Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (7): 630-637, 2007
ISSN 1816-496X

Inhibitory Effect of Methanolic Extract of *Asparagus pubescens* Bak Root in Rats and Guinea Pigs Uterine Muscle

[1]Paul A. Nwafor and [2]F.K. Okwuasaba
[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy,
University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria
[2]Department of Pharmacology, Faculty of Pharmaceutical Sciences,
University of Jos, P.M.B 2086, Jos, Plateau State, Nigeria

Abstract: The inhibitory effect of methanolic extract of *Asparagus pubescens* Bak root was studied in rat and guinea pig uterine muscles, respectively. The extract caused a concentration dependent decrease in frequency and amplitude of spontaneous activity in rat uterine muscle. It also caused a significant ($p<0.05$-0.001) dose-dependent decrease in acetylcholine-induced contraction in guinea pig uterine muscle. The sensitization of the tissue caused by 8.0×10^{-6} g mL^{-1} of extract accentuated with 1.6×10^{-5} g mL^{-1} was blocked by verapamil, an extracellular calcium antagonist. The extract dose-dependently also inhibited Ach-induced contraction in Ca^{2+} free Physiological Salt Solution (PSS). This inhibition which was statistically significant ($p<0.05$-0.001) was substantially reversed by addition of extracellular calcium. This inhibitory effect may in part involve the interference with intracellular/extracellular calcium mobilization.

Key words: Inhibitory, methanolic extract, *Asparagus pubescens*, rat, guinea pig, uterine muscle

INTRODUCTION

Asparagus pubescens (Liliaceae) is an evergreen plant which grows luxuriantly around the rocky hills and valleys of Jos metropolis in Nigeria between the months of April and September. The dried root of *Asparagus pubescens* locally called Khayan bera, has a long history of traditional medicinal use among the Rukuba community in Jos township and its neighbours where it is used for family planning, treatment of various gastrointestinal disorders and inflammatory pains (Hutchinson and Dalziel, 1968). Nwafor *et al*. (1998) had reported on the contraceptive and non-estrogenic effect of the methanolic extract of the Bak root. They have also established the antidiarrhoeal and antiulcerogenic effects of the plant as well as its antinoiceptive and anti-inflammatory effects (Nwafor and Okwuasaba, 2003; Nwafor *et al*., 2000). Recently, they reported on its effects on non-vascular muscle (Nwafor *et al*., 2005) and its effects on sexual behaviour and pituitary hormone secretion in wistar rats during pregnancy and lactation (Nwafor *et al*., 2007). Though, they showed that the mechanism of this inhibition was receptor-operated channel dependent, they did not establish the involvement of calcium dependent mechanism in calcium free Physiological Salt Solution (PSS) hence the aim of this investigation is to determine if the involvement of calcium dependent mechanism is through the extracellular or intracellular inhibition or both.

Corresponding Author: Paul A. Nwafor, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria
Tel: 234-8036778861, 234-8059153346

MATERIALS AND METHODS

Preparation of Extract

The plant material used in this study was collected from Rukuba village in Jos metropolis Plateau state, Nigeria between the months of April and September, 1995. The plant was identified and authenticated by Dr. S.S. Sanusi, Department of Botany, University of Maiduguri. Specimen vouchers (FPS. 014) were made and deposited at the herbarium of Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos. The dried root was pulverized by grinding using pestle and mortar. Then, 57 g of the ground root were subjected to exhaustive soxhlet extraction in methanol (250 mL) for 72 h at 60°C. This gave a mean yield 16±0.23 g W/W of extract. The extract was stored in -4°C from where it was used when required.

Animal Stock

Adult female guinea pigs and albino rats (weighing 350-410 and 165-200 g, respectively were used in this study. All animals were housed in a cross-ventilated room (temperature 22±2.5°C, 12 h light/12 h dark cycle) and were fed with standard mash (Feedex Nigeria, Kaduna, Nigeria) and water *ad libitum*.

Pharmacological Studies

Preparation of Isolated Uterine Horns

The isolation of uterine horn was done according to the methods of Okwuasaba and Otubu (1988) and Nwafor *et al*. (2005). The animals were killed by cervical dislocation and exsanguinated. The abdomen was opened and the uterine horns were served at the junctions with the fallopian tubes cleared of adhering mesentery and placed in a dish containing De-Jalon's solution of the following composition in millimole (mM): NaCl, 15.4; KCl, 5.63; $CaCl_2$, 0.41; $NaHCO_3$, 5.95 and glucose 2.25. Approximately, 2-3 cm of the uterine preparation was used for each experiment and was mounted in 5 mL organ bath containing De-Jalon's solution constantly gassed with 95% oxygen and 5% carbon dioxide mixture and the temperature was kept constant at 35±1°C in a thermostatically regulated water circulator (Grant instrument 51963 Cambridge). A tension of 0.5 g was applied to the tissue and it was allowed to equilibrate for at least 30 min before adding drugs or extract. Non-cumulative/cumulative responses were recorded isometrically using Ugo Basile strain guage transducer connected to microdynanometer recorder model 7080 (Uyo Basile Italy, Aqel *et al*., 1991).

Effect of Extract on Spontaneous Rhythmic Contraction of Rat Uterine Muscle

To determine the effect of extract on spontaneous movement of rat uterine muscle, 1 mg of the extract was added cumulatively into the organ bath at 5 min interval and the results were recorded.

Effect of Extract on Acetylecholine-induced Contraction on Guinea Pig Uterine Muscle

The effect of extract was investigated on non-pregnant guinea pig uterine muscle. The tissue preparation procedure was similar to that of rat uterine muscle as described earlier (Aqel *et al*., 1991). The tissue was stimulated with acetylcholine (Ach 5.0×10^{-4} -3.0×10^{-3} g mL^{-1}). Extract incubation period was 15 min while the agonist contact time was 20 sec.

Effect of Cumulative Concentration of Extract on Ach-induced Contraction on Guinea Pig Uterine Muscle

After an initial equilibration of 30 min, cumulative concentration of *A. pubescens* extract (1.0×10^{-6} -1.28×10^{-4} g mL^{-1}) was investigated on submaximal dose of acetylcholine-induced contraction on guinea pig uterine muscle and the results were recorded.

Effect of Verapamil on Extract-sustained Contraction on Guinea Pig Uterine Muscle

As a result of extract sensitization on Ach-induced contraction, verapamil, an extracellular calcium antagonist was introduced to investigate if the mechanism of this sensitization is calcium dependent. At the end of the equilibration period, 10 sec after the administration of Ach, verapamil (1.0×10^{-6} g mL^{-1}) was introduced while the extract was added cumulatively as described earlier. This procedure was repeated using promethazine, in place of verapamil, the results recorded.

Effect of Extract on Acetylcholine-induced Contraction in Ca^{2+} Free Physiological Salt Solution on Guinea Pig Uterine Muscle

As a result of *Asparagus pubescens* extract sensitization on Ach-induced contraction, further experimental investigations were carried out to ascertain if this effect is due to extracellular calcium mobilization. Calcium ion was deliberately omitted in physiological salt solution (De-Jalon) preparation and the experiment (2.3.4) was repeated in the absence of calcium. Time dependent contractile responses of Ach in Ca^{2+} free Physiological Salt Solution (PSS) was also recorded (Ebeigbe *et al*., 1986).

In another series of experiments, the submaximal dose of Ach (8.8×10^{-5} M) was used to stimulate the tissue. In the presence of Ach, increasing concentrations of extract (1.0×10^{-6}-6.4×10^{-5} g mL^{-1}) were added cumulatively. When the depressant effect of the extract was maximal, extracellular calcium (25-100 μm) was injected cumulatively to see if the inhibitory effect of the extract could be reversed.

Statistical Analysis

All the values were expressed as Mean±SEM. The data was analysed using Student's t-test. A probability level of less than 5% was considered significant.

RESULTS

At the end of equilibration period of 60 min, the effect of extract on spontaneous rhythmic contraction of rat muscle was recorded. The uterine muscle showed steady spontaneous rhythmic contractile activity. The frequency of contraction ranged from 3.0 to 3.5 per min with a tension change of 0.45±0.075 g. Incubation of the uterine muscle with extract, resulted in concentration-dependent decrease in frequency and amplitude of spontaneous activity. The inhibition was not substantially reversed by wash out (Fig. 1 and Table 1).

The extract (5.0×10^{-4}-3.0×10^{-3} g mL^{-1}) inhibited Ach (2.75×10^{-7}-1.76×10^{-4} M) induced-contractions on guinea pig uterine muscle. The inhibition was in a concentration-dependent manner ($p<0.05$-0.001). The antagonism was non-competitive since the agonist concentration response curves were displaced to the right in non-parallel fashion, with a depressed maxima (Fig. 2). The block by extract was substantially removed by wash-out for 30-60 min.

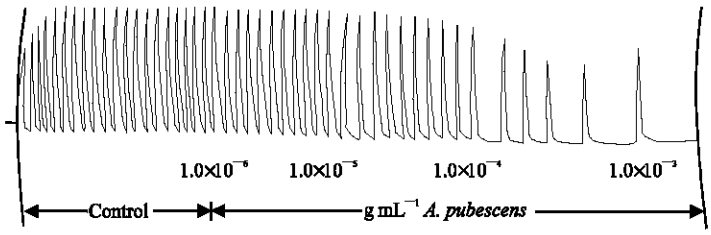

Fig. 1: Effect of *Asparagus pubescens* extract on the spontaneous rhythmic contraction of isolated rat uterine muscle. (dots show points of cumulative application of extract resulting in the final bath concentration of 1 mg mL^{-1}) (n = 5)

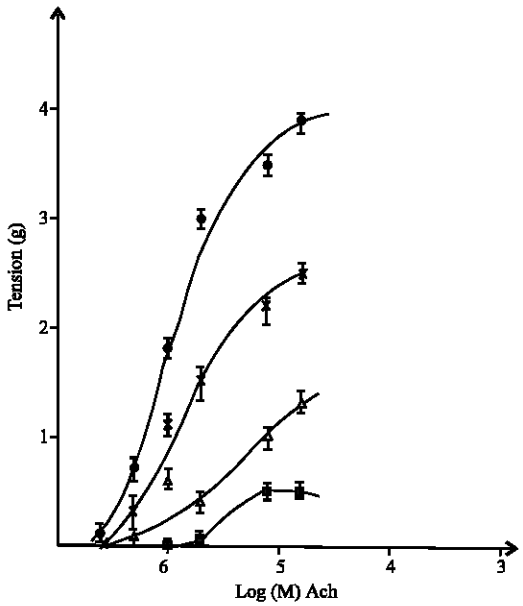

Fig. 2: Effect of Ach (●-●, 5.0×10^{-8}-3.2×10^{-5} g mL^{-1}) alone and in the presence of extract (*-*, 0.5 g mL^{-1}; Δ-Δ, 1.0×10^{-3} and ■-■ 3.0×10^{-3}g mL^{-1}) on guinea pig uterine muscle (n = 5) (*-*, $p<0.05$; Δ-Δ, $p<0.01$; ■-■, $p<0.001$)

Table 1: Concentration-inhibitory effect of *Asparagus pubescens* extract on spontaneous movement of the rat uterine smooth muscle

Extract concentration (g mL^{-1})	Mean % Max. inhibitory response
1.0×10^{-6}	4
1.0×10^{-5}	10
1.0×10^{-4}	38
1.0×10^{-3}	100

n = 5

Table 2: Reductive effect of cumulative-concentration of extract on acetylcholine-induced contraction on guinea pig uterine muscle

Concentration (g mL^{-1})	Tension reduction (g)
1.0×10^{-6}	3.2
2.0×10^{-6}	2.1
4.0×10^{-6}	1.6
8.0×10^{-6}	1.5
1.6×10^{-5}	2.5
3.2×10^{-5}	1.4
6.4×10^{-5}	0.8
1.28×10^{-4}	0.6

n = 5

The effect of cumulative concentration of extract on Ach-induced contraction on guinea pig uterine muscle is as shown in Table 2. There was a progressive inhibitory effect on Ach as the concentration of the extract increased, however, at 8.0×10^{-6} g mL^{-1} of extract, the tissue became sensitized. This sensitization by the extract was increased with higher concentration (1.6×10^{-5} g mL^{-1}) of the extract. Beyond this concentration, the effect (sensitization effect) declined progressively again but never reached the zero tension.

Table 3: Reductive effect of verapamil (1.0×10^{-6} g mL^{-1}) on *Asparagus pubescens* extract-sustained contraction on guinea pig uterine muscle

Concentration (g mL^{-1})	Tension reduction (g)
1.0×10^{-6}	1.1
2.0×10^{-6}	0.5
4.0×10^{-6}	0.3
8.0×10^{-6}	0.1
1.6×10^{-5}	0.0
3.2×10^{-5}	-0.1
6.4×10^{-5}	-0.1
1.28×10^{-4}	-0.1

n = 5

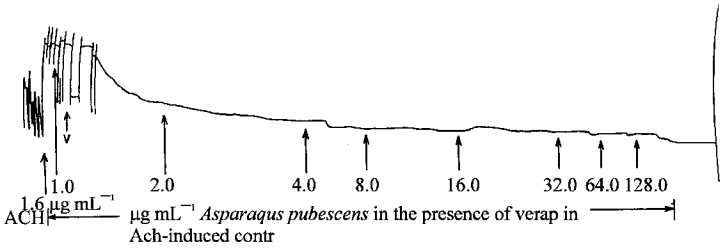

Fig. 3: Tracing showing effect of verapamil (1.0×10^{-6} g mL^{-1}) on *Asparagus pubescens* extract-sustained contraction on guinea pig uterine muscle

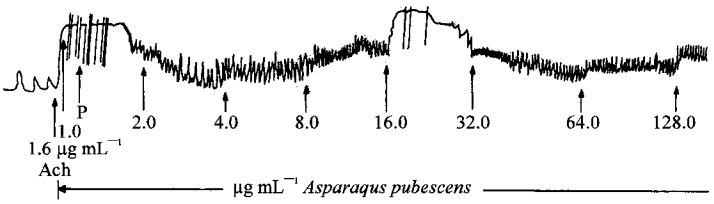

Fig. 4: Tracing showing effects of promethazine (1.0×10^{-7} g mL^{-1}) on extract-sustained contraction on guinea pig uterine muscle

The result on the effect of verapamil on extract-sustained contraction on guinea pig uterine muscle is as shown on Table 3 and Fig. 3. verapamil, an extracellular calcium ion antagonist, blocked the sensitization induced by extract. In the presence of 1.0×10^{-7} g mL^{-1}. promethazine, the effect was not inhibited which suggests that the sensitization was calcium dependent (Fig. 4).

The extract dose-dependently inhibited Ach-induced contraction in Ca^{2+} free Physiological Salt Solution (PSS). The inhibition was statistically significant ($p<0.05$-0.01). The dose-response curve was shifted to the right with a depressed maxima indicating a non-competitive antagonism (Fig. 5). In another series of experiments, the inhibitory effect of extract on Ach-induced contraction in Ca^{2+} free PSS was maximal (completely abolished Ach effect) in 20 min (Fig. 6).

Subsequently, the addition of extracellular calcium (25-100 µm) in calcium depleted guinea pig uterine muscle substantially reversed the inhibitory effect of the extract, indicating involvement of calcium (Table 4).

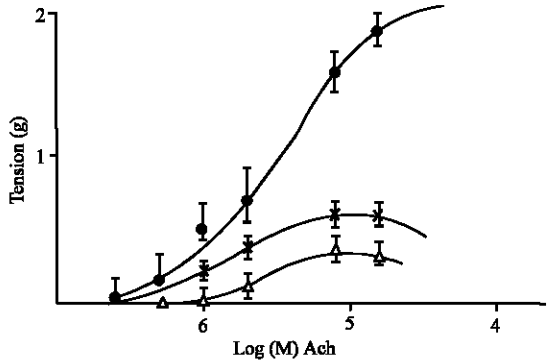

Fig. 5: Effect of extract (1.0×10^{-6}-3.0×10^{-6} g mL^{-1} on Ach-induced contraction in Ca^{2+} free Physiological Salt Solution (PSS) on guinea pig uterine muscle (*-*, $p<0.05$; Δ-Δ, $p<0.01$, ●-●, $p<0.001$)

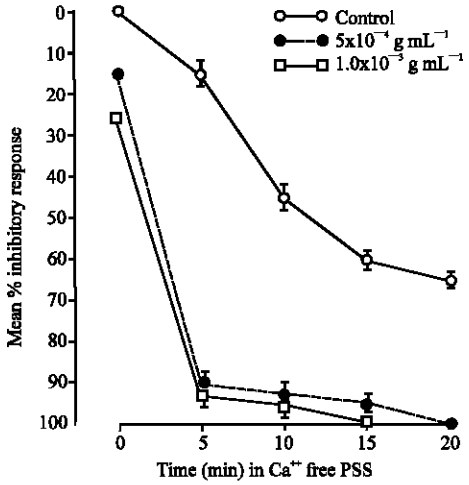

Fig. 6: Effect of extract on the time-inhibitory responses on guinea pig uterine muscle of 8.80×10^{-5} M Ach in Ca^{2+} free PSS

Table 4: Effect of extracellular Ca^{2+} (25-100 μm) addition on inhibitory effect of extract on guinea pig uterine muscle

Concentration (g mL^{-1})	Tension reduction (g)
4.0×10^{-6}	2.2
8.0×10^{-6}	1.8
1.6×10^{-5}	2.3
3.2×10^{-5}	1.9
6.4×10^{-5} + 25 μm (Ca^{2+})	1.0
64×10^{-5} + 50 μm (Ca^{2+})	2.5
64×10^{-5} + 75 μm (Ca^{2+})	3.0
64×10^{-5} + 100 μm (Ca^{2+})	3.1

n = 5

DISCUSSION

The extract inhibited the spontaneous rhythmic contraction of the rat uterine muscle. Spontaneous movement is inherent in muscle (myogenic) as a fundamental characteristics and is modified *in situ* by

hormonal and neuronal influences (Golenhofen, 1970; Nwafor *et al*., 2005). The spontaneous movement of uterine smooth muscle is regulated by the cycles of depolarization and repolarization. Action potential appears at the height of depolarization and constitute a rapid influx of Ca^{2+} via voltage dependent Ca^{2+} channel (VDCs) (Bolton, 1979). This contraction of smooth muscle is dependent on an increase in the concentration of cytoplasmic free Ca^{2+} which activates the contractile elements. The source of this activator Ca^{2+} may be extracellular or intracellular. Therefore, since the extract inhibited the spontaneous movements of rat uterine smooth muscle, it may interfere with either the depolarization process or with Ca^{2+} influx through VDCs.

The extract also inhibited the Ach-induced contraction on guinea pig uterine muscle. It is known that acetylcholine binds to muscarinic receptors on smooth muscle, thereby triggering a sequence of events resulting ultimately in smooth muscle contraction. Binding to the receptor causes the Receptor-Operated Channel (ROC) to open, thereby allowing sodium influx which causes a depolarizartion of the cell membrane. This depolarization opens the Voltage-Dependent Calcium Channels (VDC) and calcium ions enter the cell to induce the release of calcium from the sarcoplasmic reticulum (i.e., the calcium-induced release mechanism). The cytosolic calcium then binds to calmodulin and contraction is effected (Rahwan *et al*., 1977; Aloamaka *et al*., 1984; Aqel *et al*., 1991).

Verapamil, an extracellular calcium blocker, abolished the excitatory effect observed at 8.0×10^{-6} g mL^{-1} of extract. This effect was not affected by promethazine. In the presence of calcium free PSS, the effect was abolished and rejuvenated on addition of extracellular calcium.

Nwafor *et al*. (2005) had shown that the extract inhibited both the potassium chloride and barium chloride-induced contractions on rabbit fallopian tube and guinea pig ileum, respectively. It is known that potassium chloride and barium chloride elicit contractions on smooth muscle by mobilization of extracellular and intracellular calcium ions, respectively (Rahwan *et al*., 1977; Uvelius and Sigurdsson, 1981; Hazelhoff *et al*., 1982).

In conclusion therefore, that the extract inhibited both the extracellular and intracellular calcium mobilization therefore suggests that its inhibitory effect may in part be due to interference with the contractile mechanism of non-vascular smooth muscle.

ACKNOWLEDGMENT

The authors acknowledge the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, for providing Laboratory space. The study was partly sponsored by University of Maiduguri Fellowship Research Grant awarded to Dr. Paul A. Nwafor.

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