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The Pharmacological Evaluation of Cold Water Stem-Bark Extract of *Erythrophleum suaveolens* On Gastrointestinal Muscle of Guinea-Pig (*Cavia porcellus*) Ileum

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Abstract: ***The effect of cold water crude extract of stem-bark of Erythrophleum suaveolens on the activity of an isolated guinea-pig Ileum was studied. Reference drugs (Acetylcholine, Histamine, Barium Chloride, Atropine, Promethazine, and Papaverine) were used both in the presence and absence of E. suaveolens extract. Preliminary pharmacological investigation of the extract revealed inhibitory effects. Thus, investigations carried out on the isolated ileum tissue of the guinea-pig (Cavia porcellus) by running a dose-response relationship of the agonist test drugs (Acetylcholine, Histamine, and Barium Chloride) in the presence of the cold water crude extract of stem-bark of Erythrophleum suaveolens ascertained antagonist nature of the extract with a right shift. However, the degree of shift in presence of Barium Chloride is less than that with Histamine and Acetylcholine respectively.***

Keywords: Erythrophleum suaveolens, Blocking/Inhibitory effect, Cavia porcellus. Ileum, Antagonist

1. Introduction

The cliché "One drug solves it all" or "Drug for general purpose" has posed lots of arguments against traditional medicine practice. The fact that drugs do have some exaggerated effects is hardly accorded little or no concern in such practices, coupled with problems of toxicity, improvised diagnosis and unhygienic methods, lack of scientific proof and its efficiency amongst others. The problem of side effects has to be addressed, especially in Africa where preference for traditional medicine is slightly in the increase.

Drugs may affect smooth muscles either by a stimulatory or inhibitory response or by local actions on the smooth muscle cells. It is a well-established observation that inhibition in the small intestine is mediated by α- and β-adrenoreceptors especially under stressful conditions with high adrenergic activity [1], [2]. In the guinea-pig ileum, it would seem that the actions of adrenaline and nor-adrenaline are mainly on neuronal elements [3] - [5], whereas the effect of Isoprenaline is mainly on the muscle [3].Kilbinger (1980), reported that, neuronal muscarine receptors of the guinea-pig ileum contains postsynaptic muscarinic receptors which mediate the contraction of the smooth muscle [6].

The foregoing findings have been further analyzed by examining the effects of phenoxy-benzamine and propranolol on the inhibitory actions of catecholamines on acetylcholine release and on the responses of the longitudinal muscle of the guinea-pig ileum to electrical stimulation. Preliminary reports of some of the results have been made to the Pharmacological and Physiological Societies [7] - [9] and to the International Symposium on Gastro-Intestinal Motility in September, 1967 [10]. *E. suaveolens* is a perennial tree of about 30m in height, slightly buttressed, often low-branching and producing a dense spreading crown [11]. It is referred to by various names by natives [12], [13]. These include Obo/erun (Yoruba), inyi (Igbo), baska (Hausa), Kor (Tiv), lakpa(Nupe), ijin (Itsekiri), idip (Ibibio), akpa (Efik), Ovinyin (Benin), aba (Akan-Asante, Ghana), digpende (Bassari-Togo), teli (Koranko-Sierra Leone) etc [14], [15]. It is often referred to in English as sassy, sasswood, redwater tree and ordeal tree [16], [17]. Idyu *et al* (2014), concluded that, the determination of LD50 gives 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 [18]

2. Statement of Problem

Indications that the toxic effect of *E. suaveolens* was manifested through initial discomfort in form of stretching, restlessness increase in respiratory rate, prostration and loss of locomotory coordination that was followed by brief convulsion as it inhibits acetylcholinesterase activity in young Albino Mice (*Mus musculus*) indicates that some organ may have been affected. The foregoing manifestations confirmed the toxic nature of the Stem-bark extract of *Erythrophleum suaveolens* on animals and therefore justify its use as ordeal plant in the past [17].
The search of herbal preparations, that do not produce any adverse effects in the non-target organisms, and which are easily biodegradable, remains a challenge to research issue for scientists [19]. Therefore the need to explore the effect of crude cold water extract of stem-bark of E. *suaveolens* on the smooth muscle of the Gastro Intestinal Tract.

2.1 Aims and Objective

The objective of this study is to investigate the effect of cold water extract of the stem-back of *Erythrophleum suaveolens* on the activity of the Gastro Intestinal Tract, using isolated

Guinea pigs ileum and Rabbits jejunum, by way of comparing such activity with that of some standard drugs.

3. Materials and Methods

3.1 Collection and identification of plant materials

Stem-back of *Erythrophleum suaveolens* were collected from Buruku Local Government area of Benue State, Nigeria. Identification and authentication were done by Mr Okonkwo, a 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. The bark was dried under the shade, in the Pharmacology Research Laboratory of the University of Jos, Nigeria. Sample was pulverized 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 [18].

3.2 Extraction of plant material

100g of powdered stem-bark of the plant was weighed out in 1000ml capacity Pyrex glass beaker. This was dissolved in 200ml of distilled water according to the method of Audu et al. (2001). The mixture was allowed to stand for 24hours 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 ^{0}C until a reddish, sticky extract was obtained. This gave a yield of 6.125g of the extract from 100g powdered sample. The recovered extract was stored in the Refrigerator at -4 ^{o}C [18].

3.3 Crude extract preparation

1.0g of crude water extract was weighed and dissolved in 10ml of distilled water to give a stock concentration solution of 1x10^{-1}g/ml (100mg/ml). Other concentration used for the test were prepared by diluting 1ml of stock solution in 9ml of distilled water (1:9) to give 1x10^{-2}g/ml. Various concentrations were obtained through serial dilutions of the series as appropriate throughout the experiment.

3.4 Animals and tissue preparation

Average sized guinea pigs (*Cavia porcellus)* were purchased in cages from the Animal House Unit of the University of Jos, Nigeria. These were allowed to acclimatize, fed standard pelleted marsh and clean water *ad libitum* for 5 days and deprived of food 24 hours before commencement of experiment. The animals were sacrificed by stunning the head and the throat cut, thus left to bleed. The abdominal region was dissected to isolate the ileum which was quickly transferred into the freshly prepared physiological solution (Tyrode). 3cm of same tissues were carefully placed inside individual tissue bath (50ml capacity) also containing Tyrode solution, attached to a kymograph set-up which was maintained at 37^{0}C, pH(7.4) and aeration (95% oxygen and 5% CO_2), while various drugs were added as required.

3.5 Reference drugs and reagents

The drugs and the chemical reagents used were of standard analytical grade- Acetylcholine (1x10^{-2}g/ml), Atropine (1x10^{-2}g/ml), Histamine (1x10^{-3}g/ml), Promethazine (1x10^{-3}g/ml), Barium Chloride (1x10^{-2}g/ml), Papaverine (1x10^{-3}g/ml) and NaCl (8.0g), KCl (0.2g), $CaCl_2$ (0.2g),$NaHCO_3$ (1.0g), NaH_2PO_4 (0.5g), $MgCl_2$ (0.1g), $C_6H_{12}O_6$ (1.0g) and distilled water.. These were products of Sigma Chemical Company, Louis, USA, Burgoynes & Co, India, BDH Chemical Ltd. Poole, England, Kernel Chemicals, Germany and Hopkin & Williams Ltd. England. The reference drugs were prepared by weighing out and dissolving in required volume of distilled water to give desired stock concentrations.

3.6 Drugs and crude extract investigations

Various drug activities were investigated on the tissues of the guinea-pig ileum by way of arithmetic progression volume to obtain dose- responses in the following order:
Agonists in the absence and presence of antagonists as well as *E. suaveolens* extract using the isolated and mounted guinea pig ileum tissue.

- Acetylcholine (1x10^{-4}g/ml) alone: (0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml and 3.2ml) to obtain three different tracings.
- Acetylcholine (1x10^{-4}g/ml) in the presence of Atropine (1x10^{-7}g/ml) : The tissue bath was incubated with Atropine (0.5ml) for 3minutes and later administered with varying volumes of Acetylcholine as above to also obtain three different tracings.
- Acetylcholine (1x10^{-4}g/ml) in the presence of *E. suaveolens* extract (1x10^{-7}g/ml): Tissue bath was incubated with the extract (0.5ml) for 5minutes and administered with varying volumes of Acetylcholine as above.
- Histamine (1x10^{-4}g/ml) alone: Carried out as in Acetylcholine alone.
- Histamine (1x10^{-4}g/ml) in the presence of promethazine (1x10^{-7}g/ml) : Same procedure as in Acetylcholine in the presence of Atropine
- Histamine (1x10^{-4}g/ml) in the presence of *E. suaveolens* extract (1x10^{-2}g/ml): same as in Acetylcholine in the presence of *E. suaveolens* (1x10^{-7}g/ml) extract above.
- Barium Chloride (5x10^{-2}g/ml) in the presence of papaverine (1x10^{-5}g/ml): same procedure as in Acetylcholine in the presence of Atropine.
- Barium Chloride (5x10^{-2}g/ml) alone, in the presence of E. *suaveolens* extract (1x10^{-2}g/ml): done in the previous ones above.

Various tracings were obtained on the recording paper of the rotating drum.

4. Results

Dose-response for test drugs - agonists (Figs: 1, 4 & 7) and such in the presence of antagonists (Figs: 2, 5 & 9) illustrate the agonist and antagonist nature of the various test drugs. Same characteristics were exhibited by same agonists in the presence of crude extract of stem-back of *Erythrophleum suaveolens* (Figs: 3, 6 & 9). Plots of Percentage Maximum Responses against Log Concentrations (Graphs: 1, 3 & 5)

showed blocking effects of the antagonists in the presence of agonists of test drugs with a shift to the right .Same effect was exhibited by test drugs (agonists) in the presence of crude extract of stem-back of *E. suaveolens* (Graphs: 2, 4 & 6).

Tracings of dose-response relationship on guinea pig ileum

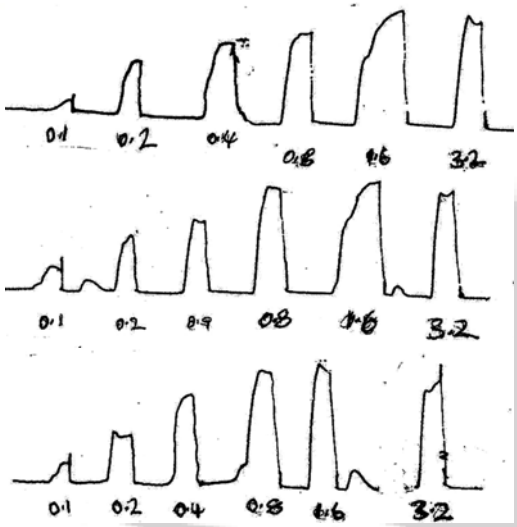


Figure 1: Acetylcholine

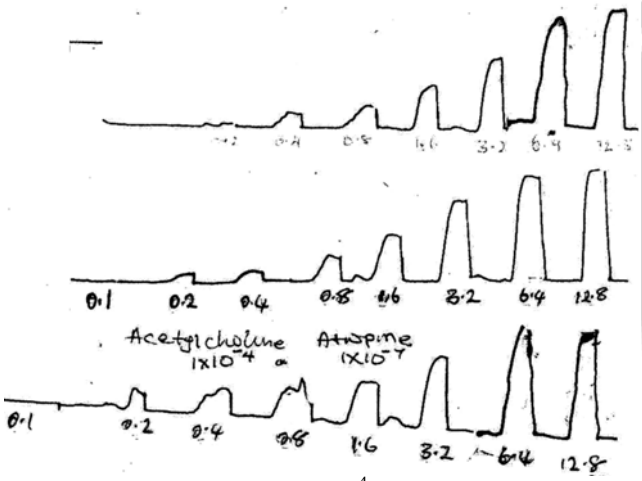


Figure 2: Acetylcholine ($1x10^{-4}$g/ml) in the presence of Atropine

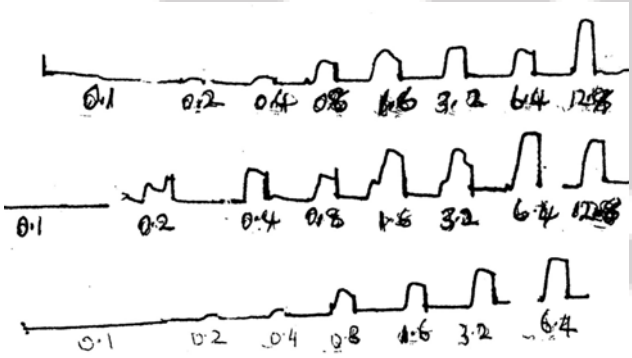


Figure 3: Acetylcholine ($1x10^{-4}$g/ml) in the presence of Extract ($1x10^{-2}$gml; 0.5ml)

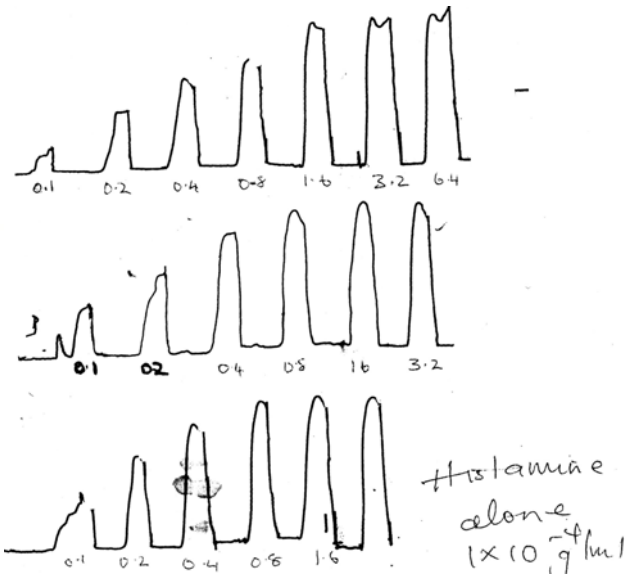


Figure 4: Histamine alone

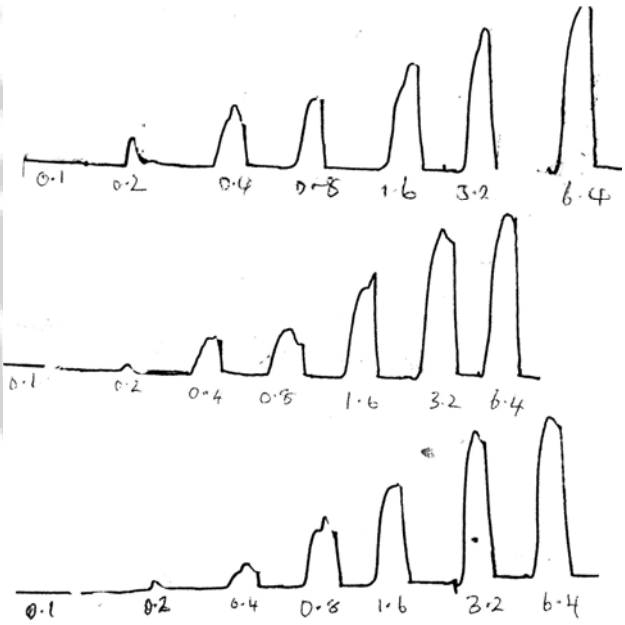


Figure 5: Histamine in the presence of promethazine

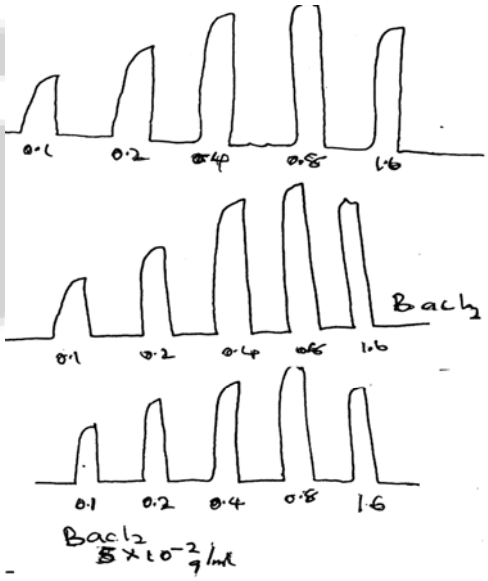


Figure 6: Effect of Histamine in the presence of Extract

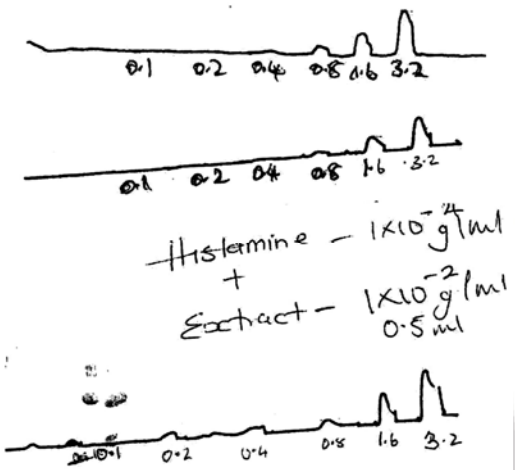


Figure 7: Effect of Barium Chloride alone

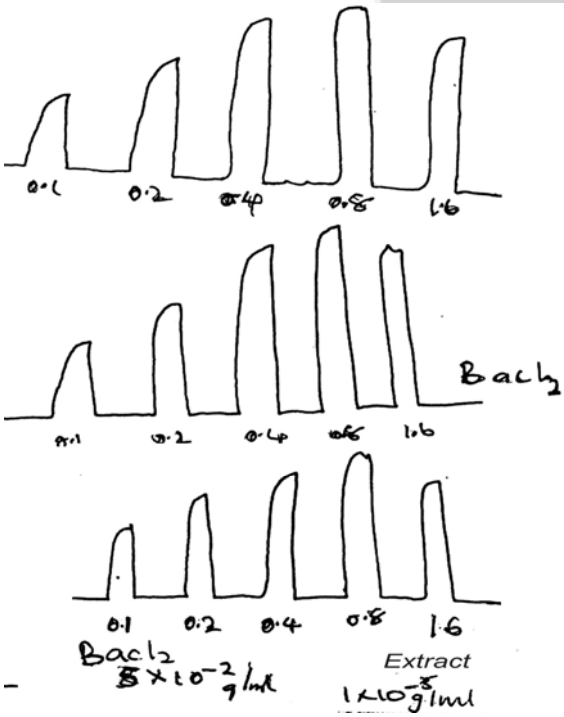


Figure 8: Effect of Barium Chloride in the presence of Papaverine

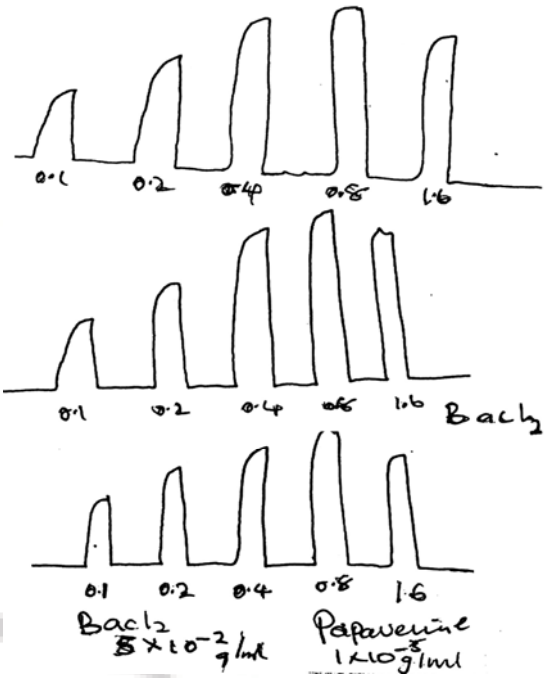
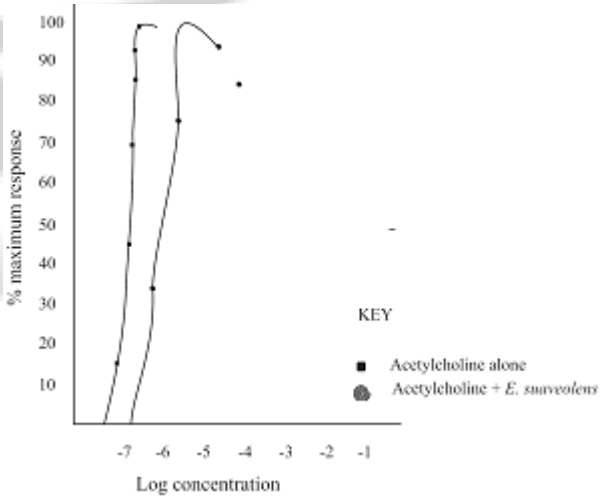
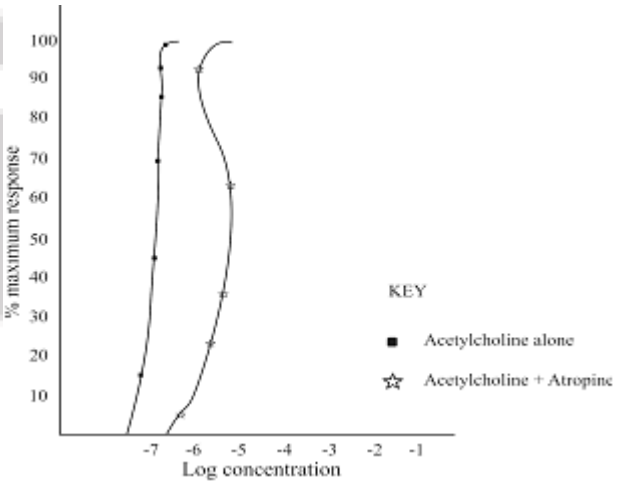


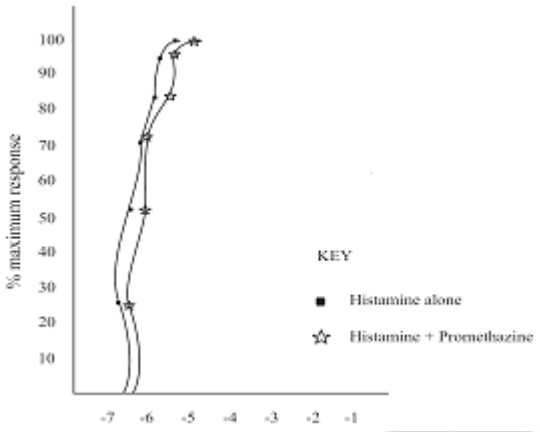
Figure 9: Effect of Barium Chloride in the presence of Extract



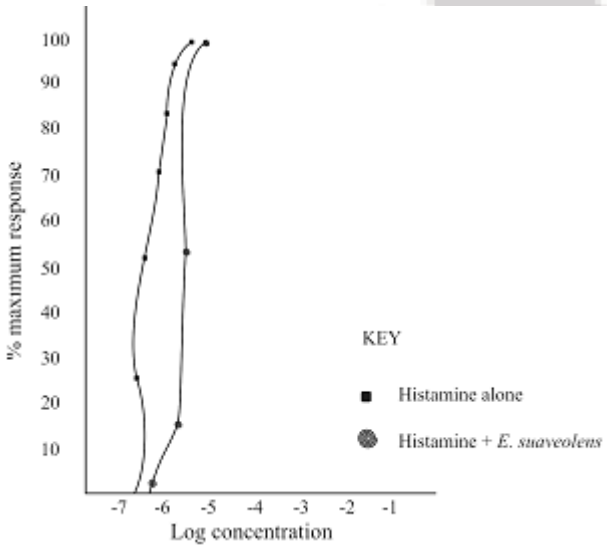
Graph 1



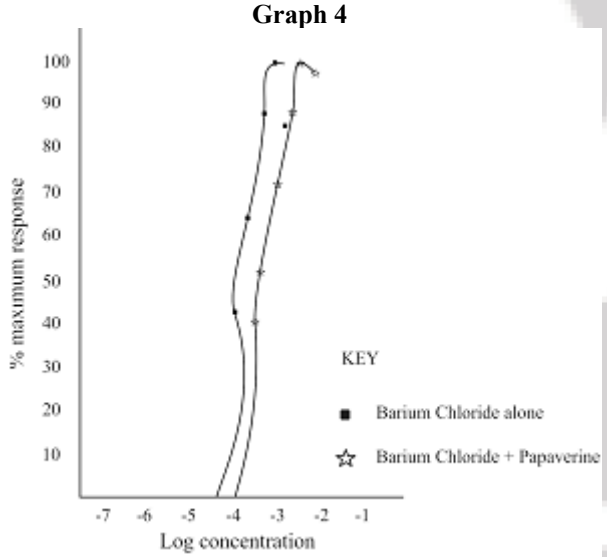
Graph 2



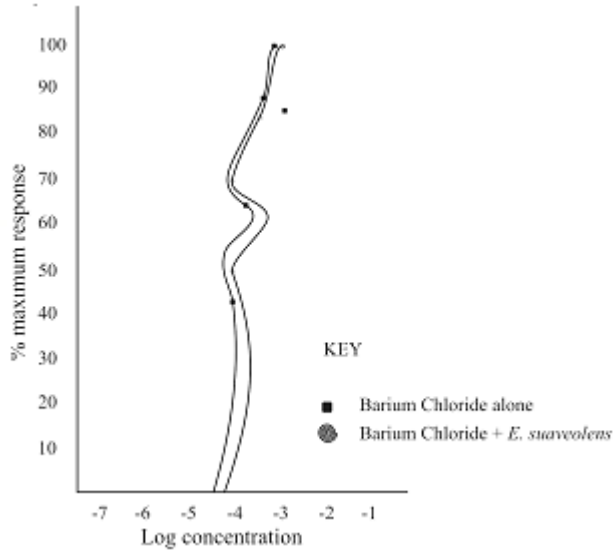
Graph 3



Graph 4



Graph 5



Graph 6

5. Discussion

Application of various volumes of working concentration of reference drugs and *E. suaveolens* extract on the isolated guinea-pig ileum tissue showed the following results:

a) Ach

This cholinergic agonist known to increase tone and amplitude of contraction and peristaltic activity of the gastrointestinal tract as well as the secretory activity of the gut is mediated through muscarinic receptors (Mactor, 1939). Results obtained are in line with increased contraction with increasing dose of Acetylcholine (max. response = $3.2x10^{-6}$g/ml). The curve showed a shift to the right in compliance with the characteristics of a competitive antagonist in the presence of Atropine (max. response = $1.28x10^{-5}$ g/ml). However, the curve obtained from that of Ach in the presence of *E. suaveolens* extract (max. response = $1x10^{-2}$ g/ml) also produced a shift to the right (Figs 1-3; Graphs 1-2).

b) Histamine:

This is an agonist, which binds to the histaminergic receptors and causes contraction of smooth muscles mediated through H1 receptors. Increased doses of Histamine also gave increased height of response (max. response = $6.4x10^{-6}$g/ml) The curve showed a shift initial discomfort in form of stretching, to the right in compliance with the characteristics of a competitive antagonist in the presence of Promethazine (max. response = $1.28x10^{-5}$ g/ml). However, the curve obtained from that of Histamine in the presence of *E. suaveolens* extract (max. response = $6.4x10^{-6}$ g/ml) also produced a shift to the right (Figs 4-6; Graphs 3-4).

c) $BaCl_2$:

This is a direct acting agonist on smooth muscles of hyperpolarization of the cell membrane, leading to opening of ion channel and thus causing influx of Ca^{2+} ions into the cell, bringing about depolarization of the membrane, resulting in muscle contraction. This was observed as recorded on the tracings obtained as $BaCl_2$ increased contraction with increasing dose (max. response = $1x10^{-4}$g/ml). The curve showed a shift initial discomfort in form of stretching, to the right in compliance with the characteristics of a competitive

antagonist in the presence of Papaverine (max. response = $3.2x10^{-3}$ g/ml). However, the curve obtained from that of $BaCl_2$ in the presence of *E. suaveolens* extract (max. response = $8x10^{-4}$ g/ml) also produced a shift to the right (Figs 7-9; Graphs 5-6)

5.1 Conclusion

Cold water stem-bark of *E. suaveolens* has blocked the activities of Acetylcholine, Histamine and $BaCl_2$ (agonists) on the isolated guinea-pig (*Cavia porcellus*) with a shift to the right, thus confirming it as a potent antagonist. The degree of shift however in the presence of Barium Chloride is less compared to that in the presence of Histamine and Acetylcholine. Yet to be ascertained mechanism of action of the extract may agree with Clague *et al* (1985) In the assessments of the action of selective agonists and antagonists at muscarinic receptors mediating ileal contractions, and the rate and force of arterial contractions as well as that of the effect of nicotinic receptor stimulation, catecholamine release and acetylcholinesterase (AChE) action on muscarinic activity, the nicotinic actions of carbachol did not affect its agonistic potency nor the antagonist affinity data obtained when this agonist was used in arterial and ileal preparations. Antagonist data indicated that muscarinic receptors mediating the rate and force of arterial contractions did not differ as differences in agonistic potencies at the two muscarinic receptors were attributable to either differences in intrinsic efficacy or susceptibility to the action of acetylcholinesterase. The small differences in agonist potency observed between arterial and ileal muscarinic receptors were considered not sufficient to indicate receptor heterogeneity [20].

5.2 Recommendation

Effect of *E. suaveolens* on other smooth muscles, mechanism of action as well as phytochemistry in order to know the exact component that is responsible for its inhibitory effect should also be explored.

6. Acknowledgement

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7. Conflicting interest

No conflict of interest.

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