

*Original Research****In-vitro* Effect of Indomethacin on Uterine Muscle Contraction Stimulated by Acetone Leaf Extract of *Anogeissus leiocarpus*****Mbursa Chiroma*, Arastus William, Peter Anjili Mshelia, Ijuptil Chiroma¹, Umar Kyari Sandabe and Chukwuka Uchendu²**

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Abstract

*This study was carried out to evaluate the inhibitory contractile effect of indomethacin on uterine muscle contraction elicited by the acetone leaf extract of *Anogeissus leiocarpus* in-vitro in Wistar rats. Adult female rats weighing 180±30g were used for this study. The rats were humanely sacrificed and the uterus isolated. Approximately 1.5 cm of the uterus was attached to the stylus of the kymograph. Graded doses of *A. leiocarpus* were used to induce contraction and indomethacin was used to abate its contractile effect. The study revealed that the acetone leaf extract of *A. leiocarpus* has the ability to induce contractions of isolated uterine muscle strips, in a concentration dependent manner with 0.53 mg/ml as the lowest active concentration. Atropine sulphate (2 µg/ml) significantly ($P<0.05$) attenuated contractions stimulated by acetylcholine (0.13 µg/ml) but had no effect on the extract mediated contractions. Indomethacin, a prostaglandin synthetase antagonist (0.05 mg/ml) was able, not only to abolish inherent spontaneous myometrial contractions but also the contractions induced by the extract. This study showed that *A. leiocarpus* has the ability to elicit uterine contraction through interactions with prostaglandin receptors and may be abortifacient. It also revealed the antagonistic effect of indomethacin on uterine contractions elicited by *A. leiocarpus*.*

Key words: *Anogeissus leiocarpus*, Contractions, Prostaglandins, Uterus

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Introduction

Medicinal plants are of great importance to the health of man and animals. The value of these plants lies in some chemical substances contained in them that produce a definite physiological action (Olajide *et al.*, 2013). Today, about 80% of the human population depend on herbal medicine for their primary health care delivery (Elujoba *et al.*, 2005). The plant, *Anogeissus leiocarpus* belongs to the family Combretaceae. It is the sole West Africa species of the genus *Anogeissus*, which is distributed from tropical Central and East Africa through tropical Southeast Asia (Steentoft, 1988). The plant is a slow growing evergreen shrub or small to medium-sized tree, reaching up to 15–30 m in height (Andaryl *et al.*, 2005). Many traditional uses have been reported for the plant, decoction of the bark is used against cough in Sudan (El Ghazali *et al.*, 2003), as an antimicrobial agent against bacterial infections and the leaves for the treatment of skin diseases and itching due to psoriasis (Ogunyemi, 1979). Rural populations use inner barks for orodental hygiene; the end of the inner bark are chewed into fibrous brush which is rubbed against teeth, tongue and gum Rotimi (1988). It is used against fungal infections such as dermatitis and mycosis, and also against stomach infections (Okpekon *et al.*, 2004). The inner bark of the tree is used as an anthelmintic in human and livestock, and for treatment of some protozoan diseases in animals such as *nagana* (trypanosomiasis) and babesiosis (Ngozi *et al.*, 2015). The ethanol extract of stem bark of *A. leiocarpus* was reported to inhibit the growth of standard strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Mann, 2008). The stem bark contains castalagin (Shuaibu *et al.*, 2008) and has antioxidant properties (Atawodi *et al.*, 2011). In addition to its numerous medicinal uses in folk medicine, local livestock farmers in North Eastern Nigeria claim that ingestion of the leaves of *A. leiocarpus* induces abortion in small ruminants, and when taken in large quantities can lead to the death of the unborn foetus and/or uterine rupture, and other longer term effects on the dam or offspring. It was therefore, considered worthwhile to evaluate the effect of the plant extract on isolated uterine smooth muscle contraction, its antagonists and possible receptors involved.

Materials and Methods

Plant Collection and Identification

The leaves of *Anogeissus leiocarpus* were collected from a rural community in Lassa, Askira/Uba Local Government Area, Borno State. It was identified and tagged by a botanist in the Department of Biological Sciences, University of Maiduguri. A voucher specimen was deposited in the herbarium of the Department of Veterinary Physiology and Biochemistry, with herbarium number Vet. Species 208 A.

Preparation and Extraction

The fresh leaves were air-dried in shade and ground into fine powder using pestle and mortar. Two hundred and twenty grams of the plant material was soxhleted, using acetone as the extracting solvent at 50 - 60°C

for 6 hours. The acetone extract was removed and filtered using Whatman filter paper (18cm). The filtrate was subsequently transferred to evaporating dish to concentrate on hot air oven at 40-50°C. After evaporation, the weight (yield) was taken and it was kept in a clean container and stored at 4°C for further analysis.

Bioassay Studies

Adult female Wister rats weighing between 180 and 230 g were used for the isolated uterine tissue studies. They were kept in plastic cages in the Department of Veterinary Physiology and Biochemistry, University of Maiduguri and fed with a suitable animal feed (Vital feeds® Nig. Ltd. Jos, Nigeria) and clean water *ad libitum*. Each rat was administered Stilbestrol at 0.1 mg/kg subcutaneously 24 hours before sacrifice. The procedure described by Uchendu and Leek (1999) was adopted for the preparation of the uterine tissue. The rats were sacrificed by stunning and decapitation, and firmly secured on the table on dorsal recumbence. The abdomen was opened by midline incision and the two uterine horns were exteriorized, dissected out and transferred to a dish containing De Jalon's physiological salt solution (PSS). The PSS had the following composition: Sodium chloride (9.0g), glucose (0.5 g), potassium chloride (0.42 g), sodium hydroxide (0.5 g), calcium chloride (0.06 g) and magnesium sulphate (0.1 g). The two horns were separated and freed from fat and extraneous tissues. A strip of about 1 - 2 cm was cut out and attached at one end (using silk thread) to an aerator and at the other end to a lever system fitted on the stylus of a kymograph. The preparation was suspended in 75 ml organ bath containing PSS that was continuously bubbled with air. The resting tension was set at 0.5 g. The temperature of the organ bath was thermostatically regulated at $37 \pm 0.50^\circ\text{C}$. During the bioassay studies, the extract was reconstituted fresh in distilled water for each study. This was because the contractile effect of the reconstituted extracts wanes with storage time. Indeed, a significant relaxation effect was observed instead. The following standard drugs were used in the course of the experiments: atropine sulphate (make); indomethacin (make); and acetylcholine (make). The concentration of extract and standard drugs given are final nutrient bath concentrations.

Statistical Analysis

The results obtained from this were presented in the forms of charts as means \pm standard error of the means (SEM).

Result and Discussion

Acetone leaf extract of *A. leiocarpus* produced a concentration - dependent increase in uterine smooth muscle contraction, with 0.53 mg/ml as the lowest active concentration. The contraction was phasic in nature and affected predominantly the amplitude with little effect on the frequency component. The calculated EC_{50} value was 0.09 mg/ml (Fig. 1).

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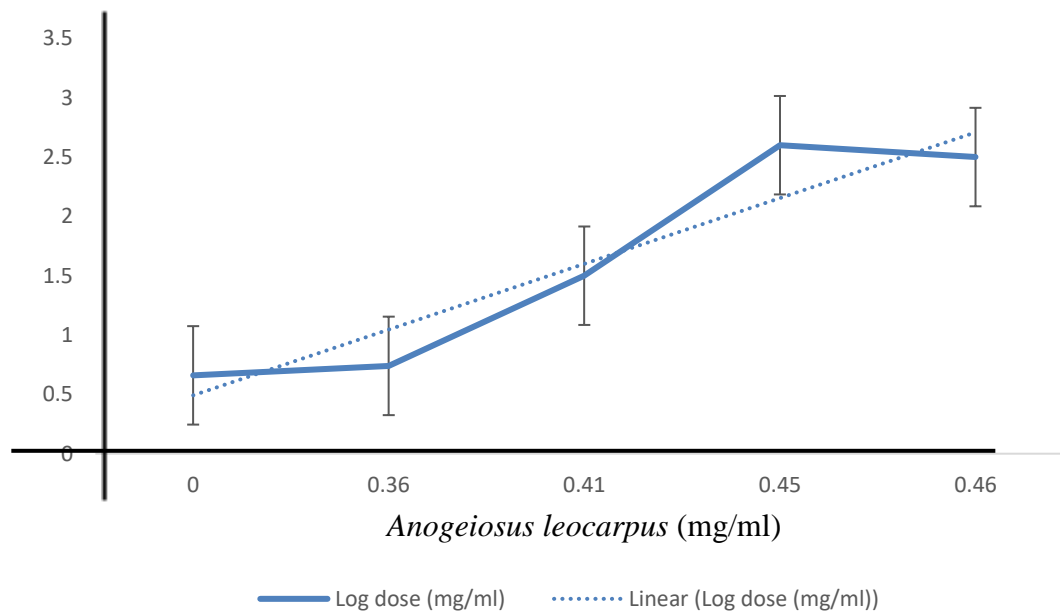


Fig. 1: Log dose of the effects of *Anogeiosus leocarpus* (DC), on uterine smooth muscles of wistar rats

Acetylcholine similarly produced a concentration-dependent contraction of the uterine muscle that was fast phasic, with significant ($P < 0.05$) increase in the frequency of contraction as the concentration of the agonist increased in the bath medium. This was also the case at concentrations beyond $0.26 \mu\text{g/ml}$. When compared with the extract, acetylcholine produced a more pronounced contractile force with $0.45 \mu\text{g/ml}$ as the lowest active concentration. The calculated EC_{50} for acetylcholine was $0.3 \mu\text{g/ml}$ (Fig. 2.). Atropine sulphate ($2 \mu\text{g/ml}$) significantly ($P < 0.05$) attenuated contractions stimulated by acetylcholine ($0.13 \mu\text{g/ml}$) but had no effect on the extract mediated contractions, while a low concentration of indomethacin (0.05 mg/ml) was sufficient not only to abolish the spontaneous myometrial contractions significantly ($P < 0.05$), but also the contractions induced by the extract (Fig. 3).

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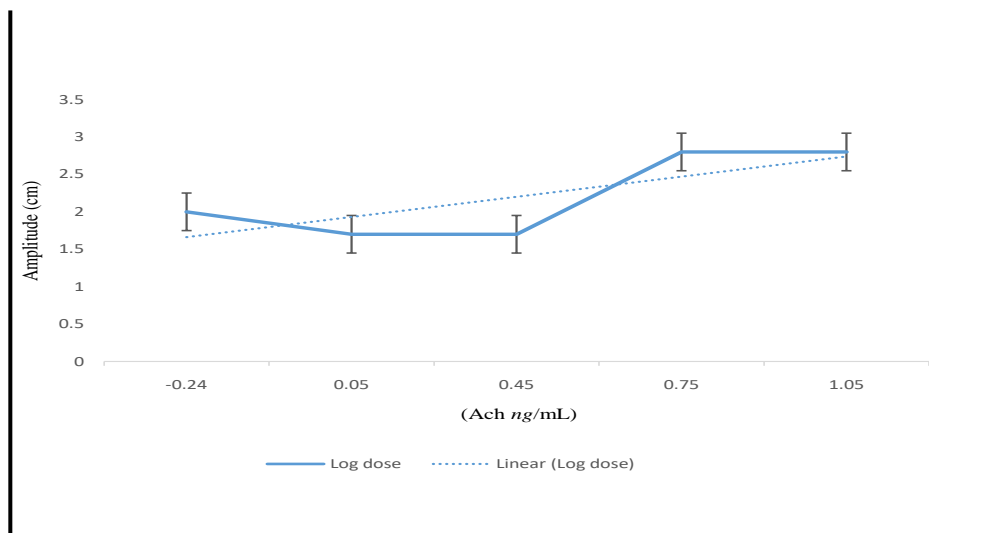
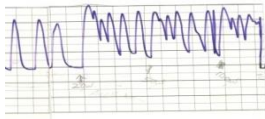


Fig. 2: Effect of acetylcholine on uterine smooth muscles of Wistar rats

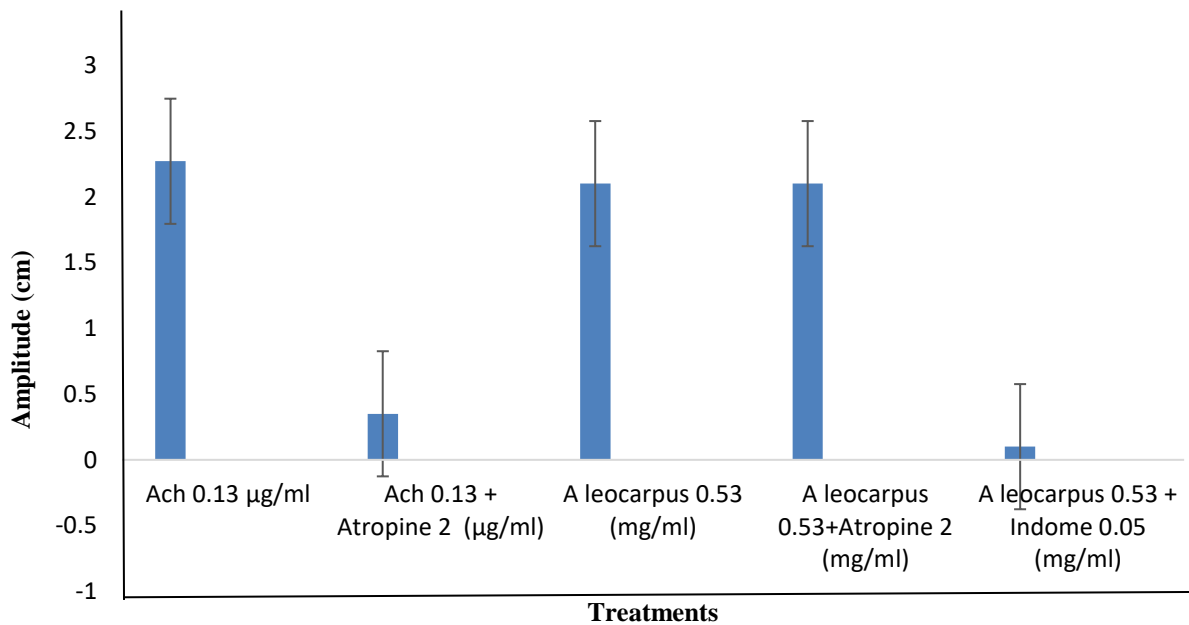


Fig. 3: Effect of atropine sulphate (2 µg/ml), acetylcholine (0.13 µg/ml), Indomethacine (0.05 mg/ml) and *A. leiocarpus* (0.53 mg/ml)

This study demonstrated clearly that acetone leaf extract of *Anogeissus leiocarpus* causes contraction of isolated uterine muscle strips of the rat in a concentration dependent manner. This represents the first reported work on the *In vitro* stimulatory effect of leaf the extract of this medicinal plant on uterine smooth muscle. El-Hassan (2004) and Belemnaba *et al.* (2013) had earlier reported the relaxation effects of the stem-bark of methanolic and dichloromethane extracts of *A. leiocarpus* on uterine and vascular smooth muscle preparation respectively. A similar relaxation effect was also observed in guinea pigs' ileum (El-Tayeb, 2004). This relaxation effect was thought to be mediated via opening of voltage-dependent K^+Na^+ ATPase channels and inhibition of cyclic nucleotide phosphodiesterases (Belemnaba *et al.*, 2013), resulting in increased intracellular cAMP, and hence relaxation of the smooth muscles (Fischer *et al.*, 1992). Our observation of spasmolytic effect of the extract of *A. leiocarpus* on the uterine muscle preparation corroborate with the findings of these earlier researchers with the difference that the antispasmodic activities reported in the present study were only observed with storage time of the reconstituted extract. The stimulatory effects of the extract reported in the current study (Fig. 1) may be explained by the analytical method employed in which acetone was used as the extracting solvent in contrast to methanol and dichloromethane used by these other investigators. It has been shown that different extraction solvents have the propensity of preferentially extracting different classes of heterogeneous compounds (Seddon and Downey, 2008; Harbertson and Downey, 2009).

The uterus is a myogenic organ that is capable of contracting by itself without any external innervations. It contains functional muscarinic receptors that are responsive to cholinergic agents such as acetylcholine, eliciting prominent myometrial contractions. Under the conditions of the present study, acetylcholine contracted the uterine muscle preparation in a graded manner (Fig. 2). This contraction was significantly ($P<0.05$) attenuated by atropine, a muscarinic receptor antagonist but without effect on the extract mediated contractions. This suggests that the extract contracted the uterus by mechanism(s) other than the stimulation of surface membrane cholinergic receptors. However, the anti-inflammatory drug, indomethacin, did not only abolished the inherent spontaneous contractions but also produced a graded inhibition of the extract-mediated contractions. We, therefore, propose that the mechanism of action of the extract may involve the prostaglandins since indomethacin is known to be a potent inhibitor of prostaglandin synthetase, the enzyme involved in prostaglandin synthesis.

Conclusion

The observed effect of indomethacin on spontaneous myometrial contraction reported in this study falls in line with the findings of Vane and William (1972) who also reported of the block of spontaneous contractions by this cyclooxygenase inhibitor in pregnant rat uterine muscle. These workers proposed that a local intramural prostaglandin generation might be responsible for the maintenance of the inherent smooth

muscle activity as was reported for other smooth muscles such as that of cat's iris (Posner, 1970); rabbit's jejunum (Ferreira *et al.*, 1972) and guinea pig's colon (Eckenfels and Vane, 1972). Evidence has also been presented linking indomethacin inhibition to diminished calcium uptake by target cells (Northover, 1971) and exerting strong effect on smooth muscle contraction. It is therefore concluded from this study, that this plant cause or induce abortion animals.

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