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Research Article

Relaxant Activity of the Methanol Extract of *Acanthus Montanus* (Nees) T Anderson (Acanthaceae) on Isolated Guinea Pig Trachea

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Abstract

Purpose: *To evaluate the effects of the methanol extract of Acanthus montanus on guinea pig trachealis muscle.*

Methods: *Guinea pig tracheae were set up in 10 ml organ baths. The effect of the methanol extract of Acanthus montanus (0.5 - 8 mg/ml) on the spontaneous tone of the trachea and carbachol-precontracted trachea in the absence and presence of propranolol, glibenclamide and procaine was studied.*

Results: *The extract (0.5 – 8.0 mg/ml) produced a concentration-dependent relaxation of the intrinsic tone in tracheal preparations which was completely blocked by propranolol (3 x 10^{-7} M), glibenclamide (10^{-7} M) and procaine (10^{-3} M). The extract (0.5 - 8 mg/ml) produced a concentration-dependent relaxation of carbachol (10^{-5}M)-precontracted trachea. This effect of the extract was partially blocked by propranolol (3 x 10^{-7}M), procaine (10^{-3}M) and glibenclamide (10^{-7}M) with a progressive increase in the median effective concentration (EC_{50}) values as follows: control, 0.66mg/ml > propranolol, 1.42mg/ml > glibenclamide, 1.54mg/ml > procaine, 2.04 mg/ml.*

Conclusion: *The results obtained suggest that the extract produces a non-specific smooth muscle relaxant effect mediated via β-adrenergic receptor mechanism or potassium channels.*

Keywords: *Acanthus montanus, Trachea, Carbachol, Potassium channels, Propranolol, Glibenclamide, Procaine.*

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INTRODUCTION

Acanthus montanus (family: Acanthaceae) is a prickly herb found in the Mediterranean, Australia and USA. It is also found in the high forests of Upper Guinea, West Cameroons, Upper Guinea as well as in southern Nigeria. In southwestern Nigeria, the leaves of *Acanthus montanus* are used in the treatment of various ailments, including cough, stomach upset, urinary disorders, chest pain, and rheumatic pains. The fresh leaves are used by the Igbos of southeastern Nigeria for the treatment of boil in the finger [1]. In Congo, a decoction of the leafy twigs is used as a purgative. In addition, the leaves pounded with a stem of *Costus* and young pineapple fruit soaked in palmwine is claimed to have diuretic action and used primarily against urethral discharge [1,2].

In our earlier studies, we demonstrated that the methanol extract of *Acanthus montanus* produced relaxation of intestinal smooth muscles [3], and possessed analgesic [4] and anti-inflammatory [5] activities. Several other pharmacological studies have shown the anti-inflammatory, antipyretic, antimicrobial and immunologic properties of the leaves of this plant [6,7]. The maternal and developmental toxicity potentials of the methanol extract of the leaves have also been evaluated [8].
The present study is aimed at evaluating the effects of the extract on the tracheal smooth muscle of the guinea pig.

EXPERIMENTAL

Plant material

The fresh leaves of the *Acathus montanus* plant were collected in Ilesin, Kwara State, Nigeria in the months of April to June and identified by Professor JD Olowokudejo of the Department of Botany & Microbiology, University of Lagos, Lagos, Nigeria. Botanical authentication was confirmed at the Forestry Research Institute of Nigeria, Ibadan, Nigeria where a voucher specimen (no. FHI 106492) was deposited for future reference.

Extraction

The air-dried leaves were cut into small bits and extracted with methanol using a Soxhlet extractor. The resulting extract was evaporated in an oven at 40 ^{o}C. This gave a yield of 12.5 %. The solid dark brown residue was suspended in gum acacia and dispersed in distilled water to form a concentration of 200 mg/ml immediately before use.

Animals

Guinea pigs (250 – 400 g) of either sex kept at the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria were used. The animals maintained under standard environmental conditions, had free access to standard diet (Oladokun Feeds Plc, Ibadan, Nigeria) and water *ad libitum*. The animals were acclimatized for one week and fasted overnight, with free access to water, prior to experiments. All animal experiments were carried out according to international guidelines [9]. Approval for the use of animals for the experiments was obtained from the Ethical Committee of the College of Medicine, University of Lagos, Lagos, Nigeria.

Isolated tissue studies

Guinea pigs of either sex were sacrificed and bled. The trachea was quickly removed and cleaned of adhering fat and connective tissue. It was then opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis muscles Segments of the trachea (4 - 6 rings each) were mounted in 10 ml isolated organ baths containing Krebs-Henseleit solution (with the following composition (mM): NaCl, 115; KCl, 4.7; $CaCl_2$. 2.52; $MgSO_4$, 1.64. $NaHCO_3$, 25; KH_2PO_4, 118; glucose, 11) bubbled with a mixture of 95 % oxygen and 5 % carbon dioxide at 37 ^{o}C and connected by means of a thread to an isometric transducer coupled to a Ugo-Basile 2-channel recorder (Gemini 7080). A tension of 2 g was applied to the preparation and the set-up was allowed to

equilibrate for 90 min with change of bathing fluid done every 15 min.

Cumulative concentration-response relationships for the extract (0.5 – 8 mg/ml) or isoprenaline (3 x 10^{-11} to 3 x 10^{-5}M) were carried out on the spontaneous tone of the trachea. Each concentration of the extract was allowed a contact time of 4 min with the tissue while isoprenaline was incubated with the tissue for 1 min.

In another set of experiments, the preparations were pre-contracted with carbachol (10^{-5}M), which produced sustained contractions. When the plateau was achieved (within 15 - 20 min), the extract (0.5 -8 mg/ml) or isoprenaline (3 x 10^{11} – 3 x 10^{-5}M) was added cumulatively to the bath. Each concentration of the extract was allowed a contact time of 4 min while isoprenaline was allowed a contact time of 1 min. Propranolol (3 x 10^{-7}M), procaine (10^{-3}M) or glibenclamide (10^{-7}M) was added to the bath for 15 min after which the tissues were precontracted for 15 min with carbachol and the concentration-response determination to the extract or isoprenaline repeated. Responses of the tissue to the extract or isoprenaline were calculated as a percentage of the maximum response obtained.

Statistical analysis

The results are expressed as mean ± SEM (standard error of mean) and n represents the number of guinea pigs used for each experiment (carried out in replicates of 2 or 3). Statistical analysis of the data was carried out using Student's t-test and the results were considered significant when $p < 0.05$.

RESULTS

The dried methanol leaf extract of *Acanthus montanus* caused a concentration-dependent relaxation of the spontaneous tone of the trachea (Fig 1A). No further relaxation was observed with the addition of isoprenaline (3 x 10^{-6}M).

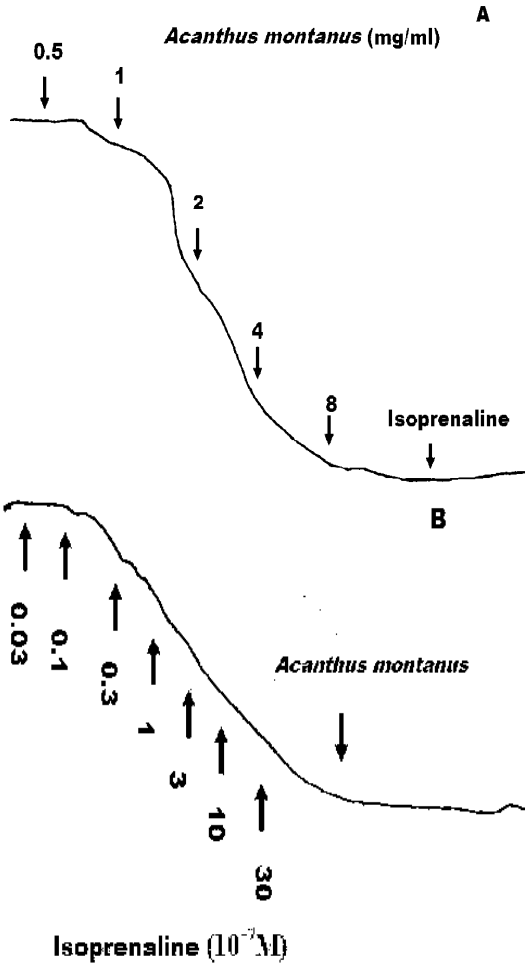

Fig 1: Representative tracings showing the direct effect of (A) *Acanthus montanus* (0.5 – 8 mg/ml) and (B) isoprenaline (3 x 10^{-9} – 3 x10^{-6}M) on isolated guinea pig tracheal preparation. Arrows (↑) depict points of addition.

In some of the trachea, isoprenaline did not cause a complete relaxation of the spontaneous tone since they were further relaxed by a submaximal concentration (4mg/ml) of the extract (Fig. 1B).

The relaxant effect of the extract on the spontaneous tone of the trachea was completely blocked by propranolol (3 x 10^{-7}M), procaine (10^{-3}M) and glibenclamide (10^{-7}M) added to separate tissues. These same

concentrations of propranolol, procaine and glibenclamide only caused significant reductions ($p < 0.05$) in the maximal effect (E_{max}) of isoprenaline on the trachea (Fig 2).

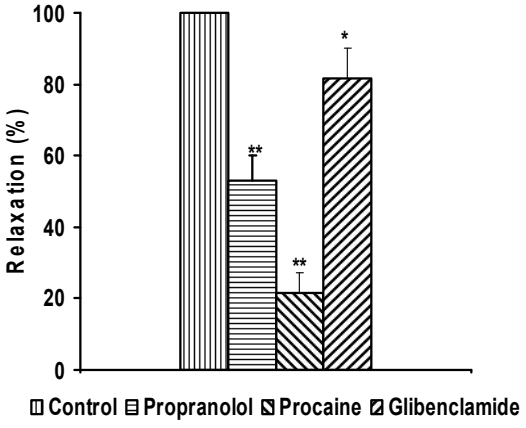

Fig 2: Direct effect of isoprenaline in the absence and presence of propranolol ($3x10^{-7}$ M), procaine (10^{-3} M) or glibenclamide (10^{-7} M). ***Note:*** Error bars represent SEM (n = 5, *$p < 0.05$, **$p < 0.001$ vs control)

A complete relaxation of the carbachol-precontracted trachea was effected by the extract (0.5 – 8 mg/ml). This effect of the extract was partially antagonized by propranolol (3×10^{-7} M), procaine (10^{-3} M) or glibenclamide (10^{-7} M) as depicted by a rightward shift of the concentration response curve (Fig 3).

Isoprenaline produced a concentration-dependent relaxation of carbachol-precontracted trachea. However, in most tissues, isoprenaline did not produce complete relaxations since addition of extract (4 mg/ml) further relaxed the tissues. While propranolol (3×10^{-7}M) produced a near complete block, procaine (10^{-3}M) and glibenclamide (10^{-7}M) did not block but rather enhanced the relaxant effect of isoprenaline on the carbachol-precontracted trachealis muscle (Fig 4).

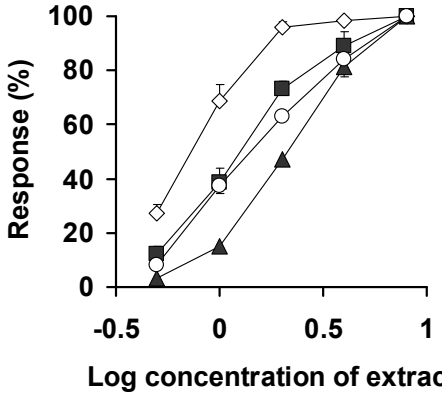

Fig 3: Mean concentration-response curve of *Acanthus montanus* (0.5-8mg/ml) addedm cumulatively to carbachol (10^{-5}M)-precontracted isolated guinea pig trachea in the absence (◊) or presence of propranolol ($3x10^{-7}$M, ■), procaine (10^{-3}M, ▲) or glibenclamide (10^{-7}M, ○). ***Note:*** Error bars represent SEM (n = 4 - 7, *$p < 0.05$ vs control

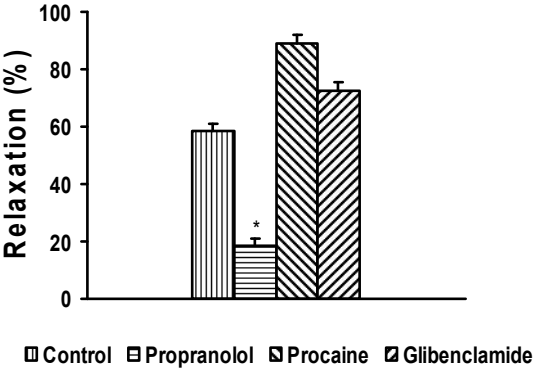

Fig 4: Effect of isoprenaline on carbachol precontracted trachea in the absence and presence of propranolol (3×10^{-7}M), procaine (10^{-3}M), or glibenclamide (10^{-7}M). ***Note:*** Error bars represent SEM (n = 5, *$p < 0.05$ vs control)

DISCUSSION

The methanol extract of *Acanthus montanus* produces a relaxation of the spontaneous tone of the trachea. This effect shows that it possesses bronchodilatory activity on the unstimulated trachea like the β-adrenergic agonist isoprenaline, which was used as the standard drug. The relaxant effect of the

extract on the unstimulated trachea was completely blocked by propranolol, procaine and glibenclamide, suggesting that the extract might be acting through β-adrenergic receptor as well as potassium channel activation. The additional effect of the extract on isoprenaline-relaxed trachea might result from its actions via the channels affected by glibenclamide and procaine since these agents completely blocked the action of the extract but not that of isoprenaline.

The extract and isoprenaline separately caused concentration-dependent relaxation of the carbachol-pre-contracted trachea. Isoprenaline and other β-adrenoceptor agonists are known to cause an enhancement of adenylyl cyclase activity and cause relaxation by several mechanisms. These mechanisms include (i) lowering of free intracellular calcium ion concentration by active removal of free calcium ions from the cell and into intracellular membrane stores, (ii) inhibition of myosin phosphorylation and, (iii) opening calcium ion activated K^+ channels [10]. On the other hand, cholinergic contraction of airway smooth muscle is associated with inhibition of adenylyl cyclase activity through the muscarinic receptors and inhibition of K^+ channels. The degree of muscarinic receptor activation is believed to be important in determining the relaxant potency of isoprenaline; hence, muscarinic M_2 receptor antagonism augments the relaxant potency of isoprenaline [11].
It is possible that the extract may act like isoprenaline in stimulating adenylyl cyclase activity and/or like a muscarinic receptor antagonist to inhibit the carbachol-precontracted tissue.

The potassium channel blockers, glibenclamide and procaine blocked the relaxant effects of the extract but potentiated the effects of isoprenaline on the precontracted trachea.

Importance of membrane potassium ion conductances for maintenance of normal electrical behaviour in smooth muscle is well known [12]. Potassium channels are prominently involved in excitation-contraction coupling processes initiated by contractile agents. Cholinergic contraction of airway and disruption of this inhibitory linkage markedly reduces functional responses of muscle segments *in* vitro [13]. Conversely, various agents that relax airway smooth muscles hyperpolarize tissues by activating potassium channels [14]. Efficacy of β-adrenergic agonists is markedly diminished when calcium activated potassium channels (K_{ca}) or adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP}) are prevented from opening with agents like procaine and glibenclamide, respectively [15]. It is not clear the mechanism by which procaine and glibenclamide produced the observed potentiation of isoprenaline in the precontracted tissues. It is possible that this effect could be mediated by mechanisms, which have nothing to do with their actions on potassium channels. For example, procaine is known to possess a surfactant effect on the membrane.

CONCLUSION

The results obtained suggest that the methanol extract of the dried leaves of *Acanthus montanus* possesses a non-specific relaxant activity on the tracheal smooth muscle. This is similar to effects earlier observed on intestinal smooth muscles [3].

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