British Journal of Pharmaceutical Research
8(1): 1-10, 2015, Article no.BJPR.19248
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

Spasmogenic Activity of the Aqueous Methanolic Extract of Unripe *Carica papaya* Fruit in Isolated Uterine Muscle of the Rat

Ebere O. Odirichukwu[1*], Nneka V. S. Uchechukwu[1], Edmund C. Mbegbu[2], Chukwuka N. Uchendu[2] and David Ogwu[3]

[1]Department of Veterinary Surgery and Theriogenology, Michael Okpara University of Agriculture, Umudike, Nigeria.
[2]Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria.
[3]Department of Veterinary Theriogenology and Animal Production, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors EOO, DO and CNU designed the study. Author EOO wrote the protocol and wrote the first draft of the manuscript. Authors ECM managed the experimental process and NVSU managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/19248
Editor(s):
(1) Elena G. Zavyalova, Chemistry Department, Moscow State University, Russia.
Reviewers:
(1) Eugenio Ragazzi, Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy.
(2) Georgios Androutsopoulos, Department of Obstetrics, Gynecology, School of Medicine, University of Patras, Rio, Greece.
(3) Ogunwande Isiaka Ajani, Department of Chemistry, Lagos State University, Ojo, Lagos, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/10438

Original Research Article

Received 1st June 2015
Accepted 7th July 2015
Published 6th August 2015

ABSTRACT

Aims: This study investigated the effect of the aqueous methanolic extract of unripe *Carica papaya* (AMEUCP) on uterine contractility and its possible mechanism(s) of action.
Place and Duration of the Study: The study was carried out in the Department of Veterinary Surgery and Theriogenology, Michael Okpara University of Agriculture, Umudike, Nigeria and the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria between February and June 2013.
Methods: Fresh mature unripe pawpaw fruits were obtained from Nsukka and extracted by cold

**Corresponding author: Email: talk2eby11@yahoo.com;*

maceration with 80% methanol for 48 hours and then concentrated to dryness. Estrogenised uterine muscle strips were harvested from sexually mature non-gravid female rats (200-250 g). In each experiment, a strip of about 1 cm was mounted in a 50 ml organ bath containing Krebs' physiological salt solution, connected to an isometric electronic force displacement transducer and an oscillograph. The following drugs were used as receptor agonists or antagonist: Salbutamol (0.2 µM), Isoprenaline (0.1 µM), Prazosin (10 nM), Propranolol (0.2 µM), cholinergic receptor antagonist; Atropine (240 nM). The extract and the standard drugs were applied at concentrations corresponding to the final bath concentration.

Results: The extract elicited forceful contraction of the uterus in a dose dependent manner and the mechanism of this contraction has been shown to involve the stimulation of myometrial cholinergic and alpha adrenoceptors, mobilization of calcium ion from the extracellular fluid and partly by release of calcium ions from the sarcoplasm.

Conclusion: These findings justify the traditional use of the plant for its uterotonic properties while deciphering its possible mechanisms of action may serve as a focal point for development of new uterotonics or oxytocics.

Keywords: Carica papaya; uterotonics; myometrium; adrenergic receptors.

1. INTRODUCTION

Abnormalities in the process of uterine muscle contractility during pregnancy and birth can have major clinical implications, including preterm labor, a leading cause of maternal and prenatal mortality [1]. In contrast, induction of labour may be necessary in certain conditions. Uterotonics or oxytocics are substances usually employed in the modulation or regulation of uterine contractility. Clinically they may be used to induce or augment labour, ripen the cervix, facilitate uterine contractions following a spontaneous abortion and prevent postpartum haemorrhage [2,3]. They are also useful in management of retained foetal membranes, contraction stress tests, treatment of cases of mis-mating (in bitches) and in cases where abortion is imperative [4]. Nature's diversity has always been, and still is one of the biggest resources of therapeutic lead compounds and a great number of medicinal plants have been shown to possess oxytocic potentials [5-8]. Currently used interventional therapies to suppress or to induce uterine contractions lack potency and/or selectivity and may possess some undesirable effects therefore necessitating the search for new oxytocis with minimal side effects.

Carica papaya, a tree of the Caricaecia family is one of the indigenous plants widely cultivated throughout the world for food and medicinal purposes. Unripe (green) *papaya* has been attributed with lots of medicinal values [9-11]. In Nigeria, it is used for management of sickle cell anaemia [12] and have been successfully incorporated into livestock feed as dietary supplement [13] while In Indian traditional medicine, green papaya is used as an emmenagogue, contraceptive and as an abortifacient [9]. The aims of the present study were therefore to assess the effects of the aqueous methanolic extact of unripe *Carica papaya* (AMEUCP) on the isolated rat uterine tissue and to propose its possible mechanism (s) of action relevant to its popular use as an abortifacient and as a contraceptive.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh sample of mature unripe pawpaw fruits were obtained from Nsukka urban region in February 2013 and authenticated by Mr A. Ozioko, a taxonomist at the International Centre for Ethno medicine and Drug Development (INTERCEDD), Nsukka.

2.2 Preparation of Extracts and Determination of Plant Yield

The fruits were sliced into thin sheets, sun-dried and pulverized into a fine powder using a dry grinder. The powder was stored in an air tight container at 23-25ºC until required. 500 g of the dried sample were subjected to cold maceration with aqueous methanol for 48 hours, stirring at intervals. The extract was concentrated to dryness in a hot air oven at a reduced temperature of 40ºC to give a yield of 15.6% (w/w). The extract was then stored at 4ºC throughout the period of the study, until used as a reconstituted aqueous-methanol solution.

2.3 Animals

Adult female Wistar albino rats (250-255 g) were obtained from the Animal House Unit of the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in rat cages in well a ventilated house, temperature of 27–30ºC, 12 h natural light and 12 hour darkness. The animals were provided commercial feed (Grand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria) and clean water *ad libitum*. Protocol for this experiment was in accordance with the guidelines on the care and well being of research animals [14]. They were also allowed to acclimatize for two weeks before the commencement of the experiment.

2.4 Physiological Salt Solution (PSS)

Fresh Krebs solution was used, having the following composition in grams per litre: NaCl 6.9 g; KCl 0.35 g; $CaCl_2$ 0.28 g; $MgSO_4.7H_2O$ 0.29 g; KH_2PO_4 0.16 g; $NaHCO_3$ 2.1 g; Glucose 2.0 g; and distilled water up to 1 litre.

2.5 Nominally Ca^{2+}- free Physiological Salt Solution

Nominally Ca^{2+}- free physiological salt solution was prepared by deliberate exclusion of calcium chloride ($CaCl_2$) from the normal composition of Krebs physiological salt solution; that is, NaCl 6.9 g; KCl 0.35 g; $MgSO_4.7H_2O$ 0.29 g; KH_2PO_4 0.16 g; $NaHCO_3$ 2.1 g; D glucose 2.0 g/L)

2.6 Tissue Preparation

The female Wistar albino rats were injected with 0.2 mg/kg stilboestrol intraperitonially 24 hours prior to the start of the experiments in order to induce oestrus in the animals. The rats were sacrificed by stunning and cervical dislocation. The uterus was isolated, freed of fats and extraneous tissues and immediately placed in petri dishes containing freshly prepared Krebs physiological salt solution (PSS). Approximately 1cm of the uterine strips were threaded and mounted up in a 50 ml organ bath containing freshly prepared Krebs PSS maintained at a temperature of 37ºC and aerated with 95% O_2 and 5% CO_2. One end of the thread was attached to a tissue holder and the other end to a smooth muscle transducer connected to a force transducer (Ugo Basile, Italy) connected to an oscilliograph (2-channel polygraph recorder, "Gemini" 7070),. The measurement set up was 2 mm/min. The preparation was then allowed to stabilize for 30 min for the development of stable rhythmic spontaneous contractions. The extracts or standard drugs at different concentrations were applied, allowed to act for 3-5 minutes and the response observed and recorded. The tissue was washed with fresh PSS before commencement of subsequent experiment.

2.7 Determination of Dose - Response Relationship

Graded concentrations (0.04–0.29 mg/ml) of AMEUCP were applied to the tissue and the responses observed and recorded after which the effective concentration (EC_{50}) was determined.

2.8 Determination of the Role of Extracellular Calcium in AMEUCP-Induced Uterine Force

The role of extracellular calcium in AMEUCP - Induced Uterine force was determined firstly by applying graded concentrations of verapamil (0.02–0.2 µM), a voltage-dependent calcium-channel blocker, to the tissue in normal physiological salt solution. The tissue responses in the presence of a fixed concentration of the extract (0.18 mg/ml) were observed and recorded.

Secondly, involvement of extracellular calcium in the contractile response expressed by AMEUCP was further investigated by applying graded concentrations (0.04-0.29 mg/ml) of the extract to the tissue in a nominally calcium free physiological salt solution. The tissue responses were equally observed, recorded and then neatly traced out.

2.9 Investigating the Possible Mechanism of Action of AMEUCP-induced Uterine Force (Cholinergic Pathways and Adrenergic Pathways)

Fixed concentrations of some standard drugs, i.e. alpha and beta adrenoceptor agonists [Salbutamol (0.2 µM), Isoprenaline (0.1 µM)] and antagonists [Prazosin (10.0 nM), Propranolol (0.2 µM)], or cholinergic receptor antagonist [Atropine (240 nM)] were applied to the isolated uterine tissue in a normal PSS. Each of the drugs was allowed to act for 3-5 minutes and the response observed and recorded.

3. RESULTS

3.1 Dose-response Relationship and EC_{50}

The extract (0.04-0.29 mg/ml) elicited a forceful contraction of rat uterine strips which increased in both frequency and amplitude in a concentration dependent manner (Fig. 1). The effective concentration (EC_{50}) of the extract was 0.06mg/ml with 0.04 mg/ml as the lowest active concentration (Fig. 2).

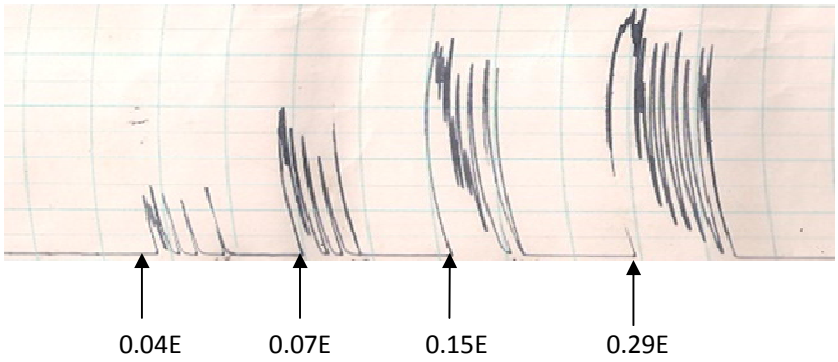

Fig. 1. Concentration response curve of AMEUCP

Key: *0.04E, 0.07E, 0.15E and 0.29E = 0.04, 0.07, 0.15, and 0.29 mg/ml of AMEUCP respectively. Direction of flow of contractile response is to the right (⟶)*

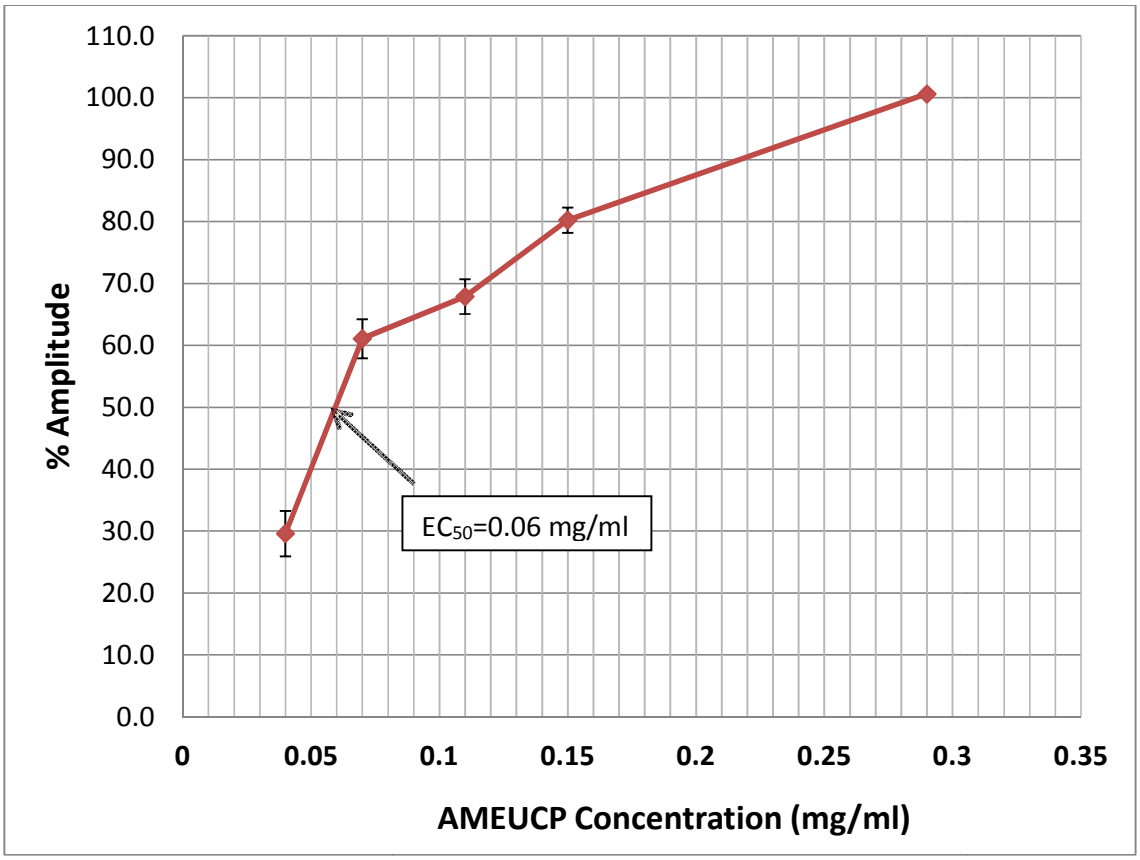

Fig. 2. Concentration-response curve of AMEUCP in uterine *in vitro* preparation showing EC_{50}

3.2 Role of Extracellular Calcium in AMEUCP-induced Uterine Force

Introduction of a voltage-dependent calcium channel blocker verapamil HCl (0.02-0.2 µM) in normal physiological salt solution greatly attenuated the AMEUCP (0.18 mg/ml) induced uterine contraction in a dose dependent manner. The contractile response of the uterine tissue to the extract (0.18 mg/ml), decreased both in the amplitude and frequency as the concentration of verapamil increased (Fig. 2) whereas, deliberate removal of Ca^{2+} from the incubation medium by incubating in a nominally Ca^{2+} free physiological salt solution resulted in a marked reduction both in frequency and amplitude of the myometrial tissue responses to the extract (Fig. 3). Application of AMEUCP initially produced a weak contractile spike after which it did not initiate any further contractile response (Fig. 3).

3.3 Involvement of Cholinergic Receptors in AMEUCP-modulated Contractility

Pre-treatment of uterine strips with a fixed concentration of atropine (240 nM); a non-specific muscarinic (cholinergic) receptor antagonist, attenuated the AMEUCP induced myometrial response. The extract induced uterine force was completely inhibited at reduced concentration of the extract (0.11 mg/ml). The response was however reinstated at the application of higher concentrations of the extract (0.15 mg/ml, 0.29 mg/ml) following pre- treatment with atropine (Fig. 4).

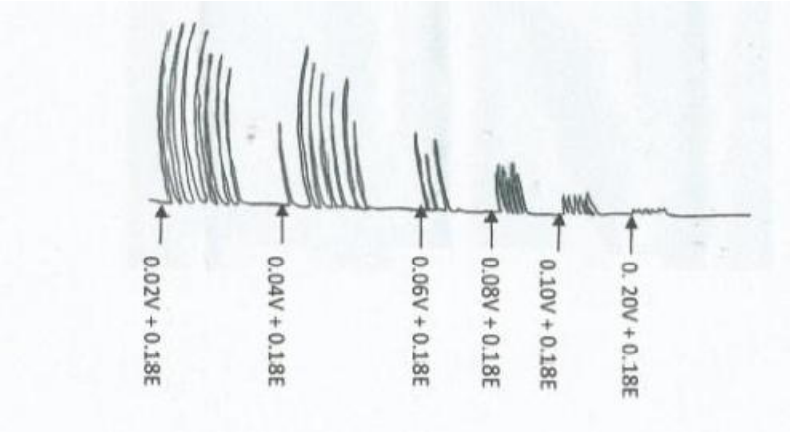

Fig. 3. Responses of AMEUCP in the presence of verapamil (a calcium-channel blocker)
Key: *0.02V, 0.04V, 0.06V, 0.08V, 0.1V 0.2V = 0.02 µM, 0.04 µM, 0.06 µM, 0.08 µM, 0.1 µM, 0.2µM of verapamil respectively; 0.18E = 0.18 mg/ml of AMEUCP.*
Direction of flow of contractile response is to the right (⟶)

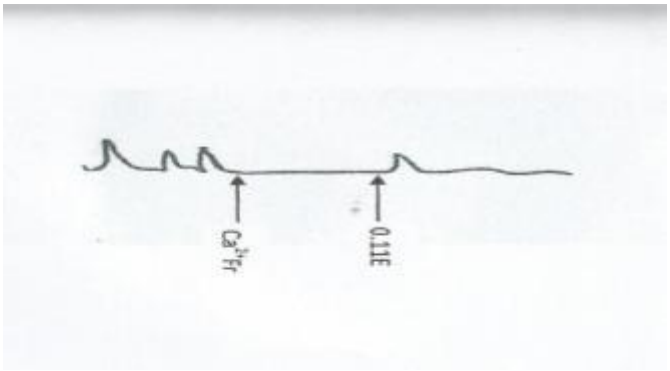

Fig. 4. Responses of AMEUCP in calcium-free medium
Key: *Ca $^{2+}$ Fr represents the point at which the nominally calcium free PSS was introduced.*
0.11E represent the concentration (mg/ml) of the extract that elicited the response (spike) in the calcium free PSS.
Direction of flow of contractile response is to the right (⟶)

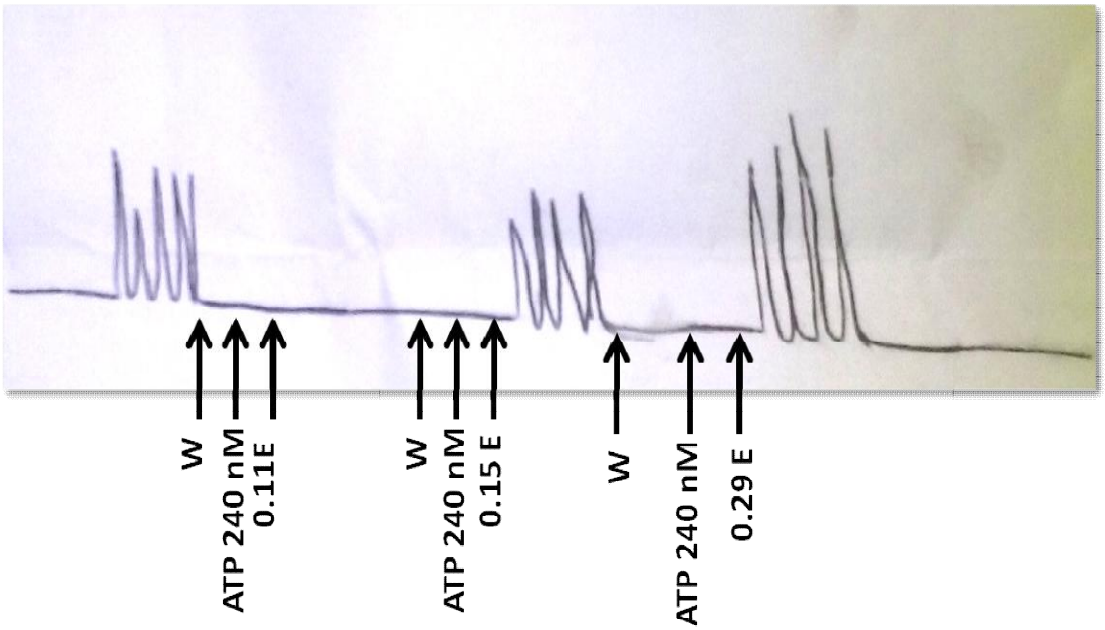

Fig. 5. Effects of cholinergic receptors in AMEUCP-modulated contractility
Key: *W = point of washing with fresh Krebs solution*
Atp 240nM = 240nM of Atropine sulphate,
0.11 E, 0.15 E and 0.29E = 0.11, 0.15 and 0.29 mg/ml of AMEUCP respectively.
Direction of flow of contractile response is to the right (⟶)

3.4 Involvement of Adrenoceptors in AMEUCP-modulated Contractility

The result of investigations made to ascertain whether the AMEUCP - induced development of uterine tone was mediated via the adrenergic receptors pathways showed that the application of a selective (Salbutamol 0.2 µM) and a non-selective (Isoprenaline 0.1 µM) β-adrenergic stimulant completely abolished (100% inhibition) the response of the uterine tissue to the extract (0.18 mg/ml) (Figs. 6a and 6b). However, Prazosin a specific alpha 1 receptor antagonist attenuated but did not completely abolish the extract induced contraction (Fig. 6c) while concomitant application of the extract and propranolol (0.2 µmol) a β-adrenoceptor antagonist, elicited a cumulative effect on the uterine tissue as compared with the response of the tissue to the extract alone (Fig. 6d) .

4. DISCUSSION

The result of this investigations showed that the aqueous methanolic extract of unripe *Carica papaya* (0.04 -0.29 mg/ml) elicited a forceful contraction of isolated rat uterine muscle strips which increased in both frequency and amplitude in a concentration dependent manner. This is in agreement with the findings of Adebiyi [9] and Saha [15] who independently reported that crude *papaya* latex from unripe fruits induced spasmodic myometrial contractions similar to oxytocin and prostaglandin $F_{2\alpha}$ in rats and guinea pig respectively. The consistent and sustained contractile activity observed in this study could be due to presence of uterotonic principle(s) which may be a combination of enzymes (papain and chymopapain), alkaloids, saponins and other phytoconstituents in the crude extract [16]. The presence of these various constituents in AMEUCP with different potency/intrinsic activity on different sites of action may have been responsible for the biphasic shape of the concentration – response curve of the extract (Fig. 2). Data from the literature indicate that some of these compound especially saponins and alkaloids possess uterine stimulating effects [17-19].

Involvement of calcium in most smooth (including uterine) muscle contraction could be by influx of calcium ions or mobilization of extracellular calcium ions through depolarization of the muscle cell membrane and opening of the calcium channels, with resultant rapid entry of

extracellular calcium into the intracellular compartment [20,21]. Calcium can also be mobilized from intracellular storage sites, such as the sarcoplasmic reticulum in the absence of extracellular Ca^{2+} so as to elicit myometrial contraction.

In this study, introduction of calcium channel blocker verapamil (0.02-0.2 µM) greatly attenuated AMEUCP (0.18 mg/ml) induced uterine contraction in a dose dependent manner. The response of the tissue markedly decreased as the concentration of verapamil increased confirming that mobilization of extracellular calcium is necessary for the extract induced uterine force. The extract however, produced only a little spike (AMEUCP 0.11 mg/ml) after which it did not initiate any further contractile response in a nominally calcium-free medium. This shows that the extract either has limited ability to mobilize calcium ions from the sarcoplasm or that the calcium ion store in the sarcoplasm is easily depleted, hence the little spike. It may therefore be said that influx of extracellular calcium, is one of the major mechanisms by which the extract induces uterine contraction while mobilization of calcium ions from the sarcoplasmic reticulum (intracellular influx) had minimal contribution to the development of uterine force.

Many studies have indicated the existence of abundant cholinergic receptors in the uterine smooth muscle and that stimulation of these receptors by agonists such as acetylcholine causes contraction of the uterus [22-24]. Abolishment of the AMEUCP (0.11 mg/ml) induced myometrial response following pre-treatment of uterine strips with atropine (240 nM) a non-specific muscarinic receptor antagonist and subsequent partial reinstatement of the contractile response as the concentration of the extract increased (0.15 mg/ml, 0.29 mg/ml) implies that stimulation of myometrial cholinergic receptors is among the possible mechanisms of action of the extract. It may however be deduced that other pathways other than stimulation of myometrial cholinergic receptors are equally involved in the mechanism of the AMEUCP induced uterine force. The involvement of muscarinic, oxytocic and H_1 receptors was also found to mediate uterine muscle contractility in response to some other plant extracts [25,26,6,7].

Alpha and beta adrenergic receptors (adrenoceptors) are appreciably present in the rat uterus and other smooth muscles. Stimulation of the α-adrenoceptors initiates, while blockage abolishes smooth muscle contractions [27]. Stimulation of α-1 receptors enhances cAMP phosphodiesterases activity, while stimulation of α-2 receptors inhibits adenylate cyclase activity, thereby decreasing the cytosolic cAMP, a condition necessary for contraction to take place [28]. Prazosin a specific alpha 1 receptor antagonist attenuated but did not completely abolish the extract induced contraction.Therefore, the extract might have elicited the observed myometrial contractility partly by stimulation of the α-1 adrenergic receptors, possibly by enhancing cAMP phosphodiesterases activity. In another study, Mbegbu [29] reported the involvement of alpha 1 receptors in ethanolic fruit extract of *Picralima nitida (Stapf)* induced uterine force while Uchendu [30] however reported mainly the involvement of alpha 2 receptors as a major mechanism of inducing smooth muscle contraction by a glycoside from the root of *Dalbegia saxatalis.*

Generally, stimulation of β-adrenergic receptors produces a relaxation effect on smooth muscles. This is mainly achieved by elevating the level of cytosolic cyclic adenosine monophosphate (cAMP) with a consequent extrusion of calcium ions hence the relaxation [31]. The administration of a selective and a non-selective β-adrenergic stimulant Salbutamol (0.1 and 0.2 µM) and isoprenaline (0.1 µ M) respectively completely abolished (100% inhibition) the normal myometrial response in a normal physiological salt solution. The administration of AMEUCP (0.18 mg/ml) could not reinstate (it was still 100% abolished) this inhibited response. This suggests that the extract may not have exerted an inhibitory effect on these agonists, either by competition for receptor sites or through reduction of the elevated levels of cytosolic cyclic adenosine monophosphate (cAMP) which they initiated [31]. Concomitant application of the extract and propranolol (0.2 µmol) a β-adrenoceptor antagonist elicited a cumulative effect on the uterine tissue as compared with the response of the tissue to the extract alone suggesting that the AMEUCP may possess a beta antagonist effect. This however requires further investigation.

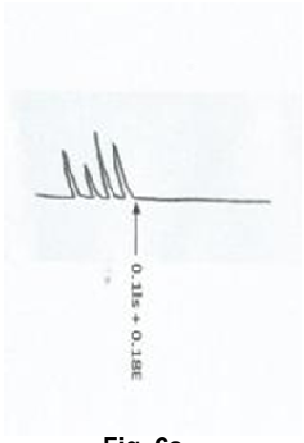

Fig. 6a

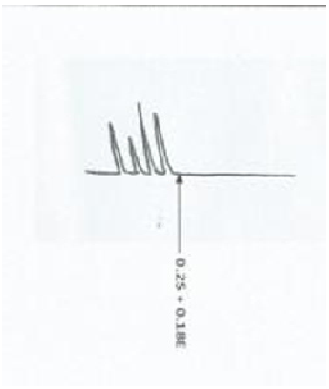

Fig. 6b

Fig. 6c

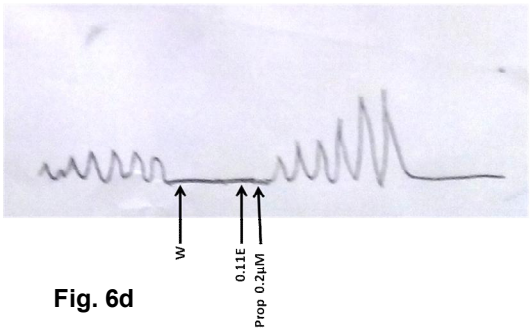

Fig. 6d

Figs. 6a, 6b, 6c and 6d. Effects of adrenoceptor stimulants and blockers

Key: *10.0Pz = 10 nM of prazosin; 0.18E = 0.18 mg/ml of AMEUCP.*
0.2S + 0.18E = 0.2 μM of salbutamol plus 0.18 mg/ml of AMEUCP;
0.1Is + 0.18E = 0.1 μM of isoprenaline plus 0.18 mg/ml of AMEUCP;
Prop 0.2 μM = 0.2 μM op Propranolol
W = point of washing with fresh Krebs solution
Direction of flow of contractile response is to the right (⟶)

5. CONCLUSION

The extract has been demonstrated to elicit forceful contraction of the uterus in a dose dependent manner and the mechanism of this contraction has been shown to involve the stimulation of cholinergic and alpha adrenoceptors, mobilization of calcium ion from the extracellular fluid and partly by release of calcium ions from the sarcoplasm. These findings lend some credence to the folkloric claim that unripe papaya possesses antifertility properties while deciphering the possible mechanism of action of this extract may serve as a focal point for development of new uterotonics or oxytocics with minimal side effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

ACKNOWLEDGMENTS

The authors are grateful to Dr A. K. Raheem for his assistance in editing the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kamatenesi-Mugisha M, Oryem-Origa H. Medicinal plants used to induce labour during childbirth in western Uganda. J Ethnopharmacol. 2007;109(1):1-9.
2. Pamplona-Roger GD. Encyclopedia of Medicinal Plants. 2000;206-18.
3. Gruber CW, O'Brien M. Uterotonic plants and their bioactive constituents. Planta medica. 2011;77(3):207-20.
4. Den Hertog CEC, De Groot ANJA, Van Dongen PWJ. History and use of oxytocics. Eur J Obstet Gynecol Reprod Biol. 2001;94:8-12.
5. Uguru MO, Okwuasaba FK, Ekwenchi EE, Uguru VE. Uterotonic properties of the methanol extract of *Monechma ciliatum.* Ethnopharmacol. 1998;62(3):203-8.
6. Veale DJ, Havlik I, Oliver DW, Dekker TG. Pharmacological effects of *Agapanthus africanus* on the isolated rat uterus. J. Ethnopharmacol. 1999;66(3):257-62.
7. Vongtau HO, Amos S, Binda L, Kapu SD, Gamaniel KS, Kunle OF, et al. Pharmacological effects of the aqueous extract of *Neorautanenia mitis* in rodents. J. Ethnopharmacol. 2000;72(1-2):207-14.
8. Omodamiro OD, Ohaeri OC, Nweke IN, J. Oxytocic. Effect of aquous, ethanolic, n-hexane and chloroform extracts of *Xylopiaaethiopica (*Anonaceae) and *Ocimum gratissium* (Labiate) on guinea pig uterus. Asian Sci Res. 2012;2(1):73-8.
9. Adebiyi A, Adaikan PG, Prasad RN. Tocolytic and toxic activity of papaya seed extract on isolated rat uterus. Life Sci. 2003;74:581-592.
10. Eno AE, Owo OI, Itam EH, Konya RS. Blood pressure depression by the fruit juice of Carica papaya (L) and DOCA-induced hypertension in rat. Phytother Res. 2000;14:235-239.
11. Ezike AC, Aka PA, Okolie CO, Ezeuchenne NA, Ezeugwu S. *Carica papaya* unripe fruits may be beneficial in ulcer. J. Med Food. 2009;12(6):268-1273.
12. Oduola T, Adeniyi FAA. Ogunyemi EO, Bello IS and Idowu TO. Antisickling agent in an extract of unripe pawpaw (*Carica papaya)*: Is it real? Afr J. Biotech. 2006; 5(20):1947-1949.
13. Bitto II, Arubi JA, Gumel AA. Reproductive tract morphometry and some haematological characteristics of female rabbits fed pawpaw peel meal based diets. Afr J. Biomed Res. 2006;9:199-204.
14. National Institute of Health (NIH). Guide for the care and use of laboratory animals, DHEW Publication. Office of Science and Health Reports, Bethesda, USA; 1985.
15. Saha JC, Savini EC, Kasinathan S. Ecbolic properties of Indian medicinal plants. Part I. Ind J. Med Res. 1961;49:130.
16. Amar NS, Zahari SS, Taib IA, Rahman MT. Effects of green and ripe papaya epicarp on wound healing and during pregnancy. Food Che Toxicol. 2008; (46):2384-89.
17. Govind K, Ravi MO, Vijai L, Artikay P, Man MS. Contraceptive and hormonal properties of the stem bark of *Dysoxylum binectariferum* in rat and docking analysis of rohitukine, the alkaloid isolated from active chloroform soluble fraction. Contraception. 2007;(76):400–407.
18. Guo L, Su J, Deng BW, Yu ZY, Kang LP, Zhao ZH. Active pharmaceutical ingre-dients and mechanisms underlying phasic myometrial contractions stimulated with the saponin extract from *Paris polyphylla Sm. var. yunnanensis* used for abnormal uterine bleeding. Human Reproductionl. 2008;23:964–971.
19. Yakubu MT, Bukoye BB. Abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. Contraception. 2009; 80(3):308–313.
20. Morgan K. The role of calcium in the control of vascular tone as assessed by the Ca^{++} indicator Aequorin. Cardiovasc Drugs Ther. 1990;4:1355-1362.
21. Uehata M, Ishizuki T, Satoh H, Ono T, Kawahara T, Morishita T, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature. 1997;(389):990–994.
22. Doods HN, Willim KD, Boddeke HW, Entzeroth M. Characterization of muscarinic receptors in guinea-pig uterus. Eur J. Pharmacol.1993;250(2):223-30.
23. Salah AM, Gathumb J, Vierling W, Wagne H. Estrogenic and cholinergic properties of the methanol extract of *Ruellia praetermissa* Sceinf. Ex. Lindau (Acanthaceae) in female rats. Phytomed, 2002;9:52–55.

24. Kurtel H, Yegen BC, Dedeoglu A, Ulusoy NB, Oktay S. Muscarinic receptor subtypes of guinea-pig gallbladder smooth muscle. Arch Int Pharmacodyn Ther. 1990;308:39-46.
25. Shi M, Chang L, He G. Stimulating action of *Carthamus tinctorius* L, *Angelica sinensis* (Oliv.) Diels and *Leonurus sibiricus* L. on the uterus. Zhongguo Zhong Yao Za Zhi.1995;20(3):173-192.
26. Veale DJH, Oliver DW, Havlik I. The effects of herbal oxytocics on the isolated "stripped" myometrium model. Life Sci. 2000;67(11):1381–1388.
27. Riemer RK, Goldfien A, Roberts JM, Rabbit rnyometrial adrenergic sensitivity is increased by estrogen but is independent of changes in alpha adrenoreceptor concentration. J Pharmacol Exp Ther. 1987;(240):44-50.
28. Yvonne YW, Alan G, James MR. Alpha adrenergic stimulation reduces cyclic adenosine 3'5'-monophosphate generation in rabbit myometrium by two mechanisms. Biol Reprod. 1988;(39):58-65.
29. Mbegbu EC, Ochiogu IS, Odirichukwu EO, Onyia CE, Nweze EC, Agbonu OA, Uchendu CN. *In vitro* uterotonic effects of ethanolic fruit extract of *Picralima nitida* (*Stapf*) on isolated uterine smooth muscles of rats. IOSR JAVS. 2014;7(3): 37-43.
30. Uchendu CN and Leek BF. Adrenergic influence of uterine muscle contractions stimulated by a glycoside from the root of *Dalbegia saxatalis*. Ind J. Expt Biol. 1999; 37:350-354.
31. Meldolesi J, Clementi E, Fasolato C, Zacchetti D. Pozzan T. Ca2+ influx following receptor activation. Trends Pharm. Sci. 1991;12:289-292.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/10438