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# Aqueous Leaf Extract of *Cissampelos owariensis* (P. Beauv) Menispermaceae Exerts Tocolytic Activity on Isolated Gravid Rat Uterus

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ABSTRACT

*Cissampelos owariensis* commonly known as velvet leaf is a dioecious climbing plant and belongs to the family Menispermaceae. It is traditionally acclaimed to be a potent herb used in the management of threatened abortion. The present study was designed to evaluate the tocolytic activity of the aqueous leaf extract of *Cissampelos owariensis* (P. Beauv.) on the isolated gravid uterine smooth muscles of albino rats. The effect of the aqueous leaf extract of *C. owariensis* on isolated gravid rat uterus was determined *in vitro* using De Jalon's method of bioassay. PowerLab 26T connected to a force transducer was used to record the contractility of the isolated gravid rat uterus when treated with different concentrations (40 µg, 80 µg, 1 mg, 2 mg, 4 mg and 8 mg/mL) of the extract while the inhibitory effect of the extract was ascertained with oxytocin. Preliminary screening and acute toxicity of the extract (2500, 5000 and 10000 mg/kg) were also conducted. The extract of *C. owariensis* displayed significant ($p < 0.05$) uterine relaxant activity by inhibiting the spontaneous contractions of the isolated gravid rat uterus and reduced the oxytocin-induced contractile activity on the smooth muscles. Thus, the results suggest that the plant extract possesses tocolytic activity that justifies its use in folkloric medicine for the treatment of threatened abortion.

***Keywords:*** *Cissampelos owariensis*, *in vitro*, tocolytic, gravid uterus.

## Introduction

Ethnopharmacological survey has been found to be one of the reliable approaches to natural and synthetic drug discovery and production.[1] Natural products and their derivatives represent over 50% of all drugs in clinical use worldwide.[2] In African traditional societies, traditional medicines are most often prepared as crude extract of medicinal plant organs (leaves, roots, flowers, bark, etc.) and used to fight many illnesses among which is infertility.[3] Traditional medicine played a crucial role in combating multiple and complex conditions affecting Africans. Because of its popularity, accessibility and affordability, more than 80% of the people in the region continued to rely on it for their health-care needs.[4]

Many modern drugs have their origin in ethnopharmacology.[5] Indeed, traditional medicine is a potential source of new drugs and a source of cheap starting products for the synthesis of known drugs. Some examples include reserpine from *Rauwolfia* species, vinblastine from *Catharanthus roseus* or the discovery of a contraceptive in *Montanoa tomentosa* (Cerv.). [6]

Preterm labour (miscarriage) is defined as a clinically recognized pregnancy loss before the 20th week of gestation.[7]Blood loss during the first half of pregnancy is usually termed threatened miscarriage, and this may or may not be accompanied by pain. Once the cervix begins to dilate, miscarriage is inevitable. Threatened miscarriage may present with minor bleeding to profound fatal shock in severe cases.[8]

Plants and herbs belonging to various families and species are used by traditional birth attendants and related practitioners to prevent premature delivery.[9] Tocolytic agents reduces the tone of myometrium and opposes contraction. Clouse *et al*,[10] demonstrated that relaxation of rat myometrium is mediated by $\beta_2$-adrenoreceptors, likewise, $\alpha_1/\beta$ – adrenoreceptors ratio determines not only the spontaneous motor activity of the rat uterus, but also the potency of the agents with tocolytic effect.[11] It has been proven that $\beta_2$-adrenoreceptor stimulants decrease the intensity and frequency of uterine contractions, and hence reduce the risk of miscarriage. According to the Cochrane Database of Systematic Reviews, an understanding of the mechanism of excitation–contraction in myometrium provides a strong ground for predicting tocolytic targets.[12] When translating this knowledge into producing therapeutic agents, several concerns are raised which focus around specificity and efficacy.[13]

*Cissampelos owariensis* is a dioecious liana (any long stemmed, woody vine that is rooted in the soil and climbs or twines around other plants) and belongs to the family of the flowering plants, Menispermaceae, which comprises over 450 species located in the Tropical climate.[14,15] In Nigeria, it is mostly found in parts of South-western and North-eastern Nigeria and has been used extensively in African folkloric medicine.[16]

It is commonly known as velvet leaf and locally known as "Ewe-jokoje" in Yoruba, "Damal gwaraaji" in Hausa, "Ebubueka enwe" in Ukwuani and "Ayovwe" in Urhobo.[17,18] The infusion of the bitter rhizome, leaves or stems have been used to cure gastrointestinal complaints such as diarrhoea, dysentery, colic, intestinal worms, and digestive complaints, and also urogenital problems such as menstrual problems, venereal diseases and infertility. Women of Benin City in Nigeria use the leaves to promote foetal growth.[19] The leaf sap is used as nose or eye drops to cure headache while the rhizome is sometimes used in the preparation

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**Figure 1:** Leaves of *Cissampelos owariensis*.

of arrow poison. A decoction of crushed leaves is used in veterinary medicine to treat diarrhea.[20] *C. owariensis* has been reported to possess antibacterial, antidiarrheal,[16] anti-diabetic effect,[18,21-23] anti-oxidant,[15,17] antilipidemic and hepatoprotective activities.[18] It has also been proven to have anti-fungal and anti-tuberculosis activity.[24] Olutayo *et al*,[15] studied the phytochemistry of *C. owariensis* and isolated two sesquiterpenes – owarienone and cissampelone, from the dried root extract of the plant, and these compounds showed antimicrobial and antiviral activity. In the present study, the aqueous leaf extract of *C. owariensis* is evaluated for its tocolytic activity on isolated gravid rat uterus.

## Materials and Methods

*Plant collection*
Samples of the matured leaves of *C. owariensis* were collected in the early hours of the morning from a residential area in Ejigbo, a suburb of Lagos State, Nigeria in January 2017. The plant material was authenticated by Mr. Kola Oyebanji, a taxonomist at the Department of Botany, University of Lagos with a voucher (LUH/7345) and the specimen was also deposited in the herbarium for future reference.

*Extraction*
The leaves of *C. owariensis* were dried in the shade for a week and finally oven-dried at a temperature of 40ºC for 6 hours before milling and weighed. The powdered leaves were macerated with 80% methanol at room temperature for 72 hours and filtered to obtain crude extract. The crude extract was then concentrated using rotary evaporator at reduced pressure.

*Phytochemical screening*
The powdered leaves and aqueous extract of the *C. owariensis* were screened for phytochemical constituents such as alkaloids, glycosides, flavonoids, tannins, triterpenes, saponins using the methods described by Sofowora.[25]

*Animals*
Twelve (12) adult female (110-120 g) and male (120 and 125 g) albino rats, ten (10) female mice (20-30 g) were procured through suppliers to the Central Animal House of the College of Medicine, University of Lagos, Idi-Araba and were kept in the Animal Unit of the College in accordance with research policy of the University. The animals were maintained in plastic cages, exposed to 12 hours dark and 12 hours light cycle and feed with food (Pelleted Commercial Feed) and water *ad libitum.*

*Acute Toxicity*
Acute toxicity was performed according to the method of Mbianctha *et al*,[26] with slight modifications. Nine mice were randomly selected, divided into three groups and starved overnight. *C. owariensis* extract doses of 2500 mg/kg, 5000 mg/kg and 10000 mg/kg were orally administered to each group respectively. The animals were kept under strict observation for up to 7 days and monitored for signs of toxicity and mortality.

*Determination of Tocolytic Effect*
To determine the tocolytic activity of the aqueous leaf extract of *C. owariensis*, De Jalon's method of bioassay on rat uterus was adopted. Two groups of matured virgin female rats (n = 4 per group) weighing 110-120 g were employed for the test. The rats were left overnight with males of proven fertility in the ratio of one male to four females. Vaginal smear was taken very early in the morning (7:00 am), washed with normal saline solution using a micropipette. The wet smear of the vaginal washing was dropped on a slide and was examined microscopically for the detection of spermatozoa. Day one of pregnancy was confirmed by the presence of spermatozoa in the vaginal smear.

Two female albino rats of 3 days gestation at a time, were sacrificed by cervical dislocation and the uterine horns were removed and placed in a dish containing physiological De Jalon's solution (NaCl 9.0 g, 10% KCl 4 2 mL, 1M $CaCl_2$ 2.7 mL, $NaHCO_3$ 5 g, Glucose 5 g, all made to 1 L in distilled water). Four uterine strips of approximately 1.5-2.0 cm long were cut, trimmed of mesenteric fat and separately mounted in a thermostatically regulated organ bath containing the solution to check for one with highest spontaneous contractility (labouring uteri). The organ bath (20 mL capacity) was maintained at 37ºC and aerated with 95% $O_2$ and 5% $CO_2$. The uterine strips were tied with a string suspended by a thread to a force transducer connected to a PowerLab 26T (AD Instruments, United Kingdom) to record isometric contractions. A tension of 1 gram was applied to the tissue and allowed to equilibrate for 30 minutes before starting the test. After equilibration, spontaneous control contraction of the uterus without any drug or extract was carried out to check for the contractility pattern. Non-cummulative doses of the extract (40 µg, 80 µg, 1 mg, 2 mg, 4 mg and 8 mg/mL) and oxytocin (standard drug) were tested and the responses observed. The effects of the extract on uterine contractions induced by 0.1 IU/mL oxytocin were determined.

*Statistical analysis*
Results were expressed as the mean ± standard error of mean. The statistical significance of the differences between means was assessed by one-way analysis of variance (ANOVA) using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California, USA). Data were considered significant at $P<0.05$.

## Results and Discussion

The present study was carried out to validate the folkloric claim of *C. owariensis* (P. Beauv.) employed in the treatment of threatened abortion. The results of the investigation show that the aqueous leaf extract of *C. owariensis* contain pharmacologically active substances capable of relaxing the smooth muscles of the rat uterus. The preliminary phytochemical analysis showed that the extract of *C. owariensis* contained secondary metabolites including saponins, phenols, glycosides, flavonoids, tannins, alkaloids, sterols and triterpens, but devoid of anthraquinones as shown in table 1. These secondary metabolites are known to exhibit various biological activities. Acute toxicity testing is very essential in chemicals and drug screening because it provides an insight into the margin of safety of a drug or plant extract. The acute toxicity study of the aqueous leaf extract of *C. owariensis* suggest it is safe (non-toxic) in rats, even at dose of 10 000 mg/kg because no visible signs of toxicity or mortality was observed.

The effect of the aqueous leaf extract of *C. owariensis* on uterine smooth muscles is consistent with the ethnopharmacological use of the plant as a uterine relaxant. It inhibited the spontaneous contractions of the isolated gravid rat uterus and reduced the contractile activity of oxytocin on the smooth muscles. The ability of the extract to inhibit contractions induced by oxytocin on 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.[27] The uterine strips developed a spontaneous contractile activity using oxytocin which served as a control in order to induce contraction of the rat uterine

muscles. According to Yallampalli *et al,* [28] an L-arginine-nitric oxide-cyclic guanosine monophosphate system is present in the uterus (self-relaxant property), and it may regulate relaxation during pregnancy and induce the lower spontaneous activity of the uterus. The addition of aqueous extract of *C. owariensis* then further decreases the amplitude of contractile activity of the uterus and the basal tonicity, as seen in table 2. Table 3 suggest the aqueous leaf extract of *C. owariensis* is a potent inhibitor of contractions induced by oxytocin, a known uterine stimulant, in a concentration-dependent manner. Since the myometrial contraction is influenced by a variety of physiological mechanisms involving intracellular signaling, calcium, ion channels, cell membrane receptors, peptides, metabolic and neuronal factors and hormones, [29] the activity of the plant extract might be attributed to one or more of the bioactive constituents present in the leaves since it is believed that phytomedicines works synergistically to address underlying imbalances in organ systems and tissue states.

To the best of our knowledge, this is the first study to report that aqueous leaf extract of *C. owariensis* shows a positive smooth muscle relaxant activity in isolated gravid rat uterus. However, another species of *Cissampelos*, (*C. mucronata*, ethanol root extract) have been mentioned to have displayed significant relaxant activity on both the isolated gravid and non-gravid rat uterus.[9]Previous studies showed that uterine relaxant effect of most studied plants might be due to the active compound(s) from the plants extract belonging mostly to the classes of flavonoids and terpenes.[30]

**Table 1**: Phytochemical Constituents of the aqueous leaf extract of *C. owariensis*.

| Constituents | Inference |
|---|---|
| Alkaloid | + |
| Saponin | + |
| Sterol and triterpenes | + |
| Cardiac glycoside | + |
| Anthraquinone | - |
| Flavonoids | + |
| Tannins | + |
| Phenols | + |

Key: Present (+), Absent (-)

**Table 2:** Effect of aqueous leaf extract of *C. owariensis* on uterine muscle of rat.

| Extract (per mL) | Relaxation(g) |
|---|---|
| Control | 0.14 ± 0.01 |
| 40 µg | -0.06 ± 0.003* |
| 80 µg | -0.14 ± 0.009*$^{\#}$ |
| 1 mg | -0.08 ± 0.003*$^{\#\alpha}$ |
| 2 mg | -0.10 ± 0.01*$^{\# \alpha}$ |
| 4 mg | -0.011 ± 0.009*$^{\# \alpha}$ |
| 8 mg | -0.13 ± 0.007*$^{\#\beta}$ |

Data are expressed as Mean ± SEM.*P < 0.05 vs Control,$^{\#}$P < 0.05 vs 40 µg, $^{\alpha}$P < 0.05 vs 80 µg, $^{\beta}$P < 0.05 vs 1 mg.

**Table 3**: Inhibitory effect of aqueous leaf extract of *C. owariensis* on contraction induced by oxytocin on rat uterus.

| Extract (per mL) | Oxytocin Contraction (g) |
|---|---|
| Control | 0.18 ± 0.01 |
| 1 mg | -0.140.01* |
| 2 mg | -0.19 ± 0.01$^{*}$ |
| 4 mg | -0.19 ± 0.01$^{*\#}$ |
| 8 mg | 0.00 ± 0.00$^{*\#\alpha\beta}$ |

Data are expressed as Mean ± SEM.$^{*}$P < 0.05 vs Control,$^{\#}$P < 0.05 vs 1 mg,$^{\alpha}$P < 0.05 vs 2 mg, $^{\beta}$ P < 0.05 vs 4 mg.

## Conclusion

The aqueous leaf extract of *Cissampelos owariensis* (P. Beauv.) showed relaxant activity by suppressing the spontaneous contractions activity on isolated gravid rat uterus and showed inhibitory action against oxytocin-induced contraction. This finding justifies the ethno-medicinal use of this plant as a tocolytic agent because the desired property of a good uterine relaxant is to decrease the amplitude and frequency of uterine smooth muscle. Further investigation is required to isolate the constituent responsible for the uterine relaxant activity of the plant.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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