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Preliminary investigation of contractile activity of *Ricinus communis* and *Euclea divinorum* extracts on isolated rabbit uterine strips

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ABSTRACT

Ricinus communis and *Euclea divinorum* of the family Euphorbiaceae and Ebenaceae, respectively, are traditionally used by Traditional Birth Attendants (TBAs) in Machakos district of Kenya to induce or augment labor, manage protracted labor, post-partum hemorrhage and retained after birth. Ethnopharmacological relevance of the study will be the provision of scientific evidence and justification for the ethnic use of both plants as oxytocic agents in the initiation of labor, treatment of prolonged labor, post-partum hemorrhage and retained placenta.

Materials and methods: The plants were harvested in the wild, identified and voucher specimens preserved. The root bark was processed to powder form, from which aqueous and ethanol extracts were obtained. Each of the extracts was separately tested on isolated uterine muscle tissue from non-pregnant and pregnant rabbits. The effect on contraction frequency (number of contractions per second) in the absence or presence of oxytocin was evaluated statistically using ANOVA. P values < 0.05 were considered significant.

Results and conclusions: All uteri exhibited a strong initial contraction following exposure to the aqueous and ethanol root bark extracts of both plants. After recovery, the resumed contraction frequencies varied with the plant extract and exogenous hormone. The results show that the extracts of both plants were able to stimulate uterine tissue contractility directly and to augment the tissue's response to oxytocin. The increase in uterine contractions as a percentage relative to negative controls was particularly significant in pregnant rabbit tissues in the presence of oxytocin, where increments of up to 245% were observed. Further pharmacological studies are however required to determine the active principles, possible mechanisms of action, efficacy and safety margins of the plant extracts.

1. Introduction

The effect of herbs on uterine tissue has been studied for several years (Bafor et al., 2009; Sullivan., 1963). A survey by McFarlin et al. (1999) revealed that in United States of America, several women employed herbal medicine for the purpose of inducing labor. The herbs most frequently named were castor oil, mentioned by 93% of the respondents, blue cohosh (64%), black cohosh (45%), red raspberry leaf (64%), and evening primrose oil (60%). However there is no compelling evidence of efficacy for inducing labor in all these products (Ernst et al., 2001). In South Africa, decoctions of *Agapanthus africanus*, *Clivia miniata* and several other herbal remedies are used traditionally as oxytocic agents in order to induce or augment labor (Veale et al., 1992, 2000; Varga and Veale, 1997) and that *Agapanthus africanus* was one of the five plants used most often to treat prolonged labor. *Ficus exasperata* has also been found to stimulate an increase in uterine contractility *in vitro* (Bafor et al., 2009). This oxytocic effect has also been utilized by traditional healers in some parts of Africa (Burundi and Nigeria) to facilitate labor and as abortifacients (Baerts and Lehmann, 1991), to hasten the expulsion of the placenta in cows after calf delivery and by TBAs to hasten child birth (Ijeh and Ukweni, 2007). The leaf extracts of *Ficus exasperata* contains tannins, flavanoids, saponins and cardiac glycosides (Bafor et al., 2009). Tannins are common constituents of medicinal plant extracts and have been reported to have pharmacological actions of their own. For example tannins have been reported to affect calcium availability for the contraction of uterine smooth muscle and cardiac muscle (Calixto et al., 1986; Polya et al., 1995). Flavanoids on the other hand have been reported to inhibit uterine contractions (Revuelta et al., 1997), while cardiac glycosides have been shown to affect the uterus of various animal species.

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The mammalian uterus comprises an outer myometrium and an inner endometrium layer (Veale et al., 2000). Uterine myometrial cells are responsible for contraction of the uterus whereas endometrial cells are secretory and non-contractile. The myometrium consists of circular and longitudinal muscles which differ in structure, function and contraction patterns. During parturition the myometrium contracts rhythmically and forcefully (Kimura et al., 1999). The contractions are induced by the secretion of oxytocin from the posterior pituitary gland. Oxytocin has clinically been used to initiate labor (Theobold et al., 1948) as well as manage cases of post-parturition hemorrhage. The levels of oxytocin and oxytocin receptors in the myometrium have been found to be higher at term than at other periods (Fuchs et al., 1982) and plays a crucial role in the expulsive stage of labor and the involution of the uterus (Mitchell et al., 1998). The uterine contraction in turn stimulates increased secretion of oxytocin. It is thought that the concentration of oxytocin receptors within the myometrium increases dramatically during gestation and consequently the sensitivity of the uterus to oxytocin increases as a result of the increased receptors whose synthesis is stimulated by estrogen.

The two plants *Ricinus communis* L. (voucher number CK001) and *Euclea divinorum* Hiern (voucher number CK018) of the family Euphorbiaceae and Ebenaceae respectively are traditionally used by TBA's in Machakos district (Kaingu et al., 2011) to induce or augment labor, to manage protracted labor, post-partum hemorrhage and retained after birth. The antenatal herbs were prepared as infusions or decoctions. The objective of this study was to investigate the contractile effect of aqueous and ethanol root bark extracts of both plants on isolated rabbit uterine tissue in the presence and absence of oxytocin.

2. Materials and methods

2.1. Plant material

The plants were harvested and brought to the University of Nairobi, School of Biological Sciences, for botanical identification. Voucher specimens were preserved for future reference. The root bark was removed while the roots were still fresh, cut into small pieces and dried at room temperature for two weeks. A Cunningham grinder was used to grind the root bark as described by Gakuya (2001). The resultant powder was packed in 200 g portions and placed in a clean airtight polythene paper and stored in a cool dark area until use.

2.2. Extract preparation

2.2.1. Aqueous extract

Two litres of distilled water was added to 200 g of *Euclea divinorum* root bark powder within a volumetric flask. The mixture was stirred at room temperature until most of the powder had dissolved. This was followed by boiling for 10 min at 100 °C (based on preliminary tests). The mixture was left to cool, and then filtered using Whatman paper and the filtrate was centrifuged at 3000 rpm for 10 min. The supernatant was filtered again on sintered glass and the filtrate lyophilized for 48 h, weighed and the extract yield calculated relative to the wet starting material (2.0% w/w). To prevent moisture uptake the resultant lyophilized aqueous *Euclea divinorum* (AED) extract was stored in labeled test tubes within a desiccator. The procedure was repeated for aqueous *Ricinus communis* (ARC) root bark powder.

2.2.2. Ethanol extract

Ethanol extraction was undertaken using a soxhlet apparatus as described by Sahin and Arsla (2008). 200 g Of *Euclea divinorum* root bark powder was packed in highly permeable cellulose 'thimble' and extracted using absolute ethanol. Exhaustive extraction was carried out by allowing refluxing for 12 h. Subsequently the ethanol extract was dried down using a rotary vacuum evaporator. The extract was left to further dry at room temperature for 2–3 days. The dry yield of ethanol *Euclea divinorum* extract (EED) was weighed and yield calculated relative to the wet starting weight (2.0%) and stored in labeled test tubes within a desiccator. The procedure was repeated for ethanol extraction of *Ricinus communis* (ERC) root bark powder.

2.2.3. Working extract solutions

The lyophilized powders of aqueous extracts as well as the dried down yield of ethanol extracts of both plants were resolubilized in de jalon solution, from which the required organ bath (40 ml capacity) concentrations of 4.0 mg/ml, 2.0 mg/ml, 1.0 mg/ml and 0.5 mg/ml were obtained.

2.3. Chemicals and equipment

Oxytocin (Batch 08D255) hormone was purchased from Agrar and Interchemie laboratories (Holland). Absolute ethanol and chloroform were purchased from British Drug House Company (England). Stilboestral cypionate (Batch 180903) was bought from Kyron Laboratories Limited (South Africa). Dimethyl sulphoxide (DMSO) came from Arkema Inc. (USA). Glucose (811214) from Pekings Chemicals (China), while sodium chloride (G211907), potassium chloride (G004206), calcium chloride (G041606), sucrose (G262207), sodium dihydrogen phosphate (G205909) and calcium hydrogen carbonate (G140706) were all bought from Lobachemie laboratory (Holland). A kymograph stimulator and organ bath came from Palmer Bioscience laboratories (USA) while the kymograph paper batch number 811-11288-0 was bought from Wenesco, Inc. (Chicago, Illinois).

2.4. Animals and welfare

Study animals were mature, female Swiss white rabbits weighing 1.5–2.0 kg, obtained from local breeders. The animals were kept in appropriate cages in the animal house, with room temperature maintained at 22 °C, and a 12:12 h light:dark cycle. Cage beddings consisted of untreated wood shavings which were changed every other day. The animals were fed on rabbit pellets from Unga feeds Limited (Kenya) and supplemented with local vegetables while water was provided *ad libitum*. All rabbits were handled humanely in accordance with the institution's Animals Welfare and Ethics Committee guideline and allowed to acclimatize for 2 weeks before commencement of the study.

2.5. Experimental design and test solutions

Swiss white female and male rabbits were used in the study and randomly assigned to the experiments. Half of the females were randomly picked and mated to yield pregnant uterine tissue. All females (non-pregnant and pregnant) received 0.1 mg/kg intra-peritoneal stilboestral injection 24–48 h before the onset of the experiment (Thomas et al., 1995). After 48 h, primed rabbits were humanely sacrificed and uterine tissue isolated for the experiments. Each experiment was accompanied by a negative (de jalon solution) treated control. Positive (oxytocin) treated controls were also included in the study for comparison.

2.6. Isolated uterine tissue preparations

Three centimeter sections were dissected from each uterine horn. The sections were freed from fat and cut open longitudinally

to give a sheet of muscle instead of a narrow tube. Each uterine strip was mounted in an organ bath containing 40 ml de jalon solution (0.5 g glucose, 9.0 g NaCl, 0.42 g KCl, 0.24 g CaCl, 4.5 g sucrose, 0.142 g NaH_2PO_4 and 2.1 g $CaHCO_3$ reconstituted with a litre of distilled water) and aerated with a mixture of 5% carbon dioxide and 95% oxygen. The organ bath temperature was kept at 33–35 °C to maintain the viability of the tissues.

2.7. The effect of Euclea divinorum and Ricinus communis extracts on non-pregnant and pregnant uterine strips in the absence and presence of oxytocin

2.7.1. Negative control contractions

Non-pregnant and pregnant uterine strips were allowed (one at a time) to stabilize for 30 min in de jalon solution alone after which its contractions were recorded on a kymograph paper at a constant kymograph speed of 20 mm per second for 5 min followed by an interval of 30 min before a second 5 min recording was made. The procedure was repeated once more using fresh strips.

2.7.2. The effect of oxytocin on uterine strips (positive control)

Fresh non-pregnant and pregnant uterine tissues were each mounted and after 5 min of negative control contractions they were exposed to 1.0 ml oxytocin (10 I.U.). The isometric contractions were recorded for 5 min, after which the strips were rinsed three times in de jalon solution, left to recover for 30 min, and the procedure repeated twice more for each tissue.

2.7.3. The effect of extracts in the absence of oxytocin

Non-pregnant uterine tissue was mounted and immediately after the 5 min of negative control contractions, the tissue was exposed to 0.5 mg/ml AED extract. The isometric contractions were recorded for 5 min, after which the strip was washed three times using de jalon solution and left to recover for 30 min or until contractions normalized. The strip was then exposed to 1.0 mg/ml AED and its contractions recorded before being rinsed three times and again allowed to recover. The process was repeated until the uterine strip had been exposed to all extract doses (0.5, 1.0, 2.0 and 4.0 mg/ml). The tests were done in triplicates using fresh strips for each dose range and the tissues rinsed well before each drug challenge. The above procedure was repeated using 'pregnant uterine strips' followed by ethanol extract (EED) on non-pregnant and pregnant strips as well as aqueous and ethanol *Ricinus communis* (ARC, ERC) extract on non-pregnant and pregnant strips.

2.7.4. The effect of extracts in the presence of oxytocin

A fresh non-pregnant uterine strip was mounted, and after 5 min of negative control contractions, it was exposed to 1.0 ml oxytocin (OXT) as well as AED extract at 0.5 mg/ml. The uterine response was recorded for 5 min and then washed three times with fresh de jalon solution and allowed to recover for 30 min. While OXT content in the organ bath remained unaltered, the uterine strip was exposed to gradually increasing organ bath concentrations of AED namely 1.0, 2.0 and 4.0 mg/ml and responses recorded. The tests were done in triplicates at each extract dose and the tissues rinsed well before each drug challenge. The above procedure was repeated using 'pregnant uterine strips' followed by ethanol extract (EED) on non-pregnant and pregnant strips as well as aqueous and ethanol *Ricinus communis* (ARC, ERC) extracts on non-pregnant and pregnant strips.

2.8. Data analysis

The frequency of uterine tissue contractions was taken as the number of contractions per second recorded over the 5 min period. All values are expressed as mean of triplicate measurements ± standard error of mean (SEM). The percent increase in contraction frequency relative to the negative controls was then calculated. The data was analyzed using one-way ANOVA and two sided Dunnett post-hoc test where significance was noted using SPSS version 11.5. *P*-values < 0.05 were considered significant. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

3. Results

Fig. 1 shows the contractile response of the uterine tissues. Upon exposure to extracts of both plants, the tissues exhibited contraction patterns that resembled (more or less) those produced by exogenous oxytocin (Fig. 1a), with closest resemblance being obtained with AED (Fig. 1b), and the least resemblance with ERC (Fig. 1e). In almost all experiments, there was a strong initial and sustained contraction after which isometric contractions resumed but at frequencies that varied with the plant extract and dose. Pregnant tissues had slightly higher negative control frequencies than non-pregnant tissues. The mean ± SEM contraction frequencies after treatment and their increase as a percentage relative to their negative controls were analyzed and compared.

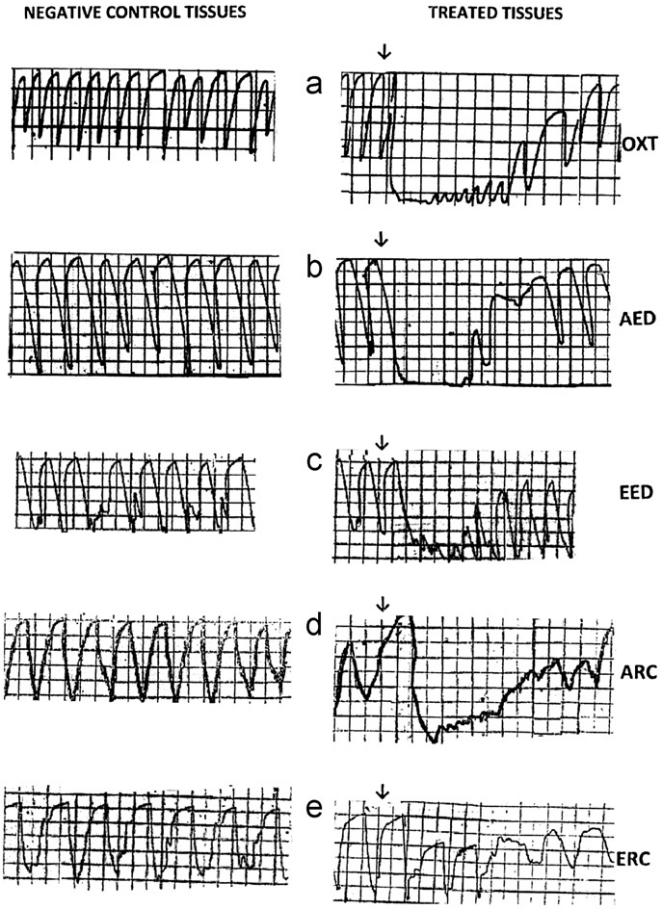

Fig. 1. Contractile response of the uterine tissues. On exposure to extracts of *Euclea divinorum* and *Ricinus communis*, (a) uterine contraction pattern exhibited by exogenous oxytocin, (b) uterine contraction pattern exhibited by AED, (c, d and e) uterine contraction patterns exhibited by EED, ARC and ERC, respectively. Contraction pattern caused by AED closely resembled that due to exogenous oxytocin while least resemblance to (a) was caused by ERC (e). *Key*: AED—aqueous *Euclea divinorum*, EED—ethanol *Euclea divinorum*, ARC—aqueous *Ricinus communis*, ERC—ethanol *Ricinus communis*. ↓ points at the extract that was infused into inner organ bath chamber containing the uterine strip.

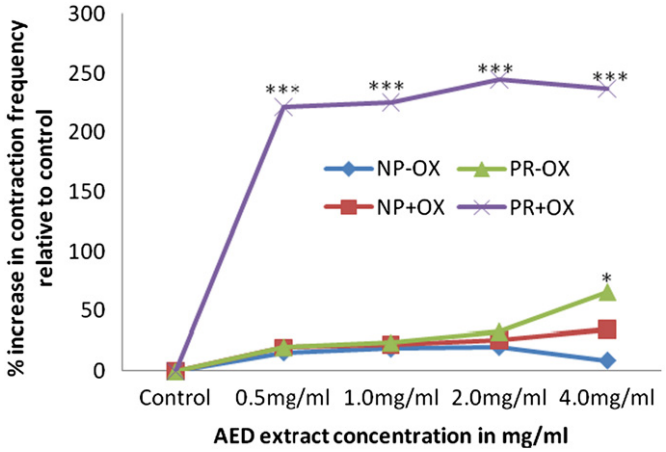

Fig. 2. Percent increase in contraction frequencies of pregnant and non-pregnant uterine strips in the presence AED extract; in the presence or absence of oxytocin. The results were not significant for non-pregnant uterine tissue in the presence or absence of OXT. With pregnant uterine strips in the absence of OXT the results were only significant at 4.0 mg/ml ($P<0.05$). However, in the presence of OXT the results were significant at all extract doses ($P<0.001$) in pregnant uterine strips. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. NP-OX non-pregnant uterine strips in the absence of OXT. NP+OX non-pregnant uterine strips in the presence of OXT. PR-OX pregnant uterine strips in the absence of OXT. PR+OX pregnant uterine strips in the presence of OXT.

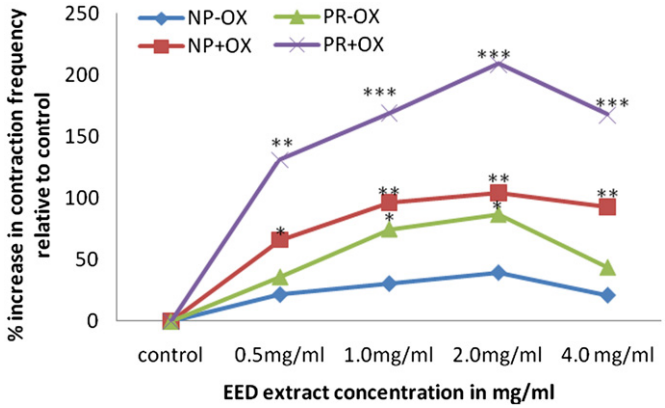

Fig. 3. Percent increase in contraction frequencies of pregnant and non-pregnant uterine strips in the presence EED extract and either presence or absence of oxytocin. The results were significant for non-pregnant uterine tissue in the presence of OXT (NP+ OX) at all extract doses (from $P<0.05$ to $P<0.01$). With pregnant uterine strips in the absence of OXT (PR-OX) the results were significant at 1.0 and 2.0 mg/ml ($P<0.05$). In the presence of OXT (PR+OX), pregnant uterine strips had the most significant results at all extract doses (from $P<0.01$ to $P<0.001$). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. NP-OX non-pregnant uterine strips in the absence of OXT. NP+OX non-pregnant uterine strips in the presence of OXT. PR-OX pregnant uterine strips in the absence of OXT. PR+OX pregnant uterine strips in the presence of OXT.

3.1. Effect of AED extract on uterine strips

The effect of AED extract on uterine tissue contraction frequencies in non-pregnant and pregnant uterine strip in the absence and presence of oxytocin is shown in Fig. 2.

The mean ± SEM contraction frequency for non-pregnant negative control tissues was 1.34 ± 0.01. With extract in the absence (NP-OXT) or presence of oxytocin (NP+OXT), the frequency of uterine contractions was not significant and ranged from 1.46 ± 0.05 to 1.81 ± 0.02, giving percent increases of between 9 and 35 respectively relative to negative controls. In pregnant uteri, the mean ± SEM contraction frequency for negative control tissues was 1.61 ± 0.01. With extracts in the absence of oxytocin (PR-OXT), the frequency of contraction increased but only significantly so ($P<0.05$) at 4.0 mg/ml extract dose and ranged from 1.94 ± 0.01 at 0.5 mg/ml extract dose to 2.67 ± 0.02 at 4.0 mg/ml extract dose giving percent increase of between 20% and 66% relative to negative controls. In the presence of oxytocin, the pregnant uterine mean frequencies increased significantly at all extract dose levels ($P<0.001$) and ranged from 5.18 ± 0.54 at 0.5 mg/ml to 5.56 ± 0.75 at 2.0 mg/ml extract doses with corresponding percent increases ranging from 222% to 245% relative to negative controls.

3.2. Effect of EED extract

Fig. 3 gives the effect of EED extract on uterine contraction frequencies in non-pregnant and pregnant uterine strip in the absence and presence of oxytocin.

The mean ± SEM contraction frequency for non-pregnant negative control tissues was 1.94 ± 0.1. In the presence of extract but absence of oxytocin, (NP-OXT) the frequencies of uterine contractions in non-pregnant uterine strips were not significantly different from negative controls and ranged from 2.36 ± 0.3 at 0.5 mg/ml to 2.7 ± 0.66 at 2.0 mg/ml extract doses, giving percent increases of between 21% and 39%. In the presence of oxytocin (NP+OXT) the frequency of non-pregnant uterine strip contractions increased significantly at all dose levels (from $P<0.05$ to $P<0.01$) and ranged from 3.22 ± 0.31 at 0.5 mg/ml to 4.13 ± 0.21 at 2.0 mg/ml extract doses, with corresponding percent increases of between 65% and 104% relative to controls. In pregnant uteri, the mean ± SEM contraction frequency for negative control tissues was 2.16 ± 0.2. In the presence of extract without oxytocin (PR-OXT), the frequencies of contractions were significantly at 1.0 and 2.0 mg/ml doses ($P<0.05$) and ranged from 2.94 ± 0.1 at 0.5 mg/ml to 4.03 ± 0.13 at 2.0 mg/ml extract doses with percent increases ranging from 36% to 86%. In the presence of oxytocin (PR+OXT) however, the frequency of contraction significantly rose (from $P<0.01$ to $P<0.001$) at all extract dose levels and ranged from 5.00 ± 0.26 at 0.5 mg/ml to 6.68 ± 0.3 at 2.0 mg/ml and percent increases of between 131% and 209% relative to controls.

3.3. Effect of ARC extract

The effect of ARC extract on uterine contraction frequencies in non-pregnant and pregnant uterine strips in the absence and presence of OXT is presented in Fig. 4.

The mean ± SEM contraction frequency for non-pregnant negative control tissues was 1.86 ± 0.34. In the presence of extract but without oxytocin, the mean ± SEM frequency of uterine contraction in non-pregnant uterine strips (NP-OXT), increased only slightly and ranged from 2.10 ± 0.41 at 0.5 mg/ml to 2.61 ± 0.53 at 2.0 mg/ml extract doses, giving percent increases of between 12% and 40% relative to controls. In the presence of extract and oxytocin (NP+OXT), the mean contraction frequency of non-pregnant uterine strip increased significantly at all dose levels (from $P<0.05$ to $P<0.01$) and ranged from 3.81 ± 0.14 to 4.47 ± 0.12 at 0.5 mg/ml and 2.0 mg/ml extract doses respectively with percent increase in contraction frequencies of between 105% and 140%. Pregnant uterine strips had a mean ± SEM negative control contraction frequency of 2.72 ± 0.12. In the presence of extract but absence of oxytocin (PR-OXT), mean contraction frequencies increased significantly (from $P<0.05$ to $P<0.01$) at 1.0, 2.0 and 4.0 mg/ml extract doses and ranged from a low value of 3.9 ± 0.51 to a high value of 5.86 ± 0.44 at 0.5 mg/ml and 2.0 mg/ml respectively, giving percent increases of between 43% and 115% relative to controls. In the presence of extract and oxytocin (PR+OXT), the pregnant uterine strips mean contraction frequency rose most significantly

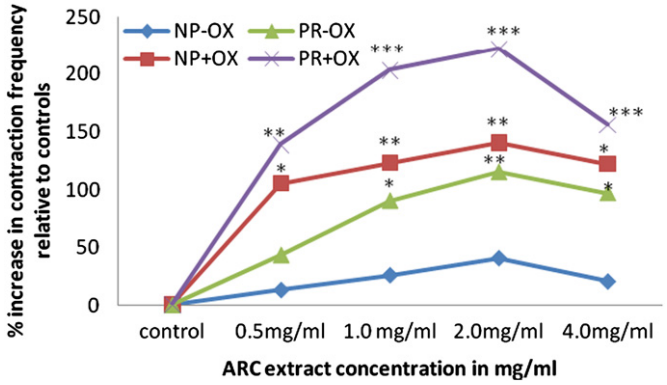

Fig. 4. Percent increase in contraction frequencies of pregnant and non-pregnant uterine strips in the presence of ARC extract and either presence or absence of oxytocin. The results were significant with non-pregnant uterine strips in the presence of oxytocin (NP+OX) at all extract doses (from $P<0.05$ to $P<0.01$). With pregnant uterine strips in the absence of OXT (PR-OX) the results were significant at 1.0, 2.0 and 4.0 mg/ml (from $P<0.05$ to $P<0.01$). The results were also significant (from $P<0.01$ to $P<0.001$) at all extract doses in pregnant uterine strips in the presence of OXT (PR+OX). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. NP-OX non-pregnant uterine strips in the absence of OXT. NP+OX non-pregnant uterine strips in the presence of OXT. PR-OX pregnant uterine strips in the absence of OXT. PR+OX pregnant uterine strips in the presence of OXT.

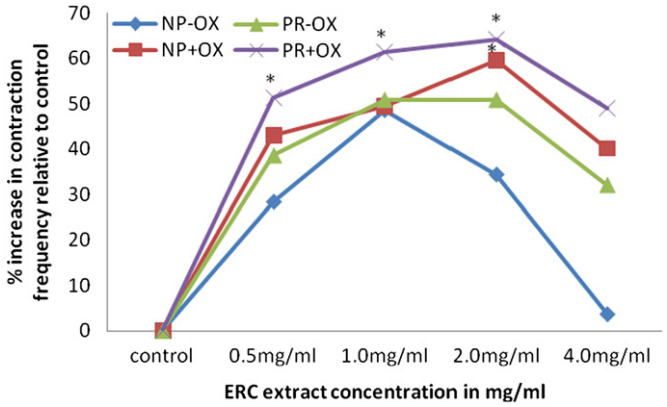

Fig. 5. Percent increase in contraction frequencies of pregnant and non-pregnant uterine strips in the presence ERC extract and either presence or absence of oxytocin. The results were significant for non-pregnant uterine strips in the presence of OXT (PR+OX) at 2.0 mg/ml ($P<0.05$) and in pregnant tissue at 0.5, 1.0 and 2.0 mg/ml ($P<0.05$) $^{*}P<0.05$ NP-OX non-pregnant uterine strips in the absence of OXT. NP+OX non-pregnant uterine strips in the presence of OXT. PR-OX pregnant uterine strips in the absence of OXT. PR+OX pregnant uterine strips in the presence of OXT.

(from $P<0.01$ to $P<0.001$) at all extract dose levels. The frequencies ranged from 6.5 ± 0.5 to 8.77 ± 0.5 at 0.5 and 2.0 mg/ml respectively, giving percent increase in contraction frequencies of between 140% and 222% relative to negative controls.

3.4. Effect of ERC extract

Fig. 5 gives the effect of ERC extract on uterine tissue contraction frequencies in non-pregnant and pregnant uterine strip in the absence and presence of oxytocin.

The mean $\pm$ SEM contraction frequencies for negative control tissues were 2.18 ± 0.18 and 2.68 ± 0.42 in non-pregnant and pregnant tissues respectively. In general, when exposed to extracts, the mean contraction frequencies ranged from a low value of 2.26 ± 0.5 in non-pregnant tissue in absence of oxytocin to a high value of 4.40 ± 0.4 in pregnant tissue in the presence of oxytocin. The percent increase in contraction frequency relative to negative control ranged from 4% to 64%. The results were significant ($P<0.05$) in non-pregnant uterine strips in the presence of oxytocin at 2.0 mg/ml extract dose and in pregnant uterine strips in the presence of oxytocin at 0.5,1.0 and 2.0 mg/ml extract doses.

4. Discussion and conclusion

4.1. Aqueous and ethanol extracts of Euclea divinorum and Ricinus communis plants

The root, bark and leaves of *Euclea divinorum* and *Ricinus communis* plants are used routinely for medicinal purposes (Kokwaro, 1993), and widely used also by TBAs in Machakos, Kenya, for pregnancy related complications (Kaingu et al., 2011). However their potency as uterotonic agents has not been documented. In the study by Kaingu et al. (2011), the herbs were reportedly administered in the latter part of gestation, during labor and the immediate post-partum period, to ease the parturition process and to manage cases of post-partum hemorrhage and retained after birth. The success of pregnancy depends on the ability of the myometrium to maintain quiescence throughout the duration of the gestation period. Premature onset of uterine contraction is often the cause of abortion. On the other hand inadequate and infrequent contractions can result in delayed, obstructed or protracted labor and retained after birth. The aim of the present study was to investigate the possible contractile effects of extracts of the two herbs on uterine tissue. The results demonstrate that both plants caused increased contraction of isolated uterine tissue, suggesting an oxytocic effect that varied with the nature of the tissues. Oxytocin is one of the most potent uterotonic agents known, and its effect on uterine contractility is of major pharmacological importance. Medicinal plants with oxytocic values that can be used to either induce labor or manage post-partum hemorrhage and retained after birth are of great importance especially in rural parts of developing countries where hospitals are not only far from rural homesteads but also have inadequate supplies of emergency medicine. Oxytocin is not only uterotonic by itself but also induces prostaglandin E_2 synthesis in uterine endometrial cells. This increase in local prostaglandin production further stimulates uterine contraction. Experiments with herbal medicines have also suggested a possible involvement of voltage operated calcium channels in *Ficus exasperata* induced uterine contractions (Bafor et al., 2010). Bafor et al. (2010) also indicated that *Ficus exasperata* may additionally stimulate uterine contractions via activation of α adreno-ceptors present in the uterus. Furthermore, the multiple components in the effect of oxytocin could be due to its action on different cell types within the myometrium. In the effect of oxytocin on human uterine smooth muscle at least three distinct components can be discerned: (1) increase in frequency of contractions; (2) initial transient increase in the base tone (incomplete relaxation); (3) long lasting increase in the amplitude and duration of phasic contractions (Shmygol et al., 2006). Shmygol et al. (2006) further identified interstitial cells of Cajal (ICC) in the myometrium which might mediate changes in contraction frequencies while smooth muscle cells are responsible for the increase in amplitude of contraction. In the present study, the effect of extracts on contraction frequencies is reported and it indicates that all extracts enhanced the frequency of contractions to a small or large extent.

Whole uterine tissue comprises an outer myometrium of longitudinal and smooth muscle cells, and inner endometrium (Veale et al., 2000). Oxytocin acts on the myometrial oxytocin receptors subtype 1a (OT_{1a}) to directly cause uterine contraction and on endometrial oxytocin receptors type 1b (OT_{1b}) to stimulate prostaglandin synthesis and release (Chan et al., 1983).

This pharmacological differentiation has been shown in both the rat uterus and pregnant human uterus (Veale et al., 2000). The two hormones (oxytocin and prostaglandin) then synergistically affect myometrial contraction. Uterine tissue is thus a complex tissue and several authors have attempted to unravel the effects of herbal extracts on it. Aqueous extract of *Agapanthus africanus* for instance was found to stimulate smooth muscle directly and to augment the initial response of the uterus to oxytocin and acetylcholine (Kaido et al., 1997; Veale et al., 1992). The results of this study indicate that the rabbit myometrium response to the extracts was stronger in the presence of oxytocin suggesting also an augmenting effect of both aqueous and ethanol extracts of *Euclea divinorum* and *Ricinus communis* although with varying degrees. Aqueous extracts of both plants as well as ethanol extract of *Euclea divinorum* were particularly effective especially on pregnant tissue and more so in the presence of oxytocin. It is also noted that for all the extracts except AED, the most effective extract dose was 2.0 mg/ml. AED on the other hand was most effective at the highest dose of 4.0 mg/ml.

4.1.1. Aqueous and ethanol extracts of Euclea divinorum plant

The results as shown in Fig. 1 indicate that aqueous extract of *Euclea divinorum* (AED) mimicked oxytocin more closely than the other extracts. AED had a dose related augmentory effect on the uterine strip's frequency of contraction where the effect was greater in pregnant compared to non-pregnant uterus and particularly so in pregnant uteri in the presence of oxytocin. These results thus support the traditional use of *Euclea divinorum* concoctions in the initiation of labor, management of protracted labor, post-partum hemorrhage and retained after birth. Physiologically during labor and the immediate post-partum period, levels of oxytocin are high within the uterus. It is possible that the crude plant extracts are able to exert maximum effect during this time as indicated by the enhanced effect on pregnant rabbit uterus in this study. Ethanol *Euclea divinorum* extract had a similar effect on uterine contraction frequency compared to aqueous *Euclea divinorum* extract. However the enhancing effect of the ethanol extract was more widespread and significant even on non-pregnant tissue be it with oxytocin.

4.1.2. Aqueous and ethanol extracts of Ricinus communis plant

Aqueous extract of *Ricinus communis* had basically similar effect to *Euclea divinorum* and also enhanced the frequency of uterine strip contraction in a dose related manner, with the highest increase in contraction frequency occurring at 2.0 mg/ml extract dose in pregnant strips in the presence of oxytocin. However, upon recovery following the strong initial contraction, the uterine tone though increased, hardly resumed the normal pattern of contraction seen prior to treatment especially at high dose. Ethanol *Ricinus communis* extract however was less effective and had no significant effect on frequency of contractions except at 0.5, 1 and 2 mg/ml doses in pregnant strips in the presence of oxytocin, where the effect was slightly significant ($P < 0.05$). Thus aqueous extract of both plants as well as ethanol extract of *Euclea divinorum* presented the most significant effect on the uterine tissue. This would suggest that the active principle is not only water soluble but also perhaps more stable in aqueous form than in polar solvents. Considering that the extracts used by TBA's are prepared in water these findings strongly support the presence/existence of an oxytocin like compound in the plants. It is tempting to argue that when consumed by pregnant women, the aqueous extract of both plants would augment endogenous oxytocin effects enough to cause or speed up parturition by inducing or augmenting labor as well as manage post-partum hemorrhage and retained after birth.

4.2. Conclusion

The study has successfully demonstrated the contractile effect of *Euclea divinorum* and *Ricinus communis* extracts on isolated rabbit uterine strips. However there is still more work to be done, for instance, this study has not isolated and studied separately the biological components of these two plants. Furthermore, the interaction of herbal components with uterine contractile elements could also be multifaceted and complex and hence require many more detailed studies. The isolated oestrogenized rat uterus preparation is a model well suited to pharmacological investigations of contractile activity in the uterine smooth muscle and is used extensively by reproductive pharmacologists. In this study oestrogenized rabbit uterine preparations were used and have so far shown that this model can also give indications as to the effect of herbal extracts. Basic initial pharmacological screening procedures such as this study, which identify direct uterine smooth muscle activity in crude plant extracts need to be followed by more detailed studies which characterize the pharmacological activity of the phytomedicine as fully as possible. For the purposes of this study for instance, whose aim was to demonstrate presence or absence of uterine contractile activity of the plant's extracts, the lowest dose was pegged at 0.5 mg/ml. Even at this dose, positive results were obtained, suggesting therefore the need for further studies on sub-threshold doses and especially their cumulative effect that would simulate the treatment regimes by TBAs. The data from this study, generated from crude extracts of *Euclea divinorum* and *Ricinus communis*, can therefore provide a basis for further investigations.

4.3. Recommendations

The results of this study are useful especially to Traditional Birth practitioners and can also provide foundational data for further research on medicinal plants used during pregnancy. The results can also be used as a guideline in planning more definitive pharmacological and biochemical tests.

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