Ex vivo Inhibitory Activity of the Ethanol Root Extract of Senna occidentalis (Labaceae) on Isolated Rat Uterus

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ABSTRACT
The ex vivo activity of the ethanol root extract of Senna occidentalis (Labaceae) on isolated rat uterus was examined in order to determine its potential in the therapy of uterine related pathologies. The ethanol root extract of S. occidentalis was investigated on the isolated uterus of rats primed with diethyl stibnoestrol (0.1 mg/kg) 24 h prior to the experiment. The extract (2.5 and 7.5 mg/mL) effect on ACh (0.1 – 1.0 µg/mL)– induced contractility, in the presence of atropine (0.12 µg/mL), propranolol (8 µg/mL), CaCl$_2$ (in Ca$^{2+}$-free medium) and in the presence of 100 mM KCl was examined. The extract significantly inhibited ACh-induced uterine contractions ($P<0.05$) and CaCl$_2$-induced uterine contractions (in Ca$^{2+}$-free medium) ($P<0.05$) in a non-competitive but concentration-dependent manner. A rightward shift of the concentration-response curve was observed in all cases. However, a bell- shaped concentration-response curve was observed for CaCl$_2$. The inhibitory effect of the extract on ACh-induced uterine contractions was unaffected by propranolol. The extract (0.1 - 0.4 mg/mL) also inhibited KCl-induced uterine contractions. The root extract of S. Occidentalis was shown to inhibit agonist-induced uterine contractions probably through interaction with voltage-operated calcium channels.

Keywords: Acetylcholine; Calcium; Propranolol; Ex vivo uterine activity; Senna occidentalis; Uterus

INTRODUCTION
Maternal morbidity and mortality due to female reproductive health disorders are serious concerns. Understanding the roles of drugs or natural products on uterine contractility will therefore contribute to the provision of useful information toward the improvement of female reproductive health in general (Lawn et al., 2006). Reproductive health issues in Nigeria are still commonly managed using herbal medicines. A large proportion of Nigerians in the rural areas at some stage in their life turn to traditional or ethno-medicinal and alternative health care systems due to the accessibility, availability, affordability and inherent trust in this method (Fakeye et al., 2014). Research into medicinal plants has thus soared through the years in the search for an efficacious means of combating reproductive health issues.

Senna occidentalis (Labaceae) also known as coffee Senna is a woody annual herb which grows to a height of about 0.5 - 2.0 m (Yadav et al., 2010). It is found native to the tropical and subtropical regions of the Americas, though globally distributed. It is distributed also in Australia, Southern and Eastern USA, Western and Eastern Africa (Yadav et al., 2010). The seeds of the plant are used as a coffee substitute (Bruere, 1942). The plant is known as ‘Albarka’ in Hausa. Different parts of the plant are popularly used traditionally for several ailments which includes as an analgesic, antibacterial, anti-inflammatory, laxative and vermifuge (Yadav et al., 2010). Toxicity studies on the aerial parts, leaves, and roots of S.occidentalis in mice doses of 250 and 500 mg/kg administered orally did not demonstrate any toxic effect or cause mortality (Aragão et al., 2009). The ethanol root extract of the plant was previously investigated and found to possess antifertility effects and weak oestrogenic activities (Govindaraj et al., 2009). However, no study has been performed on the direct effects of the ethanol root extract of S. occidentalis on uterine contractility. In the non-pregnant uterus, the myometrium (smooth muscles of the uterus) is responsible for the contractions that occur during menstruation (primates) or oestrous (mammals) and is very often responsible for the cramping observed referred to as dysmenorrhoea (Togashi, 2007). In this state, the myometrium is involved in a uterine contractility that supports and contributes to the endometrial sloughing that
occurs during menstruation (Bulletti et al., 2000). Changes in female steroid hormones released during this time also act to control the order of myometrial activity (Wray and Noble, 2008).

Uterine contractility therefore constitutes an important parameter of female reproductive health. Contractions occurring in antegrade fashion and transmitting from the fundus towards the cervical end of the uterus, is necessary for emptying or discharge of uterine content which may be menstrual blood (non-pregnant uterus) or foetus (pregnant uterus) (Lyons et al., 1991). The cervico-fundal contractions also assist in electrolyte retention and sperm transport (Kunz and Leyendecker, 2002). Myometrial retrograde contractions during pregnancy, may contribute to the maintenance of early pregnancies within the uterine cavity (de Vries et al., 1990).

Based on the preliminary study, we hypothesized that the root extracts of *S. occidentalis* exerts weak contractility effects on the isolated uterus as such may possess inhibitory effects instead. The present study was therefore aimed at investigating the effect of the ethanolic root extract of *S. occidentalis* on the isolated myometrium as a possible source of drug for female reproductive health and to additionally determine preliminary mechanism(s) of action.

**MATERIALS AND METHODS**

**Preparation of Plant Material**

Fresh roots of *S. occidentalis* were collected from the uprooted plants located in a forest at *Ekosodin* village, Benin City Nigeria. The plant was authenticated at of the Department of Phytochemistry and Plant Biotechnology, University of Benin, Nigeria. The herbarium number provided was UBHs376.

**Extraction of Plant Material**

The roots were washed to remove soil and extraneous matter and then air-dried under adequate shade for one week. On sufficient drying, the roots were chopped into smaller bits before being ground to powder using an electronic milling machine (Christy Norris, England). The powder was weighed and 2.2 L of ethanol (99.8% v/v) was added to 423g of the powder and macerated for 72 h with regular stirring as previously described (Bafor et al., 2013). The ethanol extract obtained was then filtered using Whatman filter paper and the extract concentrated under pressure using a rotary evaporator set at 40°C. The resulting semi-solid extract was then allowed to dry further in an oven set at 50°C. The dry weight of the extract obtained was 6 g; giving a percentage yield of 1.42 % w/w. The extract was stored in air-tight containers and kept in a refrigerator (4°C) until needed.

**Animals**

Female Sprague-Dawley rats 2-3 months old and within the weight range of 150-220 g were used for the study. Onset of puberty and reproductive age in rats have been reported to begin from 2 months of age (Sengupta, 2013). They were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The rats were housed in white plastic cages at environmentally controlled room temperature of approximately 27-30 ± 1°C and adequate ventilation. The rats were provided with adequate diet and water ad libitum. Adequate hygiene was maintained daily through regular cleaning and removal of faecal matter and leftover feed from the cages. The animals were handled according to Institutional Guidelines for the care and use of animals and also according to the Guide for the Care and Use of Laboratory Animals, Eighth Edition (National Research Council, 2010). Ethical permission was obtained from the Animal ethical committee, Faculty of Pharmacy, University of Benin, Nigeria.

On each day of experimentation, fresh stock solution of the dried extract was made with dimethylsulfoxide (DMSO) and distilled water and subsequent dilutions were made with distilled water. DMSO has been previously reported to have insignificant effects on uterine smooth muscle contractility, at concentrations used in this study (Bafor et al., 2013).
Isolated Tissue Experiments

Diethylstilbestrol constituted in 1:1 ethanol/water solution was administered at a dose of 0.1 mg/kg (Crankshaw, 2001) intraperitoneally as pre-treatment to the rats 24 h prior to isolation of the uterus. This was necessary in order to bring all rats to a state of oestrus, and to institute a state of hormonal homogeneity (Bafor et al., 2013; Crankshaw, 2001). On the day of the experiment, the rats were anaesthetized with chloroform in a gas chamber and ethically sacrificed. The lower abdomen was dissected and the uterine horns were located and rapidly but carefully excised and transferred to a Petri dish containing aerated De-Jalon’s solution according to methods earlier described (Bafor et al., 2010). The horns were separated, freed of connective tissue and adhering blood vessels and cut into segments of about 5 mm in length. The uterine segments were then threaded and mounted in a 10ml organ bath containing continuously aerated De-Jalon’s physiological solution of the following composition in g/5 L: NaCl 45.0, NaHCO₃ 2.5, D-glucose 2.5, KCl 2.1, and CaCl₂·2H₂O 1.32. One end of the tissue was connected to a tissue holder while the other end was connected to an isometric transducer connected to a multi-recorder (Ugo Basile, Italy). The transducer had been previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection with 1.0 g corresponding weight (Veale et al., 1999). The temperature of the organ bath was maintained at 35°C while each tissue was maintained at a resting tension of 1 g. The tissue was then allowed to equilibrate for 30 min prior to drug administration.

Studies on the Effect of Extract on Acetylcholine- induced Uterine Contraction

The effect of the extract on acetylcholine (ACh) - induced uterine contractility was determined. A concentration-response relationship for ACh (0.1 – 1.0 µg/ml) was determined in the absence and presence of extract (2.5 and 7.5 mg/ml). Concentrations were determined from preliminary experiments. The effect of ACh was repeated in the absence and presence of atropine (0.12 µg/ml). Each concentration of extract or atropine was added to the bath 10 min prior to a non-cumulative addition of ACh and the concentration-response relationship observed. After each agonist addition, the tissue was washed and the process repeated.

Studies on the Effect of the Extract on ACh- induced Uterine Contractility in the Presence of Propranolol

A concentration-response relationship for ACh (0.1 – 1.0 µg/ml) was determined in the absence and presence of extract (2.5 and 7.5 mg/ml). Prior to the addition of the extract, propranolol (8µg/ml) was added to the bath 5 min prior to the addition of extract. The extract was then left in contact for 10 min prior to the addition of ACh. After each ACh addition, the tissue was washed and the process repeated.

Studies on the Effect of the Extract on KCl- induced Uterine Contraction

In order to assess possibility of extract interaction with calcium channels, KCl solution (100 mM) was administered to pre-contract the isolated uterine segments as described by Bafor and Okunrobo (2010) with some modification. Briefly, KCl was added, at 100 mM predetermined from preliminary experiments, to the bath and left in contact for 5 min and washed. This was then repeated and to the KCl-induced uterine contraction, the extracts at 2.5 and 7.5 mg/ml were added.

Studies on the Effect of the Extract on CaCl₂- induced Uterine Contractility in Ca²⁺- free medium

In order to confirm the calcium antagonist activity, the following protocols were performed as previously described (Bafor and Okunrobo, 2010; Gilani et al., 2005). The uterine tissue was equilibrated in normal De-Jalon’s PSS and after 30 min, the physiological salt solution (PSS) was replaced with Ca²⁺-free solution containing 0.1mM Ethylenediaminetetraacetic acid (EDTA) and left to equilibrate for another 30 min. After 30 min, the PSS containing EDTA was again replaced with a K⁺- rich, Ca²⁺-free PSS of the following composition (in mM): NaCl 154, KCl50, NaHCO₃ 5.95, D-glucose 2.77, and EDTA 0.1. The new PSS was then left for a
further equilibration time of 30 min after which control concentration-response curves were performed for calcium (0.027 – 5.43 mM) in the absence and presence of extract (1.25, 2.5 mg/ml and 7.5 mg/ml).

Statistical Analysis
Data were presented as mean ± standard error of the mean (S.E.M.). Concentration- response curves were obtained for each experimental model. Statistical analysis was carried out using computer statistical software GraphPad-Prism v.6 (GraphPad Software Inc, CA, USA). One-way analysis of variance (ANOVA) with post-hoc test for linear trend and Student’s t-test were adopted for comparison. Mean percentage responses were determined and compared using unpaired T-test with SPSS. *P<0.05 was considered statistically significant in all cases. Curve fitting was applied (Bafor et al., 2013). Responses of the uterus to the different concentration of the extracts were analysed and expressed as a percentage of the control in mean ± S.E.M. Concentration–response curves were constructed from the data obtained and fitted to the equation:

\[ Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{((\text{LogEC}_{50} - X)/\text{HillSlope})}} \]

Where \( Y \) = response which starts at the Bottom and goes to the Top in sigmoid shape, \( X \) = logarithm of concentration and \( \text{EC}_{50} \) is the concentration that produces half the maximal responses. The average and maximal potencies for frequency and amplitude of contractions were calculated and this was compared after curve fitting. Comparisons were made using one-way repeated measures ANOVA with Bonferroni’s correction for multiple comparison.

RESULTS
The ethanol root extract of Senna occidentalis exhibited a concentration-dependent inhibitory effect at 2.5 and 7.5 mg/ml on ACh-induced uterine contractility (Figure 1). The contractile response to ACh was significantly inhibited by 2.5 and 7.5 mg/ml of the extract (*P< 0.05 and *P<0.001 respectively). This effect was compared to the inhibitory effect of atropine on ACh-induced uterine contraction. Atropine similarly inhibited ACh-induced contractions (Figure 2) and the concentration-response curves in both cases were shifted to the right (Figures 1 and 2). The extract and atropine also significantly increased the \( \text{EC}_{50} \) (P<0.001) of ACh (Table 1).

![Figure 1](image1.png)  
**Figure 1:** Concentration-response curves of ACh in the presence of the extract (2.5 and 7.5 mg/ml). Addition of the extract inhibited the response to ACh, shifting the curve to the right. n = 5 animals; *P < 0.05; **P < 0.001

![Figure 2](image2.png)  
**Figure 2:** Concentration-response curve of ACh in the presence of atropine (0.12 µg/ml). Addition of atropine inhibited the response to ACh, shifting the curve to the right. n = 5 animals; *P < 0.05; **P < 0.001

Table 1 shows the maximum concentrations (\( \text{E}_{\text{max}} \)) and concentrations producing 50% response (\( \text{EC}_{50} \)) of the ACh in the presence of the extract and atropine.
Table 1: Effect of the Extract and Atropine on the E\text{max} and EC\text{50} of ACh - induced Uterine Contractility

<table>
<thead>
<tr>
<th>DOSE</th>
<th>E\text{max}(%)</th>
<th>EC\text{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACH</td>
<td>99.65 ± 0.69</td>
<td>0.47 ± 1.01</td>
</tr>
<tr>
<td>ACH + ATR (0.12 µg/ml)</td>
<td>92.31 ± 16.12</td>
<td>21.38 ± 5.41*</td>
</tr>
<tr>
<td>ACH + EXT (2.5 mg/ml)</td>
<td>81.7 ± 2.45</td>
<td>5.13 ± 5.82</td>
</tr>
<tr>
<td>ACH + EXT (7.5 mg/ml)</td>
<td>51.39 ± 11.89</td>
<td>151.36 ± 3.11**</td>
</tr>
</tbody>
</table>

T = Extract; ATR = Atropine n= 5 animals *P<0.05; **P<0.001

The inhibitory effect of the extract on ACh-induced uterine contraction was not affected by the presence of 8 µg/ml propranolol as presented in Figure 3

Figure 3: Concentration-response curves of ACh and propranolol in the presence and absence of the extract n = 5 animals; *P < 0.05; **P < 0.001

The extract at all concentrations used (0.1 – 0.5 mg/ml) exhibited significant (P<0.05, P< 0.001) inhibition of KCl-induced uterine contractility (Figure 4)

Figure 4: Concentration-response curve of showing the effect of the extract on high KCl (100 mM) – induced uterine contraction. The extract concentration-dependently inhibited KCl-induced contractions. n = 5 animals; *P < 0.05; **P < 0.001.

Effect of Extract on CaCl\text{2}-induced uterine contractility in Ca\text{2+}-free Medium

CaCl\text{2}-induced contractions were also inhibited by the extract in a concentration-dependent manner in Ca\text{2+}-free medium (Figure 5). However, a bell-shaped concentration-response curve was obtained for CaCl\text{2} induced contractions in the presence of the extract (Figure 5).

The extracts inhibited the effect of CaCl\text{2} producing a bell-shaped curve. n = 4 animals.

Figure 5: Concentration-response curve of CaCl\text{2} in Ca\text{2+}-free medium alone and in the presence of extract (1.25 and 2.5 mg/ml).

DISCUSSION

The root ethanol extract of *Senna occidentalis* on rat uterine contractions exerted significant inhibitory effects on ACh-induced contractions of the isolated rat uterus. This may be due to presence of spasmolytic phytochemical constituents within the roots of the plant. Phytochemical analysis previously conducted on *S. occidentalis* reported the presence of anthraquinones, tannins, emodin and chrysarobin (Branco et al., 2011; Yadav et al.,
2010) and these have been reported to possess a relaxant effect on smooth muscles (Akubue et al., 1983; Longanga et al., 2000). Anthraquinones have been specifically reported to decrease spontaneous contractions of rat intestinal and uterine smooth muscles (Odenthal and Ziegler, 1988).

The extract in this study showed inhibitory effects on contractions induced by ACh in a concentration-dependent manner. The manner of inhibitions elicited by the extract however appeared different from that produced by atropine, a muscarinic receptor antagonist (Kumari, Sreetama and Mohanakumar, 2007). In the presence of atropine, an increase in the concentration of ACh would result in the displacement of atropine from its receptor site leading to higher maximal percentage response; hence a shift in the concentration-response curve to the right (Liu et al., 1998). However in the presence of the extract, increased concentrations of ACh failed to elevate the percentage maximum response. This suggests that the inhibitory effect of the extract on ACh-induced contraction occurs via a non-competitive antagonism. The EC_{50} of ACh in the presence of the extract was observed to be greater than that in the presence of atropine, a possible indication that the extract is more potent than atropine at concentration used. It was also observed that in the presence of propranolol, the inhibitory effect of the extract was not attenuated suggesting that the effect of the extract does not again occur via interaction with β-adrenoceptors. The extract was observed in this study to inhibit high KCl-induced uterine contractions in a concentration-dependent manner. It is well known that administration of high KCl to smooth muscles promotes the influx of extracellular Ca^{2+} into the cytosol (Kirpekar and Wakade, 1968). High KCl concentrations stimulate membrane depolarization and this results in the opening of voltage-operated Ca^{2+} channels (VOCCs) thus promoting Ca^{2+} influx (Blaustein, 1975). The Ca^{2+} current obtained in such a manner is of slow onset and long duration and has been reported to be mediated by VOCCs (Blaustein, 1975). It would therefore seem that the extract inhibits VOCCs. Several signalling processes depend on the Ca^{2+} content in the extracellular medium. It was also observed that the extract was found to inhibit CaCl_{2}-induced uterine contractions in Ca^{2+}-free medium. There was also observed an apparent shift to the right in the concentration-response curve of CaCl_{2} in the presence of the extract (Fig. 5). Calcium activated chloride channels have been reported to play a role in the excitability of the myometrium and also promote Ca^{2+} entry through VOCCs (Carl et al., 1996; Hartzell et al., 2005; Jones et al., 2004; Karaki et al., 1982; S Wray et al., 2003). The precise explanation for the bell-shaped response curve observed with CaCl_{2} in the presence of the extract is yet unknown however it is known that a bell-shaped concentration response curve can occur due to enzyme saturation (Szomolay and Shahrrezaei, 2012) and in some cases if the solution has a high affinity activation site and a low affinity inhibition site (Kasai, 1998). This however remains to be further investigated as relates to the activity of S. occidentalis.

CONCLUSION
The findings of this study indicates that the root ethanol extract of S. occidentalis inhibit ACh-induced uterine contractility possibly through interaction with voltage-operated calcium channels.

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