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Ex-Vivo Uterine Activity of *Theobroma Cacao* (Malvaceae) Aqueous Seed Extract and *Cymbopogon Citratus* (Poaceae) Aqueous Leaf Extract – A Preliminary Investigation

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ABSTRACT

There is a need to develop new drugs from natural products for the safe and effective management of female reproductive disorders. This research was therefore aimed at the investigation of two commonly consumed natural products on uterine function. The *ex-vivo* uterine activity of *Theobroma cacao* aqueous seed extract and *Cymbopogon citratus* aqueous leaf extract was investigated on non-pregnant mouse uteri. The effect of *T. cacao* (0.1 mg/mL) and *C. citratus* (0.1 mg/mL) on spontaneous uterine contractility and in the presence of oxytocin (11.54 nM) was examined. This study showed that *T. cacao* elicited a significant increase ($p < 0.05$) in the amplitude of spontaneous uterine contractions with no significant change in the frequency, which was similarly observed with *C. citratus* leaf extract. An increase in amplitude and frequency of oxytocin-induced uterine contractions was observed in the presence of *T. cacao* while with *C. citratus* there was a significant ($p< 0.05$) reduction in amplitude and non-significant reduction in frequency of oxytocin-induced uterine contractions. The uterotonic effect of both extracts in the absence of agonists observed maybe as a result of constituents with agonistic activity on intracellular calcium. This study has shown that *T. cacao* and *C. citratus* could stimulate uterine activity and may therefore be useful in the management of uterine contractility disorders.

Keywords: *Theobromacacao*, *Cymbopogoncitratus*, uterus, oxytocin, spontaneous contractions

INTRODUCTION

The use of plants for food and medicine is an age long culture. In the early years, most of the plants used as medicines were also easily affordable foodstuffs (Touwaide & Appetiti 2015). The utilization of a plant either as food or medicine largely depends on how it is prepared and what it is to be used for (Jennings et al. 2015). The use of plants both as food and medicines has been widely documented in the literatures (Etkin & Ross 1982; Sandhu & Heinrich 2005; Pieroni & Price 2006).

Theobroma cacao (T. cacao) and *Cymbopogon citratus (C. citratus)* are widely known and used both as food and as medicine. In some parts of Nigeria, both *T. cacao* and *C. citratus* are used to improve reproductive function (personal communication). *T. cacao* also known as cocoa is derived from the cocoa tree and belongs to the Theobroma family of plants which are abundant in the Amazon and tropical regions (Wood & Lass 2008). A cocoa pod skin is rough and varies between 2-3 cm in thickness. It contains 30-50 seeds which have a characteristic pale purple shade (Ishaq & Jafri 2017). For a long time, cocoa has been consumed purely for pleasure but more recently they have been shown to significantly affect health due to their high polyphenol content (Ackar et al. 2013). Studies have shown *T. cacao* contains several flavonoids which include catechins, procyanidin B2, and methylxanthines (Jalil & Ismail 2008). They are also known to contain saponins, tannins, alkaloids and terpenoids (Subhashini et al. 2010). *T. cacao* has been investigated for a wide range of health effects. Reports have shown *T. cacao* to have health benefits including potent antioxidants (Lee et al., 2003); Keen et al., 2005; , reduction of high blood pressure and improvement of endothelial function (Buijsse et al. 2006; Taubert et al. 2007), improvement of stroke and related neurologic events (Bisson et al. 2008; Sorond et al. 2008), and cytotoxic effect on cancer cells (Ferrazzano et al. 2009). *T. cacao* has also been shown to have cardioprotective properties and exert a

decrease in the levels of low density cholesterol (Santos & Macedo 2018), and improvement of symptoms of chronic fatigue and persistent cough (Usmani et al. 2004; Sathyapalan et al. 2010). Studies have also shown *T. cacao* to be potent in lowering blood sugar and therefore effective as an antidiabetic (de Oliveira & Genovese 2013). It has also been shown to have anti-malarial activities (Amponsah et al. 2012) and to inhibit platelet aggregation (Rein et al. 2000). *T. cacao* was reported to have no effect on pregnant animals and that it also does not pose any teratogenic risk when consumed (Tarka et al. 1986). In addition, it has been reported to have low estrogenic effect and therefore have no significant effect on uterus weight (Sari et al. 2017). However, it has been suggested that administration at term pregnancy may cause fetal ductus constriction (Zielinsky et al. 2014). The activity of this plant on the uterus has not been widely reported. It is therefore worthwhile to investigate its effect on uterine function. *C. citratus*also known as lemongrass is a perennial plant that belongs to the Poaceae family. It is known for its slender long leaves which are aromatic in nature with a characteristic lemon scent and can be found worldwide (Ekpenyong et al., 2014, 2015). It is commonly consumed for food purposes, however its medicinal value has long been recognized traditionally (Akande et al. 2011). Similar to *T. cacao*, it is popularized to be useful for a wide variety of health conditions (Ekpenyong et al. 2015). It has been reported to show antimicrobial activity, anti-oxidant activity, antitussive activity and cardio-protective activity (Gazola et al. 2004; Oloyede 2009; Ekpenyong et al. 2014). It also has been reported to prevent platelet aggregation (Tognolini et al. 2006), diabetes management (Mansour et al. 2002), control cholesterol levels (Leite et al. 1986), and possessing antimalarial activity (Tchoumbougnang et al. 2005). Several flavonoids and alkaloids have also been detected in *C. citratus* (Miean & Mohamed 2001; Negrelle & Gomes 2007). Again, despite its widely researched health benefits, there is paucity of data on the activity of *C. citratus* on uterine function. A study carried-out however reported that *C. citratus* leaves stimulate uterine contractions in non-pregnant rats at high concentrations but at lower concentrations, contractions were reduced. The authors however used only two animals for the entire experiment (Okpashi et al. 2014). Another study showed that *C. citratus* extract exhibit spasmolytic effects in the ileal smooth muscles (Devi et al. 2011).

This study was aimed at investigating the *ex-vivo* uterine activity of *T. cacao* seed extract and *C. citratus* leaf extract. This is to provide a proof of concept on the potential of both or either of these plants as useful options for the management of uterine contractility which is one of the parameters of female reproductive function. Additionally, to also provide evidence on effect of consumption of these natural products on uterine function, particularly when consumed by women with uterine contractility disorders for which either plant may be useful.

MATERIALS AND METHODS

Plant material

Fresh *C. citratus* leaves and *T. cacao* seeds were collected from Edo state and Ondo state respectively. They were both air-dried and then powdered. Five hundred grams (500 g) of both samples were macerated in distilled water for 72 h at room temperature with occasional stirring. The filtrate was concentrated using a rotary evaporator (BUCHI Labortechnik AG, Flawil Switzerland) at a temperature of 40°C.

Animals

Female virgin albino laboratory mice weighing between 20.0-30.0 g were purchased from the Animal House Department of Pharmacology & Toxicology, University of Benin, Nigeria. In order to ensure that the animals used were virgins, the young immature female animals were constantly separated from the male litter before they reach 15 days of age. They were handled in accordance with standards of the Public Health Service policy on humane care and use of Laboratory Animals National Institutes of Health, USA 2015. Ethical permission was sought from the ethical committee for animal use, Faculty of Pharmacy, University of Benin, Nigeria (EC/FP/016/04).

Standard diet of animal pellets and clean tap water was provided *ad libitum*. Adequate hygiene was maintained daily by regular cleaning and removal of faecal matter and leftover feed from cages.

Uterine Contractility Assay

On the day of the experiment, the animal was weighed. Animals in proestrous or oestrus stage of the oestrus cycle were selected for experiments. The stage of oestrus was confirmed via macroscopic examination of the vulva and microscopic observation of vaginal smears. The selected mouse was humanely sacrificed by cervical dislocation and the uterine horns were immediately excised and placed into a Petri dish containing warmed and aerated Ringer Locke's physiological salt solution (composition: 9 g NaCl, 0.42 g KCl, 0.32 g $CaCl_2.2H_20$, 0.2 g $NaHCO_3$ and 1.0 g D-glucose in 1 litre of distilled water). Uterine horns were carefully dissected out, freed of adhering mesenteric tissues and fat. The uterine segments (measuring 0.5 cm in length) was mounted in 10 mL organ bath containing warmed (37°C) aerated Ringer Locke's physiological salt solution (PSS). Tissues were placed under 0.5 g tension and then equilibrated for 30 min. During the period of equilibration the tension was mechanically adjusted intermittently, however no further adjustments were made during the course of the experiments. The differential force and frequency of spontaneous contractions in the longitudinal muscle layers of each uterine tissue segment were recorded via a 7003E-isometric force transducer (UgoBasile, Varise, Italy) connected to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (UgoBasile, Varese, Italy).

Experimental Protocol for the *In Vitro* Assay

The direct effect of both *C. citratus* (0.1 mg/mL) and *T. cacao* (0.1 mg/mL) were tested on spontaneous and oxytocin-induced contractions separately as described by Bafor *et al*. (2013) and single concentration additions were obtained as described below. A total of 10 animals were utilized for the entire study. Five animals for the spontaneous contractility assay and five for the oxytocin contractility study. From one animal, four uterine tissues can be obtained. We were therefore able to run experiments on same animal utilizing different sections of the uterine tissue for Theobroma cacao seeds (TCS) extract and *Cymbopogoncitratus* leaf (CCL).

Investigation of C. citratus and T. cacao on Spontaneous Uterine Contraction

After tissue equilibration for 30 min, spontaneous uterine contractions were recorded for 10 min and *C. citratus* or *T. cacao* (0.1 mg/mL) was administered to the uterine tissue in the 10 mL bath for a contact time of 10 min. The tissue was then washed sufficiently with the PSS and allowed to relax.

Investigation of C. citratus and T. cacao on Oxytocin-induced Uterine Contraction

After tissue equilibration, the effect of *C. citratus* (0.1 mg/mL) and *T. cacao* (1 mg/mL) on oxytocin-induced uterine contraction was investigated. The response of the tissue to oxytocin in the absence of the drug was first determined. Oxytocin (OT) (11. 54 nM) was added into the organ-bath for 5 min; this was immediately followed by a single concentration (0.1 mg/mL) of either *C. citratus* and *T. cacao* added to the bath separately and left in contact with the tissue for 10 min. After each set of administration, the tissue was washed sufficiently and responses recorded.

Statistical Analysis

The mean frequency and amplitude were calculated from contractions occurring within 5 min of the phasic contractions using the GraphPad Prism, (version 7.1; GraphPad software Inc, San Diego, CA, USA). Results were obtained as control vs treatment responses. All data shown were expressed as mean ± standard error of mean (SEM) and 'n' represented the number of uterine tissues from different animals. Significance was evaluated using Student two-tailed t-tests, and P values ≤ 0.05 were taken to represent maximum significance.

RESULTS

Percentage Yield of Extracts

Aqueous extract of *T. cacao* was dark brown, semisolid and non-sticky. It was soluble in water, with a percentage yield of 22.3%w/w, while the aqueous extract of *C. citratus* was a dark green, sticky pulp that was insoluble in water but soluble in Tween 80. It had a percentage yield of 16.5%w/w.

Experiments on Spontaneous Uterine Contractions

Effects of T. cacao on Spontaneous Uterine Contractions

T. cacao produced an increase in spontaneous uterine contractility (Figure 1). This was also clearly depicted in the bar plot which showed significant stimulation ($p< 0.001$) in the amplitude of spontaneous contraction by *T. cacao* at a concentration of 0.1 mg/mL (Figure 2A). However, no significant changes were observed on the frequency of spontaneous contractions, though a slight inhibition of frequency was seen (Figure 2B).

Effects of C.citratus on Spontaneous Uterine Contractions

At 0.1 mg/mL *C. citratus* caused an increase in the amplitude of spontaneous contractions with mild reductions in the frequency of uterine contractions (Figure 3). This can be clearly observed in the bar plots which showed a significant increase ($p< 0.05$) in the amplitude (Figure 4A) with no significant change in the frequency of spontaneous contractions (Figure 4B).

Experiments on Oxytocin (OT)-induced Uterine Contractions

Effect of T. cacao on OT-induced uterine contractions

T. cacao was observed in this study to augment OT-induced uterine contractions (Figure 5). Bar plots and calculations however showed no significant difference ($p > 0.05$) in the amplitude and frequency of OT-induced contractions (Figure 6A and 6B respectively) in the presence of *T. cacao* at the concentration used in this study.

Effect of C. citratus on OT-induced uterine contractions

C. citratus was observed to inhibit OT-induced uterine contractions (Figure 7). A significant reduction ($P < 0.05$) in the amplitude of OT-induced contraction was observed in the presence of *C. citratus* (Figure 8A). A reduction in frequency was also observed on OT-induced contraction in but was not statistically significant (Figure 8B).

Discussion

The present study demonstrates that the aqueous extract of *T. cacao* seed (TCS) and the leaves of *C. citratus* (CCL) stimulated uterine contractility in the absence of any agonist stimulation. However, while TCS stimulated OT-induced uterine contractility, CCL appeared to inhibit OT-induced uterine contractility in non-pregnant isolated mouse uterus.

The myometrium (smooth muscle layer of the uterus), is active throughout life and not restricted to periods of labour and delivery (Wray & Arrowsmith 2012). Uterine contraction therefore occurs throughout the menstrual cycle in non-pregnant states as well as the pregnant states, in a complex and dynamic physiological phenomenon (Aguilar & Mitchell 2010). Some of the unwanted but frequently observed results of myometrial dysfunction are contractions that are not timed leading to abortions or preterm delivery, or stronger than necessary contractions causing foetal distress, hypoxia and even death of the foetus (Wray 2007; Aguilar & Mitchell 2010). The non-pregnant myometrium exhibits contractions at different phases of the menstrual cycle; these include rhythmic, 'wave-like' contractions, oftentimes referred to as uterine peristalsis or spontaneous contractions, and the 'focal and sporadic bulging of the myometrium' leading to sustained contractions (Togashi et al. 1993; Togashi 2007). These contractions are important in endometrial sloughing and assists in sperm passage (Pehlivanoğlu et al. 2013).

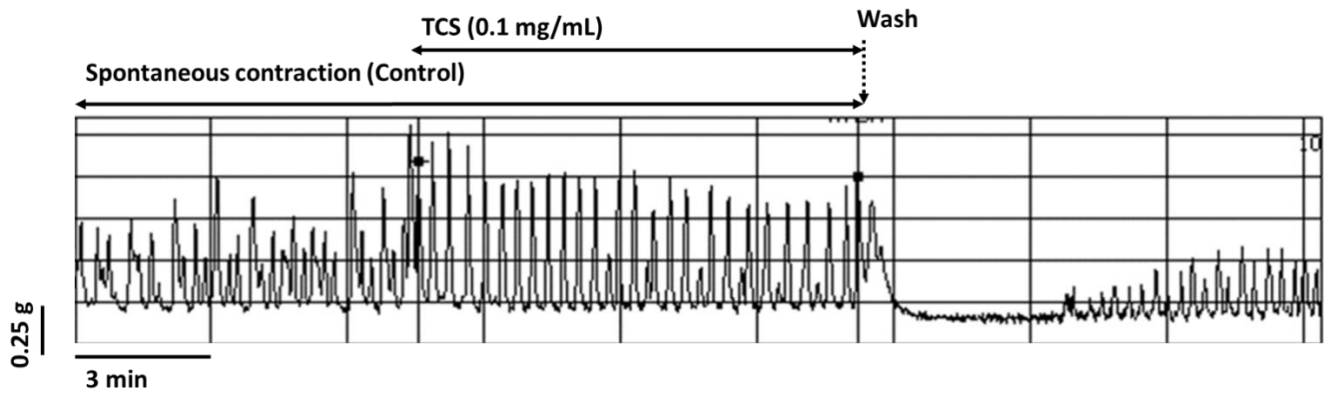

Figure 1: Representative recording showing effect of *T. cacao* seed extract (TCS) on spontaneous uterine contractions.

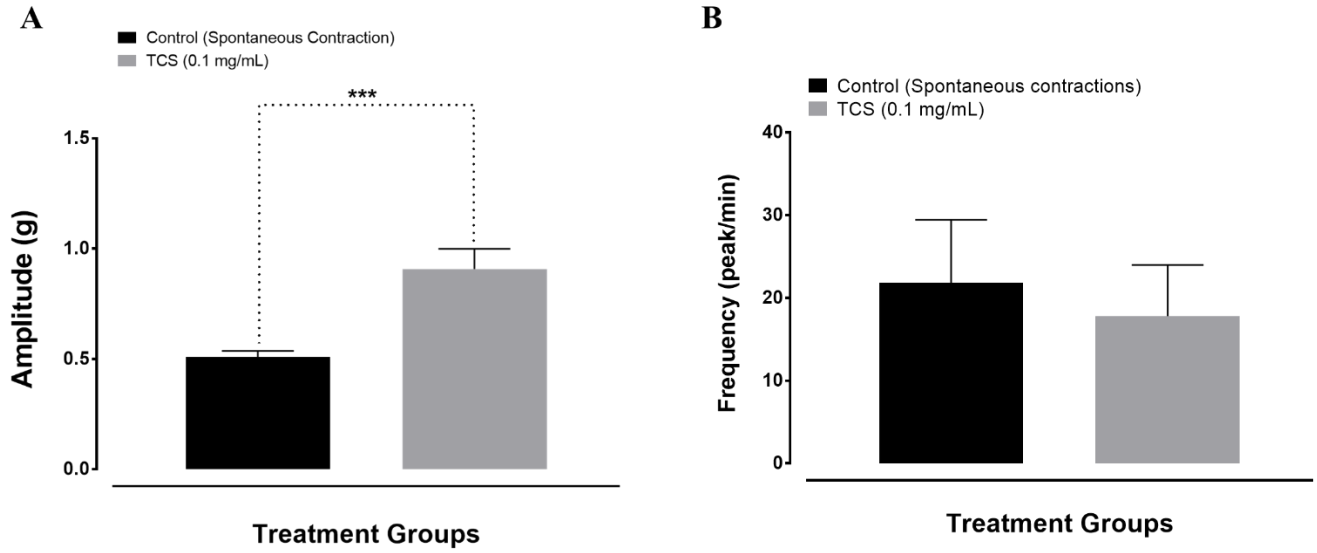

Figure 2: Bar plots showing effect of *T. cacao* seed extract (TCS) on the amplitude (A) and frequency (B) of spontaneous uterine contractions. A significant increase ($p < 0.001$) in the amplitude of contractions was observed in the presence of TCS (1 mg/mL) while no significant change in the frequency of contractions was observed in the presence of TCS n= 5 animals; ***$p<0.001$ compared to control.

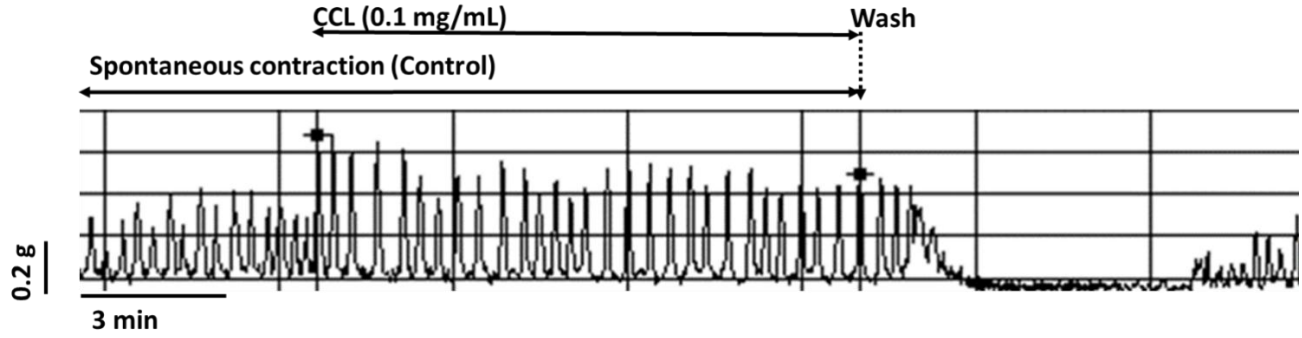

Figure 3: Representative recording showing effect of *C. citratus* leaf extract (CCL) on spontaneous uterine contractions.

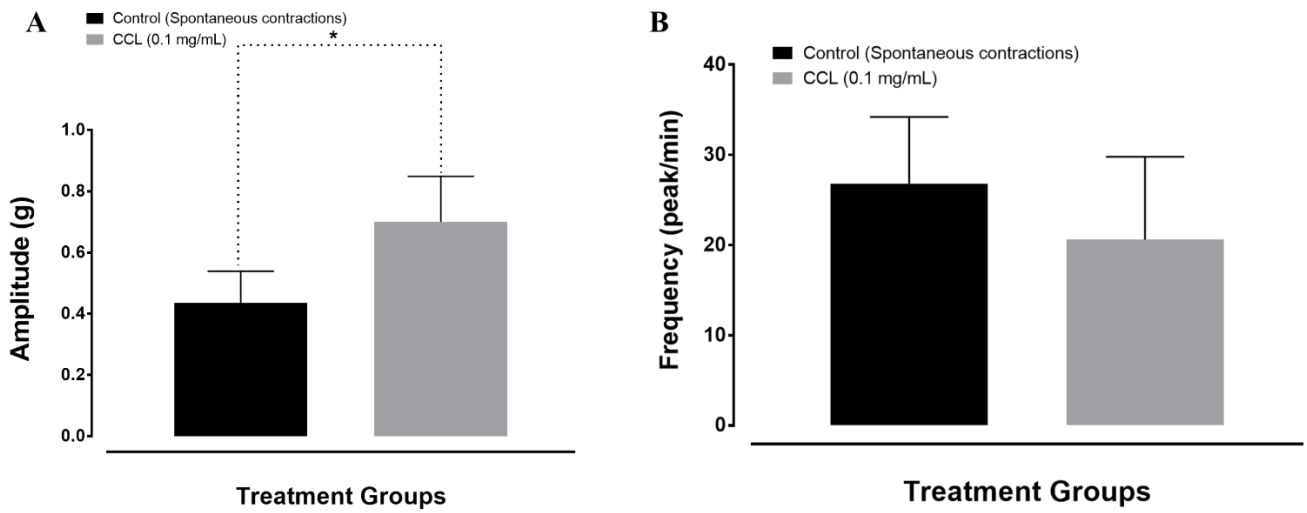

Figure 4: Bar plots showing effect of *C. citratus* leaf extract (CCL) on the amplitude (A) and frequency (B) of spontaneous uterine contractions. An increase in the amplitude of contractions was observed in the presence of CCL (0.1 mg/mL) while no significant change in the frequency of contractions was observed in the presence of CCL n= 5 animals; *p<0.05 compared to control.

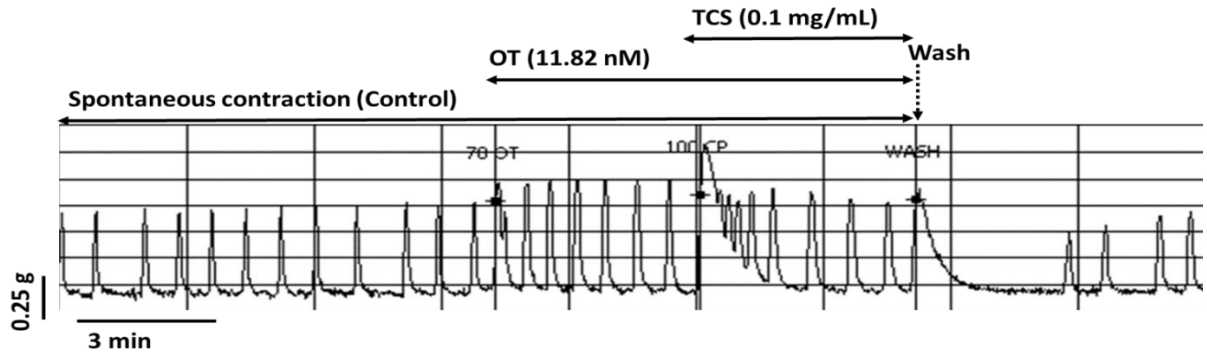

Figure 5: Representative recording showing effect of *T. cacao* seed extract (TCS) on oxytocin-induced uterine contractions.

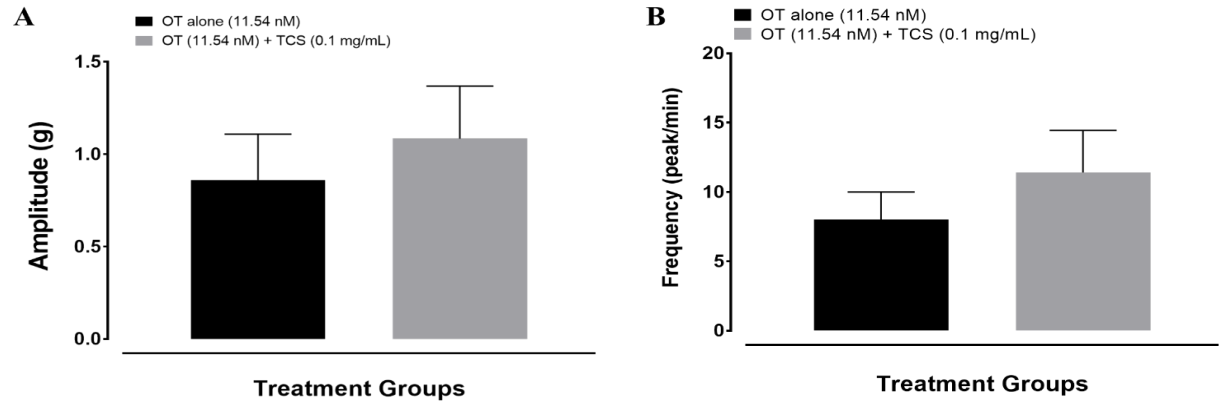

Figure 6: Bar plots showing effect of *T. cacao* seed extract (TCS) on the amplitude (A) and frequency (B) of oxytocin-induced uterine contractions. An increase in the amplitude of uterine contractions was observed in the presence of TCS (0.1 mg/mL), however an increase in the frequency of uterine contractions was observed in the presence of TCS. n= 5 animals.

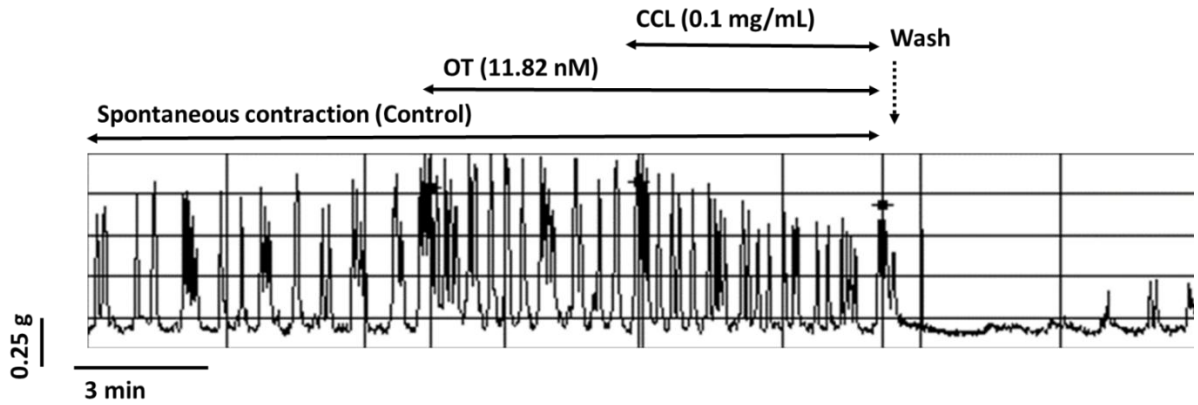

Figure 7: Representative recording showing effect of *C. citratus* leaf extract (CCL) on oxytocin-induced uterine contractions.

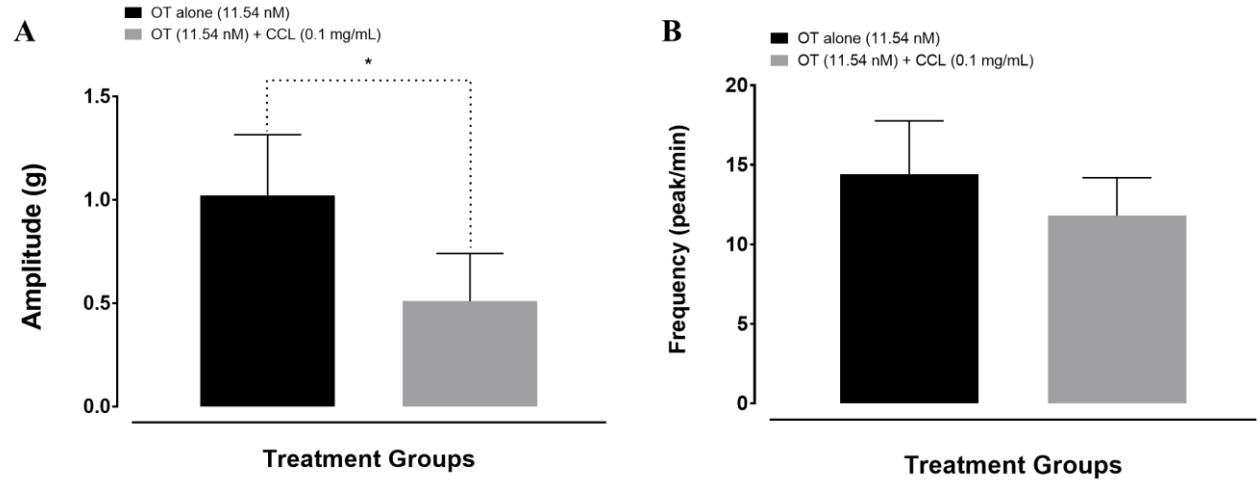

Figure 8: Bar plots showing effect of *C. citratus* leaf extract (CCL) on the amplitude (A) and frequency (B) of oxytocin-induced uterine contractions. A significant reduction ($p < 0.05$) in the amplitude of contractions was observed in the presence of CCL (1 mg/mL), and a slight decrease in the frequency of oxytocin-induced uterine contractions was observed in the presence of CCL. n= 5 animals. OT = oxytocin; * $p < 0.05$.

In late-term pregnancy, the myometrium contracts via similar mechanisms as occurs in the non-pregnant uterus with differences in morphology and concentrations of circulating hormones to systematically ensure successful expulsion of the foetus in the absence of any abnormalities (Pehlivanoğlu et al. 2013). Therefore regardless of the presence or absence of pregnancy, uterine contractions are dependent on the contractile activity of the cellular elements, the uterine myocytes. The uterine myocytes are cells of the myometrium which often exhibits a phasic contractile pattern such that the resting tone of the uterus is maintained (Aguilar & Mitchell 2010). This resting tone is often superimposed by separate intermittent set of contractions with varying frequency, amplitude and duration (Wray 2007). It is predominantly regulated by intracellular calcium concentration ($[Ca^{2+}]i$) (Wray 2007; Aguilar & Mitchell 2010). TCS and CCL were found in this study to stimulate this spontaneous intrinsic uterine contractions suggesting possible stimulation of the force and frequency of myocyte activity via stimulation of $[Ca^{2+}]_i$. An increase in $[Ca^{2+}]_i$ is reported to activate a calcium ion (Ca^{2+})-dependent cytosolic protein, calmodulin (CalM), which is capable of binding up to four Ca^{2+} ions (Johnson et al. 1996). Formation of the Ca^{2+}-CalM complex activates the enzyme, myosin light chain kinase (MLCK) resulting in an increase in the phosphorylation of myosin regulatory light chain-20 (MLC20) and

subsequent cross-bridge cycling (Shojo & Kaneko 2001). MLC20 phosphorylation by MLCK is the principal determinant of the amplitude and duration of contraction (McConnell & Wadzinski 2009; Butler et al. 2013). MLCK contains several phosphorylation target sites for protein kinase A, protein kinase C (PKC) and other kinases (Wray et al. 2001; Aguilar & Mitchell 2010) which may contribute to its activity. It may therefore seem that stimulation of the amplitude of myometrial contraction markedly observed in this study may be attributed to the augmentation of MLC20 phosphorylation by TCS and CCL. Activation of MLCK by CaIM translocation of activated MLCK towards the contractile apparatus may be the rate-limiting step of contraction (Wray et al. 2003) determining the contraction frequency of the myometrium. It would also seem that with the effect of TCS and CCL being somewhat more pronounced on the amplitude than the frequency of spontaneous contractions, both extracts may exert less activity on MLCK activation and possibly a greater effect on augmentation of MLC20 phosphorylation.

This study additionally reports the stimulation of oxytocin-induced uterine contraction by TCS and a mild inhibition by CCL. Oxytocin (OT) is known largely for its stimulatory actions on myometrial contraction (Pehlivanoğlu et al. 2013). Oxytocin stimulates calcium entry and release from the sarcoplasmic reticulum (Arrowsmith & Wray 2014). Coupling of OT to its receptor activates phospholipase-Cβ, which hydrolyses phosphatidylinositol bisphosphate (PIP_2) releasing two second messengers, inositol triphosphate (IP_3) and diacylglycerol (DAG) (Wray & Arrowsmith 2012). IP_3 activates $[Ca^{2+}]_i$ from the sarcoplasmic reticulum (SR) which proceeds to open up more extracellular calcium channels, while DAG activates protein kinase C (Wray & Arrowsmith 2012). Oxytocin has also been reported to exert a stimulatory effect on $[Ca^{2+}]_i$ entry and release from the sarcoplasmic reticulum (Soloff & Sweet 1982) while also inhibiting $[Ca^{2+}]_i$ efflux, which may result in the inhibition of myosin light chain phosphate (MLCP) (Wray & Arrowsmith 2012). The net effect is a powerful enhancement of force and slowing relaxation which could be observed in this study. The effect of TCS on oxytocin seen in this study therefore supports the uterotonic activity also seen on spontaneous contractility. Interestingly, however CCL which had initially stimulated and augmented spontaneous uterine contractility appeared to have opposing effects on OT-induced contractility where a mild inhibition was observed. The reason for this is not clearly understood at the moment, however it may well be that CCL had no effect on OT-induced contraction itself particularly since the inhibition observed was mild and insignificant. Overall, the effect of TCS and CCL on calcium mobilization requires further investigation and the possible lack of activity on OT-induced contractility by CCL also requires further investigation. From the results, TCS appeared to have a greater stimulatory effect on the spontaneous uterine contractions than CCL, suggesting that TCS may be more potent than CCL.

The findings in this study that TCS and CCL stimulates contraction of the uterus poses some potential clinical usefulness in promoting uterine contractility in conditions such as dysfunctional labour. In dysfunctional labour, uterine contractility at term is poor and requires contractility agents to augment and improve contractions and prevent caesarean sections (Wray 2007). This study is at the moment a preliminary investigation and therefore consists of some limitations. The study utilized single doses and this represent one of the limitations. Further studies are recommended where a range of concentrations are utilized and parameters of contractility such as concentration producing 50% of maximum response (EC_{50}) and concentration producing maximum response (E_{max}) are measured. This study did not also compare the pharmacological responses with existing drugs. In further studies, control drugs should be included for comparison. Furthermore, the study did not also focus on isolation of phytochemical constituents responsible for the uterine activity observed which should be investigated in future studies.

CONCLUSION

This study reports the uterine stimulatory activity of cocoa seeds and lemongrass on the non-pregnant uterus. In addition, the study reports augmentation of agonist contractile activity by cocoa seeds but a possible inhibition of agonist activity on the uterus by lemongrass. These are interesting findings and support further studies to determine the constituents responsible for the activity.

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REFERENCES

Ackar, D., Valek, L.K, Valek, M., Šubarić, D., Miličević, B., Babić, J., Nedić, I. (2013). Cocoa Polyphenols: Can We Consider Cocoa and Chocolate as Potential Functional Food? *Journal of Chemistry*, **2013** (Article ID 289392):1-7.

Aguilar, H.N., Mitchell, B.F. (2010). Physiological pathways and molecular mechanisms regulating uterine contractility. *Human ReproductionUpdate*, **16**:725–744.

Akande, I.S., Samuel, T.A., Agbazue, U., Olowolagba, B.L. (2011). Comparative proximate analysis of ethanolic and water extracts of Cymbopogoncitratus (lemon grass) and four tea brands. *Plant Science Research*, **3**:29–35.

Amponsah, S.K., Bugyei, K.A., Osei-Safo, D., Addai, F.K., Asare, G., Tsegah, E.A., Baah, J., Ofori, M., &Gyan, B.A. (2012). In Vitro Activity of Extract and Fractions of Natural Cocoa Powder on Plasmodium falciparum. *Journal of Medicinal Food*, **15**:476–482.

Arrowsmith, S., &Wray, S. (2014). Oxytocin: Its mechanism of action and receptor signaling in the myometrium. *Journal of Neuroendocrinology*,**26**:356–369.

Bafor, E.E., Rowan, E. G., &Edrada-Ebel, R. (2013). The leaves of Ficus exasperata Vahl (Moraceae) generates uterine active chemical constituents. *Journal of Ethnopharmacology*, **145**:803-812.

Bisson, J. F., Nejdi, A., Rozan, P., Hidalgo, S., Lalonde, R., &Messaoudi, M. (2008). Effects of long-term administration of a cocoa polyphenolic extract (Acticoa powder) on cognitive performances in aged rats. *British Journal of Nutrition*, **100**:94–101.

Buijsse, B., Feskens, E. J. M., Kok, F.J., Kromhout, D. (2006). Cocoa intake, blood pressure, and cardiovascular mortality: The Zutphen Elderly Study. *Archives of Internal Medicine*, **166**:411–417.

Butler, T., Paul, J., Europe-Finner, N., Smith, R., &Chan, E-C. (2013). Role of serine-threonine phosphoprotein phosphatases in smooth muscle contractility. *American Journal of Physiology and Cell Physiology*, **304**:C485-C504.

Devi, C. R., Sim, M.S., &Ismail, R. (2011). Spasmolytic effect of citral and extracts of Cymbopogoncitratus on isolated rabbit ileum. *Journal of Smooth Muscle Research*, **47**:143–156.

Ekpenyong, C. E., Akpan, E., &Nyoh, A. (2015). Ethnopharmacology, phytochemistry, and biological activities of Cymbopogoncitratus (DC.) Stapf extracts. *Chinese Journal of Natural Medicine*, **13**:0321–0337.

Ekpenyong, C. E., Akpna, E. E., &Udokang, N.E. (2014). Clinical correlates of physiochemical changes in urinary composition in subjects treated with Cymbopogoncitratus infusion. *Elixir Human Physiology*, **68**:22081–22086.

Etkin, N. L., &Ross, P.J. (1982). Food as medicine and medicine as food. An adaptive framework for the interpretation of plant utilization among the Hausa of northern Nigeria. *Social Science & Medicine*, **16**:1559–1573.

Ferrazzano, G. F., Amato, I., Ingenito, A., De Natale, A., &Pollio, A. (2009). Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). *Fitoterapia*, **80**:255–262.

Gazola, R., MacHado, D., Ruggiero, C., Singi, G., &MacEdo, A. M. (2004). Lippiaalba,

Melissa officinalis and Cymbopogoncitratus: Effects of the aqueous extracts on the isolated hearts of rats. *Pharmacological Research*, **50**:477–480.

Ishaq, S., &Jafri, L. (2017). Biomedical Importance of Cocoa (Theobroma cacao): Significance and Potential for the Maintenance of Human Health. *Matrix Science Pharma*, **1**:01–05.

Jalil, A. M. M, &Ismail, A. (2008). Polyphenols in cocoa and cocoa products: Is there a link between antioxidant properties and health? *Molecules*, **13**:2190–2219.

Jennings, H. M., Merrell, J., Thompson, J. L., &Heinrich, M. (2015). Food or medicine? the food-medicine interface in households in Sylhet. *Journal of Ethnopharmacology*, **167**:97–104.

Johnson, J. D., Snyder, C., Walsh, M., &Flynn, M. (1996). Effects of myosin light chain kinase and peptides on Ca^{2+} exchange with the N- and C-terminal Ca^{2+} binding sites of calmodulin. *Journal of Biological Chemistry*, **271**:761–767.

Keen, C. L., Holt, R. R., Oteiza, P. I., Fraga, C. G., &Schmitz, H.H. (2005). Cocoa antioxidants and cardiovascular health. *American Journal of Clinical Nutrition*, **81**:298S-303S.

Lee, K. W., Kim, Y. J., Lee, H. J., &Lee, C. Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. Journal of Agriculture and Food Chemistry, **51**:7292–7295.

Leite, J., De Lourdes, V.,Seabra, M., Maluf, E., Assolant, K., Suchecki, D., Tufik, S., Klepacz, S., Calil, H. M., &Carlini, E. A. (1986). Pharmacology of lemongrass (*Cymbopogoncitratus*Stapf). III. Assessment of eventual toxic, hypnotic and anxiolytic effects on humans. *Journal of Ethnopharmacology*, **17**:37–64.

Mansour, H. A., Newairy, A. S. A., Yousef, M. I., &Sheweita, S. A. (2002). Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. *Toxicology*, **170**:221–228.

McConnell, J. L., &Wadzinski, B. E. (2009). Targeting protein serine/threonine phosphatases for drug development. *Molecular Pharmacology*, **75**:1249–1261.

Miean, K. H., &Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of Agriculture and Food Chemistry*, **49**:30106–30112.

Negrelle, R. R. B., &Gomes, E. C. (2007). *Cymbopogoncitratus* (DC.) Stapf: Chemical composition and biological activities. *Revista*Brasileira de *Plantas*Medicinais, **9**:80–97.

Okpashi, V. E., Obi-Abang, M., &Bayim, P. R. (2014). Effect of lemongrass (*Cymbopogon*) extract on uterine smooth muscles of wistar albino rats. International Journal of Science and Engineering Research, **5**:982–988.

De Oliveira, T. B., &Genovese, M. I. (2013). Chemical composition of cupuassu (*Theobroma grandiflorum*) and cocoa (*Theobroma cacao*) liquors and their effects on streptozotocin-induced diabetic rats. *Food Research International*, **51**:929–935.

Oloyede, O. I. (2009). Chemical profile and antimicrobial activity of *Cymbopogoncitratus* leaves. Journal of Natural Product, **72**:98–103.

Pehlivanoğlu, B., Bayrak, S., &Doğan, M. (2013). A close look at the contraction and relaxation of the myometrium; the role of calcium. *Journal of the Turkish-German Gynecological Association*, **14**:230–234.

Pieroni, A., &Price, L. L. (2006). Eating and Healing: Traditional Food as Medicine. New York, London and Oxford: CRC Press.

Rein, D., Paglieroni, T. G., Wun, T., Pearson, D. A., Schmitz, H. H., Gosselin, R., &Keen, C. L. (2000). Cocoa inhibits platelet activation and function. American Journal of Clinical Nutrition, **72**:30–35.

Sandhu, D. S., &Heinrich, M. (2005). The use of health foods, spices and other botanicals in the sikh community in London. *Phytotherapy Research*, **19**:633–642.

Santos, H. O., Macedo, R. C. O. (2018). Cocoa-induced (*Theobroma cacao*) effects on cardiovascular system: HDL modulation pathways. *Clinical Nutrition ESPEN*, **27**:10–15.

Sari, B. T. A., Jati, M., Pudjiastuti, P., &Baktir A. (2017). Uterus Weight of Ovariectomized Rats Given Cocoa Powder and Extract. *Pelita Perkebunan (a Coffee Cocoa Res Journal)*, **33**:45–50.

Sathyapalan, T., Beckett, S., Rigby, A. S., Mellor, D. D., &Atkin, S. L. (2010). High cocoa polyphenol rich chocolate may reduce the burden of the symptoms in chronic fatigue syndrome. *Nutrition Journal*, **9**:55.

Shojo, H., &Kaneko, Y. (2001). Oxytocin-induced phosphorylation of myosin light chain is mediated by extracellular calcium influx in pregnant rat myometrium. *Journal of Molecular Recognition*, **14**:401–405.

Soloff, M. S., &Sweet P. (1982). Oxytocin inhibition of (Ca2+ Mg2+)-ATPase activity in rat myometrial plasma membranes. Journal of Biological Chemistry, **257**:10687–10693.

Sorond, F. A., Lipsitz, L. A., Hollenberg, N. K., &Fisher, N. D. L. (2008). Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans. Neuropsychiatric Disease and Treatment, **4**:433.

Subhashini, R., Mahadeva, R. U. S., Sumathi, P., &Gunalan, G. (2010). A comparative phytochemical analysis of cocoa and green tea. *Indian Journal of Science and Technology*, **3**:188–192.

Tarka, S. M., Applebaum, R. S., &Borzelleca, J. F. (1986). Evaluation of the perinatal, postnatal and teratogenic effects of cocoa powder and theobromine in Sprague-Dawley/CD rats. *Food and Chemical Toxicology*, **24**:375–382.

Taubert, D., Roesen, R., &Schömig, E. (2007). Effect of cocoa and tea intake on blood pressure: A meta-analysis. Archives of Internal Medicine, **167**:626–634.

Tchoumbougnang, F., Amvam, Z. P. H., Dagne, E., &Mekonnen, Y. (2005). In vivo antimalarial activity of essential oils from *Cymbopogoncitratus* and *Ocimumgratissimum* on mice infected with *Plasmodium berghei*. *Planta Medica*, **71**:20–23.

Togashi, K. (2007). Uterine contractility evaluated on cine magnetic resonance imaging. In: Annals of the New York Academy of Science, **1101**:62–71.

Togashi, K., Kawakami, S., Kimura, I., Asato, R., Takakura, K., Mori, T., &Konishi, J. (1993). Sustained uterine contractions: a cause of hypointense myometrial bulging. *Radiology*, **187**:707–710.

Tognolini, M., Barocelli, E., Ballabeni, V., Bruni, R., Bianchi, A., Chiavarini, M., &Impicciatore, M. (2006). Comparative screening of plant essential oils: Phenylpropanoid moiety as basic core for antiplatelet activity. *Life Science*, **78**:1419–1432.

Touwaide, A., &Appetiti, E. (2015). Food and medicines in the Mediterranean tradition. A systematic analysis of the earliest extant body of textual evidence. Journal ofEthnopharmacology, **167**:11–29.

Usmani, O. S., Belvisi, M. G., Patel, H. J., Crispino, N., Birrell, M. A., Korbonits, M., Korbonits, D., &Barnes, P. J. (2004). Theobromine inhibits sensory nerve activation and cough. *FASEB Journal*, **19**:231–233.

Wood, G. A. R., &Lass, R. (2008). Cocoa.4th Edition, John Wiley and Sons.

Wray, S. (2007). Insights into the uterus. *Experimental Physiology*, **92**:621–631.

Wray, S., &Arrowsmith, S. (2012). Uterine smooth muscle. *Fundamental Biology and Mechanisms of Disease*, **2**:1207–1216.

Wray, S., Jones, K., Kupittayanant, S., Li, Y., Matthew, A., Monir-Bishty, E., Noble, K., Pierce, S. J., Quenby, S., &Shmygol, A. V. (2003). Calcium signaling and uterine contractility. Journal of the Society of Gynecological Investigation, **10**:252–264.

Wray, S., Kupittayanant, S., Shmygol, A., Smith, R. D., Burdyga, T. (2001). The physiological basis of uterine contractility: a short review. Experimental Physiology, **86**:239–246.

Zielinsky, P., Martignoni, F. V., &Vian, I. (2014). Deleterious effects of maternal ingestion of cocoa upon fetal ductus arteriosus in late pregnancy. Frontiers in Pharmacology, **5**:281.