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Research Article

MECHANISTIC STUDIES OF MYORELAXANT EFFECTS OF ETHANOLIC LEAF EXTRACT OF *ERIOBOTYRA JAPONICA* ON ISOLATED RABBIT ILEUM

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ABSTRACT

Objectives: The leaves of Eriobotrya japonica are ethnopharmacologically used to treat iarrhea, asthma and hypertension in Zimbabwe. This study was aimed at determining the effect of Eriobotrya japonica on ileum smooth muscles.

Materials and methods: E. japonica plant extracts were prepared and tested on isolated rabbit ileum tissue. Plant effects were compared with histamine, Ach or KCl induced contractions to evaluate its gut modulator effects. Adrenergic activity was also tested using propranolol.

Results & Discussion: E. japonica (66-1000µg/ml) ethanolic leaf extract exhibited a dose dependant relaxant effect on spontaneously contracting isolated rabbit ileum. It also dose dependently inhibited rabbit ileum strips precontracted with histamine (0.001M), Ach (0.001M) and K+ (80 Mm) with EC50 values of 322±1.1µg/ml, 335±1.1µg/ml and 325.9 ± 1.10µg/ml respectively. The extract showed inhibition trends that were similar to the positive controls. Chlopheniramine relaxed the smooth muscle by blocking the contractile effects of Ach, EC50 value 383.3 ± 1.44µg/ml. Acetylcholine induced inhibition was similar to that caused by atropine an anticholinergic compound, EC50 value 729±1.13µg/ml. The extract inhibited potassium induced contractions in a dose dependent manner with an EC50 value of 325.9 ± 1.10µg/ml. Propranolol increased smooth muscle relaxation by 73.7% which was similar to the plant extract which increased relaxation by 77.75%.

Conclusions: The results suggest that the ethanolic leaf extract of Eriobotrya japonica has myorelaxant effects and may be acting by potentially inhibiting acetylcholine, histamine and potassium receptors on the rabbit ileum. The effect on adrenergic agonist propanolol activity was inconclusive and needs further testing

Keywords: Relaxant effects, Eriobotrya japonica, rabbit ileum.

INTRODUCTION

The study of medicinal plants is essential to promote the proper use of herbal medicine and to determine their potential as a source for new drugs. Up to 90% of all drugs used today are of plant and animal origin [1]. According to the WHO plant based traditional medicine systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care needs [2].

Eriobotyra japonica which is widely known as loquat has a long history of medicinal uses in South Eastern Asia. E. japonica is an evergreen tree growing to a height of 9 m at a medium rate [3]. E. japonica bears edible fruit and is indigenous to southeastern China and possibly southern Japan [4]. The tree grows well on a variety of soils of moderate fertility which includes light sandy loam to heavy clay or even limestone, but needs good drainage [4]. The ability of the tree to grow well in a variety of soils has

allowed it to be introduced to Zimbabwe, where an infusion of the leaves or dried powdered leaves may be taken to relieve diarrhoea or depression and to counteract intoxication from consumption of alcoholic beverages [4]. Leaf poultices can also be applied on swellings [4]. Of the numerous phytochemicals present in the leaf extracts, tannins and flavonoids are thought to be responsible for antidiarrhoeal activity achieved through increasing colonic water and electrolyte reabsorption [5]

Diarrhoea is the frequent passage of unformed, loose or watery stools, three or more times in 24 hours [6]. It is the most common clinical manifestation of gastrointestinal disease and can be caused by both infectious and non-infectious agents. Diarrhoea is a major cause of morbidity and mortality in Africa, particularly in children under the age of five [7]. It is the fourth leading cause of mortality among under-5s in Zimbabwe, contributing 9% of childhood deaths [8].

In most diarrhoeas, there is a pathological increase in intestinal motility and antimotility drugs are used clinically to manage the condition [9]. The enteric nervous system is considered to be an independent nervous system that controls and coordinates gastrointestinal motility. Gastrointestinal motility is regulated by numerous mediators, namely acetylcholine, histamine, 5-hydroxytryptamine, bradykinins, prostaglandins, substance P and cholecistokinins. These mediators achieve their contractile effects through an increase in cytosolic calcium [9]. For example histamine causes contraction of intestinal smooth muscle hence histamine-induced contraction of guinea pig ileum is a standard bioassay for this amine. The human gut is not as sensitive as that of the guinea pig, but large doses of histamine may cause diarrhoea, partly as a result of this effect. This action of histamine is mediated by H1 receptors [10]. Agents which cause smooth muscle relaxation in the gastrointestinal tract can cure the diarrhoea which is not caused by infectious agents. The development of cost effective and accessible alternative strategies such as the use of herbal medicines in the treatment of diarrhoea is encouraged [11].

For centuries, herbs have been used in traditional medicine to treat many gastrointestinal disorders. In recent times numerous scientific studies have been performed to test the potential effect of plant extracts on intestinal contractions. However, the mechanism of action by which these plants exert their therapeutic effects has not been completely elucidated [9]. Eriobotrya japonica is being used in Zimbabwe and other countries for treating diarrhoea. Previously in our laboratory we have shown that alcohol and aqueous leaf extracts of E. japonica have muscle relaxation effects on isolated rabbit ileum contractions [12]. This justifies the use of this plant in treating diarrhoea in traditional medicine however there is need to clarify the possible underlying mechanism.

MATERIALS AND METHODS

Study design

Plant material and preparation

The leaves of E.japonica were collected from a residential area in Msasa Park, Harare. The plant was identified and authenticated by a research technician from the National Herbarium and Botanical Garden of Zimbabwe. The leaves were washed, dried in shade at ambient temperature of around 25 ° C and crushed using mortar and pestle. Powdered plant material (620g) was extracted using 70% aqueous-ethanol at room temperature for 48hours, vacuum filtered through Whattman-1 filter paper and evaporated using a rotary evaporator (Rotavapor, EL130- Germany) at 40°C under vacuum. A thick dark green paste resulted. This was freeze dried (Gallen Kamp super cold freezer) and the resulting greenish coarse powder (5.5 % yield) was kept in a glass vacuum desiccator until use.

Chemicals

Acetylcholine chloride, atropine sulphate, histamine dihydrochloride, papaverine hydrochloride and epinephrine used were purchased from Sigma-Aldrich Chemical Company. Chlopheniramine maleate and propranolol tablets used were manufactured by Varichem Zimbabwe. EDTA, sodium chloride, potassium chloride, magnesium chloride, calcium chloride, NaHPO4, NaHCO3, glucose and ethanol 96% were purchased from Natchem Zimbabwe.

The Tyrode's solution which was prepared by dissolving the following salts in distilled water had the following composition (g/L) NaCl-8.0; KCl-0.2; MgCl2-0.1; CaCl-0.2; NaHPO4-0.05; NaHCO3-1.0 and glucose-1.0. The alcohol extract was dissolved in distilled water to give a stock solution of 5g/10mls. Solutions of acetylcholine, adrenaline,

atropine, papaverine, histamine, chlopheniramine and propranolol were made by dissolving the specific drugs in distilled water. All the solutions were prepared fresh on the day of the experiment.

Animals

A local breed of Chinchilla giganta rabbits of either sex were purchased from the Animal House of University of Zimbabwe and used in the study. The rabbits were kept at room temperature and given a standard pellet diet and tap water ad libitum. The standard pellet feed was withdrawn 12 hours prior to the experiment but the animals had access to water. The experiments were performed according to the OECD guidelines in compliance with the Institute of Laboratory Animal Resources, Commission on Life Sciences as well, and approved by the Joint Parirenyatwa Hospital and College of Health Sciences Research Committee (JREC), University of Zimbabwe (approval number is JREC/288/12).

Tissue preparation

The experimental protocol was adapted and modified from Walker and Scott [13]. Overnight fasted rabbits were sacrificed by a blow on the back of the head. A midline abdomen incision was made and the rabbit ileum dissected out and transferred to a petri dish containing Tyrode's solution. Chime present was removed by flushing the segment with syringe filled with Tyrode's solution. Tubular ileal segments that were 2-3 cm long were cut and mounted in a 30 ml tissue bath containing Tyrode's solution. The tissue bath was continuously aerated with carbogen gas (95 % oxygen and 5 % carbon dioxide) and maintained at around 37°C. The lower end of the ileum was tied to an aerator tube and the upper end to a DT475 isotonic transducer (iWorx,USA). This was connected to an iWorx214 Data Acquisition System (iWorx, USA) which analysed the recordings

Pharmacological Assessments

Determination of antihistamine activity and anticholinergic activity

Cumulative doses of E. japonica (66-1000 µg/ml) were added to the tissue bath in either the absence or presence of histamine (0.001M) or Ach (0.001M). The resulting dose response curves (DRCs) were recorded. Chlopheniramine (80-800µg/ml) was used as a positive control for antihistamine activity whilst atropine (100-1000 µg/ml) was used as a positive control for the anticholinergic activity.

Determination of Calcium antagonist activity

To find out whether the spasmolytic activity of E .japonica is mediated through calcium channel blockade, K+ (80 mM) was used to depolarize the ileum tissue preparations. High K+ (80 mM) was added to the tissue bath after which E.japonica (66-1000µg/ml) was added in a cumulative fashion. The relaxation of intestinal preparations, precontracted with K+ (80 mM) was expressed as a percent of the control response mediated by K+.

Determination of adrenergic agonist activity

Cumulative doses of propranolol (0.001-100 µg/ml) were added to the tissue in the presence of E. japonica (0.01 mg/ml). Adrenaline (0.1 mg/ml) was used as a positive control and dose response curves were recorded.

Data analysis

The relaxation of the tissue preparation was expressed as the percentage of the control contraction mediated by the added spasmogen and expressed as the mean ± SEM. EC50 values were determined by nonlinear regression and expressed as the mean [confidence intervals]. GraphPad Prism 5.3 software was used for the data analysis, and the values were compared using Student's t test for paired data. A probability value of $p<0.05$ was noted as indicative of significance.

RESULTS

Spontaneous contraction

The ethanolic extract of Eriobotrya japonica was tested on the spontaneously contracting isolated rabbit ileum. The extract (66-1000µg/ml) showed a dose dependent increase in relaxation of the ileum preparations with a median effective concentration (EC50) value of 203µg/ml, indicating smooth muscle relaxant activity (Fig 1).

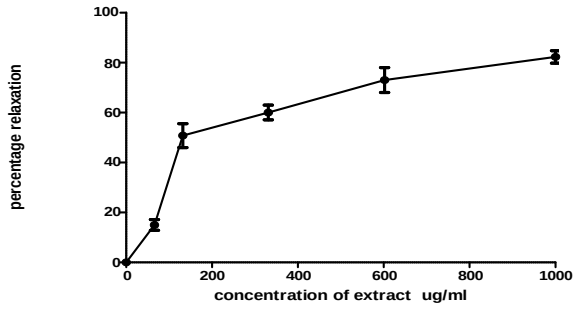

Figure 1: Effect of *E. japonica* on smooth muscle spontaneous contractions. N = 4; Data is expressed as the mean ± S.E.M.

The relaxant effects were reversible and contractility returned to normal after washing three times with Tyrodes' solution.

Histamine antagonist effect

Histamine was used as an agonist to stimulate ileum smooth muscle. The extract of E. japonica significantly inhibited the histamine induced contractions in a concentration dependent manner ($p<0.05$), with maximal relaxation of 75.75±2.5% (Figure 2).

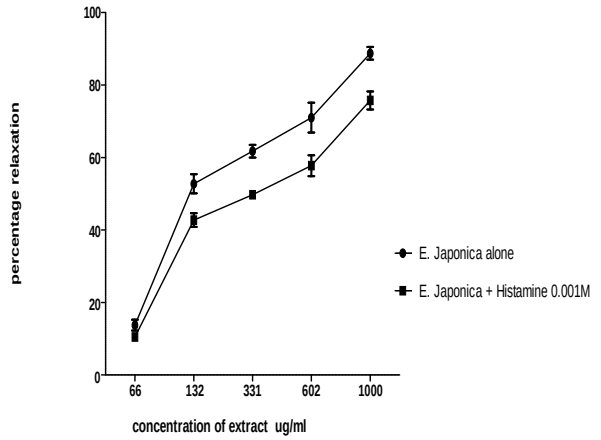

Figure 2: Effect of *E.japonica* on ileum smooth muscles in the absence and presence of histamine. N = 4; Data is expressed as the mean ± S.E.M.

The EC50 values of E .japonica (191.4 ±1.1 µg/ml) were modified by histamine to (EC50 of 322±1.1 µg/ml).

Effect of chlopheniramine

Chlopheniramine was used as a positive control to assess its action on histamine induced contractions. Chlopheniramine significantly inhibited the histamine induced contractions in a concentration dependent manner ($p < 0.05$), with maximal relaxation of 71.25±1.49% (Figure 3).

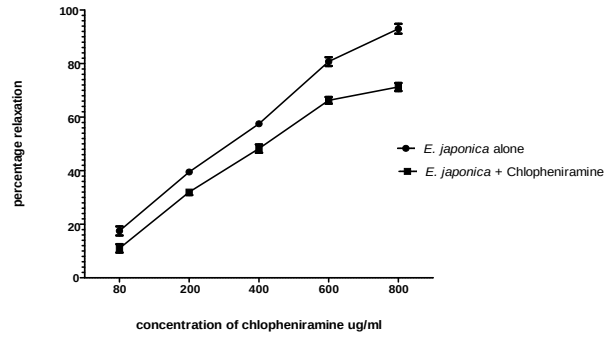

Figure 3: Effect of chlopheniramine on ileum smooth muscle in the absence and presence of histamine N = 4; Data is expressed as the mean ± S.E.M.

The EC50 values of chlopheniramine (232.7±1.11µg/ml) were modified by histamine to (EC50 of 383.3±1.44µg/ml).

Cholinergic antagonist effect

Acetylcholine was used as an agonist for stimulating ileum smooth muscle. The extract of E. japonica significantly inhibited the acetylcholine induced contractions in a concentration dependent manner ($p<0.05$), with maximal relaxation of 83.50±1.71% (Figure 4). The EC50 values of E. japonica (211.9±1.11µg/ml) were modified by histamine to (EC50 of 335±1.1µg/ml).

Acetylcholine was used as agonist for stimulating ileum smooth muscle. Atropine a well known anticholinergic agent significantly inhibited the acetylcholine induced contractions in a concentration dependent manner ($p<0.05$), with maximal relaxation of 72.25±1.80% (Figure 5).

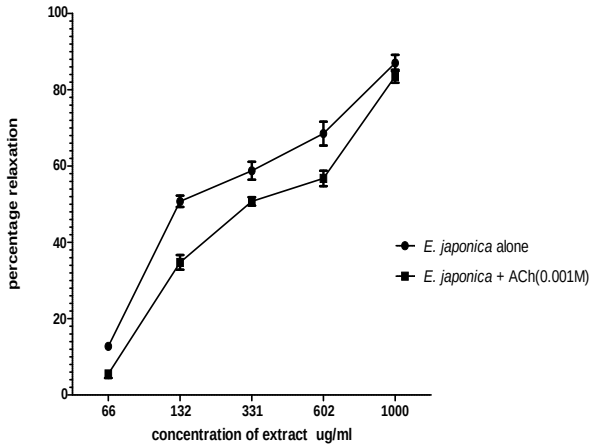

Figure 4: Effect of *E.japonica* on ileum smooth muscle in the absence and presence of Ach. N = 4; Data is expressed as the mean ± S.E.M.

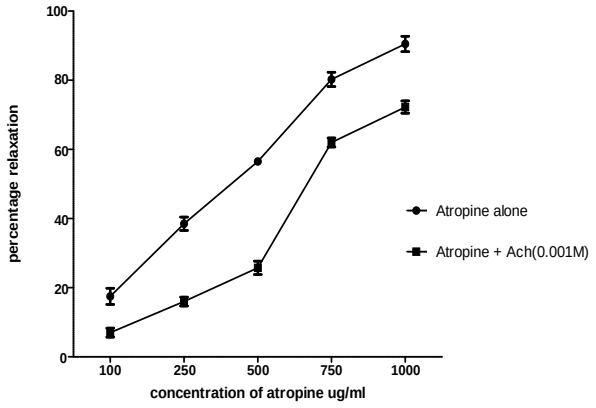

Figure 5: Effect of atropine on ileum smooth muscle in the absence and presence of ACh. N = 4; Data is expressed as the mean ± S.E.M.

Calcium antagonist effect

The effects of the E. japonica extract on KCL induced contractions are shown in Figure 6. The extract of E. japonica significantly relaxed the KCL induced contractions ($p<0.05$). The maximum relaxation was 67.75±1.38%. The EC50 values of E. japonica (183.8±1.13µg/ml) were affected by the extract of KCL to (EC50 of 325.9±1.10µg/ml).

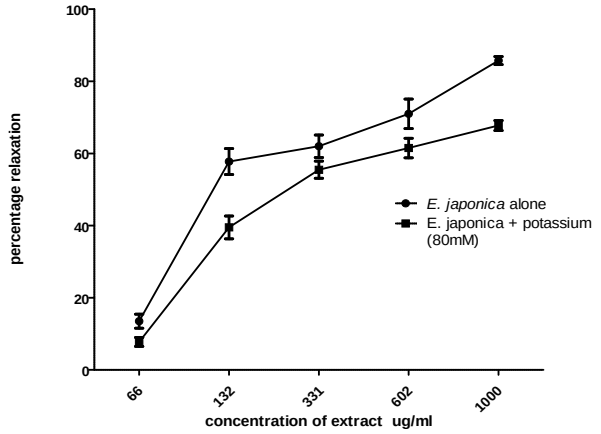

Figure 6: Effect of *E. japonica* on ileum smooth muscle in the absence and presence of potassium. N = 4; Data is expressed as the mean ± S.E.M.

Adrenegic agonist effect

In the presence of E. japonica (0.1 mg/ml) propranolol had an EC50 of 0.749 ± 0.172µg/ml. The maximum observe relaxation was 77.75±1.37%. Propranolol significantly increased relaxation caused by E. japonica and adrenaline ($p<0.05$). In the presence of adrenaline (0.1mg/ml) propranolol had an EC50 value of 0.093±0.189µg/ml with the maximum observed relaxation being 73.75±1.493%.

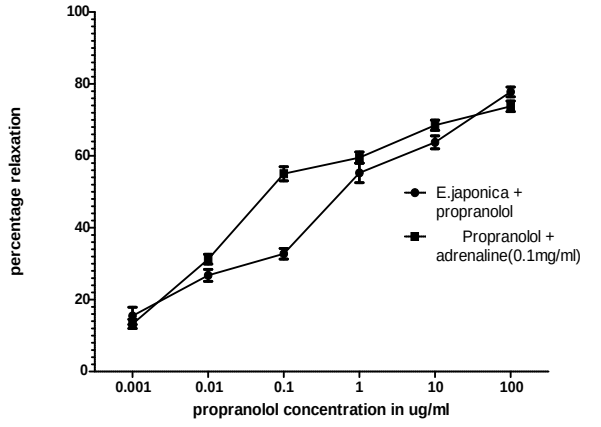

Figure 7: Effect of propranolol on ileum smooth muscle in the presence of the extract and adrenaline. N = 4; Data is expressed as the mean ± S.E.M.

DISCUSSION

Gastrointestinal tract (GIT) motor tone is modulated through multiple mediators which include neurotransmitters, inflammatory mediators and oxidative metabolites [14]. The release of these chemical modulators in the GIT causes stimulatory effect mediated though an ultimate increase in cytosolic calcium [14, 15]. The contractions of smooth muscles including that of rabbit ileum are dependent upon an increase in the cytoplasmic free calcium, which activates the contractile elements [14, 15]. The extract of Eriobotrya japonica was studied for its possible relaxant effect on rabbit ileum. Isolated ileum suspended in an organ bath showed spontaneous contractile activity and E. japonica leaf extract produced relaxant effects on these spontaneous contractions. The effects were concentration-dependent and reversible after washing the tissue with Tyrode`s solution.

Effect on histamine induced contractions

Inhibition of histaminic (H1) receptors is a possible mechanism of the plants relaxant effects. H1 and H2 histamine receptors coexist on smooth muscle cells of the gut. H1 receptors mediate contraction and H2 receptors mediate relaxation. The net effect of histamine is contraction, reflecting the dominant influence of H1 receptors [10, 16]. In this study application of histamine (0.001M) to intact smooth muscle increased the contractility of the smooth muscle. The extract of Eriobotya japonica significantly inhibited histamine (0.001M) induced contractions of the rabbit ileum in a concentration dependent manner (66-1000µg/ml). The effect of E. japonica on histamine induced contractions was similar to that produced by chlopheniramine a first generation antihistamine agent.

The relaxant effect of the extract could therefore be due to the blockade of H1 receptors. This is supported by the traditional use of the E. japonica in the treatment of inflammatory conditions [17]. The inflammatory responses resulting from the liberation of histamine are thought to be mediated through H1 receptors [18]. As a result anti-inflammatory effect can also be due to histamine H1 receptor blockade. Possible phytoconstituents mediating this effect are flavonoids. Flavonoids have been shown inhibit histamine release through the presence of two orthohydroxyl groups at C-3′ and C-4′ found in most flavanoids [19].

Effect on acetylcholine induced contractions

Acetylcholine (ACh) is a neurotransmitter released by the parasympathetic nervous system mediating its action in the GIT by stimulation of the nicotinic and muscarinic acetylcholine receptors. In the GIT M1, M2, M3, M4 and M5 receptors have been identified. However M2 and M3 receptors play some essential roles in the relaxation of the GIT [15]. Cholinergic agents are known to increase the secretory and motor activity of the gut [20], while antimuscarinic drugs reduce gastric motility [15]. The results obtained in this study indicate that the ethanolic leaf extract of Eriobotrya japonica (66-1000µg/ml) dose dependently relaxes Ach (0.001M) mediated contractility. The observed relaxant effect of the crude extract is similar to that of atropine a known anticholinegic agent that inhibits gastrointestinal motility. Atropine is used in pathological conditions in which there is increased gastrointestinal motility [21]. The possible mechanisms responsible for relaxation by medicinal plants usually includes one or more combinations of the following; inhibition of Ach release from the cholinergic nerve endings, inhibition of acetylcholinesterase enzyme at the neuro-effector junction or direct inactivation of the muscarinic receptors of smooth muscles of the GIT [15].

Calcium antagonist effect

Exposure to high tissue bath concentrations, K+ (80 mM) is reported to cause smooth muscle contraction through opening of voltage dependent L-type Ca2+ channels, thus allowing influx of extra-cellular Ca2+ and hence, causing contractile effect . Substances that inhibit K+ induced contractions are considered to be blocker of Ca2+ influx [14, 15, 22]. The Eriobotrya japonica leaf extract showed relaxant activity when tested on K+ (80 mM) induced contractions, suggesting that the spasmolytic effect is possibly mediated through calcium channel blockage. There is growing evidence that the spasmolytic effect of plant extracts is associated with the presence of phenolic compounds [23]. Flavonoids are one of the most numerous and widespread group of phenolics in higher plants and are found in E. japonica [13]. Some flavonoids that inhibit intestinal motility in vitro have been reported to exhibit calcium antagonist and anticholinergic activities [23]. The anticholinergic effect of flavonoids could be the one mediating the anticholinergic effects of the extract. Quercetin one of the flavonoids found in E. japonica leaves [17] was found to produce relaxation in ileum contracted by KCl [23]. Therefore quercetin and other related flavonoids may be responsible for calcium channel blockade in E. japonica.

Adrenergic receptor blockade

Adrenaline and noradrenaline are chemical transmitters' at most post-ganglionic sympathetic nerve endings. They relax all muscle strips with two exceptions: the cardiac and ileocaecal sphincters [24]. In man there are two types of adrenergic receptors (alpha and beta) in the gut wall, both of which cause relaxation when stimulated [23]. The responses to adrenaline are partly blocked by adrenergic receptor antagonists that prevent relaxation. These can be alpha adrenergic receptor antagonist for example prazosin or beta-adrenergic receptor antagonist for example propranolol [24]. Propranolol is the prototype beta aderenergic antagonist and blocks beta 1 and beta 2 receptors [25]. In this study propranolol increased the relaxation caused by E. japonica or adrenaline. This is inconsistent with the available literature since it is supposed to reverse the relaxant effect of adrenaline. As a result, the results on adrenergic agonist activity are inconclusive. Further work can be done to investigate other mechanisms and pure chemical compounds which are free from excipients found in tablets could be used.

CONCLUSIONS

The study showed that the ethanolic leaf extract of Eriobotrya japonica possesses relaxant activity that may be mediated through histamine receptor (H1) blockade as the extract caused relaxation of histamine induced contractions. It could also possibly be through acetylcholine antagonism since the extract relaxed acetylcholine induced contractions or through calcium channel blockade since the extract caused relaxation of potassium induced contractions. The adrenergic agonist activity was inconclusive. Additional mechanism(s) cannot be ruled out since they were not investigated in this study.

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Abbreviations

Ach	- Acetylcholine
Ca	- Calcium
K^+	- Potassium ion
Ca^{2+}	- Calcium ion
KCl	- Potassium Chloride
EC_{50}	- Concentration of drug required to produce 50% of that drug's maximal effect
DRC	- Dose Response Curve
GIT	- Gastrointestinal Tract
WHO	- World Health Organisation

