ORIGINAL PAPER

https://dx.doi.org/10.4314/njpr.v19i1.9

Nig. J. Pharm. Res. 2023, 19 (1) pp 93-101
ISSN 0189-8434 e-ISSN 2635-3555

Available online at http://www.nigipharmres.com

Fruit Extract of Oil Palm (*Elaeis Guineensis* (Jacq.)) Inhibits Uterine Contractility in *Ex-Vivo* Mouse Models

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Different parts of *Elaeis guineensis* (EG) plants are used ethno-medicinally for treating numerous illnesses including abdominal contraction.

Objectives: Thus, this study evaluated the inhibitory potential of the fruit-extract of EG on the uterine contractility in *ex-vivo* mouse models.

Material and Methods: The effect of EG extract on spontaneous uterine contractions, oxytocin (OT)-induced uterine contractions, high potassium chloride induced uterine contractions and oxytocin-induced calcium free contraction were evaluated in adult non-pregnant female albino mice.

Results: EG in a concentration dependent mode diminished spontaneous uterine contractions, while amplitude and frequency of contractions were inhibited significantly, but the frequency of contraction was at the highest concentration of EG. EG also produced a significant inhibition of the frequency of OT-induced contraction in calcium-free media.

Conclusion: The aqueous fruit extracts of EG inhibited uterine contraction in the uterus through inhibition of intracellular calcium stores.

Keywords: *Elaeis guineensis* fruit, Oxytocin-induced uterine contractility, Potassium chloride induced uterine contraction, Oxytocin induced calcium free contraction

INTRODUCTION

Elaeis guineensis (EG) Jacq, (Arecaceae) is a perennial, monocotyledonous, monoecious tropical crop mainly grown exclusively by seeds for the oils from the palm fruit and palm kernel (Muniran *et al.*, 2008; Mgbeze and Iserhienrhien, 2014). It is thought to have its origin in Africa (Obahiagbon, 2012). It has an extended stout single stem of about 20 to 30 m high, terminating in a crown of about 20 to 100 leaves (Dransfield *et al*., 2005). Different parts of the plant have been utilized ethno-medicinally for several therapeutic purposes (Ekwenye and Ijeomah, 2005).

EG has been reportedly used as an anti-poison (Irvin, 1985), in the relief of headaches, pains and rheumatism. It is also used in treating and managing atherosclerosis, heart illnesses and arterial blockage (Sasidharan *et al*., 2012; Ekwenye and Ijeomah, 2005; Honstra, 1986). In infant and children, it is effective against diarrhoea, dysentery (Ekpa and Ebana, 1996) and in the regulation of body temperature during convulsion.

Pharmacologically, EG has been reported to inhibit the growth of MCF-7 cell line and reduce the incidence of colon cancer (Nesaretnam *et al*., 2004, 2008; Boateng

et al., 2006; McIntyre *et al*., 2000). It is known to modulate cellular functions and scavenges for free radicals due to considerable high content of vitamins A and E (Van Rooyen *et al*., 2008; Rao, 2000). Also fresh palm oil lowers the level of serum lipids (Kritchevsky, 2000) and improves intestinal absorption of protein and digestion of selected amino acids and stimulates procreative ability (Ebong *et al*., 1999). Its anti-inflammatory properties were exhibited by significantly reducing cellular inflammatory mediators (Wu *et al*., 2008), while its wound healing activity (Irvin, 1985) can be seen from the better sore closure, tissue restoration at the spot of injury and associated histopathological values relating to injury curing (Sasidharan *et al*., 2012).

Ethno-medicinal usage and pharmacological potentials of the fruit of EG could be linked to its high fatty acids content, these fatty acids could alter the state of uterus, which is made up of smooth muscle to a large extent. The uterus is a pear-like shaped empty organ, found in the female lower abdomen, in between the bladder and the rectum. It has a tiny lower portion called the cervix and wider upper part called the corpus. The uterus has a length of 6 to 8cm, is 2 to 3cm thick in its walls. The width of the uterus changes, but ranges from 6cm wide at the topmost part to only half the fundus width at the neck. It is made of muscles that take care of the growth and development of the embryo or foetus by providing nourishment during gestation (Lee *et al.,* 2019). The hole of the uterus opens into the opening of the vagina, and both construct the birth canal (Rogers *et al.,* 2019).

The endometrium is the wet mucous membrane lining of the uterine cavity. During the menstrual cycle, the thickness of the lining changes and it is thickest at ovulation. When a released egg is fertilized, it adheres to the endometrium of the uterus and starts to develop. When the egg is not fertilized, the outer layer of the wall of the uterus is shed; the unfertilized egg and accumulated tissue now leave the body through the vagina at menstruation. The endometrium of the uterus also releases secretions which help to preserve the egg and sperm cells. Its fluid consists of water, iron, potassium, chloride, glucose and proteins. Glucose provides nutrient for the reproductive cells, and protein helps with implantation of the fertilized egg. The other constituents provide a well-suited setting for the egg and sperm cells (Rogers *et al.,* 2019).

The physiology of the uterus can be altered by both utero-tonic and tocolytic agents. Utero-tonic agents cause contraction or increase the tone of the uterus. In the process stimulating labour and reducing postpartum haemorrhage. Some of these agents are analogues of oxytocin while others may act as indirect oxytocinergics. Tocolytic agents are used to suppress premature labour thus helping to delay delivery for a few days, even though manifestations may take hours before been noticed. However, suppression of contractions is often not in totality and monitoring of the mother and foetus may be required.

The use of herbs as medicine dates back to pre-historic time (Awuchi, 2019). It is readily available especially in Africa, relatively cheap and are believe to be free from side effects (Talalay, 2001; Vickers, 2007). Medicinal plants have also served as the starting materials for the development of many orthodox drugs and many more still remain untapped (Ahn, 2017). Some of these medicinal plants are still used as food and in the process of being consumed, phytochemicals are added to our diet, which may alter normal cellular function in human. Oil palm is normally added to meal in most part of Nigeria in order to enhance the nutrition value and enrich the colour and taste of the food. In some traditional setting, the fresh palm oil fruit is use in making soup in addition with other condiments. Physiological, human tissues are made of lipoproteins that can be penetrated by fatty compounds such as those from extract from EG. The movement of these phytochemicals into the cell is believe to alter the normal functioning of tissue. Literature search showed the absence of information pertaining to the uterine contractility potential of EG fruit extracts, thus this study was aimed at determining the inhibitory potential of the fruit extracts of EG on the uterine contractility in *ex-vivo* mouse models.

METHODOLOGY

Drugs, chemicals and reagents

Oxytocin (Anhui medipharm Co Ltd, China), Tween 80 (Kermel-kn®, China), Sodium Chloride, Potassium Chloride, and Sodium Bicarbonate (Lobacheme PVT Ltd, India), Calcium Chloride- $CaCl_2$ (BDH chemicals Ltd Pook, England), D-Glucose and Potassium Chloride (Guangdong GuanghuaSci-Tech Co. Ltd. China), and Ethylene diamine tetra-acetic acid (Kermel, China). Solvents used were of analytical grade and concentrations of drugs used were expressed as final bath concentrations.

Plant collection and preparation

Fresh fruits of *E. guineensis* were purchased in September, 2019 from Oba Market in Benin City, Edo State, Nigeria. Identification and authentication were done by Prof. H.A. Akinnibosun of Department of Plant and Animal Biotechnology, University of Benin. A herbarium number UBH-E444 was assigned and a voucher specimen was generated. The fruits of *E. guineensis* (500 g) were cleaned and boiled at 100 ˚C for 45 min in distilled water. The softened fruits were

first mashed in distilled water with the aid of a mortar and pestle and subsequently filtered to separate the mesocarp from the endocarp. The resulting aqueous extract of *E. guineensis* (EG) was filtered again. The filtrate was concentrated to dryness and stored at 4 °C until needed. The extract yield was 19 % (95 g).

Animals

Adult non-pregnant female mice weighing between 20.0-30.0 g, aged 3-4 months were utilized in this study. The mice were purchased and housed at the animal unit of the Department of Pharmacology and Toxicology, University of Benin. Animals were sheltered in an environmentally controlled room temperature of approximately 27 ± 5 ˚C and natural light/dark cycle. All experiments were carried out as approved by the Ethics Committee, Faculty of Pharmacy, University of Benin (EC/FP/022/12). Animals were handled according to standards of the Public Health Service policy on humane care and use of laboratory animals. The animals were fed on standard diet of animal pellets and clean tap water *ad libitum.*

Experimental procedures

Isolation of the mouse uterus

Mice confirmed to be in oestrus stage of the oestrus cycle via visual assessment of the vulva and microscopic assessment of vaginal smears were utilized (Bafor *et al*., 2019). Mice were humanely sacrificed by cervical dislocation. The uterine horns were isolated and immediately transferred onto a dissecting petri dishes containing previously warmed and aerated physiological solution (PSS) of the composition (mM/L); NaCl 154.0, KCl 5.63, $CaCl_2.2H_2O$ 2.05, $NaHCO_3$ 5.95 and D-glucose 2.78 (Bafor *et al*., 2019). A segment of the uterine horn (approximately 0.5 mm) was cut, freed of connective tissues and mounted longitudinally in an organ bath (10 mL) containing aerated PSS. The uterine tissue setup was maintained at 37˚C. The organ bath was directly connected to an isometric force transducer (7003E, Ugo Basile, Varese, Italy) attached to a 17400 data capsule digital recorder (Ugo Basile, Varese, Italy). The uterine tissue was placed under a resting tension of 4.90 mN, and allowed to equilibrate for 30 min or develop regular spontaneous contractions. The amplitude and frequency of the uterine contractions were recorded.

Effect of EG on spontaneous uterine contractions

The spontaneous contractions were recorded for 10 min, and immediately followed by the cumulative addition of EG (0.01 – 12.21 mg/mL). Each concentration of EG was left in contact with the tissue for 5 min during which the amplitude and frequency were recorded.

Effect of EG on oxytocin (OT) -induced uterine contractions.

Spontaneous contractions of the uterine tissues were recorded for 10 min prior to the addition of OT (11.62 nM). OT was then added and observed for 10 min and the tissue was subsequently washed and allowed to recover. OT was added again to the bath and recorded for 5 min. In the continued presence of the OT, EG (5.21 mg/mL) was then added for 5 min.

Effect of EG on high potassium chloride (KCl) -induced uterine contractions

KCl (80 mM) was added to the organ bath in order to induce tonic uterine contractions. The concentration-response of high KCl was observed for 10 min and the tissue was washed and allowed to recover. KCl was added again to the uterine tissues and the effect was recorded for 5 min, thereafter, EG (5.21 mg/mL) was added for 5 min in the continued presence of KCl. The responses were recorded.

Effect of EG on oxytocin-induced calcium free contraction

On development of regular uterine contractions, the PSS was replaced with another PSS containing zero calcium and 0.1 mM EDTA for 5 min. OT (11.62 nM) was then added to the bath for 5 min before the application of EG (5.21 mg/mL) to the bath for 5 min.

Data analysis

Data obtained are expressed as mean ± standard error of mean (SEM) and "n" represents the number of animals. Analysis was carried out using the GraphPad Prism, (version 8.1.1, GraphPad software Inc, San Diego, CA, USA). Significance was evaluated using appropriate t-tests and one-way ANOVA with Dunnet's multiple comparison test. The limit of significance was set at $p \leq 0.05$. Contractions occurring a t the last 5 min of the phasic contractions were used to calculate the mean frequency and amplitude.

In data sets from spontaneous contractility experiments mean log concentration-response curves were analyzed by fitting data to a four-parameter logistic equation, using non-linear regression with GraphPad Prism 8.0 (GraphPad software, San Diego, CA, USA) using the following equation values (Y = Bottom + (Top-Bottom)/ (1 + 10^(LogIC_{50}-X)*HillSlope). Where Y = response which starts at the bottom and goes to the top in sigmoid shape, X = logarithm of concentration and IC_{50} is the concentration that produces half the maximal.

RESULTS AND DISCUSSION

EG on spontaneous uterine contractions

EG (0.01 – 12.21 mg/mL) decreased spontaneous uterine contractions in a concentration-dependent manner (Fig. 1a). The amplitude of contractions were significantly inhibited ($p < 0.001$) in the presence of EG (Fig. 1b). The frequency of contractions were also inhibited in the presence of EG but this was significant ($p < 0.01$) at the highest concentration of EG (Fig. 1c).

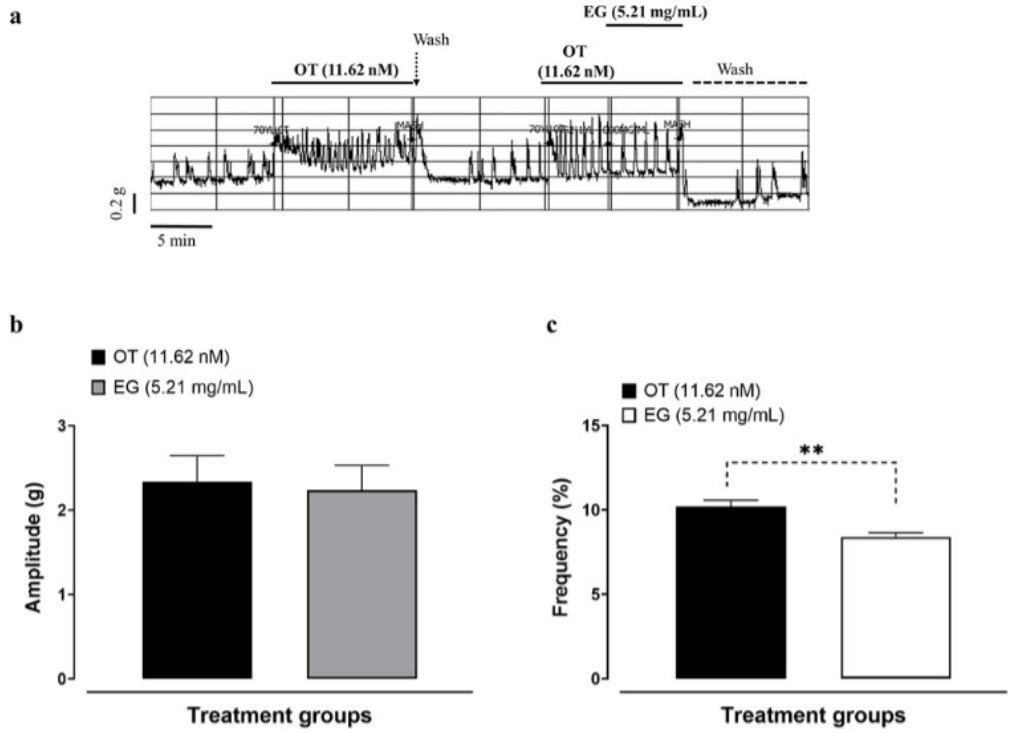

Figure 1. Effect of EG on spontaneous uterine contractions. (a) Original recording showing the cumulative effect of EG on spontaneous uterine contractions; (b) EG inhibited the amplitude of spontaneous uterine contractions; (c) EG inhibited the frequency of spontaneous uterine contractions. n = 5 animals. **p< 0.01; ***p<0.001 compared to control (spontaneous contraction in absence of extract).

EG on OT-induced uterine contractions

EG (5.21 mg/mL) had minimal effect on OT-induced contraction (Fig. 2a). The amplitude of OT-induced contraction was unaffected (Fig. 2b) however, the frequency was significantly reduced ($p < 0.01$) by EG (Fig. 2c).

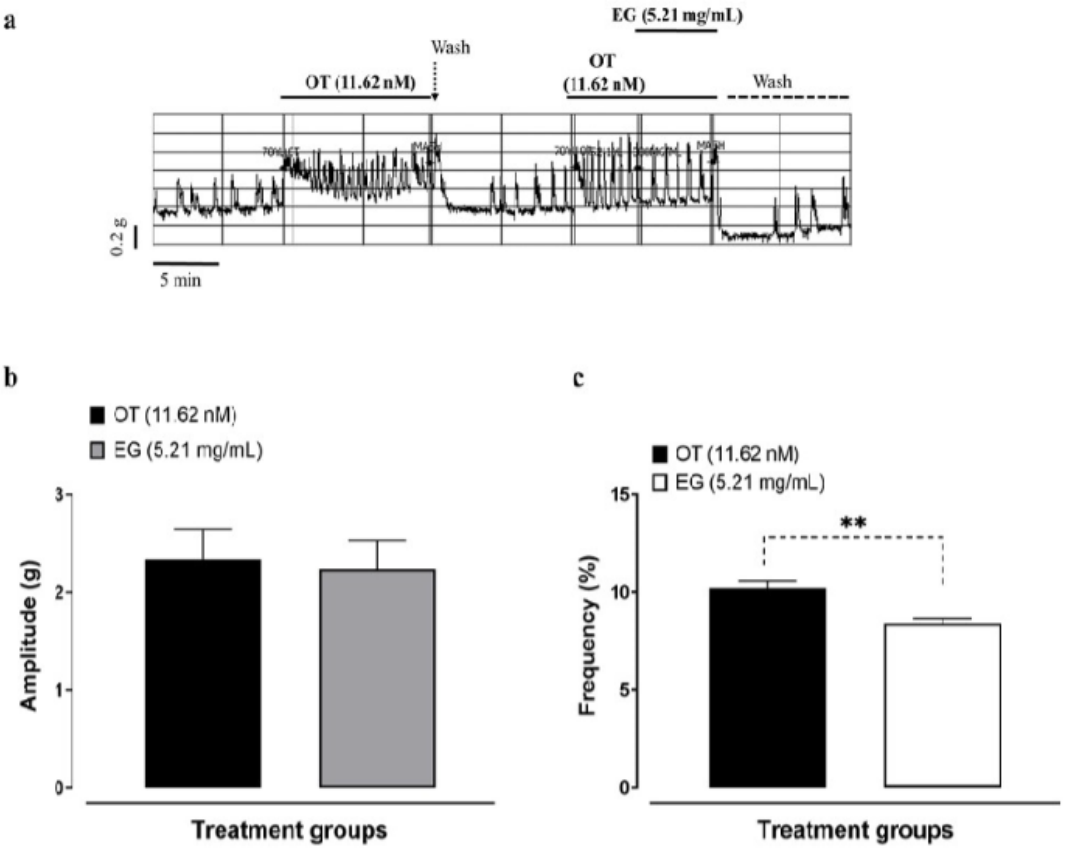

Figure 2. Effect of EG (5.21 mg/mL) on OT-induced uterine contractions. (a) Original recording of the response of OT in the presence of EG; (b) Effect of EG on the amplitude of OT-induced uterine contraction; (b) Effect of EG on the frequency of OT-induced contraction. n = 5 animals; **p<0.01 compared to OT alone.

EG on high KCl-induced tonic uterine contractions

EG (5.21 mg/mL) had no effect on high KCl-induced contractions (Fig. 3a). This was also observed on analysis of the amplitude of contractions induced by KCl in the absence and presence of EG (Fig. 3b).

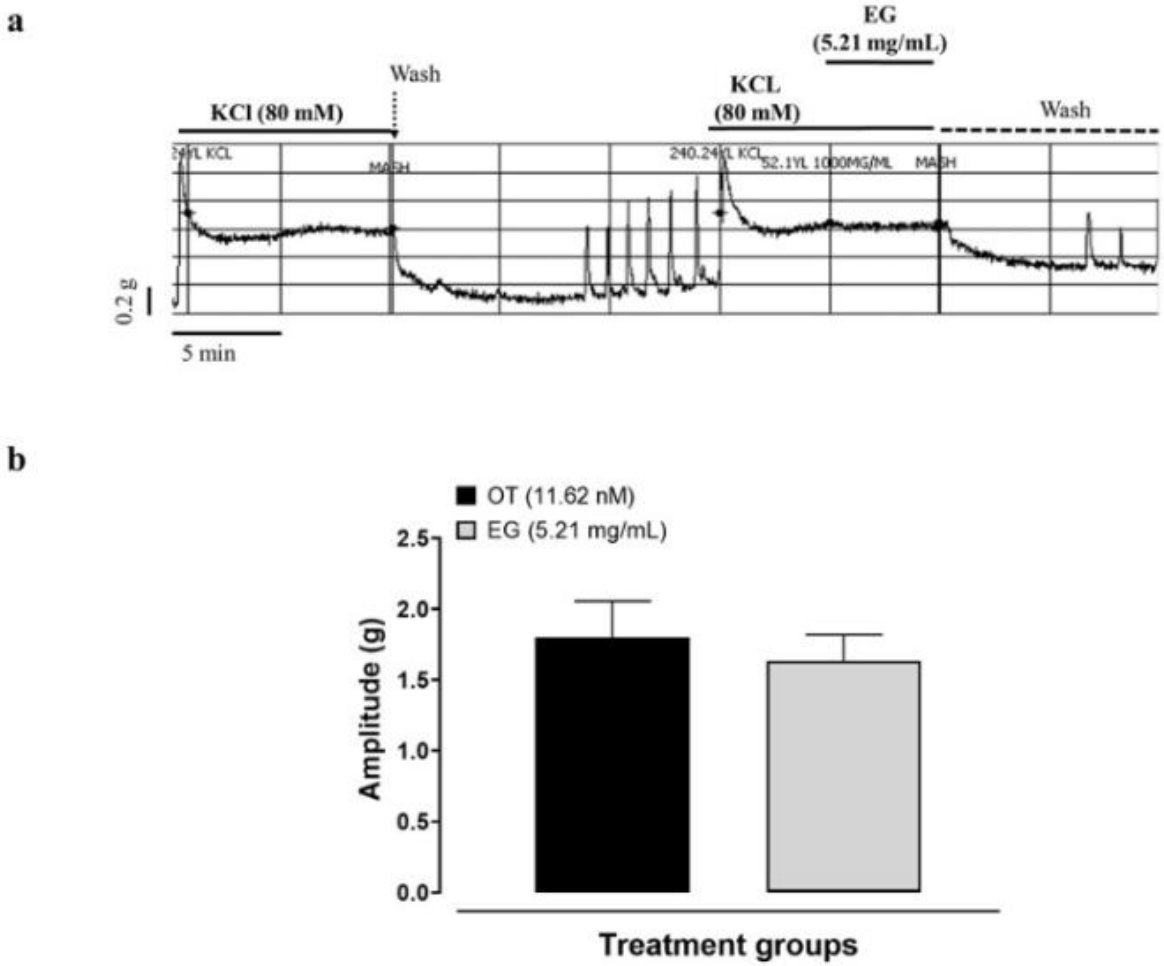

Figure 3. Effect of EG (5.21 mg/mL) on high KCl-induced (80 mM) uterine contraction. (a) Original recording showing the effect of EG on KCl-induced tonic contractions in the isolated mouse uterus; (b) EG did not alter the amplitude of KCl-induced contraction. n = 5 animals.

EG on OT-induced uterine contractions in calcium-free media

The activity of OT was reduced by EG in calcium-free media (Fig. 4a). In calcium-free media, EG inhibited the amplitude of OT-induced contraction, though it was statistically non-significant (Fig. 4b). However EG produced a significant inhibition of the frequency of OT-induced contraction in calcium-free media (Fig. 4c).

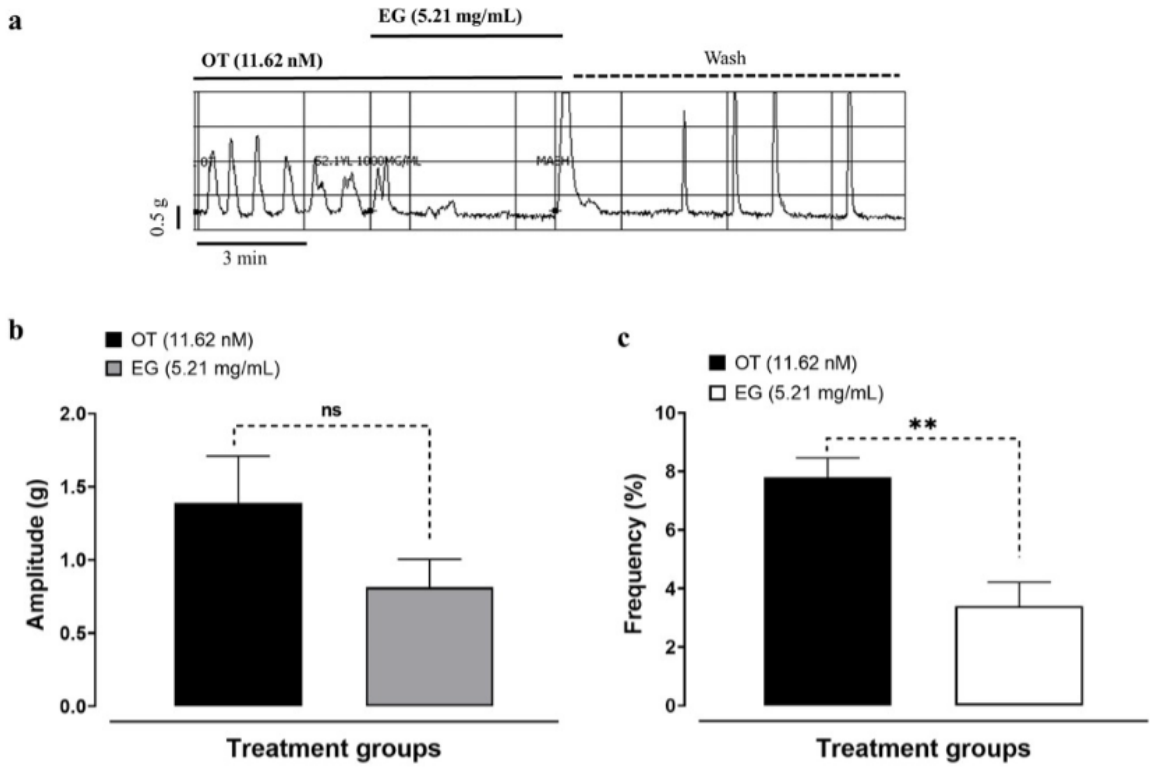

Figure 4. Effect of EG (5.21 mg/mL) on OT-induced (11.62 nM) in calcium-free media. (a) Original recording showing the effect of EG on OT in calcium-free media; (b) EG reduced the amplitude of OT-induced contraction; (c) EG inhibited the frequency of OT-induced contraction. n= 5 animals; **p<0.01 compared to OT alone.

EG was observed in this study to exert inhibitory effects on uterine contraction. This was clearly observed on the activity of EG on spontaneous uterine contractions in the isolated non-pregnant mouse uterus. Uterine contractions in the non-pregnant uterus are essential during post ovulation to promote sperm transport, embedding of the expelled ovum and also to ensure removal of sloughed endometrial material in the absence of fertilization (Kuijsters *et al.*, 2017). These contractions contribute to the spontaneous contractions observed in the non-pregnant uterus (Wray and Arrowsmith, 2012) and are controlled by intracellular and extracellular Ca^{2+}- contributions (Floyd and Wray, 2007). This may therefore suggest that the effect of EG may be mediated via inhibition of either or both Ca^{2+}- contribution sites. Interestingly, however EG failed to inhibit the amplitude of OT-induced contraction but did have an inhibitory effect on the frequency of OT-induced contraction. OT is a potent uterine contracting agent which acts via interaction with oxytocinergic receptors to induce activation of phospholipase C pathway which eventually leads to Ca^{2+}-mobilization from intracellular and then extracellular stores. OT has also been recently discovered to interact with other pathways besides phospholipase C and these include gamma aminobutyric acid, sphingosine and nicotinamide adenine dinucleotide pathways which ultimately results in Ca^{2+}- mobilization as well (Bafor *et al*., 2016). This suggests that EG may have failed to block any of these pathways or may have failed to block Ca^{2+}- mobilization from either the extracellular or intracellular pathway. Further investigation showed that EG lacked activity on extracellular calcium channels. This was clearly observed when EG was unable to inhibit tonic contractions induced by high KCl. High KCl is known to open voltage-gated calcium channels causing sustained depolarization (Bafor *et al*., 2019; Granger *et al*., 1986; Little *et al*., 1985) which was observed as tonic contractions in this study. However, EG inhibited OT-induced contraction in calcium-free media suggesting that EG inhibits or blocks intracellular Ca^{2+}- release from intracellular stores. The major source of calcium in calcium-free media originates from intracellular stores (Kupittayanant *et al*., 2001) which if blocked will prevent response of drugs that depend on such stores such as OT. Overall, it appears that EG acts through inhibition of intracellular Ca^{2+}-release.

CONCLUSION

This study has shown that the aqueous fruit extracts of EG inhibits uterine contraction in the non-pregnant uterus. The study has also shown that EG acts through inhibition of intracellular calcium stores but has no effect on extracellular calcium stores.

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Conflict of Interest: None declared
Received: January 15, 2023
Accepted: March 21, 2023