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Full Length Research Article

ASSESSMENT OF CONTRACTILE EFFECT OF *TREMA ORIENTALE* (CANNABACEAE) ROOT BARK EXTRACTED WITH METHANOL ON UTERUS OF NON-PREGNANT RAT

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ABSTRACT
Charcoal-tree, *Trema orientale* (L.) Blume (Cannabaceae) (syn. *T. orientalis*) has a range of uses in African traditional medicine such as the treatment of respiratory ailments, gonorrhea, yellow fever, and toothache, while the root bark is used traditionally to induce labour. This study evaluated the possible contractile effect of methanol (MeOH) crude extract of the root bark of *T. orientale* on the isolated uterus of oestrogenised non-pregnant female rats suspended in physiological salt solution (PSS). The effect of MeOH extract (0.2-4.0 mg/mL) on spontaneous uterine tissue contraction in physiological salt solution (PSS) was determined. Similarly, 1.0 mg/mL extract was tested on uterine tissues pre-contracted with oxytocin (6.7 µg/mL) in PSS and in the calcium-free medium. Finally, tissue was precontracted with high KCL concentration (80 mM, 1 mg/mL) followed by adding extract (10 mg/mL). Amplitude and frequency of contraction were measured in all cases. Extract elicited dose-dependent increases in amplitude (0.347 – 1.252 gr) and frequency (8 – 19 peaks/min) of spontaneous uterine contraction. For the oxytocin pre-contracted uteri in normal PSS and calcium-free-PSS, as well as high KCL concentration-induced contraction, decreases in amplitude and frequency of contractions were recorded, suggesting the plant did not potentiate the effects of these agonists. *Trema orientale* root bark methanol extract demonstrated tocolytic properties on a preliminary basis, not contractile.

Keywords: *Trema orientale,* root bark, contractile effect, rat uterus, methanol extract

INTRODUCTION

Trema orientale (L.) Blume (Cannabaceae) (syn. *T. orientalis*) is universally distributed in tropical and warm temperate climates ranging from South Africa through the Middle East, the Indian subcontinent up to China and Australia (Niranjan *et al*., 2023). It is employed in African traditional medicine as a vermifuge, bark and leaf decoctions are used as inhalation, drink, lotion or gargle as remedy for coughs, sore throat, asthma, bronchitis, toothache and gonorrhea (Adinortey *et al*, 2013; Niranjan *et al*., 2023; Appau *et al*., 2024). *T. orientale* is commonly used to facilitate childbirth in both Tanzanian (Shangali *et al*., 2008) and Ivorian (Koman *et al*., 2021) traditional ethnomedicines. Various bioactivity reports including antibacterial, antidiabetic, anti-inflammatory, anticancer, antiulcer and antipyretic, were documented in reviews by Parvez *et al.* (2019), Niranjan *et al* (2023) and Appau *et al* (2024) for *T. orientale*.

Reviews of literature on plants used to induce labour during pregnancy in Sub-Saharan Africa such as *Abelmoschus esculentus Ricinus communis, Pouzolzia mixta* and *Fadogia ancylantha* (El Hajj and Holst, 2020) and elsewhere, for example *Aspilia mossambicensis* (Tanzania), *Clivia miniata* (South Africa) and *Excoecaria cochinchinensis* (Thailand) (Gruber and O'Brien, 2011) have been published. In addition, the uterotonic effect of *Psidium guajava* stem bark was recently reported (Gbolade *et al*., 2023). Furthermore, Alade *et al.* (2018) have documented folkloric labour inducing practices with medicinal plants, including *T. orientale* in Bayelsa State, Nigeria. Among the nine traditional uterotonic plants from the northern and southern Nigeria investigated at cellular level, *Commelina africana, Sida corymbosa, Duranta repens, Vernonia amygdalina* were found to be highly active (Attah *et al*., 2012). Other less active plants were: *Calotropis procera, Hyptis suaveolens, Ocimum gratissimum, Saba comorensis* and *Sclerocarya birrea.* Furthermore, bioactive tocolytic compounds including 1,3 dilinolein and 3-O-glycerol monoacetate have been isolated from only few Nigerian plants such as *Ficus exasperata* (Bafor *et al*., 2013). They are primarily useful in treating preterm labour.

Root decoction of *T. orientale* is taken orally to facilitate childbirth in Tanzanian (Shangali *et al*., 2008) and Cote d'Ivoirean (Koman *et al*., 2021) ethnomedicines, but its pharmacological potential on isolated animal uterus has not been previously reported. We therefore investigated the methanol extract of the root bark of this folkloric labour-inducing plant for possible uterotonic effect in non-pregnant female rats, and herein report our findings.

MATERIALS AND METHODS

Drugs and reagents

Stilboesterol and oxytocin (Sigma-Aldrich, USA); methanol, NaCl, $NaClO_3$, KCL, $CaCl_2$, $MgCl_2$, $NaHCO_3$ and D-glucose (BDH Chemicals, England).

Plant collection and extraction

Root bark peels of *T. orientale* were harvested from Forestry Research Institute of Nigeria garden, Ibadan in Oyo State, Nigeria in January 2022, and authenticated (voucher no. IUO/17/146) at the Department of Pharmacognosy herbarium, Igbinedion University Okada (IUO). Bark peels were cut into small pieces, air-dried for seven days on concrete floor and grounded into coarse powder using a locally fabricated grinder. Powdered plant (1 kg) was exhaustively macerated with absolute methanol (MeOH), filtered, and extract concentrated on electric water bath (40^{o}C). The extract had a total yield of 1.29 % residue and it was refrigerated at 4^{o}C until needed.

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Experimental animals

Healthy non-pregnant female albino rats (120–150 g) were utilized for this study. They were housed under standard conditions (27±5 °C and natural light and dark cycles) in the Central Animal House, University of Benin, and fed with standard animal pellets (Bendel Foods and Flower Meal, Edo State, Nigeria) and water *ad libitum.* Animals were handled in accordance with the Public Health Service policy on humane care and use of Laboratory Animals (National Research Council of the National Academies, 2011).

Preparation of physiological salt solution and uterine tissue

The biological assay was carried out in Pharmacology laboratory, University of Benin, Edo State, Nigeria. Firstly, the physiological salt solution (PSS) was prepared according to Ijioma *et al*. (2020) such that 1 L contained: NaCl (9g), KCl (0.42g), $CaCl2.2H_2O$ (0.06g), NaHCO3 (0.5g), and Glucose (0.5g).

This experiment was performed using Bafor *et al* (2019) formula after an initial approval of the Animal Ethics Committee of College of Pharmacy, Igbinedion University Okada (IUO/ETHICS/024). Female rats previously primed with stilboesterol (1 mg/kg) for 24 h were used for the study. Animals were humanely killed by cervical dislocation and the uterine horns were immediately removed and placed in a petri dish containing previously warmed and aerated PSS. Connective and adhering tissues were removed from the isolated uterus and one horn was dissected in half to obtain a segment of the uterine horn of approximately 1–2 mm in length. Uterine segment obtained was mounted in a warmed tissue organ bath (10 mL) maintained at 37°C and containing aerated PSS. Organ bath was connected to an isometric force transducer (7003E- Ugo Basile, Varise, Italy) linked to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (Ugo Basile, Varese, Italy). The tissue was equilibrated under resting tensions of 4.90 mN for 30–45 min or till regular contractions were obtained.

Determination of uterine contractility

Varying doses (0.2-4.0 mg/mL) of MeOH extract based on routine experimentations in our laboratory, were added to the organ bath containing isolated uterine tissue suspended in PSS to obtain concentration-response relationships (Bafor *et al.,* 2019). Effects of 1.0 mg/mL extract on spontaneous tissue contraction on tissues pre-contracted with oxytocin (6.7 µg/mL), and high KCL concentration (80 mM, 1 mg/mL), were similarly determined. Concentrations used were previously determined in our laboratory constituting the total effect of the extract. Each concentration was allowed a contact time of 5 min before measuring the amplitude and frequency of contraction.

Determination of effect of extract in Ca^{2+}-free medium

In this experiment, calcium in the PSS was substituted with ethylene diamine tetra-acetic acid (EDTA) (Bafor *et al.,* 2019). Uterine tissue was initially equilibrated for 30 min with former PSS and replaced with EDTA (1 mM). Tissue was then equilibrated in this Ca^{2+}-free solution for 3–5 min (it was essential that contractions were not totally diminished during the experiment to allow for measurements). After equilibration, oxytocin (6.7 µg/mL) was added and a contact time of 5 min was allowed before adding extract (1 mg/mL). A contact time of 5 min was allowed for each sample before measuring amplitude and frequency of contraction.

RESULTS

Addition of MeOH extract to isolated rat uterus at 0.2 – 4.0 mg/mL (Plate A: Fig. 1A) resulted in dose-dependent increases in amplitude from the basal value of 0.347 gr at 0.2 mg/ml to peak at 1.252 gr (260.8% increase) at the highest concentration of 4 mg/ml in the spontaneous contraction model (Plate A: Figures 1B). Corresponding frequency data were 8 peaks/min and 19 peaks/min (137.5% increase) (Fig. 1C), respectively.

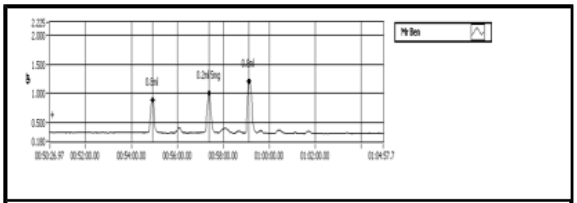


Figure 1A: Recording chart of methanol extract of *Tyrema orientale* (TO) on spontaneous contraction of non-pregnant rat uterus

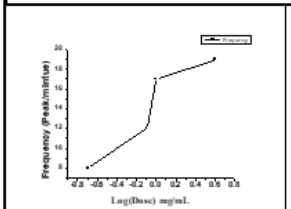


Figure 1C: Effect of methanol extract of TO on frequency of spontaneous contraction of non-pregnant rat uterus

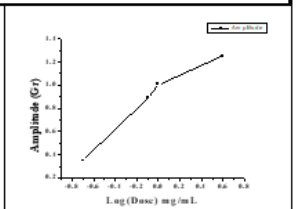


Figure 1B: Effect of methanol extract of TO on amplitude of spontaneous contraction of non-pregnant rat uterus

PLATE A: Effect on *Trema orientale* on spontaneous contraction of non-pregnant rat uterus

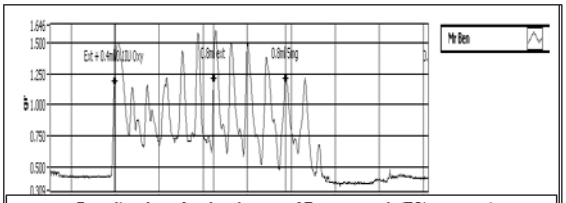
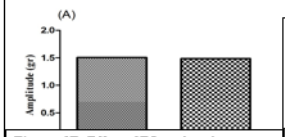


Figure 2A: Recording chart of methanol extract of *Trema orientale* (TO) on oxytocin induced uterine contraction in normal medium



Figures 2B: Effect of TO methanol extract on amplitude of oxytocin-induced uterus in normal medium

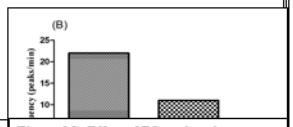


Figure 2C: Effect of TO methanol extract on frequency of oxytocin-induced uterus in normal medium

PLATE B: Effect of *Trema orientale* on oxytocin-induced uterine contraction in normal medium

From this study, it was found that *T. orientale* extract (1 mg/mL) had no appreciable effect on contraction amplitude (1.486 gr, 1.39% reduction) in the oxytocin-pretreated uterus experiment (Plate B: Fig. 2B) in normal PSS. However, it inhibited frequency by 54.54% (from 22 to 11 peaks/min) (Plate B: Figures 2C). Exclusion of calcium ions from the medium (Plate C: Fig 3A) and with the same concentration of extract, led to 16.90% inhibition in contraction amplitude in the uterus pretreated with oxytocin (6.7 µg/mL) (1.505vs1.250 gr,) (Plate C: Fig 3B).

In the high KCL (80 mM, 1 mg/mL) concentration experiment (Plate D: Fig 4A), extract was found to produce 19.90% inhibition of contraction amplitude (1.321vs1.058 gr) (Fig 4B).

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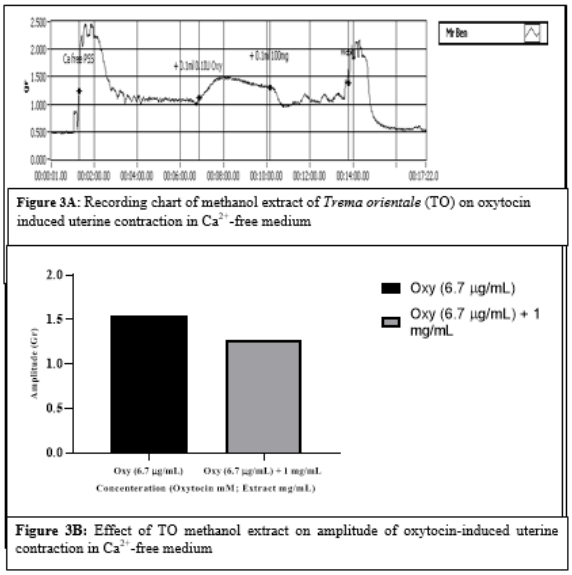

Figure 3A: Recording chart of methanol extract of *Trema orientale* (TO) on oxytocin induced uterine contraction in Ca^{2+}-free medium

Figure 3B: Effect of TO methanol extract on amplitude of oxytocin-induced uterine contraction in Ca^{2+}-free medium

PLATE C: Effect of *Trema orientale* on oxytocin-induced uterine contraction in Ca^{2+}-free medium

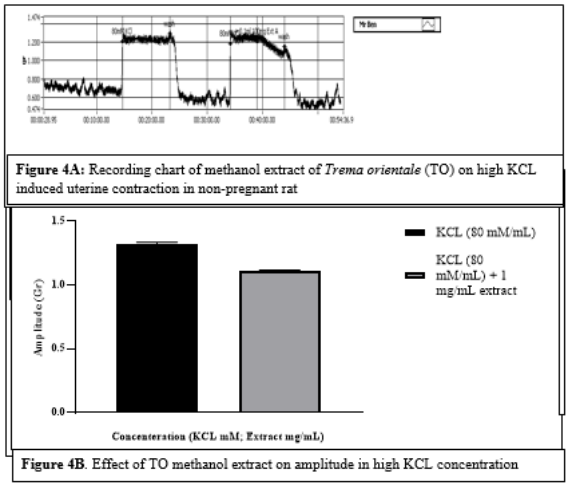

Figure 4A: Recording chart of methanol extract of *Trema orientale* (TO) on high KCL induced uterine contraction in non-pregnant rat

Figure 4B. Effect of TO methanol extract on amplitude in high KCL concentration

PLATE D: Effect on *Trema orientale* on high KCL concentration-induced uterine contraction

DISCUSSION

The observed response of *T. orientale* extract on uterine contraction in normal PSS is in consonance with published information on some other medicinal plants (Gruber and O'Brien, 2011; Kupittayanant *et al*., 2014; Gbolade *et al*, 2022), and is indicative of uterotonic potential. It is also possible that *T. orientale* extract acted by a similar mechanism of action to oxytocin which stimulates myometrial contractility and promotes the expression of labour-associated genes. Kupittayanant *et al*. (2014) postulated mechanism for spontaneous contraction of uterine smooth muscle to involve inhibition of K^+ channels and sarcoplasmic reticulum Ca^{2+} ATPase, leading to release of prostaglandins, while Watcho *et al* (2011) suggested the release of prostaglandins as a key factor.

Inhibition of contraction frequency in normal PSS medium may indicate tocolytic action by the extract. Suppression of uterine contraction in calcium-free medium indicates possible relevance of Ca^{2+} (Rezaeizaseh *et al*., 2016; Carreiro *et al*., 2017) in uterine contractility. According to Bafor *et al.* (2019), intracellular Ca^{2+} contributes to uterine contractility. Our findings in this regard possibly suggests a different mechanism of action on uterine smooth muscle by *T. orientale* extract compared with standard agonist, oxytocin. This agrees with reports of other workers (Bafor *et al*., 2019; Carreiro *et al., 2017*). Ability of medicinal plants to inhibit oxytocin in Ca^{2+}-deficient PSS is suggestive of non-specific uterine relaxant activity (Razaeizadeh *et al*., 2016). Apart from *T. orientale* investigated in this study, other plant-based tocolytic agents such as *Barteria fistulosa, Napoleonaea vogelii, Spondias mombin, Euphorbia convolvuloides* and *Ceiba pentandra* have been reportedly used in the traditional herbal recipe of Southeast Nigeria (Ijioma *et al*., 2020). Similarly, *Ficus capensis* (Owolabi *et al*., 2009) and *Justicia flava* (Bafor *et al*., 2019) have been found to relax rat uterine smooth muscle pre-contracted with oxytocin *in vitro* suggesting tocolytic action.

The action of the extract in antagonising KCL-induced contraction possibly points to the fact that *T. orientale* acted by a mechanism different from that of KCL. This inhibition may further strengthen the tocolytic proposal for *T. orientale*, and agrees with reports of other workers on certain plant extracts (Carreiro *et al*., 2017; Bafor *et al,* 2019). High KCL is believed to act by voltage-gated calcium channels leading to sustained tissue depolarization (Bafor *et al.*, 2019).

Certain phytoconstituents such as palmitic and oleic acids (Ijioma *et al*., 2020), and flavonoids (Carreiro *et al.,* 2017) have been reported to produce inhibitory effects on the rat's uterine smooth muscle tissue. Isolation of bioactive tocolytic compounds is not frequently encountered in literature. However, Rezaeizadeh *et al* (2016) have reviewed uterine relaxant effects of plants, and documented limited isolation of bioactive tocolytic compounds predominantly flavonoids and terpenes. Furthermore, Bafor *et al* (2019) reported isolation of lignans and alkaloids as bioactive tocolytic components from the Nigerian *Justicia flava.* Tocolytic agents have been useful in the management of pre-term labour and preventing immature birth (Ijioma *et al*., 2020). Evidence of the presence of some phytometabolites such as saponins, terpenes, flavonoids, tannins and cardiac glycosides in *T. orientale* has been documented (Adinortey *et al.,* 2013; Parvez *et al.,* 2019), and some of these phytoconstituents may be responsible for the observed effects of *T. orientale* root bark MeOH extract on isolated uterine smooth muscle. Involvement of phytometabolites in uterine contractility has been published (Gruber and O'Brien, 2011). Hence, root bark MeOH extract of *T. orientale* in this study may be of value in this direction.

Conclusion and Recommendation

Trema orientale root bark investigated in this study for the first time on a preliminary scale, produced positive effects on spontaneous contraction but inhibited agonist-induced contractions in the non-pregnant rat uterus *in vitro*. Tocolytic potential rather than inducer of labour as used in traditional medicine, is thus proposed. Further detailed study and characterization of the bioactive tocolytic components in the MeOH extract will form the focus of future research.

Conflict of Interest: Authors have declared that no competing interests exist in this work

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