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Uterine relaxant property of the ethanolic root extract of *Cissampelos mucronata*

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Abstract

Objective: To investigate the uterine smooth muscle relaxant property of the root extract of *Cissampelos mucronata* and relate it to its traditional use in the prevention of pre-term labour. **Materials and methods:** Phytochemical and pharmacological screenings were carried out using standard procedures. In addition to investigating the effects of the extract on non-gravid and gravid rat uterus, its effects on contractions induced by known uterine stimulants were assessed. The effects of the extract on the amplitude and frequency of contractions of gravid rat uterus were also determined. With the use of glibenclamide, an ATP-sensitive potassium channel blocker, the effect of the extract on potassium channel opening was studied. **Result:** Phytochemical constituents present in the root include carbohydrates, glycosides, sterols/triterpens, flavonoids, tannins and alkaloids. The extract relaxed the non-gravid rat uterus in a concentration- and time- dependent fashion. It also antagonized contractions evoked by serotonin, oxytocin, acetylcholine and prostaglandin E₂ (known uterine spasmogens). The uterine relaxant effect of terbutaline (a selective β_2 -receptor agonist used as a tocolytic agent) was potentiated by the extract in a concentration-related manner while the contractions induced by propranolol (a non-selective β -receptor antagonist) were inhibited by the extract. The frequency and amplitude of contractions of the gravid uterine strips in the absence and presence of the extract were significantly different ($p < 0.05$). Glibenclamide antagonized the uterine relaxant effect of the extract, an indication of possible participation of potassium channel in the actions of the extract. The contractions evoked by calcium chloride in uterine smooth muscles suspended in Ca²⁺-free K⁺-depolarizing solution were inhibited by the extract, suggesting that the activities of the extract may be non-specific in origin. **Conclusion:** Ethanolic root extract of *C. mucronata* displayed significant ($p < 0.05$) relaxant activity on the isolated gravid and non-gravid rat uterine smooth muscles. The results justify the use of the plant in traditional medicine as a tocolytic (uterine relaxant) agent.

Key words: *Cissampelos mucronata*, Uterine relaxant activity

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1. Introduction

The goal of treatment of preterm labour is to reduce perinatal mortality and decrease the rate of prematurity [1]. Despite the liberal use of tocolytic agents (uterine relaxants) in the recent decade, the incidence of premature delivery has not declined and it has continued to be a therapeutic dilemma for the health-care professionals [1]. Few of the medications available for the treatment of premature labour have proven to be effective for long-term suppression of uterine contractions.

Risks of these drugs are considerable and may be life-threatening [2-6]. Pharmacoeconomic considerations have not been favourable in the prospects of procuring these drugs by patients in developing countries. Consequently, a major priority in obstetric research is the prevention of prematurity [1].

In many parts of the world, the use of plants and plant products have been an integral part of traditional practice in the treatment of preterm labour. Traditional medicine is an important component of Nigerian health care system and plants and herbs belonging to various families and species are used by traditional birth attendants and native healers to prevent premature delivery. Success rates claimed with some of these plants raise considerable hope in the prospect of finding a novel tocolytic agent.

One such plant is *Cissampelos mucronata*, traditionally acclaimed as a potent tocolytic agent in the Eastern parts of Nigeria. The root or leaf macerated in local gin or water is taken orally to prevent premature labour. *C. mucronata* is a climbing shrub that is widespread in dry parts of Africa. The leaves are entire, thickly papery, alternate and about 8 cm long [7] while the root is fibrous in nature.

In our effort to evaluate Nigerian traditional medicines [8-11], a screening study was carried out *in vitro* to evaluate the ethanolic root extract of *C. mucronata* for uterine relaxant property.

2. Materials and methods

2.1. Collection of plant material

Fresh roots of *C. mucronata* were collected in May 2000, from Mr. Goddy Mbonu, a herbalist/traditional birth attendant, in Isuofia. Anambra State, Nigeria. Mr. A.O.Ozioko of the Department of Botany, University of Nigeria, Nsukka (UNN) confirmed botanical identification, and voucher specimen has been deposited in the University Herbarium.

2.2. Extraction

The roots were washed, cut into smaller pieces, air-dried for seven days and reduced to coarse powder using mortar and pestle. About 90 g of the coarse powder was macerated for 24 h in 500 ml of 70% ethanol.

This was filtered and freeze-drying of the filtrate gave a solid yield of 8.13 %. Samples of the dried filtrate were suspended in 3% Tween 85 to derive the appropriate concentrations used in the study.

2.3. Phytochemical analysis

The root was screened for phytochemical constituents using the methods described by Evans [12].

2.4. Animals

Adult female white albino rats (110-170 g) in bred and maintained in the Animal Unit of the Department of Pharmacology and Toxicology, UNN, were used in the study.

They were allowed free access to food (guinea feed PLC, Nigeria) and water prior to the commencement of the experiment. The animals used in compliance to the local ethical standard.

2.5. Effect on non-gravid rat uterus

The non-gravid rat uteri were stimulated into oestrus by pretreating the animals with 0.1 mg/kg of stilboesterol subcutaneously 24 h before use and the isolated tissue preparation was set up using standard procedures [13]. The animals were killed by a blow on the head and exsanguinated. The uterus was isolated and each horn cut open longitudinally into a sheet. Each sheet was cut into two strips.

Each strip was suspended in a 30 ml organ bath containing De-Jalon's solution aerated with 95% O₂ and 5% CO₂ and maintained at 37 ± 1°C. With a resting tension of 0.5 g, contractions were recorded using an isotonic transducer, 7006 (Ugo Basile, Italy), connected to a 2-channel recorder 'Gemini' 7070.

After 60 min equilibration period, responses of the preparation to the extract and standard drugs (prostaglandin E₂, serotonin, acetylcholine, glibenclamide, calcium chloride and oxytocin) were established. The effects of the extract on the responses elicited by the standard drugs were also evaluated.

The activity of the extract against propranolol-induced contractions as well as the extract's action on the uterine relaxant effect of terbutaline were evaluated. The inhibitory effects of the extract on uterine contractions induced by oxytocin and acetylcholine were determined in time-dependent manner.

For the study of the effect of calcium ion on the uterine relaxant activity of the extract, De-Jalon's solution was replaced with Ca²⁺-free, K⁺-depolarizing solution of the following composition (g/L): NaCl 1.58, NaHCO₃ 1.26, KCl 7.46, MgCl₂·7H₂O 0.25 and glucose 1.98.

The uterine strips were washed for 30 min with the calcium ion-deficit depolarizing solution to remove intra- and extra-cellular calcium ions.

Dose-response curves were obtained by non-cumulative addition of calcium chloride and the effect of the extract on the contractile effects of calcium chloride in the uterine strips were investigated.

2.6. Effect on gravid rat uterus

The experiment was set up as described above with strips of gravid rat uterus. The influence of the extract on the spontaneous contractions of isolated gravid rat uterine

strips were determined with respect to the amplitude and frequency of contractions. The initial values (taken as control) were determined at the first 10 min after 60 min equilibration.

Subsequently, the nature of the spontaneous contractions was evaluated for the following 10 min in the presence of the extract (76 µg/ml). Three separate determinations were made in each case and the results compared with the control.

2.7. Statistical analysis

Results were expressed where appropriate as mean ± standard error of mean. Means of the control trials were compared to those of the test trials using Student's *t* - test and results were regarded as significant at P<0.05.

Table 1
Effect of ethanolic root extract of *C.mucronata* on smooth muscle of non-gravid rat uterus.

Extract (µg/ml)	Relaxation (cm)
40	0.38 ± 0.11
80	1.62 ± 0.15
120	1.94 ± 0.17
160	2.37 ± 0.10

n=3; Values are Mean±SEM

3. Results

The result of the phytochemical analysis showed that the root contained carbohydrate, glycosides, sterols/triterpens, flavonoids, tannins and alkaloids. Organoleptic examination indicated that the root tasted bitter with persistent minty taste. The extract concentration-dependently relaxed the isolated non-gravid uterine preparation (Table 1). The extract, 80, 120 and 160 $\mu\text{g/ml}$, potentiated the relaxant effect of terbutaline (100 $\mu\text{g/ml}$) in non-gravid rat uterus in the order of 19.75 ± 1.75 , 72.84 ± 2.56 , and 85.19 ± 1.98 % respectively.

Moreover, the maximal contractions induced by propanolol (13.3 $\mu\text{g/ml}$) was inhibited to the tone of 61.11% by 115 $\mu\text{g/ml}$ of the extract. The extract inhibited the contractions produced by known uterine stimulants such as oxytocin, serotonin, acetylcholine and prostaglandin E_2 in a concentration-related manner.

The concentration of the extract producing 50% inhibition of the maximal contractions produced by the spasmogens (ID_{50}) is shown in table 2. The table indicates that the extract was most potent at inhibiting contractions induced by oxytocin while that evoked by prostaglandin E_2 was least affected. Glibenclamide, an ATP-sensitive potassium channel blocker, competitively blocked the relaxant effect of the extract (Table 3).

Table 2
Concentrations of the *C. mucronata* extract that inhibited 50% (ID_{50}) of the maximal contractions elicited by different uterine spasmogens.

Uterine spasmogens	ID_{50} value ($\mu\text{g/ml}$)
Oxytocin	56.67
Serotonin	71.33
Acetylcholine	87.66
Prostaglandin E_2	103.00

In addition, the extract was found to inhibit in a time-dependent pattern, the contractions produced by oxytocin and acetylcholine (Table 4).

Maximal contractions produced in the presence of the spasmogens alone were re-established in the presence of 192 $\beta\text{g/ml}$ of the extract by non-cumulative addition of increasing concentration of the spasmogens, pointing to surmountable antagonism. CaCl_2 elicited contractions in non-gravid uterine smooth muscles suspended in calcium ion-deficit K^+ -depolarized solution. These contractions were antagonized by the extract (Table 5).

Additionally, the extract completely blocked the spontaneous rhythmic movement of the isolated gravid rat uterus. The amplitude of contractions of gravid rat uterine strip monitored for 10 min each were 9.0 ± 0.2 cm and 5.7 ± 0.9 cm while the frequency were 14 ± 3 per 10 min and 7 ± 2 per 10 min in the absence and presence of the extract (76.67 $\mu\text{g/ml}$) respectively.

4. Discussion

The results of this study show that the ethanolic root extract of *C. mucronata* contain pharmacologically active substances capable of relaxing smooth muscles of the isolated gravid and non-gravid rat uterus.

Table 3
Percentage inhibition of the relaxant activity of the extract (180 $\mu\text{g/ml}$) by glibenclamide in non-gravid rat uterus

Glibenclamide ($\mu\text{g/ml}$)	Percentage inhibition of relaxation
1.67	20.00 ± 1.21
3.33	46.76 ± 2.43
6.67	66.64 ± 1.76
13.33	85.89 ± 2.23

n=3; Values are Means \pm SEM

Table 4
Time-dependent inhibitory effect of the extract (76.67 µg/ml) on contractions produced by oxytocin and acetylcholine (2.67 µg/ml each) in non-gravid rat uterus.

Time (min)	Percentage inhibition of maximal contractions	
	Oxytocin	Acetylcholine
0.0	30.00 ± 2.15	2.03 ± 0.31
2.5	74.12 ± 3.98	4.40 ± 0.86
5.0	83.63 ± 6.72	60.32 ± 3.54
10.0	94.42 ± 5.68	100.00 ± 0.00
15.0	100.00 ± 0.00	-

n=3; Values are Means±SEM

The ability of the extract to inhibit contractions induced by acetylcholine in the rat uterine smooth muscle probably indicated inhibitory action through the parasympathetic (cholinergic) nervous pathway since the uterus has been shown to be partly innervated through the parasympathetic axis [14]. Serotonin can directly stimulate the smooth muscles of the uterus to contract [15].

Hence, the blockade of serotonin-induced contraction is a likely indication of anti-serotonergic activity. Prostaglandin E₂ causes contraction of the myometrium especially from the late second trimester [16].

On the other hand, oxytocin stimulates both the frequency and the force of contractile activity in uterine smooth muscle [14]. Deriving from these, the inhibitory activity of the extract on the uterine contraction elicited by oxytocin and prostaglandin E₂ may point to inhibitory activity at the respective receptors or blockade in one of the pathways through which they exert their contractile effects.

Furthermore, the extract significantly decreased the amplitude and frequency of rat uterine smooth muscle. Such activities are the desired properties of a good uterine relaxant.

Myometrial relaxation is partly mediated by stimulation of the β₂-receptors. This stimulation ultimately decreases myometrial contractility by decreasing intracellular calcium [17,18]. This may explain the potentiation of uterine relaxant effect of terbutaline (a typical β₂-receptor agonist) by the extract and antagonism of propranolol (non-selective β-receptor antagonist)-induced contractions by the extract.

It is not known whether the observed uterine relaxant effect of the extract was partially as a result of direct effect on µ₂-receptors or due to influence on intracellular calcium concentration.

Potassium channel opening would hyperpolarize the plasma membrane, which in turn would prevent activation of voltage-dependent calcium

Table 5
Effect of increasing concentration of the extract on maximal contractions produced by CaCl₂ (0.04 µg/ml) in non-gravid rat uterus.

Increasing concentration of the extract (µg/ml)	Percentage inhibition
76.67	60.2 ± 3.6*
115.00	77.4 ± 2.4*
153.34	100.0 ± 0.0*

n=3; * (P<0.05) (maximal contraction induced by CaCl₂ vs contraction produced by the presence of ext.)

channels by spasmogens and lead to inhibition of tension development [19]. Glibenclamide, a blocker of ATP-sensitive potassium channel [19], was found to antagonize the uterine relaxant effect of the extract.

Consequently, it is possible that the uterine relaxant effect of the extract may be partly attributed to enhancement in potassium channel opening events. Inhibition of calcium-induced contractions in potassium-depolarized tissues is commonly accepted as a test for agents that act non-specifically by inhibiting calcium ion participation in excitation–contraction-coupling process [20-22].

Therefore the inhibitory activity of the extract against contractions induced by CaCl_2 in non-

gravid rat uterine smooth muscle suspended in Ca^{2+} -deficit K^+ -depolarizing solution is an evidence of non-specific uterine relaxant activity.

The ethanolic root extract of *C. mucronata* exhibited potent uterine relaxant property. The activity may be attributed to one or more of the bioactive constituents present in the root. The mechanism(s) responsible for the observed effects has not been elucidated but may most likely be multifactorial.

This suggestion is supported by the efficacy of the extract at inhibiting the uterine contractile activity of various spasmogens acting through distinct receptors. The results confirm the local use of this plant as a tocolytic agent.

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