

Determining the *In vitro* Effects of Aqueous Elephant Dung and *Fadogia aencylantha* Leaf Extracts on Isolated Rat Pregnant Uterus Horns

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Abstract

Background: In Zimbabwe, elephant dung and *Fadogia aencylantha* are widely used in the third trimester of pregnancy. They are believed to facilitate childbirth because they open the birth canal during delivery. However, the effects of these medicines in pregnancy have not been investigated. This study aimed to determine the effects of elephant dung and *F. aencylantha* leaf extracts on isolated pregnant and non-pregnant uterine horns.

Materials and methods: Elephant dung and *Fadogia aencylantha* leaves were air-dried, macerated in water, filtered, evaporated and freeze dried. Uterine horns from 72 non-pregnant and 72 pregnant female Sprague-Dawley rats were used. The 'test' strips were separately exposed to elephant dung (20-640 mg/ml) or *F. aencylantha* (25-800 mg/ml) while the 'controls' received distilled water only. Effects of oxytocin on tissues exposed to these extracts were measured.

Results: Elephant dung aqueous extract contracted the uterus in a dose-dependent manner whilst *Fadogia aencylantha* relaxed uterine tissue in a dose-dependent manner. The effects of both the Elephant dung and *Fadogia aencylantha* extracts were higher in pregnant uteri versus non-pregnant uteri. Oxytocin potentiated elephant dung-induced contraction, but only slightly shifted baseline upwards in tissues exposed to *Fadogia aencylantha*.

Conclusion: Elephant dung possessed oxytoxic effects and contracted uteri in a dose-dependent manner. *Fadogia aencylantha* relaxed the uterus in a dose-dependent manner, suggesting that it might slow down labour progression since uterine contraction will not be occurring at a sufficient enough pace to facilitate labour and delivery. Pregnant women and health care workers need education to understand the possible effects of using these traditional medicines in pregnancy.

Keywords: Elephant dung; *Fadogia aencylantha*; Pregnancy; Uterus; Oxytoxic

Abbreviations: GD: Gestation Day; ANOVA: Analysis of Variance; IP3: Inositol-1,4,5-Triphosphate; FAE: *Fadogia aencylantha* Extract.

Introduction

Traditional medicines are used to treat and manage a number of health conditions in many African countries, including Zimbabwe. This has been attributed to the belief that the medicinal effects help to normalize physiological function and correct the underlying cause of the disorder [1]. Different plants and animal matter have also been used as medicine during pregnancy, birth, and for postpartum care in many areas around the world for generations [2]. Pregnant women use these medicines to prevent miscarriages, ease morning sickness, reduce swelling of the legs and ankles, and treat abdominal pains [3]. They are also used during childbirth to speed up labour contractions, prevent post-partum haemorrhage, cleanse the womb and encourage lactation [4].

In Zimbabwe, local women use a range of traditional medicines during pregnancy including *Pouzolzia mixta* (Soap nettle/*Munhanzva*), *Abelmoschus esculentus* (Okra/*Derere*), and *Dicerocaryum zanguebarium* (Devil thorn/*Ruredzo*) [5,6]. These

plants are mainly used in the third trimester to induce labour, avoid perineal tearing and ensure safe delivery [5-7]. Soil from a burrowing mole and holy water, to mention a few, are also used to precipitate or facilitate labour and protect the unborn child, respectively. Zimbabwean women also use elephant dung (*Ndoveyenzou*) and *Fadogia aencylantha* leaves (*Makoni* tea) during pregnancy, mainly to widen the birth canal and to prevent perineal tearing.

Despite the widespread use of such traditional medicines, there is insufficient scientific data to justify its use in pregnancy. The bodies of pregnant women are extremely vulnerable to the effects of drugs due to the physiological changes that occur in pregnancy [8], with some drugs being harmful to both the mother and foetus. In addition, understanding the pharmacological effects of some of these traditional medicines might eventually lead to the development of novel drugs. This study was thus conducted to determine the *in vitro* effects of two traditional medicines that are commonly used by Zimbabwean women during pregnancy: elephant dung and *Fadogia aencylantha*.

Materials and Methods

This was an experimental *in vitro* study.

Ethical consideration

This study was approved by the Joint Research and Ethics Committee of the University of Zimbabwe and Parirenyatwa group of hospitals and the Medical Research Council of Zimbabwe. The study conformed to the "Handbook of laboratory animal management and welfare" [9].

Materials

Elephant dung was identified and collected by a game park ranger from Hwange Game Park which is located 19.1241°S, 26.5926°E, in December 2017. A voucher specimen of the Elephant dung was stored in the Clinical Pharmacology Department (TM/ED-01/17). *Fadogia aencylantha* was collected from Domboshava in January 2018 (17.6118°S, 31.1432°E) after it had been identified by a botanist from the Harare Botanical gardens. A voucher specimen was stored in the Clinical Pharmacology department (TM/FD-01/18).

Preparation of the elephant dung aqueous extract and *Fadogia aencylantha* leaf aqueous extract

One kilogram of fresh Elephant dung and one kilogram of *Fadogia aencylantha* leaves were separately air-dried at room temperature (26 ± 1°C) for one week. The dried products were milled (using a mortar and pestle) into a fine powder. The powders were separately macerated in 2.5 liters of distilled water for 48 hours at room temperature (26 ± 1°C). The mixtures were filtered and the supernatants were collected. A rotary evaporator was then used to concentrate the aqueous extracts by drying them at 60 ± 1°C. Freeze-drying gave 107.4 g (10.74% yield) of a dark green, powdery, crude extract of the Elephant dung, and 92.3 g (9.23% yield) of a dark brown powdery extract of the *Fadogia aencylantha*. Aliquot portions of the two extract residues were weighed and dissolved in distilled water (at room temperature) for use on each day of the experiments.

Animals

12 healthy young male and 154 young healthy female Sprague-Dawley rats weighing between 160-200 g were purchased from the University of Zimbabwe college of Health Sciences Animal House. The animals were housed in the Department of Clinical Pharmacology under standard laboratory conditions, with free access to standard pellets for food and drinking tap water. Male animals were housed in a separate cage whilst the females were divided into two groups i.e. Elephant dung and *Fadogia aencylantha* treatment groups containing 72 females each. Of the 72 animals in a group, 36 were used in the non-pregnant state, whilst 36 rats were allowed to mate with the males to ensure pregnancy. The pregnant animals used in the experiments were between 15-18 days pregnant. Prior to the experiment, animals were fasted for 16 hours, but they had access to drinking water.

Mating

To ensure pregnancy, 12 male and 36 female animals were allowed to mate. 3 female rats were put into a cage and a single male was introduced into each cage at a ratio of 3:1. The animals were left to mate over a six-day period. Female rats were then checked for a copulation plug daily and when a plug was observed the females were separated from the males. The day the plug was observed was recorded as gestation day zero (GD0) and the female rats weights were recorded. Animals with no plug were left in the cages with the male for

mating to continue and were removed only when a plug was observed. To confirm pregnancy, the female rats were weighed every morning and their weights were recorded (pregnant rats gain more than 1.75 g by gestation day 8).

Determining the pharmacological effects of elephant dung and *Fadogia aencylantha* aqueous leaf extracts

Pregnant and non-pregnant rats were quickly excised after being euthanized *via* CO₂ inhalation just before the uteri were removed. Segments of 2 cm in length were dissected rapidly and adhering connective tissues and fat were removed. Uteri segments were mounted in 25 ml Iworx tissue organ-baths containing Tyrode's physiological solution of the following composition NaCl₂ g, KCL 10% 0.5 ml, CaCl₂ 0.5 ml, MgCl₂ 0.25 ml, NaHCO₃ 0.25 g, and NaH₂PO₄ 0.125 ml. Organ bath temperature was maintained at 37°C. The lower ends of the uterine tissues were attached using silk sutures to tissue holders and the upper ends to force transducers connected to Iworx-IWX214. Two tissues from the same animal were set up i.e. the 'test' and the 'control' segments. The uterine segments were given time to equilibrate in Tyrode's solution for 30 minutes with the bath solution being changed every 15 minutes.

After the equilibration period, graded concentrations of Elephant dung (20, 40, 80, 160, 320, 640 mg/ml) or *Fadogia aencylantha* (25, 50,100,200,400,800 mg/ml) (n=6) were added to the bath-fluid at different times. (These doses were selected based on reported doses that the women used during pregnancy). In addition, dose response effects were determined by incubating tissues with oxytocin (2 µU/ml). After exposing the tissues to the Elephant dung or *Fadogia aencylantha* for about 5 minutes, bath-applied concentrations were washed out 4-5 times, and the tissue was allowed to rest for 5-10 minutes, or until its basal tone returned to baseline point. The 'control' smooth muscle strips were treated with distilled water (0.1-2 ml only equivalent to the volume of bath applied Elephant dung or *Fadogia aencylantha*. Each of the 'test' smooth muscle preparations were used for one concentration-response curve only, with extract-induced responses on the smooth muscle strips being recorded using LabScribe[®] software.

Data analysis

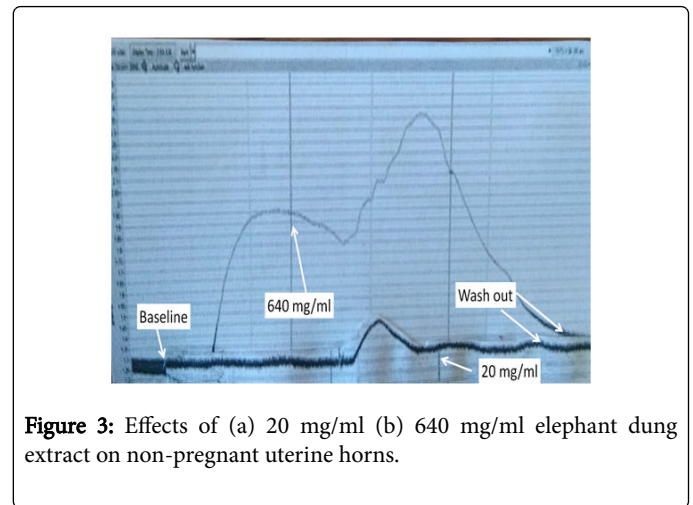
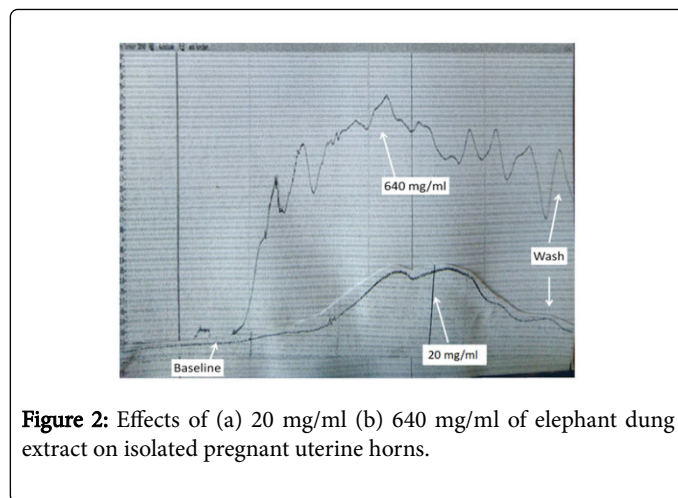
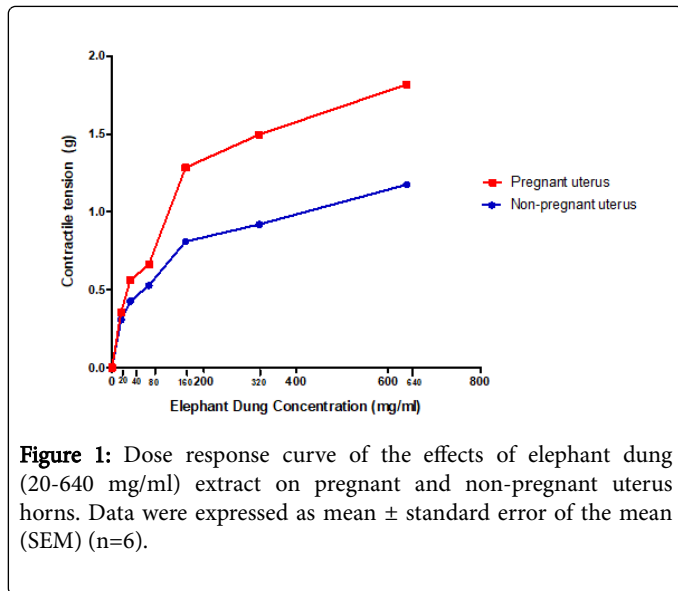
The contraction/relaxation produced by each concentration of either elephant dung extract or *Fadogia aencylantha* was measured and expressed as a percentage of contraction/relaxation of the maximum tension (100%). Experimental data were expressed as mean ± standard error of the mean. Statistical comparison was performed using Graphpad Prism[®] Version 7 using Student's t-test and one way ANOVA between 'tests' data and 'control' data. When ANOVA exhibited a statistical difference, Dunnett's post hoc tests were applied. p-values of <0.05 were considered to be statistically significant.

Results

Effect of elephant dung on the uterus

Elephant dung extract significantly contracted both pregnant and non-pregnant uteri in a dose-dependent manner (Figure 1). The effects of the extract were 0.68 g (59%) greater in the pregnant uterus as opposed to the non-pregnant one. Significant (p<0.05-0.001) dose-dependent increases in amplitude and frequency of contractions were observed after the extract was added to pregnant uteri, with an upward shift in baseline contractions (Figure 2). Although an upward shift in

baseline and an increase in contractile amplitude were also observed in the non-pregnant uterus, elephant dung extract had no effect on the frequency of contractions (Figure 3). Oxytocin significantly ($p < 0.05$) potentiated the contractile effects of elephant dung extract on pregnant uteri (Table 1).



Concentration mg/ml	Elephant dung extract alone		Elephant dung extract +Oxytocin 2 μ U/ml	
	Contractile tension (%)	N	Contractile tension (%)	N
20	32.9	6	44.9	6
40	33.9	6	63.6	6
80	39.5	6	72.2	6
160	44.3	6	81.5	6
320	48.1	6	89.9	6
640	50.1	6	92.6	6

Table 1: Effect on pregnant uterus of elephant dung extract alone and elephant dung+Oxytocin.

Oxytocin potentiated elephant dung-induced contractions in a dose-dependent manner.

Effect of *Fadogia ancylantha* extract on the uterus

Fadogia ancylantha aqueous leaf extracts (25-800 mg/ml) relaxed uterine tissues in a dose-dependent manner (Figure 4). The extract significantly ($p < 0.05-0.001$) reduced the frequency and amplitude of contractions in pregnant uterus with a downward shift in baseline (Figure 5). This effect was pronounced in the pregnant uterus. In non-pregnant uteri *Fadogia ancylantha* aqueous extracts significantly shifted baseline downwards (Figure 6). Adding oxytocin to pregnant tissues pre-incubated with *Fadogia ancylantha* slightly shifted baseline upwards, although no changes in frequency or amplitude of contraction were recorded (Table 2).

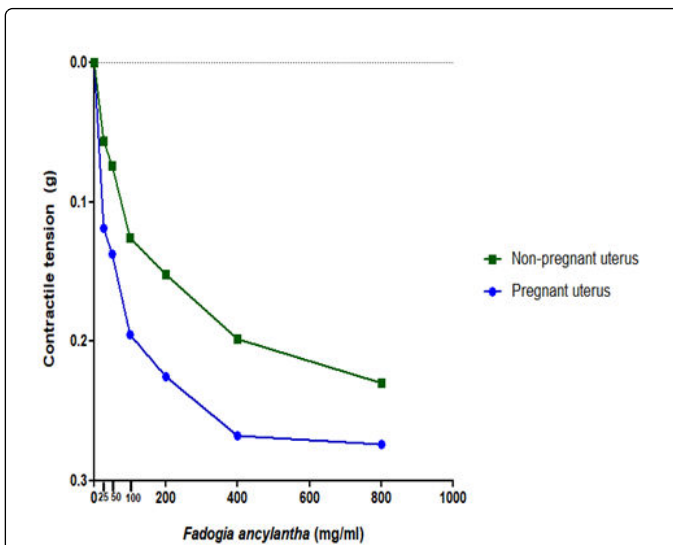


Figure 4: Dose response curve of the effects of *Fadogia aencylantha* extract on pregnant and non-pregnant uterus horns. Data were expressed as mean \pm standard error of the mean (SEM) (n=6).

Concentration mg/ml	<i>Fadogia aencylantha</i> extract alone			<i>Fadogia aencylantha</i> extract +Oxytocin		
	Mean relaxatory tension %	SEM	N	Mean relaxatory tension %	SEM	N
25	8.1	0.5	6	5.7	1.2	6
50	13.5	1.1	6	9.1	2.1	6
100	32.7	2.4	6	26.7	2.7	6
200	54.4	2.6	6	48.8	2.5	6
400	82.2	2.1	6	73.5	1.4	6
800	84.3	2.9	6	76.9	2.8	6

Table 2: Effect on pregnant uterus of *Fadogia aencylantha* alone and *Fadogia aencylantha* extract+Oxytocin.

Oxytocin slightly shifted the baseline upwards in pregnant tissues that were pre-incubated with *Fadogia aencylantha*. Data were expressed as mean \pm standard error of the mean (SEM) (n=6).

Discussion

We found that elephant dung extract (20-640 mg/ml) contracted the uterus in a dose dependent manner in both pregnant and non-pregnant uteri. The contractile effects of the extract were more pronounced in the pregnant uteri as opposed to the non-pregnant ones. *Fadogia aencylantha* aqueous leaf extract relaxed the uterus in a dose-dependent manner with the effects also being more prevalent in pregnant versus non-pregnant uteri. Oxytocin potentiated the contractile effects of elephant dung extracts whilst it reduced the relaxatory effects of *F. aencylantha*.

Elephant dung aqueous extract

The uterine changes associated with pregnancy could account for the differences observed between the pregnant and non-pregnant uterine horns after exposure to the elephant dung extract [10]. Under normal circumstances, phasic contractions of the uterus occur due to spontaneous changes in electrical activity within the myometrial cells [11]. This results in action potential generation and depolarization. Depolarization causes the opening of voltage-gated, L-type calcium channels, rapid calcium entry and an elevation in intracellular calcium. During pregnancy however, uterine muscle fibres undergo muscular hypertrophy and multiplication due to the effects of both oestrogen and progesterone. These increases in hormone levels and their receptors increase the contractility of the uterus.

In the presence of elephant dung extract, oxytocin potentiated the contractile effects of the extract in a dose dependent manner. It has been reported that during pregnancy the concentration of oxytocin in the blood is slightly elevated and the number of oxytocin receptors also increases [12]. When oxytocin or oxytocin agonists bind to the receptors, contraction of the uterus occurs *via* the release of inositol-1,4,5-triphosphate (IP₃) from the hydrolysis of phosphoinositide. IP₃ binds to and opens ligand gated ion channel that allow Ca²⁺ to move into the cytosol, where it activates various cellular processes such as: mobilization of Ca²⁺ from storage organelles, release of prostaglandins, and regulation of cell proliferation. These processes all increase contractile tension as they also increase the sensitivity of

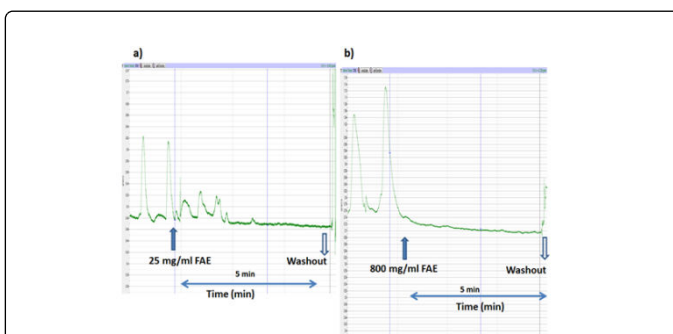


Figure 5: Effects of (a) 25 mg/ml (b) 800 mg/ml *Fadogia aencylantha* extract on pregnant uterine horns.

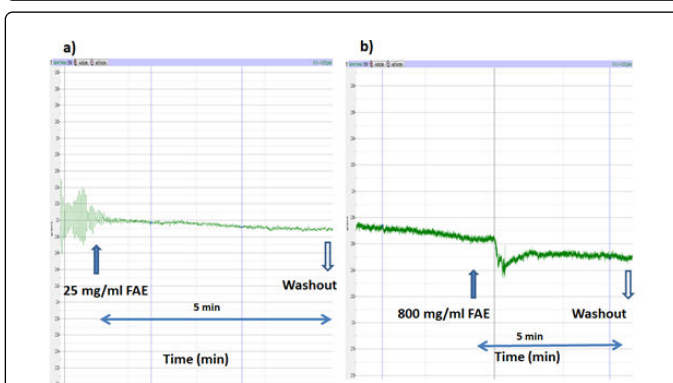


Figure 6: Effects of (a) 25 mg/ml (b) 800 mg/ml *Fadogia aencylantha* extract on non-pregnant uterine horns.

tissues to calcium [13], which could account for the potentiation that was observed when elephant dung aqueous extract was mixed with oxytocin.

The *in vitro* effects of elephant dung could also be attributed to the different foods that are eaten by the elephants. Numerous studies analysing the feeding patterns of elephants showed that elephants consume over 307 food and non-food items, with 70% of their food coming from trees [14]. Some of the food trees consumed by elephants include *Acacia xanthophloea*, *Azelia bipindensis*, *Colon coba welwitschii*, *Bridelia ferruginea* and *Terminalia superba* [15]. A number of these trees contain anthraquinones, flavonoids, saponins, terpenoids and condensed tannins, which are known to facilitate uterine contraction. The phytochemicals present in the trees could thus be responsible for the observed effects [16].

***Fadogia aencylantha* aqueous leaf extract**

Traditionally *Fadogia aencylantha* is used to treat a variety of ailments including abdominal and chest pain, asthma, toning muscles and diabetes [17]. Some of these conditions arise as a result of smooth muscle contraction. The ability of the plant to treat these illnesses suggests that *F. aencylantha* extracts treat disease by relaxing the smooth muscle, consistent with the results observed in this study.

Different authors have attributed the effects of the plant to its phenolic components [18,19]. These include phytochemicals such as acetylchalcone, glucosidichalcone, hydrocoumarin, glucosidicoumarin, saponins and triterpenoids [18,19]. Saponins and polyphenolic compounds present in *F. aencylantha* act as free radicals scavengers, whilst triterpenoids inhibit various enzymes involved in tissue contraction [17,20,21]. The combined effect of these phytochemical products could thus be promoting tissue relaxation, which could explain the study results.

In addition, as pregnancy progresses myometrial response to various agonists and antagonists is enhanced [22]. Tocolytic agents however, can stop the ability of the uterus to contract thus temporarily delaying delivery. These agents are mainly calcium receptor/channel antagonists, -adrenergic agents, oxytocin antagonists, prostaglandin antagonists, and magnesium sulphate [23]. In addition, nitric oxide donors can also decrease uterine contractility in pregnant rats. Study results therefore suggest that *Fadogia aencylantha* might be acting as a tocolytic agent at different receptors, since contractile tension decreased significantly after the extract was added, and no significant increases in tension were recorded when oxytocin was added.

Although these traditional medicines are commonly used in pregnancy, we found varying effects on pregnancy and child birth. The contractile effects of elephant dung could facilitate childbirth when used during labour, but could also induce premature labour if used earlier in pregnancy. The relaxation effects of *Fadogia aencylantha* could slow down labour during childbirth, which could result in complications to both the mother and the unborn baby. Women therefore need to be educated during antenatal care visits on the effects of using these medicines during pregnancy. Similarly, healthcare workers need to be sensitized on these results so as to ensure that they ask relevant questions when women register for antenatal care.

Limitations

The observed effects were mainly *in vitro* effects. *In vivo* effects might produce different results as the two extracts might be converted

into different products when ingested orally. Research is needed to determine the effects of these extracts *in vivo*. In addition, phytochemical analysis of the extracts was not conducted. Understanding the phytochemistry of these two crude extracts could have helped to explain the observed effects.

Conclusion

In our study, we found that elephant dung possessed oxytocic effects and contracted the uterus in a dose dependent manner, whilst *Fadogia aencylantha* relaxed the uterus in a dose dependent manner. Using these remedies could either delay or speed up natural labour progression and complicate the childbirth process. Pregnant women and healthcare workers should therefore be educated and informed on the possible complications that can arise from using these traditional medicines in pregnancy.

Conflict of Interest

The authors declare there is no conflict of interest.

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