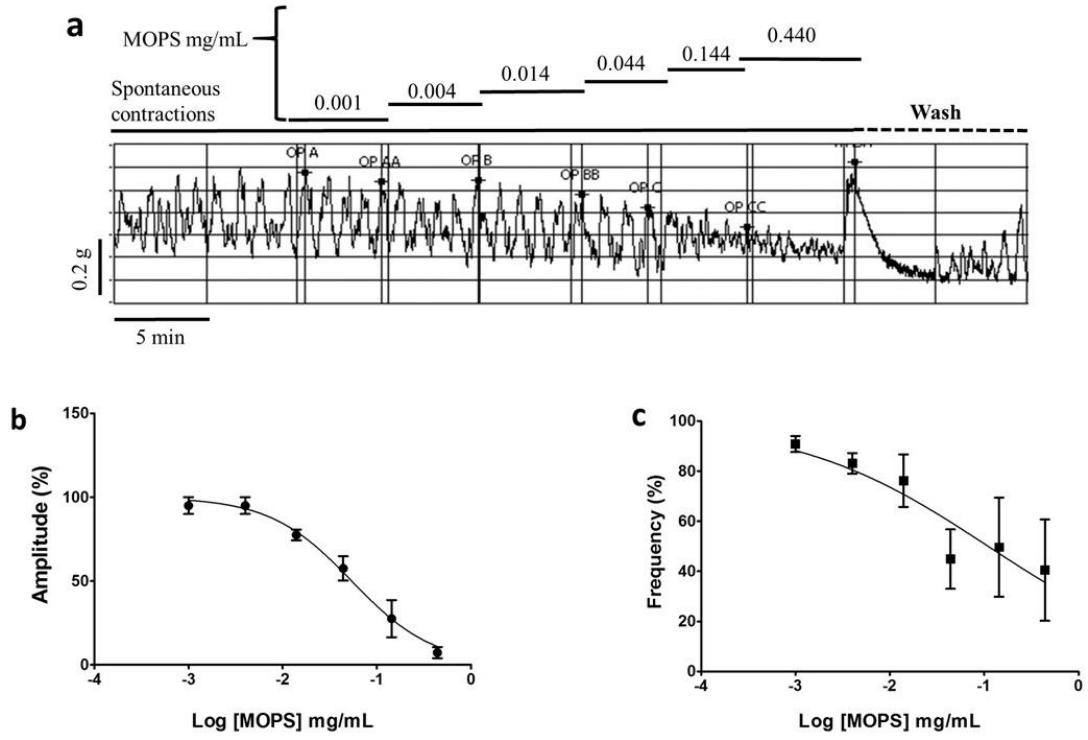

Figure 2. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on spontaneous uterine contractility of pregnant animals.

(a) Original representative recording of the effect of MOPS on spontaneous contractility in the pregnant uterus; **(b)** MOPS inhibited the amplitude of spontaneous uterine contraction; **(c)** MOPS also inhibited the frequency of the spontaneous uterine contractions in the pregnant uterus; Values are mean ± SEM, n = 4 animals.

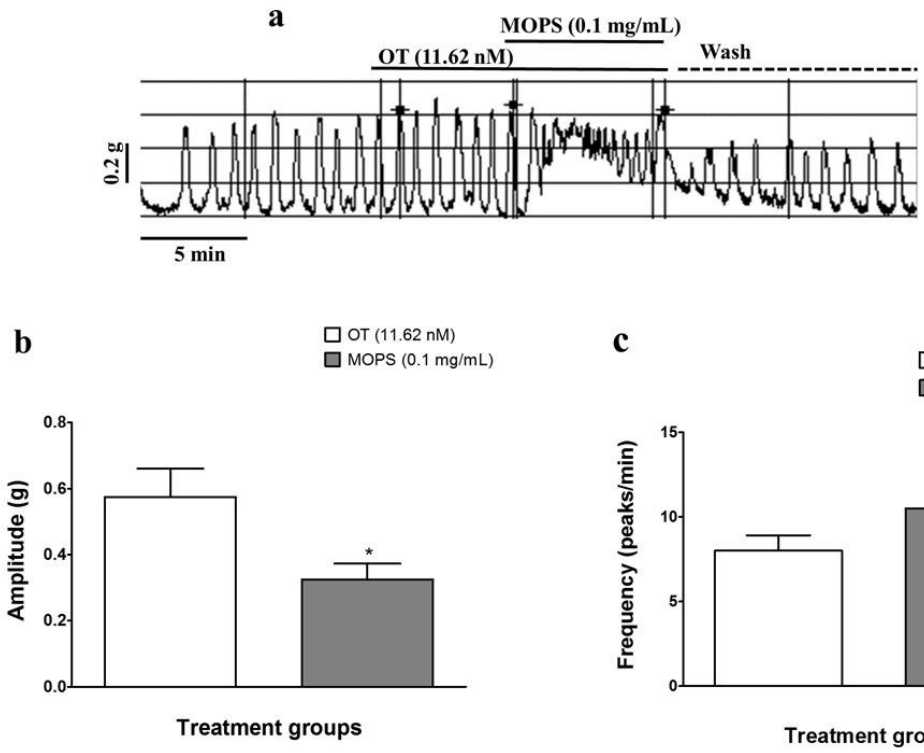

Figure 3. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on oxytocin (OT)-induced uterine contractions in non-pregnant animals.

(a) Original representative recording showing the effect of MOPS on OT-induced uterine contractions; **(b)** MOPS significantly ($p<0.05$) inhibited the amplitude of OT; **(c)** an increase in frequency OT-induced contractions was observed in the presence of MOPS; Values are mean ± SEM,*$p<0.05$ compared to control; n= 5 animals.

MOPS was shown to also inhibit OT-induced uterine contractions. OT is an agonist of uterine contractions and acts through the stimulation of phospholipase C and activation of the second messengers, inositol triphosphate (IP_3) and diacylglycerol (DAG), which ultimately lead to the mobilization of calcium from both extracellular and intracellular stores (Arrowsmith and Wray, 2014; Wray, 2007). OT has also recently been reported to be involved in the utilization of nicotinamide adenine dinucleotide (NAD), gamma-aminobutyric acid (GABA), and sphingosine pathways for uterine contraction (Bafor et al., 2016). By extrapolation, inhibition of OT-induced uterine contraction by MOPS may be associated with the direct or indirect interaction with any one or more of IP_3, DAG, NAD, GABA and sphingosine pathways.

To investigate the interaction of MOPS with extracellular calcium influx, MOPS activity on high KCl was investigated. At the concentration used, MOPS was found to have no effect on KCl-induce tonic uterine contractions. High KCl activates the opening of voltage-gated calcium channels, which lead to a massive influx of calcium ions (Wray et al., 2001). It therefore implies that MOPS does not interact with VGCCs in calcium regulation of uterine contraction. MOPS however inhibited OT-induced uterine contraction in calcium free solution, suggesting that MOPS inhibited calcium release from intracellular stores, which is the primary source of calcium when a calcium free solution is used (Kupittayanant et al., 2002).

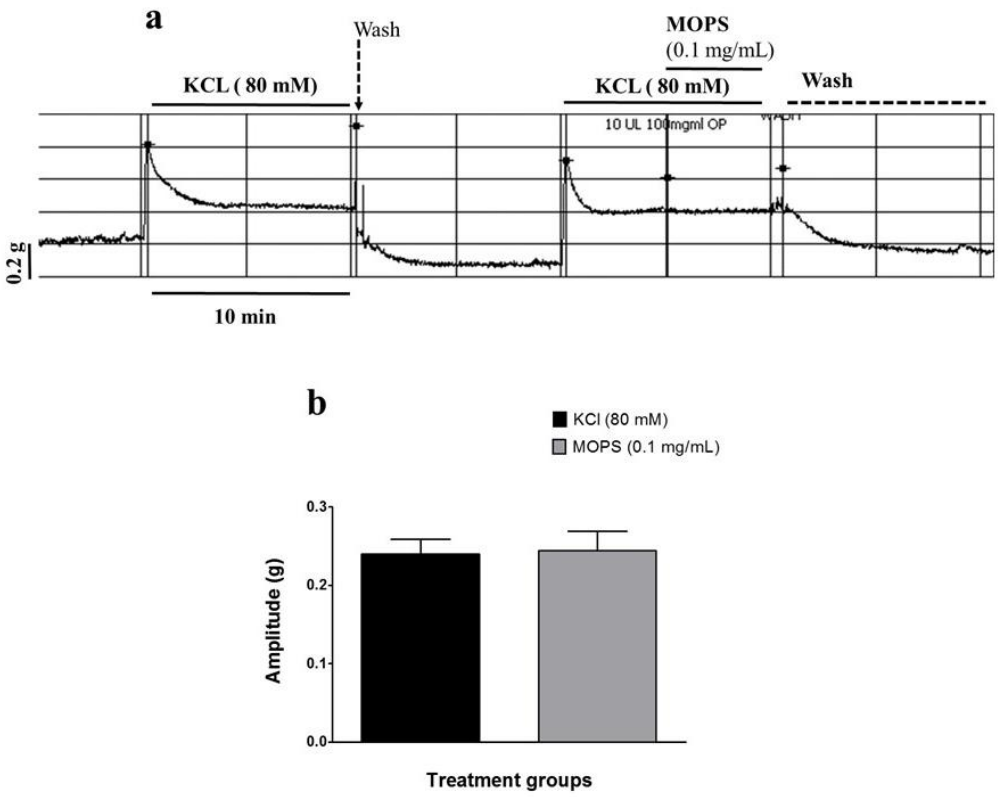

Figure 4. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on high KCl-induced uterine contractions in the non-pregnant uterus.

(a) Original representative recording showing the effect of MOPS on KCl-induced uterine contractions; **(b)** MOPs was observed to have no effect on the amplitude of KCl-induced contractions. Values are mean ± SEM, n = 5 animals. One-way ANOVA followed by Dunnett's post hoc test.

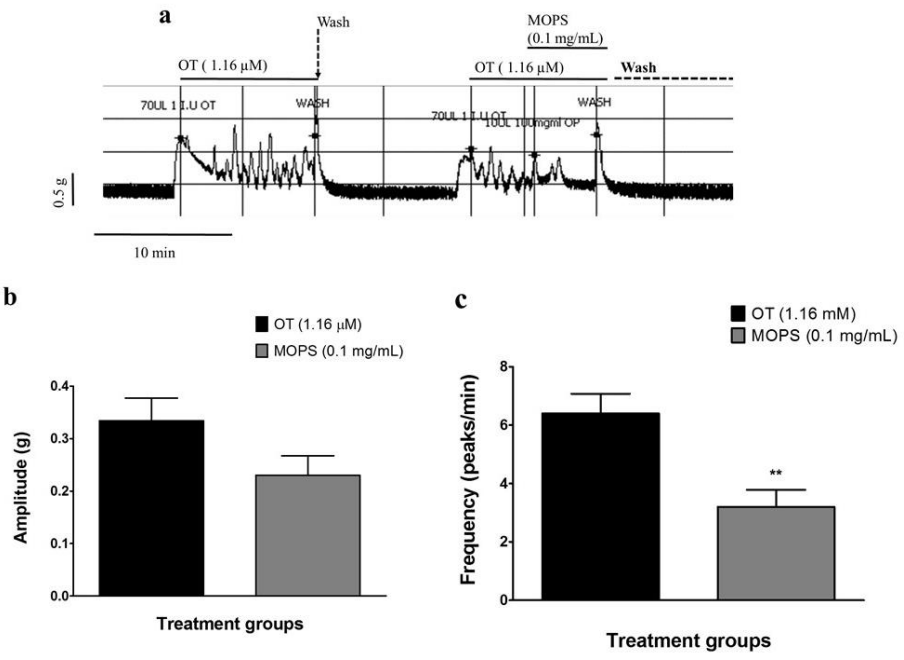

Figure 5. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on oxytocin (OT)-induced uterine contractions in Ca^{2+}- free media in the non-pregnant uterus.

(a) Representative recording of the responses of MOPS in Ca^{2+}- free solution; **(b)** MOPS inhibited the amplitude of OT-induced contraction in Ca^{2+}- free PSS; however, MOPS significantly inhibited ($p<0.01$) the frequency of OT-induced contraction **(c)**. Values are mean ± SEM, n = 5 animals; **$p<0.01$ compared to OT alone. One-way ANOVA followed by Dunnett's post hoc test.

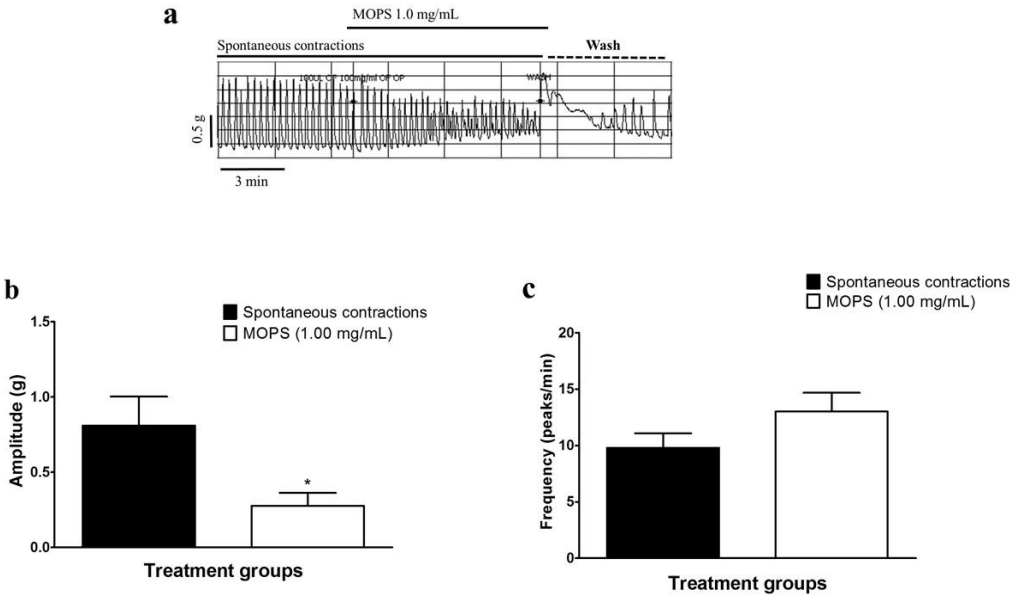

Figure 6. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on spontaneous contractions of the non-pregnant uterus.

(a) Representative recording showing the effect of MOPS on spontaneous contraction; **(b)** MOPS significantly inhibited ($p<0.01$) the amplitude of contractions; **(c)** while an increase in the frequency was observed; Values are mean ± SEM, *$p<0.01$; n = 5 animals. One-way ANOVA followed by Dunnett's post hoc test.

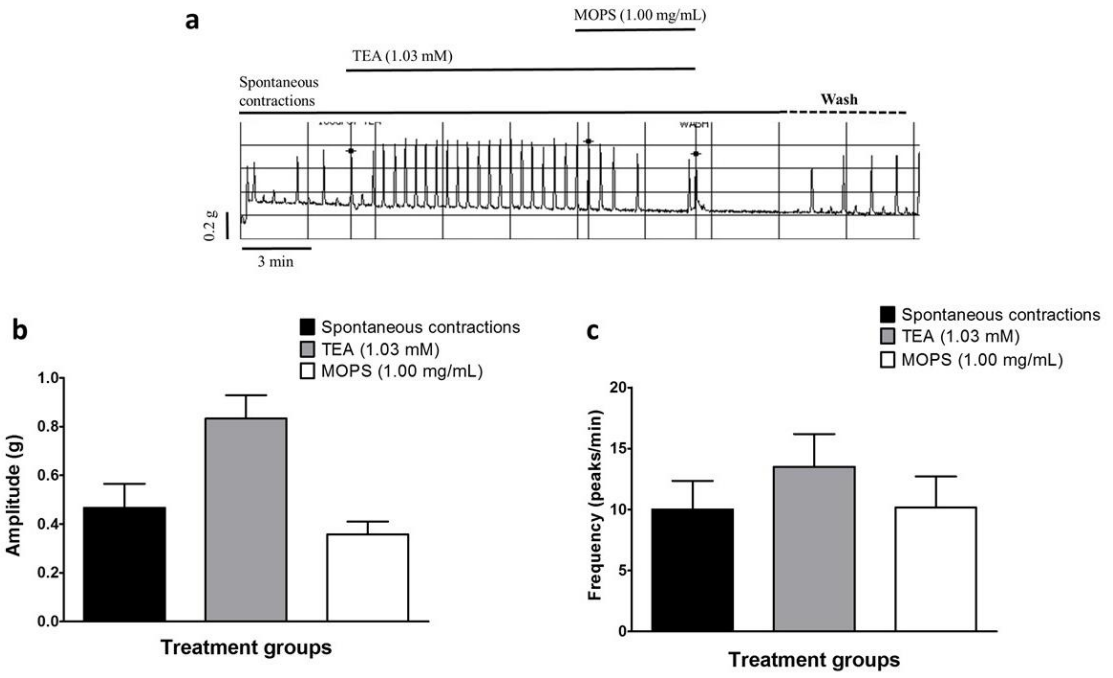

Figure 7. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on spontaneous uterine contraction in the presence of tetraethylammonium chloride (TEA).

(a) Representative recording showing the effect of TEA on MOPS' activity on the uterus; **(b)** In the presence of TEA, MOPS' inhibitory activity on the amplitude of spontaneous contractions was slightly attenuated; **(c)** and MOPS activity on the frequency of contractions was also slightly attenuated. Values are mean ± SEM, n = 5 animals.

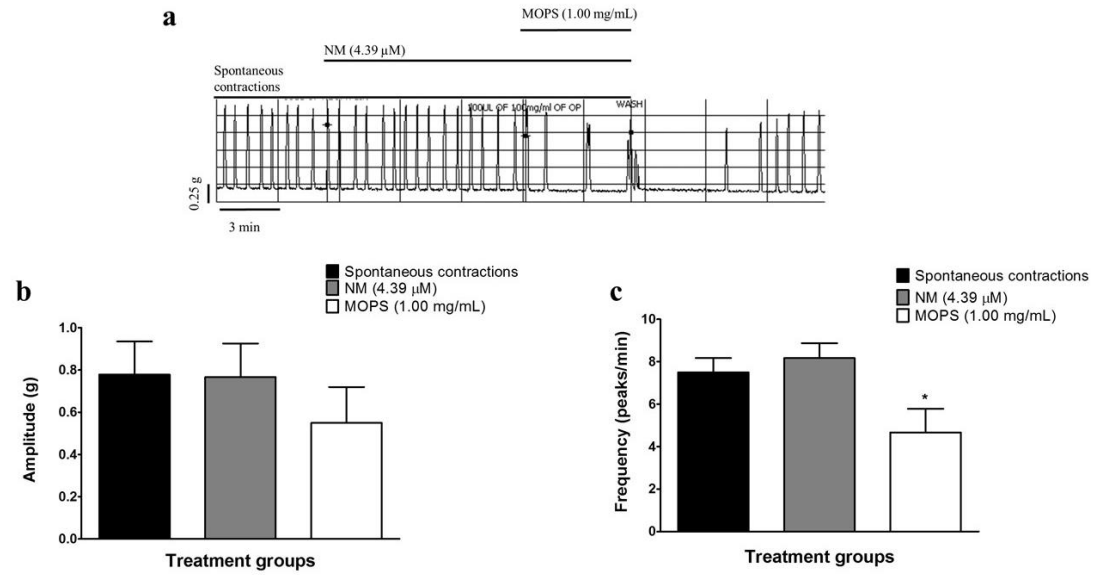

Figure 8. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on spontaneous uterine contraction in the presence of neomycin (NM).

(a) Representative recording showing the effect of NM on MOPS' activity on the uterus; **(b)** In the presence of NM, MOPS' inhibitory activity on the amplitude of spontaneous contractions was slightly attenuated; **(c)** and MOPS significantly inhibited ($p<0.05$) the frequency of contractions. Values are mean ± SEM, n = 5 animals; *$p<0.05$ compared to spontaneous contraction alone. One-way ANOVA followed by Dunnett's post hoc test.

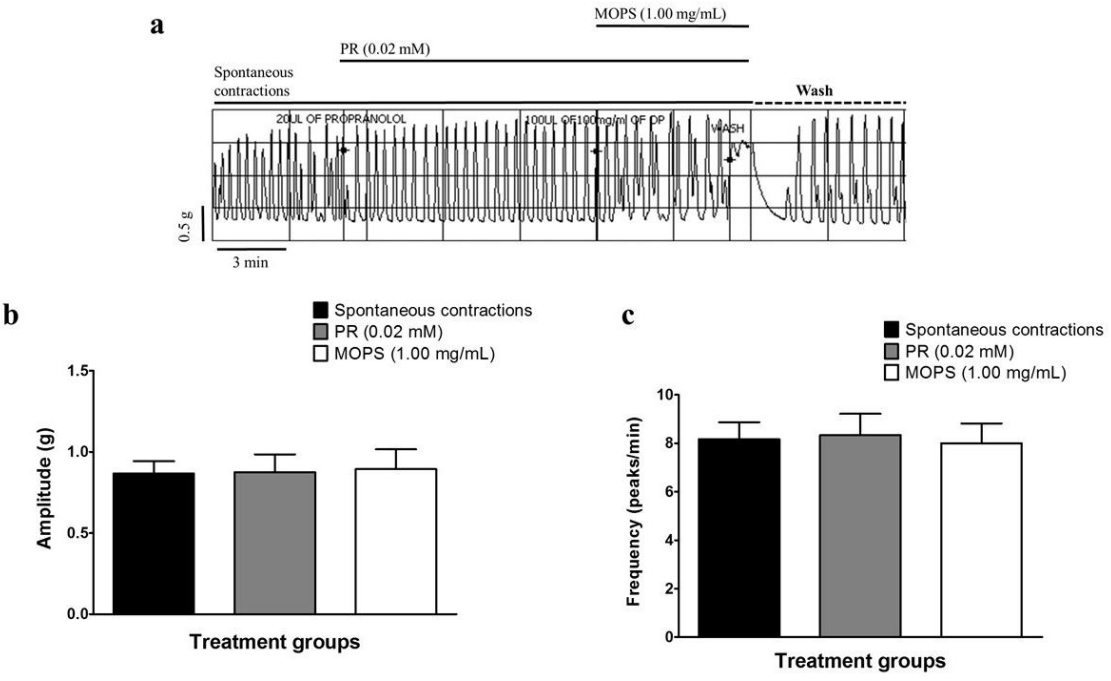

Figure 9. Effect of the methanolic extract of *O. procerum* stem bark (MOPS) on spontaneous uterine contraction in the presence of propranolol (PR).

(a) Representative recording showing the effect of PR on MOPS' activity on the uterus; **(b)** In the presence of PR (0.02 mM), MOPS' inhibitory activity on the amplitude of spontaneous contractions was attenuated and; **(c)** MOPS activity on the frequency of contractions was attenuated. Values are mean ± SEM, n = 5 animals. One-way ANOVA followed by Dunnett's post hoc test.

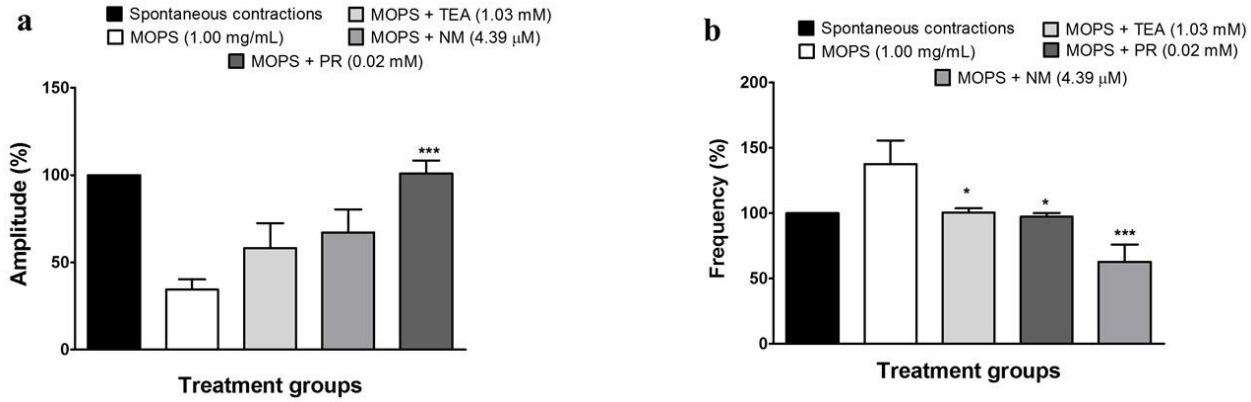

Figure 10. Effect of some antagonists on methanolic extract of *O. procerum* stem bark (MOPS)-mediated inhibition of uterine contractility in the non-pregnant uterus.

(a) PR produced the greatest attenuation of MOPS-induced inhibition of the amplitude of contraction, while **(b)** NM PR, and TEA in decreasing order of potency showed greater attenuation of the frequency increase induced by MOPS. Values are mean ± SEM, n=5 animals; *p<0.05; **p<0.01, ***p<0.001 compared to MOPS alone; PR= propranolol; TEA = tetraethylammonium chloride; NM = neomycin. Values are mean ± SEM, One-way ANOVA followed by Dunnett's post hoc test.

Table 2. Summary of the mechanism(s) of the methanolic extract of *O. procerum* stem bark on uterine contractility.

Drug/activity	Pharmacological mechanism	Response in the presence of MOPS	Proposed mechanism of MOPS
OT	Stimulation of Ca^{2+} mobilization through oxytocin receptor activation	Inhibition of OT-induced uterine contractility	Antagonism of OT receptors/ inhibition of calcium mobilization
KCl	Activates extracellular VGCCs	No effect on high KCl-induced tonic contractions	Does not inhibit VGCCs
OT in calcium-free solution	Utilization of Ca^{2+}-released from intracellular stores	Inhibition of OT in calcium free solution	Inhibition of calcium released from intracellular stores
TEA	Blocks potassium channels non-selectively	Slight attenuation of the inhibitory effect of MOPS	May open potassium channels
NM	RyR blocker	Slight attenuation of the inhibitory activity of MOPS	May block RyRs
PR	β-adrenoceptor antagonist	Overcomes the inhibitory effect of MOPS	Agonist of β-adrenoceptors in the uterus

OT – oxytocin; KCl – potassium chloride; VGCCs- voltage operated calcium channels; PR – propranolol; TEA – tetraethylammonium chloride; NM – neomycin; RyR – ryanodine receptor.

To investigate the contribution of receptor activity to the intracellular calcium blocking effect of MOPS, the receptor antagonists, PR and NM as well as the channel blocker, TEA were utilized. TEA non-selectively blocks potassium channels in the uterus. In this study, TEA only slightly attenuated the inhibitory effect of MOPS, which was followed closely by NM. NM blocks the activity of ryanodine (RyR) in the uterus (Laver et al., 2007). RyR is situated on the sarcoplasmic reticulum (SR) (Mackrill et al., 2015) where it acts as a calcium release channel. However, PR a β-adrenoceptor agonist resulted in a significant attenuation ($p<0.01$) of MOPS' inhibitory activity on the uter-

us. Agonists of β-adrenoceptors activates cyclic adenosine monophosphate (cAMP) and/or guanosine triphosphate (GTP), which results in the phosphorylation of adenosine triphosphate (K_{ATP}) and large conductance potassium channels (BKCa) (Barata et al., 2004). In addition, our recent study found that β-adrenoceptor agonists inhibits the phosphatidylinositol signaling and the activity of prostaglandin F1 (Bafor et al., 2016). This cascade of events inhibits calcium influx and causes relaxation of the uterus. PR is a potent β-adrenoceptor blocker and by overcoming the inhibitory effect of MOPS, it clearly suggests that MOPS has β-adrenoceptor agonist activity, however interaction with phoshatidylinositol signaling is also suggested.

CONCLUSIONS

This study has shown that the stem bark of *Omphalocarpum procerum* inhibits uterine contraction in the pregnant and non-pregnant uterus. The inhibitory effect of *O. procerum* stem bark on the uterus appears to occur through the activation of β-adrenergic receptors in the uterus, which promote uterine relaxation. To the best of our knowledge this is the first report showing the activity and possible mechanism of *O. procerum* on the uterus. Further studies are however required to isolate the active constituents responsible for the activity of *O. procerum*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to acknowledge Mr. Kingsley of Pharmacognosy department, Faculty of Pharmacy for his assistance with initial extraction of the plant. We would also like to acknowledge Mrs Osemelomen Omoruyi, Mrs Uloma Elvis-Offiah and Ms. Faith Ukpebor for their assistance during the course of the experimental studies. This study did not receive any funding.

REFERENCES

Akihisa T, Kojima N, Katoh N, Kikuchi T, Fukatsu M, Shimizu N, Masters ET (2011) Triacylglycerol and triterpene ester composition of shea nuts from seven African countries. J Oleo Sci 60: 385–386.

Akihisa T, Kojima N, Kikuchi T, Yasukawa K, Tokuda H, Masters ET, Manosroi A, Manosroi J (2010) Anti-inflammatory and chemopreventive effects of triterpene cinnamates and acetates from shea fat. J Oleo Sci 59: 273–280.

Arrowsmith S, Wray S (2014) Oxytocin: Its mechanism of action and receptor signalling in the myometrium. J Neuroendocrinol 26: 356–369.

Bafor EE, Ebidame VO, Elvis-Offiah UB, Omoruyi O, Eze GI, Igbinuwen O, Braimoh KP (2017) A role of alpha-tocopherol and phylloquinone in the modulation of uterine contractility and reproductive function in mouse models. Medicina 53: 190–202.

Bafor EE, Eniye M, Ayinde BA, Nworgu ZA (2015) *In vitro* evaluation of the effect of *Corchorus olitorius* (Tiliaceae) on isolated mouse uterus. J Pharm Bioresour 12: 120–128.

Bafor EE, Rowan EG, Edrada-Ebel R (2016) Towards understanding myometrial regulation: metabolomic investigation reveals possible new pathways of oxytocin and ritodrine activity on the myometrium. Reprod Sci 24: 691–705.

Bafor EE, Ukpebor F, Omoruyi O, Ochoyama E, Ekufu J, 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. J Ethnopharmacol 243: 112087.

Baliga MS, Pai RJ, Bhat HP, Palatty PL, Boloor R (2011) Chemistry and medicinal properties of the bakul (*Mimusops elengi* Linn): A review. Food Res Int 44: 1823–1829.

Barata H, Thompson M, Zielinska W, Han YS, Mantilla CB, Prakash YS, Feitoza S, Sieck G, Chini EN (2004) The role of cyclic-ADP-ribose-signaling pathway in oxytocin-induced Ca^{2+} transients in human myometrium cells. Endocrinol 145: 881–889.

Bulletti C, Ziegler D, De Polli V, Diotallevi L, Ferro ED, Flamigni C (2000) Uterine contractility during the menstrual cycle. Hum Reprod 15: 81–89.

Chen C-H, Lin Y-H, Heitkemper MM, Wu K-M (2006) The self-care strategies of girls with primary dysmenorrhea: a focus group study in Taiwan. Health Care Women Int 27: 418–427.

Cumming G, Fidler F, Vaux DL (2007) Error bars in experimental biology. J Cell Biol 177: 7–11.

De Vries K, Lyons EA, Ballard G, Levi CS, Lindsay DJ (1990) Contractions of the inner third of the myometrium. Am J Obstet Gynecol 162: 679–682.

Floyd R, Wray S (2007) Calcium transporters and signalling in smooth muscles. Cell Calcium 42: 467–476.

Gruber CW, O'Brien M (2011) Uterotonic plants and their bioactive constituents. Planta Med 77: 207-220.

Kelly JV (1962) Myometrial participation in human sperm transport: A dilemma. Fertil Steril 13: 84–92.

Kupittayanant S, Luckas MJM, Wray S (2002) Effect of inhibiting the sarcoplasmic reticulum on spontaneous and oxytocin-induced contractions of human myometrium. Int J Obstet Gynaecol 109: 289–296.

Laver DR, Hamada T, Fessenden JD, Ikemoto N (2007) The ryanodine receptor pore blocker neomycin also inhibits channel activity via a previously undescribed high-affinity Ca^{2+} binding site. J Membr Biol 220: 11–20.

Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE (2015) Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: An updated systematic analysis. Lancet 385: 430–440.

Mackrill JJ, Challis RA, O'Connell DA, Lai FA, Nahorski SR (2015) Differential expression and regulation of ryanodine receptor and myo -inositol 1,4,5-trisphosphate receptor Ca^{2+} release channels in mammalian tissues and cell lines. Biochem J 327: 251–258.

Maltaris T, Dragonas C, Hoffmann I, Mueller A, Schild RL, Schmidt W, Beckmann MW, Dittrich R (2006) The extracorporeal perfusion of the swine uterus as an experimental model: The effect of tocolytic drugs. Eur J Obstet Gynecol Reprod Biol 126: 56–62.

National Research Council (2010) Guide for the Care and Use of Laboratory Animals: Eighth Edition, in: Guide for the Care and Use of Laboratory Animals. National Academies Press; p. 118. doi:10.2307/1525495

Ngamgwe RF, Yankam R, Chouna JR, Lanz C, Furrer J, Schurch S, Kaiser M, Lenta BN, Ngouela S, Tsamo E, Brenneisen R (2014) Procerenone: A fatty acid triterpenoid from the fruit pericarp of *Omphalocarpum procerum* (Sapotaceae). Iran J Pharm Res 13: 1423–1428.

Park K-S, Park K-I, Hwang D-S, Lee J-M, Jang J-B, Lee C-H (2014) A review of *in vitro* and *in vivo* studies on the efficacy of herbal medicines for primary dysmenorrhea. Evidence-Based Complement Altern Med 2014: 1–11.

Quiroz D, Sosef M, Van Andel T (2016) Why ritual plant use has ethnopharmacological relevance. J Ethnopharmacol 188: 48–56.

Wray S (2007) Insights into the uterus. Exp Physiol 92: 621–631.

Wray S, Arrowsmith S (2012) Uterine Smooth Muscle in: Muscle. New York: Academic Press; p. 1207–1216.

Wray S, Jones K, Kupittayanant S, Li Y, Matthew A, Monir-Bishty E, Noble K, Pierce SJ, Quenby S, Shmygol AV (2003) Calcium signaling and uterine contractility. J Soc Gynecol Investig 10: 252–264.

Wray S, Kupittayanant S, Shmygol A, Smith RD, Burdyga T (2001) The physiological basis of uterine contractility: a short review. Exp Physiol 86: 239–246.

AUTHOR CONTRIBUTION:

Contribution	Bafor E	Eyegbagharen T	Ochoyama E	Idu M
Concepts or ideas	x			x
Design	x			
Definition of intellectual content	x			x
Literature search	x	x		
Experimental studies	x	x	x	
Data acquisition		x	x	
Data analysis	x	x		
Statistical analysis	x	x		
Manuscript preparation	x	x	x	x
Manuscript editing	x	x	x	x
Manuscript review	x	x	x	x

Citation Format: Bafor E, Eyegbagharen T, Ochoyama E, Idu M (2020) *Ex-vivo* inhibition of uterine contractility by the stem bark extracts of *Omphalocarpum procerum* P.Beauv. (*Sapotaceae*) in mouse models. J Pharm Pharmacogn Res 8(2): 92–104.