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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