Effect of lemongrass (cymbopogon) extract on uterine smooth muscles of wistar albino rats

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ABSTRACT: The effect of lemon grass (Cymbopogon) on uterine smooth muscles of wistar albino rats was investigated. Two female albino with weight 180g and 200g were sacrificed by decapitation after they have being subjected to 14 hours fasting. 2cm of uterine strip was mounted on a 10ml organ bath with tyrode solution. Results showed that at 0.01 to 0.1mg/ml concentration, a dose dependent contraction was produced. Oxytocin which was used as standard control, showed 0.1mg/ml and volume 0.1ml at 0.100 \pm 0.25 percentage contraction, While maximum contraction was 14.88%. But at 1mg/ml, 10mg/ml and 20mg/ml, the increase contraction was 2.0 \pm 0.25%, 5.0 \pm 0.025% and 6.75 \pm 0.25% respectively; while percentage contraction for oxytocin was 29.76%, 74.40% and 100% respectively. On the other hand, lemongrass showed that at concentration 100mg/ml, with 0.1ml, increase contraction was 12.75 \pm 0.25% and 100% maximum contractions. In order to keep constant concentration and lower volume 0.25ml and 0.3ml, produced 9.75 \pm 0.25% and 4.75 \pm 0.25% increase contractions respectively. Upon reducing concentration of extracts and keeping volume at constant level, motility (contraction) was reduced. 0.01mg/ml and 0.1mg/ml concentration, showed the increase contraction response 1.00 \pm 0.25% and 15.12 \pm 0.25%. Thus, these extract showed significant effect on the uterine motility based on concentration dose-dependent rather than volume administered as well as potentials for alternative oxytocin.

Keywords: Lemon Grass, Oxytocin Concentration, Traditional Medicine and Smooth Muscles Contraction.

Introduction

Traditional use of medicine has been identified as a means of studying the potentiality of future medicine. In the year 2001, about 122 compounds used in conventional medicine were identified by researchers as derivatives of "ethno-medical" plants sources, with 80% of these compounds used in the same or similar ways as traditional ethno-medical (Fabricant and Farnsworth, 2001). Plants are capable of synthesizing chemical compounds known as metabolites, which are classified biochemically as primary metabolites. Examples are carbohydrates, proteins, fats and oils, minerals and vitamins, and biologically active complex chemical substances called secondary metabolites. Although some of these metabolites are used for defensive purposes against predators, fortunately, some of them have non-toxic effects on humans, thus they are

beneficial in treating human diseases (Fabricant and Farnsworth, 2001).

Medicinal plants contain secondary metabolites that vary highly in structure such as saponins, tannins, essential oils, alkaloids, flavonoids etc (Hagman et al, 1998, Sofowora, 1993) those posses' curative properties. They have been shown to contribute to the treatment of diseases such as HIV/AIDS, Malaria, Diabetes, Sicklecell, Anaemia, Mental disorders (Elujoba et al, 2005) and Microbial, infections (Iwu et al., 1999; Okigbo et al., 2005). (Iwu et al, 1999) reported that the main benefits of using plant derived medicine are their relative safety compared to synthetic alternatives, thus providing intense therapeutic benefits and more affordable treatments. Secondary metabolites of plants are also known as phytochemicals, which commonly posses a wide range of pharmacological activities or actions (Trease

and Evans, 1989; Sokeng, *et al*, 2007), examples are Alkaloids, the most important of this group. They play a role in autonomic nervous system, blood vessels, promoting diuresis, respiratory system, gastrointestinal tract, malignant diseases, infections, uterus and malaria (Trease and Evans, 1989; Omotayo and Omoyeni,2009). Others are Tannins,well recognized to have both antioxidant and antimicrobial properties. Saponins, which have anticarcinogenic and anti diabetic effect, also are capable of reducing cholesterol level (Trease and Evans, 1989).

Lemongrass is an aromatic tropical plant with long slender blade that can grow to a height of 15m. It is water loving plant, strived better in environment support its hydrophilic properties (Kains, 2007). It is a rich substance called citral. The active ingredient in lemongrass is not known to interact adversely with any drug or dietary supplement. It is green plant and photosynthetic in nature because of the green pigment. Lemongrass is mildly diuretic and a stimulant tonic herb when drink as tae or used for cooking. The herb promote digestion of fat by cutting the level of cholesterol, fat and toxins from once body along with aiding the stimulation of blood circulation in the body. Because of these therapeutic properties of lemongrass and it essential oil, it has been prescribed for edema, varicose veins, hemorrhoids and retention of body fluid (Ameyaw and Owusu-Ansah, 2007).

Lemongrass (*cymbopogon*) is known to have various therapeutic and pharmacologic effect on body tissue such as aiding in digestion, relieving spasms, muscles cramp, rheumatism and headache (Guyton, and Hall, 2006). Lemongrass has been used for centuries in South America and India as folk medicine and a favorite ingredient in Thai cuisines and dishes that boost a targy, Asain flavor (Kains, 2007). However, in-depth studies of its effect on uterine smooth muscles may not have

being carried out. Lemongrass essential applications include: antiseptic, antifungal, antimicrobial and antidepressant effects. It extracts is just apt to relax tired body. It also possessed properties that help cleaning of oily skin acne and athletes foot. It alleviates excessive perspiration and a stimulant to tonic herbs. While they are different species of lemongrass, (cymbopogon) citrates is the variety more recommended for medicinal purpose. Research has also bolstered the herbs reputation as an analgesic and sedative therapy. Effective against 22 strains of bacterial and 12 types of fungi (Bennette et al, 2003) Uterine smooth muscles are just like other smooth muscles of the body (occurring in sheets). The muscles are involved in the expulsion of the fetus during delivery by contraction and relaxation of the uterine epithelium and small amounts of blood during sexual intercourse (Ebong, and Shode, 2000).

Oxytocin is a mammalian hormone that acts as a neurotransmitter. It is best known for its role in female reproduction. It is released in large amount as after distention of the cervix and vagina during labor and after stimulation of nipples, facilitating birth and breastfeeding (Abbiu, 1990). During the first two weeks of lactation, oxtocine served to assist in the uterus by clotting the placental attachment point postpartum (Young, 2009). The stimulation causes neurons that make oxytocin to fire action potentials in intermittent busts. These burst result in the secretion of pulses of oxytocin from the neurosecretory nerve terminals of the pituitary gland (Macbeth, 2009). Oxytocine contract the uterus, oestrogen induces oxytocine receptors synthesis and consequently, the uterus at term is highly sensitive to this hormone (Bagozzi, 2003). Study has showed that both amplitude and frequency of contraction are related to dose. Therefore, uterus relaxing completely between contractions during low-dose infusion. High doses increase the frequency of contractions and there is incomplete relaxation between them (Braide, 2006).

Oxytocin has peripheral actions in the brain, mediated by specific high affinity oxytocin receptors. The oxytocin receptor is a G-protein, coupled receptor which requires mg^{2+} and cholesterol (Wouters *et al*, 2007)l. It belongs to rhodopsin type 1 group of Gprotein coupled receptors. The actions and functions of oxytocin is seen below letdown reflex-in lactating mothers. It acts at the mammary glands, causing milk to be "letdown" into a collecting chamber from where it can be extracted by compressing the areola and sucking at the nipples. Sucking by the infant at the nipple is relayed by spinal nerve to the hypothalamus (Murray, 2005). The stimulation causes neurons that make oxytocin to fire action potentials in intermittent burst. These result in the secretion of pulses of oxytoxin from neurosecretory nerve terminals of the pituitary gland (Wouters, et al 2007). The research is designed to investigate the effect of lemongrass extracts on uterine smooth muscles as an alternative oxytocin.

Methodology

Plant Materials

These plants lemongrass (*cymbopogon*) were collected from the premises of Cross River University of Technology, Okuku campus. A quantity of 560g of lemongrass was obtained fresh and air dried 35°c-38°c for 4-7 days, the low temperature was to prevent denaturation of phyto-chemicals. The resulting dried leaves was beaten to coarse form using a pistle and morta and taken for extraction by sochlex methods.

Results

Animals

The experimental animals used in this study were female Wistar albino rats of between 12-14 weeks old, with body weight of 180g and 200g. They were obtained from the Faculty of Veterinary Medicine, University of Calabar. They were sacrifice by decapitation and dissected to remove the uterus. The uterus were used to investigate the contractile effect of oxytocin as well as to observed if the lemongrass extracts has any motility or contractile effect.

Chemicals/Reagents

All chemicals used for this research were of analytical grade and products from Serva, Heidelberg limited, New York.

Statistical Analysis

All investigations were carried out in triplicate and data obtained were presented as mean \pm standard deviation using descriptive statistics. Analysis was conducted using SSPSS version v16, for the determination of mean values.

Table 1 Effect of graded concentration (high) of lemongrass (cymbopogon) extracts on uterine contraction.

Concentration	Vol. of	Final bath	Log	Increase in	% increase	%
of extracts	extracts(concentration	FBC	contraction	in	maximum
(mg/ml)	ml)	(mg/ml)	(gm/ml)	(mm)	contraction	contraction
100	0.1	10	1.0000	$12.75{\pm}0.25$	164.73±2.23	100.00
100	0.2	20	1.3010	9.75±0.25	125.89±0.85	76.42
100	0.3	30	1.4771	4.75 ± 0.25	61.16±1.34	9.81
100	0.5	50	1.6990	4.75±0.25	61.16±1.34	9.89

Results are mean \pm SD for three determinations

Table 2 Effect of graded concentration (low) of lemongrass (cymbopogon) extracts on uterine contraction.

Concentration	Vol. of	Final bath	h Log	Increase in	% increase	%
of extracts	extracts	concentration	FBC	contraction	in	maximum
(g/ml)	(ml)	(mg/ml)	(gm/ml)	Mm	contraction	contraction
10 ⁻²	0.1	0.001	-3.000	2.00 ± 0.25	32.14±1.19	15.12
10-1	0.1	0.01	-2.000	2.00 ± 0.25	32.14±1.19	15.12
1	0.1	0.1	-1.000	5.25 ± 0.25	83.93±0.60	39.50
10	0.1	1	0.000	7.25 ± 0.25	116.07±0.6	54.62
20	0.1	2	0.3010	13.25±0.25	212.50 ± 4.17	100.00

Results are mean \pm SD for three determinations

Table 3 Effect of graded concentrations 0f Oxytocin on uterine contraction.

Concentrati	Vol. o	of Final bath	Log FBC	Increase in	% increase	%
on of	extracts	concentrati	(gm/ml)	contraction	in	maximum
extracts	(ml)	on (mg/ml)		(mm)	contraction	contraction
(mg/ml) 10 ⁻²						
10-2	0.1	0.001	-3.000	1.00 ± 0.25	14.88 ± 1.19	14.88
10-1	0.1	0.01	-2.000	1.00 ± 0.25	14.88 ± 1.19	14.88
1	0.1	0.1	-1.000	2.00 ± 0.25	29.76 ± 0.60	29.76
10	0.1	1	0.000	$5.00\pm0,25$	74.40 ± 0.60	74.40
20	0.1	2	0.3010	6.75 ± 0.25	100.00 ± 4.1	100.00
					7	

Results are mean \pm SD for three determinations

Discussion

In contrast to the skeletal muscles, movement, motility or contractility in uterus is usually very slow as such, the experiment was time consuming. The effect of lemon grass (Cymbopogon) on uterine smooth muscles of wistar albino rats was investigated. Two female albino rats with weight 180g and 200g were sacrificed by decapitation after they have being subjected to 14 hours fasting. 2cm of uterine strip was mounted on a 10ml organ bath with tyrode solution. Results showed that at 0.01 to 0.1mg/ml concentration, a dose dependent contraction was produced. Oxytocin was used as standard control, showed 0.1mg/ml and volume 0.1ml at 0.100 \pm 0.25 while percentage contraction was 14.88%. The results obtained showed the effect of graded concentrations 10mg/ml to 50mg/ml of final bath concentration of lemongrass (cymbopogon) extract on uterine contraction as shown in table 1, log of final bath concentration of lemongrass extracts and

percentage maximum contraction graded to concentration of 1mg/ml to 15mgml on uterine muscle (Ameyaw and Owusu-Ansah, 2007). From this study, cymbopogon at low doses (concentration and volume), produced a dose dependent decreased contraction 0.01mg/ml-1.0mg/ml. but at 10.0mg/ml, cymbopogon further invoked a contraction of uterine smooth muscles of the rat. The results obtained strongly suggest that very low doses of cymbopogon induced relaxant effect while moderately lower concentration and lower volume, lemongrass could illicit uterine contraction. There is little or no literature about the effects of lemongrass on pregnant uterine smooth muscles at very low doses, which will illicit motility at very low doses. It could be suggested that at low doses (volume) used in this work, the action of *cymbopogon* is similar to that of oxytocin, and therefore may accelerate parturition and placenta expulsion (Kurmar, et al 2008).

Oxytocin as a hormone has been known to induce motility of the uterus. Thus, in these work, the result showed that contraction occurred at 0.01mg/ml and 1mg/ml at 0.1ml, contraction increased to 1.00±0.25. Percentage increase in contraction was 14.88% as showed in table 2 and 3 respectively. However, a volume of 0.1ml and increased concentration 1mg/ml, 10mg/ml and 20mg/ml, contraction motility was 2.00±0.25, 5.00±0.25 and 6.75±0.25 respectively in table 3. While percentage increase in contraction was 29.76 $\pm 0.60\%$, 74.40 $\pm 0.60\%$ and 100. 00 \pm 4.17% respectively. From these data, it has showed that oxytocin concentration has greater effect on contraction than the volume of oxytocin, where a corresponding` percentage increase will lead to smooth muscle contraction in the uterus (Rose et al, 2009).

On the other hand, lemongrass that has not being known to induce motility effect at the uterine smooth muscle showed upon it administration that at high concentrations with variable volumes, contractions (motility) occurs (Bagozzi, 2003). Concentration 100mg/ml and volume 0.1ml produced greater contraction 12.75±0.25 and percentage increase of 100%. According to (Guyton, and Hall, 2006), (Prestwich, 2003) by maintaining concentration and lower volume of extracts 0.1mg/ml, 0.3mg/ml and 0.5 mg/mlproduced contractions of 9.75±0.25, 4.25±0.25 and percentage increase, 76.42%, and 9.81% respectively in table 1. Upon reduction of concentration of the extract, while keeping the volume at constant level, contraction (motility) levels were greatly reduced. Concentration of 10⁻²mg/ml and 10⁻¹mg/ml increased 1.00±0.25% and 15.12±0.25%. contraction by However, concentration of 1.10mg/ml and 20mg/ml increase contraction to $2.00\pm0.52\%$, $5.00\pm0.25\%$ and $6.75\pm0.25\%$ respectively as can be seen in table 2.

From these, it can be deduced that concentration of lemongrass extracts has a greater influence on the uterine smooth muscle motility than the administered volume, though the volume of extracts tends to induce a relaxant effect. Increased concentrations upon low volume of the extract gave higher contractions, similar to the effect of oxytocin on uterine smooth muscle. But increased concentration and volume caused a decreased in motility (Murray, 2005). Both concentration and volume influence contractile motility, acute administration of low concentration 0.01mg/ml-1.0mg/ml of extracts at low volume causes little motility on the non pregnant uterine smooth muscles in rats (Prestwich, 2003). We therefore states that lemongrass is and can serve as alternate stimulant, relaxant muscles diuretic product if properly harnessed.

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