Original article

BIPHASIC ACTIVITY OF *ACANTHUS MONTANUS* AQUEOUS EXTRACT, ITS FRACTIONS AND A NEW ISOLATED COMPOUND ON RAT UTERINE SMOOTH MUSCLE.

Asongalem EA[1*], Foyet HS[2], Folefoc GN[3], Eyong KO[3], Kamtchouing P[2].
[1] *Pharmacology and Toxicology Unit, Department of Physiological Sciences, Faculty of Medicine & Biomedical Sciences, University of Yaounde 1, Yaounde, Cameroon, P.O. Box 8283, Yaounde Email: cpehw@yahoo.com., Fax: 237 2221873*
[2] *Department of Animal Biology & Physiology, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon.*
[3] *Department of Organic Chemistry, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon.*
* Correspondence to: cpehw@yahoo.com or Tel: 237 7855728, P.O. Box 8283, Yaounde

ABSTRACT

In this study, we report for the first time the effects of *Acanthus montanus* (Acanthaceae) aqueous extract, its fractions and Acanthus sulphate ester (ASE) – a newly isolated sulphate ester, on rat uterus. This in vitro research involved an organ bath containing an isolated unpregnant rat uterus suspended in De Jalons physiological solution and aerated with Carbogen ($95\%O_2$ +5% CO_2). Following equilibration, the extract, fraction and ASE were individually added to the tissue in the absence and presence of standard antagonists and compared with standard uterine agonists. The responses were recorded on a 2-channel Ugo Basile recorder.

The extract had a biphasic action with an initial relaxation (IC50 = 3.00 mM) of spontaneous uterine contractions followed by stimulation (IC50 = 0.63 mM) of the same tissue. Its fraction showed both relaxation and contraction depending on the polarity of extraction solvent. 100% methanolic fraction (IC50 = 150.0 μM) and ASE (131.0 μM) contracted the uterus. The IC50s of the other fractions were variable. These stimulatory activities were less than those of PGF2α, acetylcholine, diazoxide, oxytocine and histamine.

The extract, methanolic fraction and ASE were inhibited by phentolamine, prazosin, pyrilamine, indomethacine, verapamil but not atropine. The extract was unaffected by propranolol while tetraethylammonium had slight stimulation. Quinacrine did not affect the extract and MeOH fraction activities. Verapamil was the most potent inhibitor. The involvement of both extracellular and intracellular ionic channels of calcium and potassium were observed; they are probably involved in the mechanism of action of ASE.

Keywords: *Acanthus montanus*, uterus, contraction, relaxation

RÉSUMÉ

Dans cette étude, nous montrons pour la première fois les effets de l'extrait aqueux de *Acanthus montanus* (Acanthaceae), de ses fractions et de Acanthus sulphate ester (ASE), une nouvelle molécule isolée, sur l'utérus de ratte. Ces travaux, *in vitro* ont été réalisés à l'aide d'une cuve à organe contenant une solution physiologique type de Jalons aérée au carbongène (95% O_2+5% CO_2). L'utérus isolé d'une rate non gravide y était suspendu. Après équilibration, l'extrait, les fractions et l'ASE ont été ajoutés sur l'organe, séparément, en absence et en présence d'antagonistes standards et leurs effets ont été comparés à ceux des agonistes standards de l'utérus. Les réponses ont été enregistrées à l'aide d'un enregistreur type Ugo Basile à deux canaux.

L'extrait a révélé une action biphasique avec une relaxation initiale (IC50=300mM) des contractions spontanées, suivies d'une stimulation (IC50=0,63mM) du même organe. Ses fractions ont provoqués une relaxation et une contraction, en fonction de la polarité du solvant d'extraction. La fraction au méthanol (100%) (IC50 = 150,0μM) et l'ASE (131,0μM) ont provoqué la contraction de l'utérus. Les IC50 des autres fractions ont été variables. Ces actions stimulantes ont été plus faibles que celles de la PGF2α, de l'acétycholine, du diazoxide, de l'ocytocine et de l'histamine.

Les effets de l'extrait, de la fraction au méthanol et de l'ASE ont été inhibés par la phentolamine, la prazosine, la pyrilamine, l'indomethacine, le vérapamil et pas atropine. Les effets de l'extrait n'ont pas été affectés par le propranolol, tandis que le TEA a eu une légère activité stimulante. Les effets de l'extrait et de la fraction au méthanol n'ont pas été affectés par la quinacrine. Le vérapamil a été l'inhibiteur le plus jouissant. Une intervention des canaux calciques et potassiques extracellulaires et intracellulaires a été observée, élucidant probablement un mécanisme d'action de l'ASE.

Mots clés: *Acanthus montanus*, utérus, contraction, relaxation

INTRODUCTION:

Stretching from the forest of Benin in West Africa to Congo Basin and Angola in Central Africa is *Acanthus montanus* (Nees) T. Anderson (Acanthaceae), one of the 50 species of Acanthus found in the family Acanthaceae. In Nigeria, the leaves are used for cough, rheumatism, hypertension, skin infection, boil and witches (Igoli et al., 2005); in Cameroon, it is used for cough, dysmenorrhoea, pain, epilepsy and miscarriages (Noumi & Fozi, 2003), while in Gabon, it is used for cough, heart troubles, rheumatic pains and syphilis (Burkill, 1985; Adjanohoun et al., 1996).

In previous studies, the plant caused relaxation of intestine (Adeyemi et al., 1999) and uterus due to the presence of beta-sitosterol (Asongalem et al., 2005). This sterol has been shown to possess anticancer, anti-inflammatory, cholesterol lowering, anti-microbial and anti-fungal effects (Ovesna et al., 2004). The beta-sitosterol probably gave the plant, the credence to be used traditionally in treating most of these ailments. In the course of testing for the relaxant properties of the plant, stimulations were observed in extracts and fractions, especially the aqueous extract and its methanol extracted fractions.

Our aim was to determine which substance was at the center of the stimulations, its antagonists, relative potency with standard stimulants and the role of calcium ions.

MATERIALS AND METHODS:

Plant collection and identification:

Acanthus montanus (Nees) T. Anderson (Acanthaceae) plants were collected in Nsimeyong area of Yaounde, Cameroon in October 2004 and identified in the National Herbarium, Yaounde. The voucher number was 1652SRFCAM.

Preparation of extracts:

The aqueous extract was prepared by maceration of 3.8 kg in 10L of boiled distilled water for 24h. The extract was later filtered, and the solvents eliminated by concentration in a rotor evaporator to give 400g (10.5%) of aqueous extract.

Preparation of fractions:

380g of aqueous extract was subjected to flash chromatographic fractionation on a silica gel column eluted with hexane followed by gradient mixtures of hexane-ethylacetate-methanol. Several fractions were obtained and combined based on their thin layer chromatographic (Sigma-silica gel precoated TLC plates) resemblance. 9 fractions were grouped as: F_1 (Hexane 100%); F_2 (hexane–EtOAc 90:10); F_3 (hexane–EtOAc 75:25); F_4 (hexane–EtOAc 50:50); F_5 (EtOAc 100); F_6 (EtOAc-MeOH 90:10); F_7 (EtOAc-MeOH 75:25); F_8 (EtOAc-MeOH 50:50); F_9 (MeOH 100%). Each fraction was tested on the tissue.

Isolated compounds:

Fraction F_9 (60g) which showed very strong smooth muscle contracting activity, was subjected to column chromatographic fractionation over silica gel (GF_2 Merck) and eluted with gradient mixtures of hexane-ethylacetate, ethylacetate-methanol and finally with 100% methanol.

Reagents:

Atropine sulphate, propranolol HCl, phentolamine HCl, prazosin HCl, pyrilamine HCl, verapamil HCl, indomethacine, quinacrine 2HCl, tetraethylammonium acetate (TEA), ryanodine, isoproterenol HCl, oxytocin acetate, caffeine, histamine HCl, acetylcholine HCl, EDTA, and $PGF2\alpha$ were obtained from Sigma-Aldrich Chemie GmbH, Kappelweg 1, Germany. All chemicals used in the preparation of physiological solutions were of reagent grade.

Animals and isolated tissue preparation:

Twenty adult Wistar non-pregnant rats (180-212g) were used. They were raised in the Animal House of the Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1 and fed with standard laboratory chow with water made *ad libitum*. The animals were handled based on US NIH publication #85-23, revised in 1985. Following clearance from the Institutional Ethical Committee, the rats were sacrificed by CO_2 axpyxiation, the uterine horns excised, removed and placed on De Jalon's solution aerated by 100% O_2.

Measurement of responses on rat uterine smooth muscles:

Effects of the aqueous extract, its fractions, isolated compound and some standard agonists on rat uterus.

The rat uterus was cut into 2cm long segments. Two pieces were suspended in inner organ baths of a 2-chamber Ugo Basile 4050 isolated organ bath which contained De Jalons physiological solution or De Jalons solution in which $CaCl_2$ had been omitted with or without EDTA. The temperature of the bath was kept at $30\pm0.5°C$ and aerated with 100% O_2. A preload of 0.7g was used on the tissue connected to an isometric transducer (Ugo Basile cat. 7010) coupled to a Ugo Basile 2-channel Gemini 7070 recorder. The tissue was washed every 15mins during 60 min equilibration time. Following this equilibration, the cumulative dose-effects of the extract was tested on uterine smooth muscle. 30

mins after washing the tissue, the extract was tested again. Also tested similarly were the fractions and the isolated compound – Acanthus sulphate ester (ASE).

Effects of standard antagonists on contractions induced by the plant preparations:

Various doses of phentolamine HCl (1 mM), Prazosin HCl (1 mM), propranolol HCl. (1 mM), atropine sulphate (1 mM), pyrilamine maleate ((1 mM), indomethacine (2 mM), quinacrine diHCl (1 mM) verapamil HCl (1 mM) and TEA (1 mM) were used to antagonize the contraction induced by single doses of extract (32 mM), 100% methanolic fraction (0.8 mM) and the new compound – ASE (16 μM). The speed of the paper was 2mm/min. Each experiment was performed at least six times.

Similarly, ryanodine, verapamil, isoproterenol and tetraethylammonium acetate at various doses were used to antagonize contractions induced by the new compound in the absence and presence of Calcium ions. The aim was to determine the role of intracellular Calcium on contractions induced by the new compound.

Statistics: The effective mean concentrations (ED50) were obtained using both the linear regression analysis and Litchfield & Wilcoxon (1949) methods. The results were expressed as mean ± SEM.

RESULTS:

The 100% methanol elution afforded a compound which was cream-coloured solid with melting point of >300°C and soluble only in water. Its structure was determined as disodium propanoic acid 1,2 disulphate ester renamed Acanthus sulphate ester (ASE) (Fig. 1). This was a new compound. The structural determination included the use of spectral analysis (MS, ^{1}H- and ^{13}C-NMR) and 2D-experiments (COSY, HMQC and HMBC).
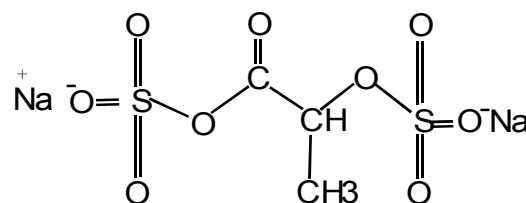
Figure 1: 1, 2-disodium sulphate-propan-1-one (Acanthus sulphate ester). It is a new compound isolated from *Acanthus montanus*.

The aqueous extract had a biphasic action on the uterine smooth muscle (Fig. 2A & 2B). In phase 1, a dose dependent (0.2 – 6.4 mM), inhibition of the spontaneous uterine contractions through depolarisation of the muscle was noted. Its median inhibitory concentration (IC50) was 3.0 mM (2.3 – 3.7). At the peak, the sustained contraction was brief and the tissue relaxed completely (indicated by the return of the pen to its baseline). This was immediately followed by an absolute refractory period of at least 20 mins in which the tissue was not responsive to any agonist stimulation. Beyond this time, the quiescent uterus responded again to the same doses of the extract by contracting (Phase 2) dose dependently (Fig. 2B). The effective median concentration (EC50) of contraction was 0.63 mM (0.25 – 0.96)

Table I shows the IC50s of fractions of *Acanthus montanus* aqueous extract on spontaneous rat uterine contractions. 100% methanolic (MeOH) fraction (50 - 320 μM) also caused relaxation of spontaneous contractions through depolarization (Fig 2C). The relaxation pattern was similar to that of the aqueous extract and diazoxide (Fig 2F).

However, at the peak, the contraction was sustained with no further spontaneous activity observed and was abolished only after washing the tissue. A resumption of the spontaneous muscular activity occurred immediately compared to the aqueous extract which had an absolute refractory period. When the dose-response process for the fraction was repeated, a similar response pattern was obtained. Its IC50 was 150.1 µM.

Table I: IC50ₛ of fractions from aqueous extract of *Acanthus montanus* on rat uterine smooth muscle.

| Fraction	Mean Inhibitory Concentration (IC50) (µM)
Hexane (100%)	320.3 (298.2 - 342.2)
Hexane-EtOAc (90:10)	19.2 (14.6 – 23.6)
Hexane-EtOAc (50:50)	12.3 (09.8 – 14.8)
Hexane-EtOAc (25:75)	13.1 (11.5 – 14.7)
EtOAc (100%)	30.3 (24.2 – 36.4)
EtOAc-MeOH (90:10)	83.2 (76.2 – 90.1)
EtOAc-MeOH (75:25)	126.9 (114.6 – 137.5)
EtOAc-MeOH (50:50)	123.3 (104.1 – 132.4)
MeOH(100%)	**150.1 (130 – 170)**

The potency of the extract decreased with increasing polarity of the extracting solvent.

n = 6-7

MeOH:EtOAc (50:50) fraction (10 – 320 µM) initiated contraction of the tissue each time it was added into the inner organ bath but the overall outcome was a classic relaxation pattern, unlike 100% methanolic fraction. IC50 was 123.3 µM. Washing of the tissue led to the resumption of its spontaneous activity. MeOH:EtOAc (25:75) fraction (50 to 320 µM) also showed a similar trend like the 50:50 fraction but the relaxation was more pronounced with the presence of weaker fraction-induced contractions as the doses increased. The IC50 was 126.9 µM. MeOH:EtOAc (10:90) fraction (10 – 320 µM) also showed a similar effect as 100% ethylacetate (EtOAc) fraction with IC50 of 83.2 µM.

Figure 2. Tracings showing the biphasic effects of aqueous extract (A & B), its 100% MeOH (C) and 50:50 MeOH:EtOAc (D) fractions, ASE (E) and diazoxide (F) on rat uterine contractility.

A   B   C   D   E   F
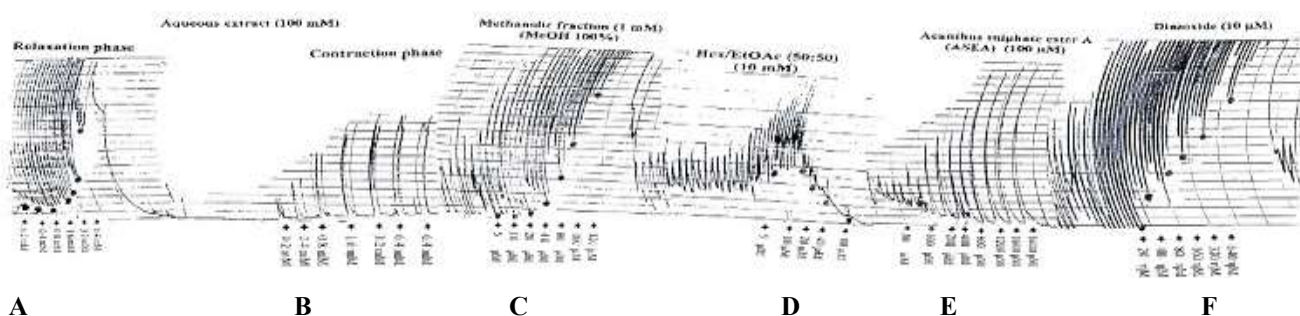
Relaxation induced by MeOH (C) and

MeOH/EtoAc (**D**)fractions were achieved through different mechanisms. ASE (**E**) failed to produce a similar pattern of relaxation like the extract and MeOH fraction on the rat uterus.

Using 5 to 100 μM of 100% EtOAc fraction, tissue relaxed with further weaker fraction-induced contractions. An IC50 of 30.3 μM was obtained. Doses of 1 – 50 μM of EtOAc:Hex (25:75) fraction were capable of relaxing the tissue with loss of its spontaneous activity. This meant that the maximum amount of the relaxant substances was extracted by this solvent mixture with an IC50 of 13.1 μM. The EtOAc:Hex (50:50) (Fig 2D) had a concentration dependent reduction in amplitude of contraction with loss of the tissue spontaneity. This effect was achieved with doses between 5 and 80 μM. On repeating the dose-response process of the fraction after absolute refractory period, contractions were induced. The IC50 was 12.3 μM. EtOAc:Hex (90:10) had similar trend to 100% hexane fraction but more potent as a relaxant. Its IC50 was 19.2 μM. Hexane (100%) had the least content of the relaxant constituent. The IC50 was 320.3 μM.

Table II: Relative stimulatory potency of standard uterotonics and ASE on rat uterine smooth muscle.

Drug	Mean Effective Concentration (EC50)
PGF2α	1.1 ηM (0.96 – 1.3)
Acetylcholine	68.5 ηM (60.5 – 77.5)
Diazoxide	89.0 ηM (10.6 – 74.4)
Oxytocine	276.9 ηM (181.1 – 396.2)
Histamine	19.5 μM (16.5 – 23.2)
ASE	131.0 μM (82. 4 – 190.2)

n = 6-7; EC50 = Mean effective concentration required to stimulate the spontaneous contraction of the uterus. The strength of contraction by ASE was more than histamine, comparable to acetylcholine but less that $PGF_{2\alpha}$.

Acanthus sulphate ester (ASE), isolated from 100% methanolic fraction showed principally stimulatory effect (Fig 2E) with pattern which differed from those of the extract and methanolic fraction. This stimulatory effect did not matter if the tissue had spontaneous or quiescent activity. Table II shows the relative potency of ASE and some utero-stimulants. The order of potency based on their EC50s was PGF2α > acetylcholine > diazoxide > oxytocine > histamine > ASE.

Table III: IC50s of standard antagonists required to inhibit contractions induced by *Acanthus montanus* aqueous extract, its methanolic fraction and ASE on rat uterus.

Antagonist	*Acanthus montanus* aqueous extract	Methanolic fraction	Acanthus sulphate ester (ASE)
EC50 (absence of antagonist)	0.63 mM (0.25-0.93)	150.11 µM (120.01 – 170.23)	0.13 µM (0.08 – 0.19)
Pyrilamine	76.11 µM (7.28 - 430.0)	2.22 µM (1.73 – 2.74)	0.37 µM (0.08 - 1.73)
Propranolol	Unaffected	0.57 µM (0.26 – 1.24)	48.81 µM (34.82 – 68.33)
Phentolamine	3.84 mM (0.58 – 250)	0.15 mM (0.028 – 0.85)	0.23 µM (0.02 – 2.56)
Prazosin	121.63 µM (113.3 – 128.9)	2.93 µM (0.15 - 3.56)	0.71 µM (0.25 - 1.96)
Atropine	Unaffected	Unaffected	Unaffected
Indomethacine	2.54 mM (0.86 – 7.47)	98.44 µM (89.40 - 108.44)	95.54 µM (43.93 – 147.14)
Quinacrine	Unaffected	Unaffected	1.97 µM (0.14 – 28.07)
Verapamil	785.01 ηM (643.31 – 926.91)	69.82 ηM (59.33 – 75.43)	55.0 ηM (9 – 305.0)
Tetraethylamonium (TEA)	Unaffected	Slightly stimulatory	9.86 µM (6.32 – 11.99)

n = 6-7; Data expressed as Mean ± SEM); EC50 = Mean effective concentration of extract, fraction and ASE required to stimulate the spontaneous contraction of the uterus. The plant had sympathetic and histaminergic but not parasympathetic effects.

Table III shows the IC50s of antagonists which were capable of inhibiting by 50%, the maximal contractions induced by aqueous extract, methanolic fraction and ASE. Pyrilamine (H_1 antagonist) inhibited the three preparations: extract (IC50 = 76.45 µM), methanolic fraction (IC50 = 2.22 µM) and isolated compound (IC50 = 0.37 µM), Figure 3A. Propranolol, non specific β adrenoceptor antagonist, did not inhibit the extract but did for fraction (IC50 = 0.57 µM) and ASE (IC50 = 48.84 µM) induced contractions (Figure 3B). It had a weaker effect on ASE induced contraction, than phentolamine. Phentolamine decreased partially the contractions induced by the extract (IC50s = 3.84 mM) and fraction (IC50s = 0.15 mM) but potently for ASE (IC50s = 0.23 µM). The inhibitory effect by prazosin (an α_1-adrenergic antagonist) was more powerful than phentolamine on

contractions induced by the extract (IC50 = 121.6 µM), fraction (IC50 = 2.93 µM) but similar in strength with ASE (IC50 = 0.71 µM) (Figure 3C).
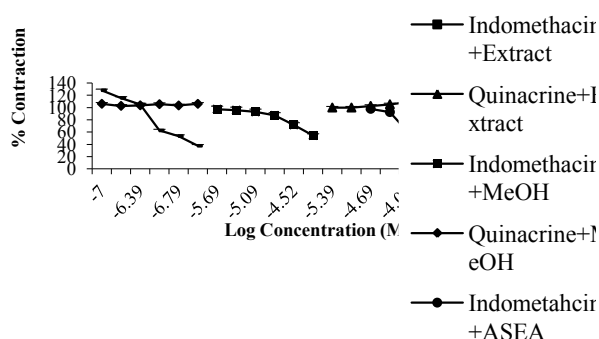
Figure 3: Effects of antagonists on contractions induced by aqueous extract of *A. montanus*, methanolic fraction and Acanthus sulphate ester.

Figure 3A: Effect of pyrilamine on *A. montanus* aqueous extract, MeOH fraction and ASE on rat uterus,
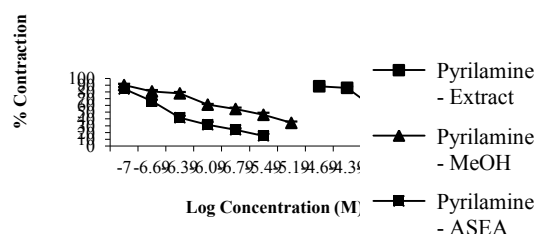
Figure 3B: Effect propranolol on *A. montanus* aqueous extract, MeOH fraction and ASE on rat uterus.
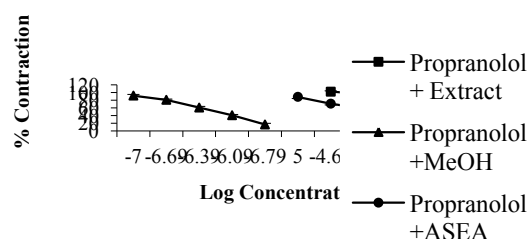
Figure 3C: Effect of prazosin and phentolamine on *A. montanus* aqueous extract, MeOH fraction and ASE on rat uterus.
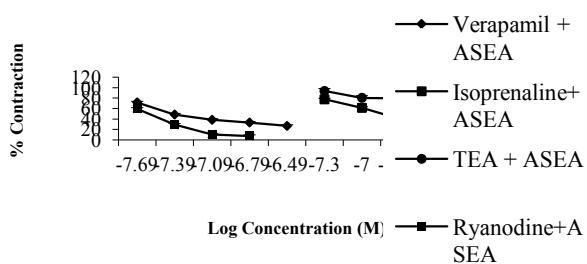

- Prazosin + Extract
- Phentolamine+Extract
- Prazosin+ MeOH
- Phentolamine + MeOH
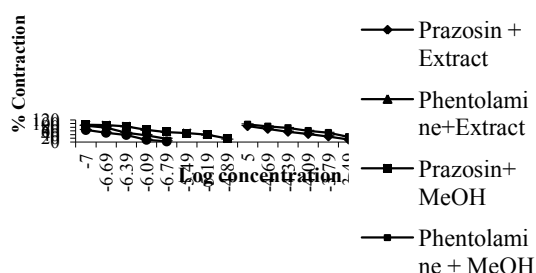
Figure 3D: Effect of Indomethacine and quinacrine on *A. montanus* aqueous extract, MeOH fraction and ASE on rat uterus.

- Indomethacin +Extract
- Quinacrine+Extract
- Indomethacin +MeOH
- Quinacrine+MeOH
- Indometahcin +ASEA

Figure 3E: Effect of verapamil on *Acanthus montanus* aqueous extract, MeOH fraction and ASE on rat uterus.

- Verapamil+ Extract
- Verapamil+MeOH
- Verapmil+ASEA
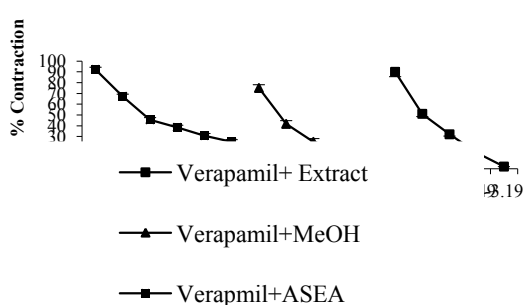
Figure 3F: Effect of some antagonists on ASE in the absence of calcium ions.

- Verapamil + ASEA
- Isoprenaline+ ASEA
- TEA + ASEA
- Ryanodine+ASEA

Atropine was found not to affect extract, MeOH fraction and ASE induced contractions. Quinacrine did not affect contractions of extract and fraction but inhibited ASE induced contraction with an IC50 of 1.97 µM. Indomethacine did inhibit the extract partially (IC50 2.54 mM) but did equipotently inhibit fraction (IC50 = 98.44 µM) and ASE (IC50 = 95.5 µM) contractions (Figure 3D). Tetraethylammonium (TEA) failed to affect the contractions of the extract and methanolic fraction but did partially antagonize the ASE (IC50 = 9.86µM). Meanwhile verapamil caused antagonism of extract (IC50 = 785.4 µM), methanolic fraction (IC50 = 69.85 µM) and isolated compound (IC50 = 55.50 ηM) contractions (Figure 3E).

Table IV: Role of extracellular and intracellular calcium on ASE induced contraction.

	Milieu with Ca^{2+}	Milieu without Ca^{2+}
Acanthus sulphate ester (ASE) only	131 µM (81 – 181)	1.38 mM (1.15 – 1.60)
Verapamil + ASE	72.11 ηM (98. 99 – 160.12)	53.07 ηM (38.91 – 72.78)
Tetraethylammonium (TEA)+ ASE	9.86 µM(6.32 – 11.99)	19.43 µM (17.64 – 21.51)
Ryanodine + ASE	39.93 ηM (2.87-5.55)	24.88 ηM (22.66 – 27.20)
Isoproterenol + ASE	0.29 µM (0.25 – 0.34	0.17 µM (0.14 – 0.20)

The values represent IC50s of the antagonists required to antagonist the various stimulants. n = 6-7; Both extracellular and intracellular Ca^{2+} played roles in the ASE induced contractions.

Table IV shows the importance of extracellular (EC) and intracellular (IC) Ca^{2+} in mediating the contraction of ASE. Its EC50 to induce contraction in the absence of calcium was low (10^{-3} M) as compared to the presence (10^{-6} M). In the physiological solutions, verapamil, isoprenaline, ryanodine but not TEA were very potent and each had almost equivalent strength required to reduce the ASE contraction by half (Figure 3F). In EC Ca^{2+} milieu, ryanodine and not TEA inhibited PGF2α (IC50 = 83.8±5.3 ηM). The ryanodine also inhibited oxytocine (99.8±6.4 ηM) and caffeine (128.1±10.1 ηM) (data not shown).

DISCUSSION:

The relaxant properties of methanolic extract (Adeyemi et al., 1999), methanol/methylene chloride and aqueous extracts (Asongalem et al., 2005) of *Acanthus montanus* have been reported earlier. The present study revealed that the aqueous extract had a biphasic effect on the uterus. It caused

relaxation of the tissue spontaneous activity followed by stimulation when the same doses were used. A similar relaxation pattern was seen with the methanolic fraction and not ASE. This means the plant contained both relaxing and stimulating active ingredients as confirmed by results obtained with other fractions. The stimulatory substances were more in the methanol containing extracting solvents whereas hexane/ethylacetate solvent had more relaxing substances. It was the interactions of these compounds that led to the observed depolarising relaxation patterns. As the stimulating substance was isolated, purified and tested, only contractions were recorded, thus, confirming the presence of stimulants in the extract and the MeOH fraction. The stimulants caused initial mobilization of extracellular calcium ions (Taggart et al., 1996) which culminated into contraction, but the relaxant substance (s) prevented the calcium re-uptake into its intracellular stores, therefore leading to prolonged contraction. The MeOH fraction and diazoxide had different mechanisms of action, since the former lost its pattern as a result of purification. We have identified this relaxant substance to be β-sitosterol (Asongalem et al., 2005). A dose dependent combined relaxation and contraction effects of some plants have been recorded with *Harpagophytum procumbens* DC (Ismail et al., 2004), a Chinese plant *Angelica sinensis* (Dong quai) (Shi et al., 1995), and acetycholine acting on bovine trachea (Clarke & Kirkpatrick, 1995).

The extract-induced contractions were clearly seen only in quiescent tissue resulting from the loss of the uterine spontaneity but ASE did cause contraction independent of the uterine activity. The degree of the stimulation was comparable to acetylcholine and much less than PGF2α.

The stimulation induced by the extract, fraction and ASE was not mediated by the parasympathetic or cholinergic pathway since atropine had no effect on the contraction. The sympathetic system was involved in the mediation of the stimulation. Phentolamine, a sympathetic α-adrenergic non-selective antagonist, was less active against the extract and fraction induced contractions but more potent on ASE along with prazosin. This meant that the new compound was more potent on α_1 adrenergic receptors – a receptor implicated in the contraction of smooth muscles. This sympathetic effect was not only restricted to the alpha receptors but also the β-adrenoceptors. Propranolol did not affect the extract but did inhibit ASE contractions with less potency compared to methanolic fraction. Adeyemi et al. (1999) had earlier reported the inability of propranolol to inhibit the relaxation induced by methanolic extract of this plant. Therefore, effect of ASE is mediated by both the α and β adrenorceptors. Another receptor apart from prazosin which was highly implicated in the mediation of extract, fraction and ASE contractions was the H_1 histaminergic receptor. Pyrilamine at low doses, was able to inhibit the contractions.

Quinacrine (phospolipase A_2 inhibitor) and indomethacine (cyclooxygenase inhibitor) normally prevent the formation of prostaglandin from arachidonic acid. Quinacrine did not reduce the contractions of the extract and fraction but did for the ASE, whereas, endomethacin did for all the preparations. The import of this finding is that ASE interferes with the prostaglandin biosynthesis. ASE pharmacological activities can be said to involve autacoids such as histamine and prostaglandins (Vanheel et al., 1999). The involvement of prostaglandin in the pharmacology of the plant was found in its ability to act as an anti-inflammatory, analgesic and antipyretic agent (Asongalem et al., 2004).

Membrane potential is essential in smooth muscle contraction and is largely achieved by the L-type voltage-gated Ca^{2+} channels (Seban et al., 2004). This channel allows extracellular (EC) Ca^{2+} entry into the cell to contribute in the rise of cytosolic Ca^{2+} concentration which is responsible for the muscle contractility (Mironneau, 1994; Taggart & Susan, 1998). Verapamil blocks this channel. This explains why ASE was very potent in the presence of EC Ca^{2+} with verapamil inhibiting it. The verapamil also blocked ASE in Ca2+- free milieu with similar potency to Ca^{2+} containing milieu. This resulted from its ability to produced time-, voltage- and concentration-dependent "inactivation" or block of open K^+ channels during depolarizing pulses, with negligible block of closed channels at negative holding potentials (Jacob & Decoursey , 1990; Avdonin et al., 1997).

Ca^{2+} sensitive K^+ channel extrudes K^+ from the cell in exchange for Ca^{2+} and is blocked by TEA in EC (Perez et al., 1993) and IC Ca^{2+} milieux (Anwer et al., 1993; Kutluay et al., 2005). TEA blocked ASE induced contractions in Ca^{2+} - containing and Ca^{2+} free physiological solutions. In Ca^{2+} free solution, agonist like ASE, induced spontaneous calcium release from stores through ryanodine-sensitive channel (Taggart et al., 1997) resulting in activation of Ca^{2+} sensitive K^+ current and cell hyperpolarisation (Nelson et al., 1995). TEA blocked this activation thus, reduction in ASE induced contraction. In the presence of EDTA, these contractions were completely abolished, demonstrating the importance of calcium ions in the medium.

Agonists such as caffeine are unable to release Ca^{2+} from caffeine-sensitive stores in Ca^{2+} free solution whereas oxytocin and carbachol can (Taggart et al., 1998). Like oxytocin and carbachol, ASE stimulates. The released Ca^{2+} can lead to stimulation of the smooth muscles. Ryanodine-

sensitive and IP3-sensitive channels are involved in releasing the intracellular (IC) stores. Different agonists are sensitive to different IC stores. ASE was blocked by ryanodine to the same extent in the presence and absence of EC Ca^{2+}. The sensitivity of ASE blockade by ryanodine was similar to $PGF_{2\alpha}$ and oxytocin, but less than caffeine.

Isoprenaline is a non-selective beta agonist whose pharmacological action is inhibitory, and acts through cAMP dependent mechanisms. Downstream effectors activated via a cAMP – dependent mechanism(s) include plasma membrane K^+ channels and Ca^{2+} sensitive K^+ channel (Yoshio et al., 2005). ASE was blocked by isoprenaline in both Ca^{2+} solutions probably through these K^+ channels.

CONCLUSION:

Acanthus montanus extract induced contraction was mediated by a new isolated substance called Acanthus sulphate ester (ASE). The ASE did this through α_1-adrenoceptors, H_1 histamine receptor and arachidonic acid metabolism. It lacked cholinergic activity. Its stimulatory action was less compared to $PGF_{2\alpha}$, acetylcholine, diazoxide, oxytocin and histamine. Calcium and potassium channels were implicated in both EC and IC Ca^{2+} milieux.

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