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RESEARCH ARTICLE

Relaxative Effect of Cold Water Stem-Bark Extract of *Erythrophleum suaveolens* on Frog *Rectus Abdominis*

Effet relaxant de l'eau froide Extrait de la tige-écorce d'Erythrophleum suaveolens sur le Rectus Abdominus de la grenouille

T. O. Ogundeko, M. I. Builders*, E. A. Ogbole

ABSTRACT

BACKGROUND: ***Erythrophleum suaveolens* is a folkloric plant claimed to be a muscle relaxant. The frog rectus abdominus muscle is a tissue that is apt for the study of neuromuscular junction activities.**

OBJECTIVE: **To determine the muscle relaxant effect of *Erythrophleum suaveolens* on the isolated frog rectus abdominis muscle in the presence of acetylcholine.**

MATERIALS AND METHODS: **Dose- response relationships (DRC) of cold water bark extract of *Erythrophleum suaveolens*, acetylcholine (Ach) only in the presence of *E. suaveolens* at varying concentrations and volumes from given stocks of $1x10^{-4}$ in each case were studied. Responses were obtained isotonically and recorded via a dynamometer.**

RESULTS: **Stem-bark water extract of E. *suaveolens* blocked the contracture effect of acetylcholine. The relaxing effect of the extract on the isolated rectus abdominis muscle was slow, though blockade effect on the acetylcholine-induced contractions decreased with increasing dose of acetylcholine. However, the effect of Ach on frog rectus abdominis muscle was dose-dependent. Effective concentrations (EC50) of Ach and E. suaveolens plus Ach were $3.16 x10^{-9}$ and $2.58 x10^{-7}$ g/ml respectively.**

CONCLUSION: ***Erythrophleum suaveolens* is a skeletal muscle relaxant,which appears to be a potent non-depolarizing neuromuscular blocker. BJM 2017; 1(1): 15–18.**

Keywords: *Erythrophleum suaveolens*, Frog rectus Abdominis, Acetylcholine, Inhibition, Muscle Relaxant.

ABSTRAIT

CONTEXTE: Erythrophleum suaveolens est une plante folklorique prétendu être un myorelaxant. Le rectus grenouille est abdominus muscle est un tissu qui est apte pour l'étude des études de jonction neuromusculaire.

OBJECTIF: Déterminer l'effet myorelaxant de Erythrophleum suaveolens sur la grenouille isolé rectus abdominis muscle en présence d'acétylcholine.

MATÉRIELS ET MÉTHODES: relation dose-réponse (RDC) de froid extrait d'écorce de l'eau de Erythrophleum suaveolens, acétylcholine (Ach) seulement en présence de E. suaveolens à des concentrations et des volumes variant de stocks de données de 1x10-4 dans chaque cas ont été obtenus avec réponses isotonique enregistrées (1mm / sec) par l'intermédiaire d'un dynamomètre.

RÉSULTATS: Stem écorce extrait de l'eau de E. de l'extrait bloqué l'effet de contracture de Ach. L'effet relaxant de l'extrait isolé sur le muscle droit de l'abdomen a été lente, bien que l'effet de blocage sur les contractions induites par l'acétylcholine a diminué avec l'augmentation de la dose d'acétylcholine. Cependant, l'effet de l'Ach sur le rectus abdominis muscle grenouille est dose-dépendante. Les concentrations efficaces (CE50) de Ach et E. suaveolens ainsi Ach étaient 3.16 x10-9and 2,58 x10-7g / ml, respectivement.

CONCLUSION: Erythrophleum suaveolens est un relaxant musculaire squelettique, et semble être un non-dépolarisants bloquant neuromusculaire puissant. **BJM 2017; 1(1): 15–18.**

Mots-clés: Erythrophleum suaveolens, grenouille rectus abdominis, acétylcholine, Inhibition, Relaxation.

Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Bingham University, Jos Campus.

**Correspondence:* Dr. Modupe Builders. Pharmacology and Therapeutics, College of Medicine and Health Sciences, Bingham University, Jos Campus. Private Mail Bag 2172, Jos, modupebuilders@yahoo.com

Abbreviations: Ach, Acetylcholine; **DRC,** Dose-response curve; **EC_{50}**, Effective concentration at 50%; **NMJ,** Neuromuscular junction.

INTRODUCTION

The rectus abdominus muscle of the frog though striated does not behave like normal voluntary muscle and responds to acetylcholine (Ach) by giving a slow contraction. It is an extremely useful preparation for showing the actions of those compounds that block transmission at the neuromuscular junction (NMJ) by acting the same way as an excess of Ach. Such compounds will also block transmission in the rat phrenic nerve diaphragm and stimulate the slow fibres of the frog rectus.[1–2]

Drugs like diltiazem and verapamil have been reported to inhibit contractions of frog rectus abdominus muscles in the presence of Ach.[3] The relaxing effect of cold water stem-bark extract of *E. suaveolens* is evident on skeletal muscle such as the rat phrenic nerve-diaphragm. An observed relaxation-pattern of *E. suaveolens*on rats phrenic nerve diaphragm preparation showed it as closely related with that of hexamethonium thus suggesting as an explorable potent- muscle relaxant as claimed by traditional healers.[4] The determination of LD_{50} on albino mice gave an insight into the safety margin of E. *suaveolens* (223.8±0.05mg/kg body weight) falling within the very toxic range as defined by Hodge and Sterner categorization.[5] Investiga-tions carried out on isolated ileum tissue of the guinea-pig (*Caviaporcellus*) and smooth muscle of the rabbit jejunum (*Oryctolagus-cuniculus*) by running a dose-response relationship of agonist test drugs (acetylcholine, histamine, and barium chloride; isoprenaline and adrenaline) in the presence of the cold water crude extract of stem-bark of *Erythrophleum suaveolens* showed an antagonist effect with a right shift and inhibitory nature of *Erythrophleum suaveolens.*[6]

Detailed investigations on plant materials especially such that are already in use especially by trado-medical practitioners should not be taken with levity. *Erythrophleum suaveolens* is used for diverse purposes: as drinks, the bark is used as alcoholic and stimulant as well as laxative, abortifacient, antibiotics, and in the treatment of oedema, gout, rheumatism amongst others in the area of medicine.[7–9]

This study aimed to further confirm the muscle relaxant effect of *Erythrophleum suaveolens* on the isolated frog rectus abdominis muscle in the presence of acetylcholine.

MATERIALS AND METHODS

Thr stem-back of *Erythrophleum suaveolens* was collected from Buruku Local Government Area, Benue State central Nigeria. This was identified and classified by Professor Hussaini (Botanist with the University of Jos Nigeria) and Dr Okonkwo (Taxonomist with the College of Forestry, Jos, Nigeria). Plant sample was dried in the Pharmacology Laboratory of Bingham University, Jos while the process of extraction was done as described earlier.[4–7]

Freshly-prepared isolated frog r*ectus abdominis* muscle was attached to a lever (0.5–1g load) and mounted on an organ bath (50–100ml) containing freshly prepared frog ringer solution (NaCl–6.50g, KCl –0.75g, $CaCl_2$ –1.00g, $NaHCO_3$–0.40g – Sigma Chemical Company, Louis, USA, Kernel Chemicals, Germany). Contraction and relaxation responses were isotonically recorded (1mm/sec) via a dynamometer (UgoBasile, Comerio, Italy) (5volts), frequency (0.5Hz), pulse width (1.4mls), (1). pH (7.4), aeration (air) and temperature (320C). Acetylcholine - Sigma Chemical Company, Louis, USA was used as the reference drug.

Dose- response of relationships (DRC) of cold water bark extract of *Erythrophleum suaveolens*, Acetyl-choline and Ach in the presence of *E. suaveolens* at varying concentrations and volumes from given stocks of $1x10^{-4}$ in each case were obtained. Responses were isotonically recorded (1mm/sec) via a dynamometer.

RESULTS

Erythrophleum. suaveolens extract produced no response on the isolated tissue of frog rectus abdominis muscle, thus no amplitude of response was observed (Table 1). Tissue in the presence of Ach exhibited contractile response with threshold and maximum heights response of 1.5cm and 3.2cm respectively, at $4.0x10^{-7}$ g/ml, percentage maximum response was 100 as shown in Table 2.

Table 3 indicates that *E. suaveolens* extract in the presence of Ach reduced tissue response (lowest height–0.6cm) at $2.2 x10^{-8}$g/ml with percentage maximum response of 70 and (highest–0.9cm) at $1.0x10^{-6}$g/ml with percentage maximum response of 100.

A dose-dependent graph indicating effective eoncentration (EC_{50}) of Ach and E. *suaveolens* plus Ach $3.16 x10^{-9}$ and $2.58 x 10^{-7}$g/ml was obtained by plotting percentage maximum responses against log concentrations (Figure 1). The curve showed a shift to the right in compliance with the characteristics of an agonist in the presence of Ach (percentage maximum response = 100). However, the curve obtained from that of Ach in the presence of *E. suaveolens* extract (percentage maximum response = 100) also produced a shift to the right.

Table 1: Dose Effect of *E. suaveolens* Extract on Frog Rectus Abdominis Muscle

Ach (g/ml)	*ESE*	FBC (g/ml)	Log FBC	MR (cm)	Responses	MedR (cm)	MMR %
$1 x 10^{-4}$	0.2	$4.0 x 10^{-6}$	–6.0	0.0	3	0.0	0.0
$1 x 10^{-4}$	0.4	$8.0 x 10^{-6}$	–9.0	0.0	3	0.0	0.0
$1 x 10^{-4}$	0.6	$1.2 x 10^{-5}$	–7.9	0.0	3	0.0	0.0
$1 x 10^{-4}$	0.8	$1.6 x 10^{-5}$	–2.0	0,0	3	0,0	0,0
$1 x 10^{-4}$	1.0	$2.0 x 10^{-5}$	–3.0	0.0	3	0.0	0.0
$1 x 10^{-4}$	2.0	$4.0 x 10^{-7}$	–7.4	0.0	3	0.0	0.0

Ach, Acetylcholine; ESE. E. suaveolens extract (ml); FBC, final bath concentration; MedR, median response (cm); MMR, Mean Maximum Response (%) ; MR, Mean Response (cm).

Table 2: Effect of Acetylcholine Concentration on Frog Rectus Abdominis Muscle

Ach (g/ml)	*ESE*	FBC (g/ml)	Log FBC	MR (cm)	Responses	MedR (cm)	MMR %
1×10^{-4}	0.2	4.0×10^{-7}	–6.40	1.5	3	1.5	46.9
1×10^{-4}	0.4	8.0×10^{-7}	–6.1	2.0	3	2.0	62.5
1×10^{-4}	0.6	1.2×10^{-7}	–5.92	2.7	3	2.7	84.4
1×10^{-4}	0.8	1.6×10^{-6}	–5.80	2.8	3	2.8	87.5
1×10^{-4}	1.0	2.0×10^{-5}	–3.01	3.2	3	3.2	100.0
1×10^{-4}	2.0	4.0×10^{-7}	–7.4	3.2	3	3.2	100.0

Ach, Acetylcholine; ESE. E. suaveolens extract (ml); FBC, final bath concentration;MedR, Median response; MMR, Mean Maximum Response; MR, Mean Response.

Table 3: Dose-response Relationships of Acetylcholine in the Presence of *E. suaveolens* extract on Frog Rectus Abdominis Muscle

Ach (g/ml)	*ESE*	FBC (g/ml)	Log FBC	MR (cm)	Responses	MedR (cm)	MMR %
1×10^{-4}	0.2 + 0.5	1.4×10^{-8}	–7.85	0.0	3	0.0	0.0
1×10^{-4}	0.4 + 0.5	1.8×10^{-8}	–7.74	0.0	3	0.0	0.0
1×10^{-4}	0.6 + 0.5	2.2×10^{-8}	–7.70	0.63	3	0.63	70.0
1×10^{-4}	0.8 + 0.5	2.6×10^{-8}	–7.60	0.7	3	0.7	77.8
1×10^{-4}	1.0 + 0.5	3.0×10^{-8}	–7.52	0.8	3	0.8	88.9
1×10^{-4}	0.5 + 0.5	1.0×10^{-6}	–6.0	0.9	3	0.9	100.0

Ach, Acetylcholine; ESE. E. suaveolens extract (ml); FBC, final bath concentration; MedR, Median response; MMR, Mean Maximum Response; MR, Mean Response.

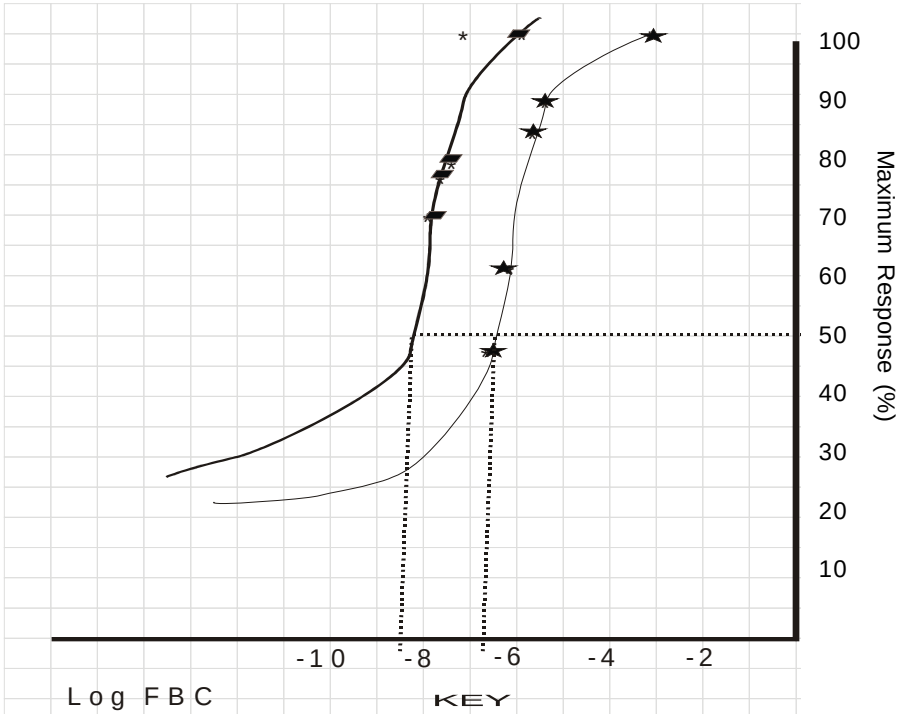

Fig. 1: Maximum Response against Log Final Bath Concentration of Acetylcholine alone and Acetylcholine in the presence of *E. suaveolens.* ★ Acetylcholine alone ■ Acetylcholine + *E. suaveolens.*

DISCUSSION

The fact that muscle relaxants are very powerful drugs which may produce negative effects has left one to explore the use of *Erythrophleum suaveolens* for same purpose in traditional medicine practices. This is also useful for drug development.

***Erythrophleum suaveolens* extract alone**: *Erythrophleum suaveolens* extract blocked the nicotinic actions of endogenous ligand acetylcholine by way of inhibiting flow of nerve impulses within the tissue.[10] Acetylcholine is a natural endogenous ligand and one of the synaptic transmitters. The acetylcholine receptor transmits its signal across the plasma membrane, increases transmembrane conductance of the relevant ion and thereby altering the electrical potential across it membrane. Acetylcholine causes the opening of the ion channel in the nicotinic acetylcholine receptor (Ach R), which allows Na^+ ions to flow down its concentration gradient into cells, producing a localized excitatory postsynaptic potential- a depolarization, and excitation.[10]

***Erythrophleum suaveolens* in the presence of acetylcholine:** The inhibitory effect of the extract of *Erythrophleum suaveolens* may be due to blockage of the receptor and thus preventing the entry of ions during depolarization of skeletal muscle by acetylcholine. The results of the present investigation suggest that an extract of *Erythrophleum suaveolens* may exert an inhibitory effect on skeletal muscle contraction and this may be due to inhibition of the effect of acetylcholine at the receptor site.[10]

Neuromuscular blocking drugs inhibit neuromuscular transmission from nerves to muscles by competitively blocking the binding of acetylcholine to its postsynaptic receptors at the motor end plate, thereby causing paralysis of the muscle.[11] *E. suaveolens* extract exhibited non-depolarizing muscle relaxation by preventing access of acetylcholine to the receptor protein. This results in no depolarization and prevents a change in resting potential of the motor end-plate. The result is lack of muscular contraction or paralysis.[12]

E*rythrophleum suaveolens* extract blocked the contracture effect of Ach. The relaxing effect of the extract on the isolated rectus abdominus muscle was slow, though blockade effect on the acetylcholine-induced contractions decreased with increased dose of acetylcholine. Paralysis is increased when using either non-depolarizing or depolarizing relaxants by substances such as halogenated volatile anesthetic agents, ether, lidocaine, digitalis glycosides, quinidine, diuretics, and procaine. Also non-depolarizing blocks are increased by additional non-depolarizers.[13]

Muscle relaxation is the mainstay of modern anaesthesia and intensive care. Through manipulation of the traditional structure-action relationships, many new and improved muscle relaxants have been created, and several have been brought to clinical use.[14] Research in the field of neuromuscular-blocking drugs such as the isolation of tubocurarine and *Malouetiabe quaertiana* through improved understanding of the physiology of neurons and receptors over the years[15–16] suggests similar compounds may also be responsible for non-depolarizing blocking activity of *E. suaveolens.*

Conclusion

The cold water stem-bark extract of *E. suaveolens* exhibited antagonizing effect on the Ach-induced contraction of the isolated frog rectus abdominis muscle. This study corroborates the fact that *E. suaveolens* is a skeletal muscle relaxant. There is therefore need to proffer better approach to the use of *E. suaveolens* as skeletal a muscle relaxantin tradio-medicine as well as in drug development.

Conflicts of interest:

No conflict of interest.

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