



Uterine Contractile Activity of Extract of *Icacina trichantha* on Albino Non-Pregnant Rat Uterus

Ebhodaghe F. Okieimen¹, Omonkhelin J. Owolabi², Osaro Iyekowa¹, Eribe M. Jonathan^{1*}¹Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria.²Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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ABSTRACT

The use of medicinal plants to facilitate births and control abortion among rural women is a common practice in Sub-Saharan Africa. *Icacina trichantha* is one of such plants used in Edo and Delta States of Nigeria for the treatment and management of threatened abortion and uterine pain by herbal practitioners. The present study was conducted to determine the uterine contractile activity of *Icacina trichantha* on albino non-pregnant rat uterus.

The powdered plant leaves were extracted with methanol in a soxhlet apparatus. The crude extract was concentrated in a rotary evaporator at 50°C. Phytochemical screening was conducted according to standard methods and contractile activity was determined using non-pregnant female albino rat with the aid of Ugo Basile channel recorder.

Alkaloids, phenolics, eugenols and steroids were present while tannin was absent. The uterine contractile results revealed that the methanol extract of *I. trichantha* produced a significant ($p < 0.05$) decrease in both oxytocin and acetylcholine-induced contractions of the rat uterus.

The activity of the extract was comparable to that of salbutamol and atropine; two positive controls that significantly ($p < 0.05$) relaxed the uterus.

Keywords: *Icacina trichantha*, uterine contraction, phytochemicals, non-pregnant rat.

Introduction

The use of medicinal plants to facilitate birth or protect the young foetus is a common practice in Edo and Delta states of Nigeria. *Icacina trichantha* has been reported to have anti-inflammatory, antioxidants and antimicrobial activities while saponins, chalcones, anthocyanidins have been reported as phytoconstituents.¹

The 'Ibo' tribe of Eastern Nigeria considers the plant to be an aphrodisiac, and they use it on soft tumors.² In most rural communities in Southern Nigeria, the tuber is used extensively by herbalists and traditional doctors in the treatment of constipation, poisoning, to induce emesis, and to treat malaria.³ In the work of Otun,⁴ isolated oil of *Icacina trichantha* has been reported to constitute stearolic acid, a monounsaturated fatty acid which is an acetylenic analogue of oleic acid that has been implicated for the hypotensive effect of olive oils. Ethanol extract of *I. trichantha* had been reported to significantly protect the liver and the kidneys of carbon tetrachloride-poisoned rats.⁵ In another study, *I. trichantha* extract was observed to exhibit significant analgesic effect⁶ and Ferreira corroborated the analgesic activity by implying that the effect is an anti-inflammatory, analgesic, like aspirin or indomethacin, which block the cyclooxygenase pathway of arachidonic acid metabolism.⁷ The research of Umoh⁸ has shown that *I. trichantha*, a root/tuber crop contains antinutritional factors like hydrogen cyanide, soluble and total oxalate, tannin, phytate and alkaloids. While the root of *I. trichantha* is edible, with a strong presence

of alkaloid and benzophenone.⁹ Meanwhile the chemistry of "*Icacina*" has it that 0.08% icacinone and 0.03% Icacinol were isolated from the plant.⁸ *I. trichantha* is also rich in (9βH)-pimarane, 17-nor-(9βH)-pimarane and 17-nor-primarane diterpenoids.¹⁰⁻¹² Recent studies had shown the presence of 12-hydroxyicacinolactone.¹³ The isolates, (9βH)-pimarane and 17-nor-primarane derivatives have indicated cytotoxicity against cancer cell lines.¹⁴⁻¹⁶

Icacina trichantha oliv, (Icacinaceae) is a perennial shrub which grows up to about 2 m above the soil surface. It is native to most part of Nigeria especially in the rainforest zone. The leaves are simple, alternate and broadly-elliptic while the stem is straggling, semiwoody, round in cross-section with soft brown hairs and arises from an underground tuber with soft brown hairs.¹⁷ The plant is locally called 'Ebe-kpowo', 'Ebosan' and 'Ukpasan' in Edo State, Nigeria.¹⁸ According to Gills,¹⁸ the plant part used by most traditional healers is the leaves whose decoction is used to cure mental disorders and relieve pain. The plant is extensively used in the rural areas and thus regarded as a major handy household medicine for emergency treatment; hence, virtually all household have the macerated tuber in ethanol which is stored in corked bottle.¹⁹ The presence of bioactive constituents like saponin and tannins;¹⁸ alkaloids, saponin, phenols and tannins;¹⁹ alkaloids, steroids and cardiac glycosides²⁰ have been reported on the leaves of the plant. While antimicrobial,²¹ antioxidant,¹⁹ and anti-inflammatory activities²² have been reported for the plant leaves. Natural products from most plants are used in pharmaceutical preparations either as crude-extracts, tinctures, pure compounds or analogous compounds from highly active isolated compounds.²³ The use of plants and their derived substances is increasing daily owing to their importance as a potential source for the discovery of new therapeutic agents.

Materials and Methods

Collection of Plant sample

Fresh leaves of *I. trichantha* were collected in Egor Local Government Area of Edo State, Nigeria in June 2017. The plant was identified and authenticated by a taxonomist Dr. Aigbokhan, with herbarium voucher

*Corresponding author. E mail: eribemary@yahoo.com
Tel: +2348066232465

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number (UBHm 0174) deposited in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The fresh leaves were rinsed with distilled water, dried under shade for four weeks and pulverized to a fine powder.

Drugs and Chemicals

The following drugs were used: diethylstilbestrol (Merck), Oxytocin (Rotex Medica), Acetylcholine (Sigma Aldrich) and Atropine (Sisbu Xierkang, Pharm. Co. Ltd).

Extraction

A total of 500 g of the plant material was extracted with a soxhlet apparatus using 1.5 L of methanol (BDH, England) as the solvent. The extract was concentrated in vacuo at 50°C. The extract was stored in a refrigerator at 4°C until needed for the experiment.

Phytochemical Screening

The extract was subjected to preliminary phytochemical screening for the detection of glycosides, saponins, eugenols, tannins, phenolics, terpenoids, steroids, flavonoids and alkaloids.^{24, 25}

Animals

Non-pregnant female albino rats (210-230 g) were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were maintained under standard environmental conditions and had free access to standard rat feeds (Broiler mash) and water. The rats were allowed an acclimatized period of two weeks before the experiment. The animals were handled according to standard guidelines for the use and care of experimental animals.

Acute toxicity test

The acute toxicity of *I. tridaantha* in rat was estimated using the method described by Lorke.²⁶ Animals received oral administration of 10, 100, 1000, 1600, 2900 and 5000 mg/kg of methanol extract of *I. trichantha*. Control group received distilled water orally. Animals were observed for 24 hours for death and other toxic signs/symptoms.

Preparation of physiological salt solution (De-Jalon)

Sodium chloride (45.0g), KCl (2.10 g), NaHCO₃ (2.50 g) and D-glucose (2.50g) were weighed and made into a solution of 3.5 L distilled water. Calcium chloride (0.40 g) was made into a solution of 1.5 L distilled water in a separate beaker. Both solutions were then added to give 5 L.

In vivo Contractile activity

Five female non-pregnant rats (210-220 g) were pretreated and primed with 0.2 mg/kg of diethylstilbestrol intraperitoneally for 24 hours.²⁷ The rats were sacrificed under chloroform anaesthesia. The uterus was identified and the two horns of the uterus cut out and transferred to a petri-dish containing the physiological salt solution (PSS). One centimeters (1 cm) length of the uterus was cut out and threaded using a surgical silk. The channel recorder of Ugo Basile was calibrated, threaded with the uterus and connected to a transducer. The uterus tissue was mounted in 10 mL of organ bath containing De-Jalon solution and allowed to equilibrate for 30 minutes with periodic changing of the physiological solution every ten minutes. The tissue was aerated with air via an aerator and temperature maintained at 37°C with a pH of 7.4. The spontaneous contraction of the uterus was recorded with FT 03 transducer connected to an interval until constant responses were recorded via a force displacement transducer (model FT03) coupled with bridge amplifier and power Lab 4/25 data acquisition system connected to a computer running Lab-Chart 6 software. The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (0.5 g). The tissue was put under 0.5 g tension. The two standard drugs for uterine contraction used were oxytocin and acetylcholine. For oxytocin, concentrations of 0.1 i.u/ml and 1.0 i.u/mL were prepared and volumes of 0.1 mL, 0.2 mL, 0.4 mL, 0.8 mL and 1.0 mL of each of these concentrations were used to construct the concentration-response curve. A contact time of 30 seconds was allowed after which the tissue was washed and allowed to relax for 90 seconds before the administration of the next dose.

Acetylcholine (Ach) of 1.0, 100 and 1000 ug/mL concentrations were used at volumes of 0.1, 0.2 and 0.4 mL to construct the concentration-response curve similar to what was used for oxytocin. The effect of the extract (10 and 20 mg/mL) on the concentration-response curves for oxytocin and

acetylcholine was determined. Hence, responses to different doses of oxytocin and acetylcholine alone and in the presence of the extract were obtained. The effect of two positive controls (salbutamol and atropine) were also determined.

Statistical analysis

All results are expressed as the mean of five experiments \pm SEM (standard error of mean) and continuous line graph. The data were analyzed statistically by student's t-test using GraphPad instat version 2.05a. The level of significance was $P < 0.05$.

Results and Discussion

The phytochemical analysis of the methanol extract of *I. trichantha* leaves is shown in table 1. This result corroborates the findings of Shagal and Kubmarawa²¹ for ethanol extract of the plant except for the absence of flavonoid and glycoside.

Acute toxicity

The absence of death at 5000 mg/kg of the extract shows that the lethal dose of the methanol extract of the plant is higher than 5000 mg/kg which may be an indication of the safety of the plant.

Effect on the isolated rat uterus

The traces and the results of the effect of the methanol extract of *Icacina trichantha* on oxytocin and acetylcholine-induced contraction in the non-pregnant rat uterus are shown in Figures 1, 2 and 3.

Figures 2 and 3 indicate the effects of the methanol extract of *Icacina trichantha* on oxytocin and acetylcholine-induced contractions, respectively on the non-pregnant rat uterus.

The results obtained for the uterine contractile activity indicate that the methanol extract of *I. trichantha* produced a significant ($p < 0.05$) decrease in both oxytocin and acetylcholine-induced contractions of the rat uterus (figures 2 and 3).

The results in figure 2 also show a comparative inhibitory activity produced by the extract and salbutamol which is used clinically in the treatment of threatened abortion in pregnant uterus, while figure 3 shows a comparative inhibitory activity produced by the extract and atropine on acetylcholine-induced contraction. The activity of the extract is similar to that of salbutamol and atropine, two positive controls that significantly ($p < 0.05$) relaxed the uterus (Figures 2 and 3, respectively).

Results obtained from this study clearly indicate a shift of the concentration-response curve to the right produced by both doses of the extract. This shift was significant and similar to that produced by salbutamol and atropine.

Conversely, a significant decrease in the E-max was also seen due to the administration of the extract.

The uterus is spontaneously active, which means that, with or without any nervous/hormonal stimulation, a piece of isolated, pregnant or non-pregnant, uterus will produce regular spontaneous contractions.²⁸

Table 1: Phytochemical screening of methanol extract of *I. trichantha*.

Phytochemical constituents	Methanol extract
Glycosides	+
Saponin	+
Flavonoid	+
Phenolics	+
Tannins	-
Eugenol	+
Steroid	+
Terpenoid	+
Alkaloid	+

+ = present - = absent

Table 2: The acute toxicity test of methanol extract of *I. trichantha*

Group	Number of rats	Dosage (mg/kg)	Clinical signs	Mortality
1	5	0(Control)	None	0/5
2	5	10	None	0/5
3	5	100	None	0/5
4	5	1000	None	0/5
5	5	1600	None	0/5
6	5	2900	None	0/5
7	5	5000	None	0/5

Our results showed that the extract at both doses produced significant inhibition of oxytocin and acetylcholine-induced contractions of the uterine smooth muscle in non-pregnant rats. An increase in the EC₅₀ was also observed in the presence of the extract. The inhibitory effect of the extract was more prominent on oxytocin than acetylcholine. On its effect on oxytocin, it may be due to physiologic antagonism, while the effect on acetylcholine-induced contractions may be due to muscarinic receptor blockade.²⁸

Conclusion

It can be concluded that the methanol extract of *Icacina trichantha* possesses inhibitory activity on the uterine smooth muscles in non-pregnant rats, which is consistent with the literature report of its use in the treatment of spontaneous abortion.

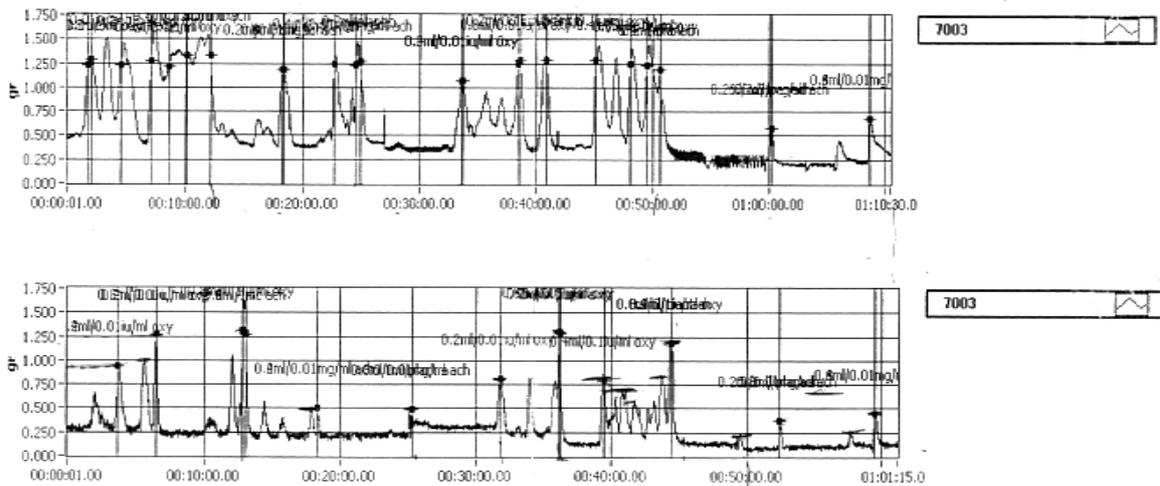


Figure 1: Chart of tracing of the contractile activity in non-pregnant rat uterus.

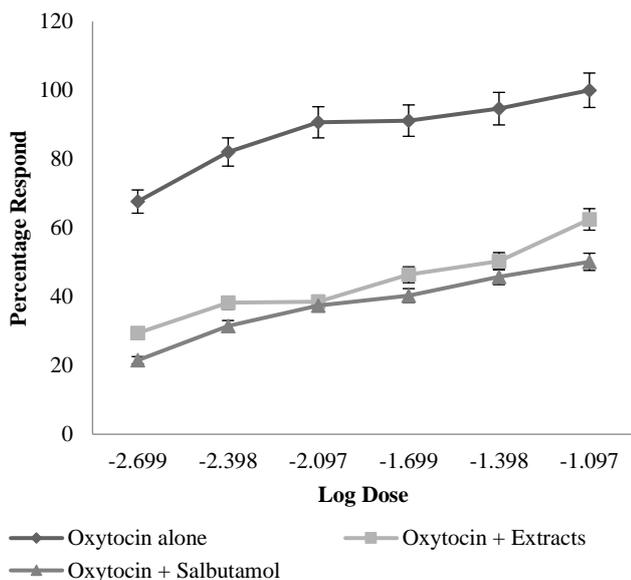


Figure 2: Effects of the methanol extract of *Icacina trichantha* on oxytocin-induced contraction in the non-pregnant rat uterus. Values are mean percentage response ± SEM (n = 5). *P < 0.05 significantly different from oxytocin-induced contraction alone.

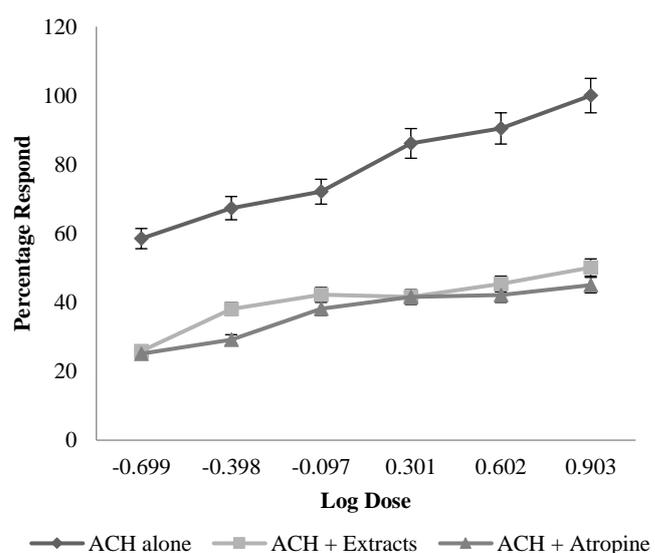


Figure 3: Effects of the methanol extract of *Icacina trichantha* on acetylcholine-induced contraction in the non-pregnant rat uterus. Values are mean percentage response ± SEM (n = 5). *P < 0.05 significantly different from acetylcholine induced contraction alone.

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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