

Original Research Article**INVESTIGATION OF RELAXATIVE EFFECT OF STEM-BARK EXTRACT OF
Erythrophleum suaveolens ON RAT PHRENIC NERVE-DIAPHRAGM MUSCLE****ABSTRACT**

The effect of water soluble fraction macerated stem-bark extract of *Erythrophleum suaveolens* was studied on isolated tissues of the Rat's phrenic nerve diaphragm muscles. Reference drugs (Acetylcholine, Pancuronium bromide and Suxamethonium Chloride) were used on the isolated tissues. Extract blocked the effect of Acetylcholine-induced contractions, thus a relaxing effect. 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 Pancuronium. 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, Pancuronium.

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 Barstad et al, (1965) 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. 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 [1], [2]. There have been a considerable

number of investigations on the influence of drug on fusimotor functions and spindle afferent activity [3], [4], [5], [6]. The determination of LD₅₀ 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 [7]. 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* [8], [9].

E. suaveolens is a perennial tree of about 30m 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 [10], [11] – [15]. 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 [8]. 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 [16].

Detailed investigations on plant materials especially such that are already in use especially by tradio-practitionals should not be taken with levity. As a result of need for further confirmation of the blockade/inhibitory effect of cold water bark extract of *E. suaveolens* on other smooth muscles of the GIT [9].

1.1 Statement of Problem

Some African Traditional healers claim that extract of *Erythrophleum suaveolens* is a potent muscle relaxant. This they also use in children.

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 using 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 was done by Mr. Okonkwo - a plant taxonomist with

the Federal School of Forestry, Jos Plateau State, Nigeria and authenticated by Professor S.W Husseni of the Department of Botany, University of Jos, Nigeria.

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 according to the method of Ibrahim *et al.* (1984); Audu *et al.* (2001). The pulverized was stored at room temperature until required Idyu *et al.*, (2014). Maceration method of extraction was established by weighing 200g of powder was weighed and dissolved in 300ml distilled water and stirred thoroughly and left for 24 hours. Mixture was allowed to stand for 24hours at ambient room temperature, 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 $80 \pm 1.0^{\circ}\text{C}$ until a reddish, sticky extract was obtained. The recovered extract was stored in the Refrigerator at -4°C according to Idyu *et al.* (2014). 0.5g of crude water extract was weighed with a Mettler balance and dissolved in 5ml of distilled water to give a stock concentration solution of $1 \times 10^{-2}\text{g/ml}$ and $1 \times 10^{-3}\text{g/ml}$. which were used throughout the experiment.

2.2 Animals and Tissue Preparation

2.2.1 Rat's Phrenic Nerve

Adult sized Wister strain rat sized rabbits (3.8-4.5kg) were purchased in cages from the Animal House Unit of the University of Jos, Nigeria. These were allowed to acclimatize, fed with pelleted feeds and clean water *ad libitum* for 3 days, maintained at NEV temperature ($26-28^{\circ}\text{C}$) and deprived of food 24 hours before commencement of experiment. Rat diaphragm together with the left phrenic nerve was dissected out according to the method of Bulbring (1946). 2-4cm 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 [DK Ganguly, 1978]. 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 = 5V, Frequency = 0.5Hz, Pulse Width = 1.4mls 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% CO₂ and maintained at 32⁰C. Pancuronium, Suxamethonium and *E. suaveolens* Extract were tested on the isolated preparations through nerve and muscle stimulations and effects obtained thereafter.

2.3 Reference Drugs and Reagents

2.3.1 Drugs

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

2.3.2 Reagents

NaCl (6.9g), KCl (0.35g), CaCl₂ (0.28g), NaHCO₃ (2.10g), KH₂PO₄ (0.16g), MgSO₄·7H₂O (0.29g) and C₆H₁₂O₆ (2.00g) - Sigma Chemical Company, Louis, USA, 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

Drug activities were investigated on the isolated muscle and nerve tissues at various concentration and volume to obtain graded dose-responses from prepared stocks [*E. suaveolens* extract (1x10⁻¹g/ml); Pancuronium (1x10⁻³g/ml) and Suxamethonium (1x10⁻³g/ml)] in same order.

3. RESULTS

Figures 1, 2 and 3 show various drugs effect of graded concentrations on the isolated phrenic nerve-diaphragm tissue:

Fig. 1: *Erythrophleum suaveolens* (extract) 1X10⁻¹g/ml

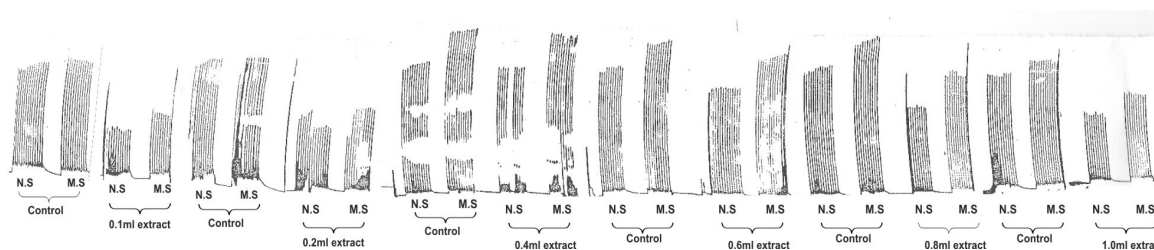


Fig. 2: Pancuronium (Ach induced) 1×10^{-3} g/ml

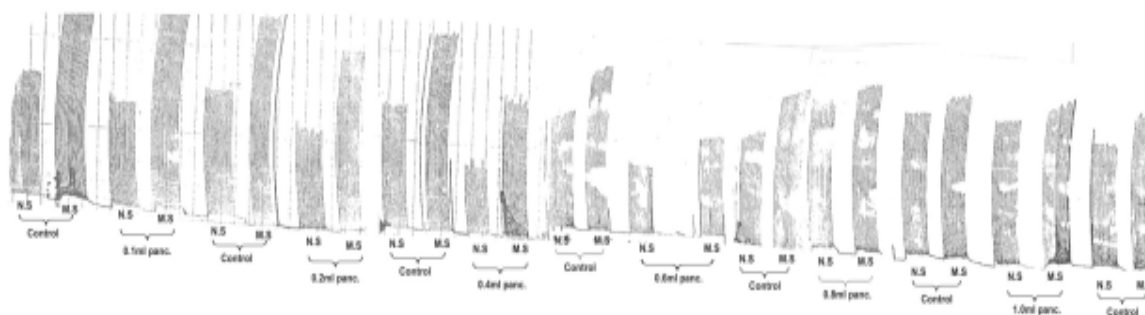
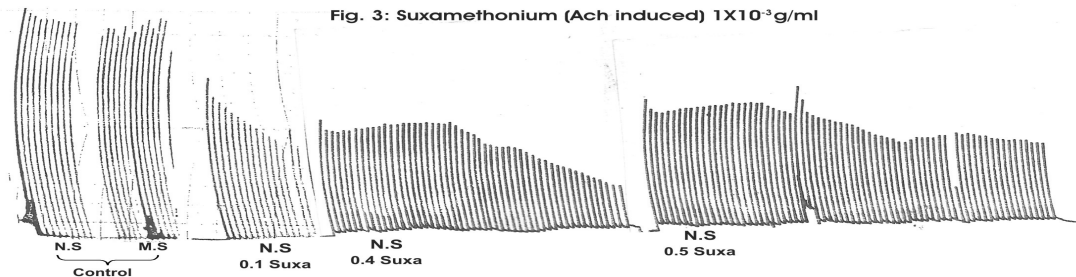


Fig. 3: Suxamethonium (Ach induced) 1×10^{-3} g/ml



KEY:
NS - Nerve Stimulation
MS - Muscle Stimulation

4. DISCUSSION:

Effects of *E. suaveolens* extract and reference drugs on the isolated rat phrenic nerve-diaphragm are highlighted as follows:

4.1 *E. suaveolens* on extract

The extract blocked both the muscle and nerve twitches. Both effects were dose-dependent. However, blocking effect was more on the nerve than muscle (direct stimulation) (Fig. 1).

4.2 Suxamethonium

There was initial excitation followed by a rapid fall in tissue response. The tissue recovered from the effect of the drug (Fig. 2).

4.3 Pancuronium

A relaxing effect was observed in the presence of this drug than that obtained from nerve stimulation. The effect was due to the fact that Pancuronium is a muscle relaxant (Fig. 3)

Inhibitory effect of *E. suaveolens* extract (100μ) was observed to be close to that of Pancuronium (10μ). The blocking effect as seen more on the nerve than muscle in the presence of the extract is an indication that *Erythrophleum suaveolens* could be acting on receptor-

operated channel than voltage operated channel.

5. CONCLUSION:

The relaxing effect of cold water stem-bark extract of *E. suaveolens* is evident on skeletal muscle of rat phrenic nerve-diaphragm as observed relaxation-pattern is closely related to that observed with Pancuronium. Cold water stem-bark extract of *E. suaveolens* is therefore a potent muscle relaxant as claimed by traditional healers, hence could be used as a muscle relaxant.

5.1 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.

Conflicting interest: No conflict of interest.

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