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# Investigation of Relaxative Effect of Stem-bark Extract of *Erythrophleum suaveolens* on rat Phrenic Nerve-diaphragm Muscle

**T. O. Ogundeko[1], I. I. Idyu[2], M. T. Ojo[3], V. C. Idyu[4], E. A. Ogbole[1], M. I. Builders[1], M. S. Ramyil[5*], B. O. Toma[6] and A. F. Asalu[1]**

*[1]Department of Pharmacology and Therapeutics, College of Health Sciences, Bingham University, Jos, Nigeria.*
*[2]Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.*
*[3]Department of Laboratory Technology, Faculty of Natural Sciences, University of Jos, Nigeria.*
*[4]Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.*
*[5]Department of Medical Microbiology, College of Health Sciences, Bingham University, Jos, Nigeria.*
*[6]Department of Paediatrics, Faculty of Medical Sciences, University of Jos, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Author III designed the study and wrote the protocol. Authors MTO, MSR, and VCI managed the animals, and collected all data. Authors TOO, MSR and BOT performed the statistical analysis, and wrote the first draft of the manuscript. Authors TOO, III and EAO did the literature search, Authors TOO and MIB wrote part of the manuscript while Author AFA did the final review of manuscript. All authors read and approved the final manuscript.*

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**Corresponding author: E-mail: crownramyil@yahoo.com;*

## ABSTRACT

**Background:** The effect of crude extract of *Erythrophleum suaveolens* was studied on isolated tissues of the Rat's phrenic nerve-diaphragm muscles. Study aimed to investigate the relaxant effect of crude extract of *E. suaveolens* on the skeletal muscle using isolated rat phrenic nerve-diaphragm preparation in comparison with selected test drugs activity.
**Methods:** Reference drugs (Hexamethonium, Pancuronium and Suxamethonium) were used on the isolated tissues. Crude extract blocked the effect of Acetylcholine-induced contractions, thus a relaxing effect.
**Results:** The relaxing effect of cold water stem-bark extract of *E. suaveolens* is evident on skeletal muscle of rat phrenic nerve-diaphragm. The observed relaxation-pattern is closely related with that of Hexamethonium.
**Conclusions:** Cold water stem-bark extract of *Erythrophleum suaveolens* is therefore a potent muscle relaxant as claimed by traditional healers, hence could be explored as a muscle relaxant.

*Keywords: Erythrophleum suaveolens; Rat's phrenic nerve-diaphragm; skeletal muscle; relaxation; Hexamethonium.*

## 1. INTRODUCTION

Scope for development of a simple *in vitro* preparation for study of pharmacological aspect of proprioceptive activity in mammalian striated muscle have extended the work of [1] on muscle spindle efferents in the rat phrenic nerve-diaphragm preparation with particular purpose of testing the validity of this preparation for drug effect evaluation [1]. Muscle spindle afferent discharges exhibiting an approximately linear length-frequency relation could be recorded from the phrenic nerve in the isolated phrenic nerve diaphragm preparation of the rat [2–3]. There have been a considerable number of investigations on the influence of drug on fusimotor functions and spindle afferent activity [4–7]. The determination of $LD_{50}$ on albino mice gave an insight into safety margin of E. suaveolens (223.8±0.05mg/kg body weight) falling within the very toxic range as defined by Hodge and Sterner (1947) categorization [8]. Extract from Investigations carried out on the isolated ileum tissue of the guinea-pig (*Cavia porcellus*) and GIT 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* ascertained antagonist effect with a right shift and inhibitory nature of *Erythrophleum suaveolens* [9-10].

*E. suaveolens* is a perennial tree of about 30 m in height, slightly buttressed, often low-branching and producing a dense spreading crown. The plant is one of the useful plants of west tropical Africa which is referred to by various names by natives [11-16]. 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 [9]. The knowledge of the effect(s) of substances to be used as medicines on different body systems is very vital in drug development viz–a viz drug safety [17].

### 1.1 Statement of Problem

Some African Traditional healers claim that extract of *Erythrophleum suaveolens* is a potent muscle relaxant for adults and children. Detailed investigations on plant materials especially such that are already in use by tradio-practitionals with the current increase in infant mortality rate due to the illicit use of local preparation in Africa and some other parts of the world should not be taken with levity. As a result, there is need for further investigation of the blockade/inhibitory effect of cold water bark extract of *E. suaveolens* on the skeletal muscle.

### 1.2 Aims and Objective

To investigate the relaxant effect of cold water extract of the stem-back of *E. suaveolens* on the skeletal muscle using isolated rat phrenic nerve diaphragm preparation in comparison with selected test drugs activity.

## 2. MATERIALS AND METHODS

### 2.1 Plant Preparation

#### 2.1.1 Collection

Stem-back of *Erythrophleum suaveolens* were collected from Buruku Local Government Area of Benue State, Nigeria. Plant Identification and authentication were done by Mr. Okonkwo - a plant taxonomist with the Federal School of Forestry, Jos Plateau State, Nigeria and Professor S.W Husseni of the Department of Botany, University of Jos, Nigeria respectively.

#### 2.1.2 Extraction

Plant bark was dried under shade, in the Pharmacology Research Laboratory of the University of Jos, Nigeria. Sample was pounded into powder using wooden Mortar and Pestle. The pulverized was stored at room temperature until required. Maceration method of extraction was established by weighing 200 g of powder, weighed and dissolved in 300 ml distilled water and stirred thoroughly then allowed to stand for 24 hours. After 24 hours at ambient room temperature, mixture was stirred with a glass rod and then filtered through Whatman number one filter paper using suction pump. The filtrate was concentrated in a water bath at a temperature of 80±1.0°C until a reddish, sticky extract was obtained. The recovered extract was stored in the Refrigerator at 4°C. 0.5 g of crude water extract was weighed with a Mettler balance and dissolved in 5ml of distilled water to give a stock concentration solution of $1x10^{-1}$ g/ml and further diluted to $1x10^{-3}$ g/ml to obtain suitable amplitude. However, $1x10^{-1}$ g/ml concentration was used throughout the experiment.

### 2.2 Animals and Tissue Preparation

#### 2.2.1 Rat's phrenic nerve

Adult sized albino rats (Wister strain) (160.5 g body weight) were purchased in cages from the Animal House Unit of the University of Jos, Nigeria.

#### 2.2.2 Ethical issues

Two of the authors that handled the animals are licensed to handle laboratory animals.

The animals were allowed to acclimatize, fed with pelleted feeds and clean water *ad libitum* for 3 days, maintained at natural environmental temperature (NEV) of 26-28°C and deprived of food for 24 hours before commencement of experiment. Rat diaphragm together with the left phrenic nerve was dissected out accordingly to the method of Bulbring [18]. 2-4 cm of the muscle was taken out around the point of entry of the phrenic nerve. Care was taken to exclude the tendonous portion and to incise the muscle along the direction of its fibers [2]. One end of the muscle was rigidly fixed and the other end was attached to a metal hook for applying stretch on the electrode while the phrenic nerve was placed on a bipolar electrode with ends connected to a student physiograph stimulator (Labotech ss-700 BD instrumentation India) and responses obtained isometrically on recording paper via student the physiograph stimulator at Voltage = 5 V, Frequency = 0.5 Hz, Pulse Width = 1.4 mls and Sensitivity = 1. The fresh preparation was secured into an isolated tissue bath containing freshly prepared Krebs solution, pH 7.4, aerated with 95% Oxygen and 5% $CO_2$ and maintained at 32°C. Crude extract of *E. suaveolens*, Hexamethonium, Pancuronium and Suxamethonium were tested on the isolated preparations through nerve and direct muscle stimulations and effects obtained thereafter.

### 2.3 Reference Drugs and Reagents

#### 2.3.1 Reference drugs

* Pancuronium Bromide – Faulding.
* Suxamethonium Chloride - Faulding.
* Physostigmine - Sigma Chemical Company, Louis, USA.
* Hexamethonium Chloride – Spectrum Chemica, USA.
* Acetylcholine - Sigma Chemical Company, Louis, USA.

#### 2.3.2 Reagents

NaCl (6.9 g), KCl (0.35 g), $CaCl_2$ (0.28 g), $NaHCO_3$ (2.10 g), $KH_2PO_4$ (0.16 g), $MgSO_4 7H_2O$ (0.29 g) and $C_6H_{12}O_6$ (2.00 g) - Sigma Chemical Company, Louis, USA and Kernel Chemicals, Germany.

The reference drugs were prepared by weighing out and dissolving in required volume of distilled water to give desired stock concentration. Various dilutions from stock were made for each experiment.

### 2.4 Drugs and Crude Extract Investigations

The effect of drugs on the twitch response was done by obtaining initial control tracing for both nerve and direct muscle stimulations. Subsequently, drug activities were investigated on the isolated tissues at various concentration and volume to obtain graded dose-responses in the following order: Incubation with Ach ($1x10^{-2}$ g/ml) for 3 minutes; Hexamethonium ($1x10^{-3}$ g/ml); Pancuronium ($1x10^{-3}$ g/ml); Physostigmine ($1x10^{-5}$ g/ml); Suxamethonium ($1x10^{-3}$ g/ml) and Physostigmine ($1x10^{-5}$ g/ml).

Having established tracings for the effects of the reference drugs on the isolated tissues, graded dose-responses for the crude extract of *Erythrophleum suaveolens* alone ($1x10^{-1}$ g/ml) and that of Hexamethonium ($1x10^{-3}$ g/ml) alone were also established and heights of responses of both compared.

## 3. RESULTS

Addition of Acetylcholine produced a response with both nerve and muscle stimulation with height of response (2.6 cm); (4.8 cm). The addition of Hexamethonium showed a reduced tissue response was with nerve and muscle stimulation producing reduced height of response (1.9 cm); (3.4 cm) thus blocking the activity of Ach. Tissue exhibited a decreased response to both nerve and muscle stimulations in the presence of Pancuronium, as the there was a gradual reduction in height of response, thereby further blocking the effect of Ach but recovered in the presence of Physostigmine. Suxamethonium showed an initial increase in response, then a progressive and erratic decrease without recovery even in the presence of Physostigmine. This also establishes the fact that Suxamethonium shows an irreversible, non-competitive blockade (Fig. 1).

Crude extract of *Erythrophleum suaveolens* showed a blocking effect like that of Hexamethonium as observed in Fig 1. Both effects were dose-dependent. However, blocking effect was more on the nerve than muscle (direct stimulation) (Fig. 2).

Tissue exhibited a relaxing effect in the presence of this already established muscle relaxant. Higher blocking effect was also obtained from muscle stimulation than that obtained from nerve stimulation (Fig. 3).

The height of contraction of crude extract of *E. suaveolens* ($1x10^{-1}$ g/ml) obtained from nerve and muscle stimulations at 0.1ml and at 0.8ml were 2.6 cm: 4.0 cm and 3.4 cm: 4.25 cm respectively (Figs. 2, 4 and Table 1). That of Hexamethonium ($1x10^{-3}$g/ml) at same volumes was 2.9 cm: 4.7cm and 5.25 cm: 5.5 cm respectively (Figs. 3, 5 and Table 1).

Inhibitory effect of E. suaveolens extract ($1x10^{-1}$ g/ml) was observed to be close to that of Pancuronium ($1x10^{-3}$ g/ml).

## 4. DISCUSSION

Observed activities of the selected neuromuscular blocking agents from the tissue response established their known characteristics. Hexamethonium, a non-depolarizing ganglion blocker and an ACh (NN) receptor antagonist, Pancuronium, that showed observable reduced height of nerve and muscle response, thereby further blocking the effect of ACh, thus a competitive blocking effect and Suxamethonium which exhibited an irreversible, non-competitive blockade.

**Table 1. Height of twitches of crude extract of *E. suaveolens* and Hexamethonium**

| Volume administered (mls) | Height of response (cm) | | | |
|---|---|---|---|---|
| | Crude Extract of *E. suaveolens* ($1x10^{-1}$ g/ml) | | Hexamethonium ($1x10^{-3}$ g/ml) | |
| | NS | MS | NS | MS |
| 0.1 | 2.6 | 4.0 | 2.9 | 4.7 |
| 0.2 | 3.0 | 3.9 | 3.0 | 3.9 |
| 0.4 | 3.3 | 4.1 | 5.2 | 5.4 |
| 0.6 | 3.4 | 4.3 | 5.3 | 5.5 |
| 0.8 | 3.4 | 4.25 | 5.3 | 5.5 |
| 1.0 | 2.3 | 2.4 | 5.25 | 5.5 |

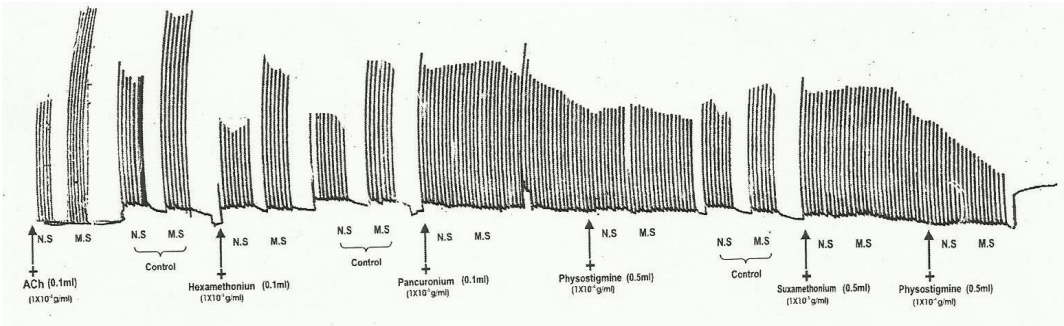


**Fig. 1. Twitches of reference drugs on isolated rat's phrenic nerve-diaphragm**

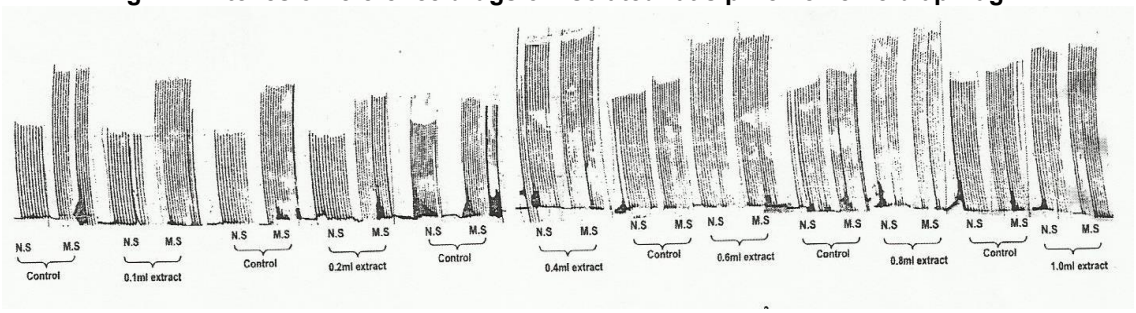


**Fig. 2. Twitches of E. suaveolens crude extract (1x10-1 g/ml) on isolated rat's phrenic nerve-diaphragm**

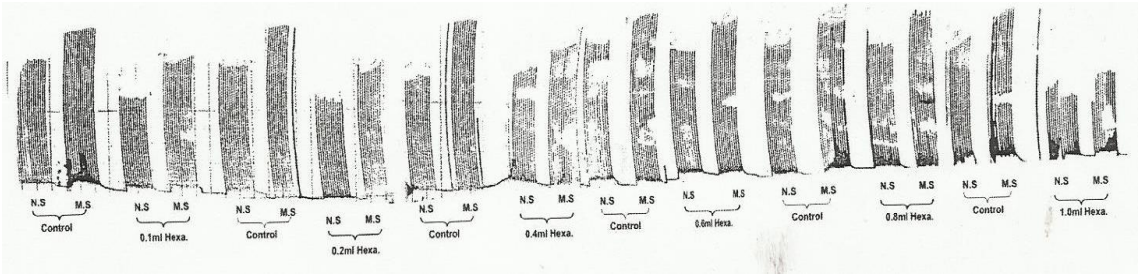


**Fig. 3. Twitches of Hexamethonium (1x10-3 g/ml) on isolated rat's phrenic nerve- diaphragm**
*Key: NS= Nerve Stimulation MS= Muscle Stimulation*

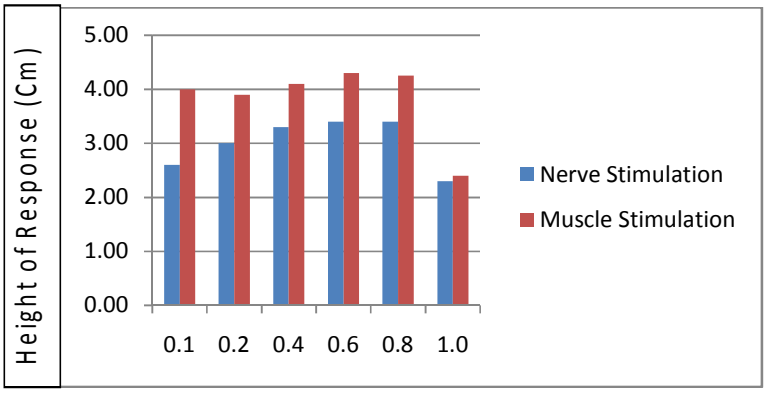


**Fig. 4. Activity of crude extract of *E. suaveolens* (1 X $10^{-1}$ g/ml)**
*Volume Administered (mls)*

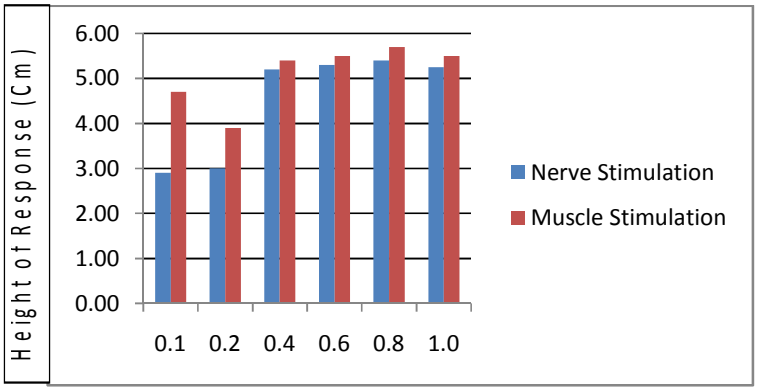


**Fig. 5. Activity of Hexamethonium (1 X $10^{-3}$ g/ml)**
*Volume administered (mls)*

The twitches of crude extract of E. suaveolens on the rat's phrenic nerve diaphragm followed the same trend with that of Hexamethonium as both drugs exhibited dose-dependent effect. Study evidently showed height of contraction of crude extract of E. suaveolens ($1x10^{-1}$ g/ml) at 0.1ml and 0.8 ml compared with that of Hexamethonium ($1x10^{-3}$ g/ml). Crude extract of E. suaveolens has a lower height of response (Figs. 2, 4 and Table 1) despite administered at a little higher concentration than Hexamethonium (Figs. 3, 5 and Table 1).

The dose-dependent blocking effect exhibited by the crude extract as seen more on the nerve than muscle is an indication that *Erythrophleum suaveolens* could be acting on receptor-operated channel rather than voltage operated channel. As a result, the extract may most probably acts on the autonomic ganglia by binding on the $N_N$ receptor.

## 5. CONCLUSION

The relaxing effect of crude extract of *E. suaveolens* fairly compares with that of Hexamethonium on skeletal muscle of rat phrenic nerve-diaphragm as observed in the relaxation-pattern and twitches thus, possesses the characteristic of a non-depolarizing ganglion blocker despite its mechanism of action not appearing at par with that of Hexamethonium. Cold water stem-bark extract of *E. suaveolens* is therefore a potent muscle relaxant as claimed by traditional healers; hence its use as a muscle relaxant could be explored.

## 6. RECOMMENDATION

Further studies on muscle relaxant properties with other skeletal tissues in order to enable the usage of *E. suaveolens* as a muscle relaxant both for adults and children in the near future is recommended.

## CONSENT

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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