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Studies on Antagonistic Action of Methanolic Extract of *Ledebouria ovaltifolia* on Isolated Rat Stomach Strip and Rabbit Jejunum Preparations

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Abstract: The inhibitory and analgesic effects of aqueous extract of *Ledebouria ovaltifolia* has been reported in a previous study. In the present study, the effects of methanolic root extract was examined on Ach-induced contractions in Rat Stomach Strip (RSS) and on the pendular movement of Rabbit jejunum in order to examine the time and pattern of antagonistic action of the extract in these muscles. The action of the extract on Ach-induced contractions was non competitive, since maximum contractions were suppressed and right wand shift of the curve. As extract concentration increases, a pronounced time dependent inhibition was observed. In the isolated rabbit pendular movement, the inhibitory action of the extract mimicked Adrenaline like effect in the gut. These results show that *L. ovaltifolia* possesses, antispasmodic actions similar to that of biological amines.

Key words: *Ledebouria ovaltifolia*, rat stomach strip, contraction inhibition, rabbit pendular movement

INTRODUCTION

Traditional herbal practice is well utilized in Swaziland, a tiny kingdom in Southern Africa. Over 50,000 estimated plant species are used in traditional medicine. The rich biodiversity of the flora in the Kingdom has been shown by traditional practitioners (inyanga or sangoma) to possess medicinal remedies; also the socio-cultural affinity of the people (Makhubu, 2003) support traditional medicine, as a potential therapeutic alternative medicine. The prohibitive cost of orthodox medicine, poverty of the vast majority of the people and the affordability of herbal decoction is promoting the use of herbal medicine in the kingdom. The present study is aimed at examining the antagonistic actions of the extract as a result of non competitive actions previously reported and also to see if the medium of extraction significantly affected pattern of action.

MATERIALS AND METHODS

Plant collection and extraction: The root of *Ledebouria ovaltifolia* (Hhyacithaceae) was collected from fields in the Malkerns Research Station in Swaziland in January 2006. The sample was authenticated by the curator Mr. Dlamini and a voucher specimen No. LORT 2257T was deposited for keeps in the herbarium. The root (200 g) was pounded using mortar and pestle and then blended using (moulinex) blender, a brownish extract was soaked in mixture of methanol and dichloromethane (1:1, V/V). The

methanolic extract was evaporated using the rota-vapour to dryness. The filtrate was collected and preserved in the refrigerator -20°C for subsequent use.

Phytochemical screening: A phytochemical analysis of the extract of *L. ovaltifolia* was conducted according to the method of Trease and Evans (1983). Positive tests were obtained for saponin, glycosides, tannins and flavonoids.

Animals: Male albino rats (200-300 g), rabbit (300-500 g) were raised in the institute animal house under standard environmental conditions. All animals had free access to rodent pellets and water.

Experimental procedures (rat stomach strip): The rats were killed by cutting one of the common carotid arteries. The stomach strip excised and placed in a Petri dish containing a physiological salt solution at room temperature. The strip was carefully cleaned off connective tissues. Circular muscle strips were then prepared from the stomach according to the technique used by Vane (1957). Both ends of the circular muscle strip were tied with cotton threads and mounted in an organ bath (capacity 15 mL) which was filled with a physiological salt solution maintained at 37°C and aerated with a gas mixture containing 95 and 5% CO₂. The preparation was equilibrated for 1 h in the organ bath in an unstretched condition and the bathing solution renewed every 25 min. After equilibration the contractile

responses were recorded isometrically using a force transducer model (7010, Ugo Basil) and the signal amplified with a 2-channel Dynanometer (Gemini 7070).

The physiological salt solution used in this experiment was Tyrode solution, with the following ionic composition in (mM^L⁻¹), NaCl 137, KCl 2.8, MgCl₂ · 6H₂O 1.3, NaHCO₃ 11, NaH₂ PO₄ 1.2, CaCl₂ · 2H₂O 1.8 and glucose. The solution was aerated with 95-5% CO₂ gas mixture which maintained the solution at pH of between 7.2-7.4. In some experiments various concentrations of the extract were added.

Isolated rabbit preparations IRJ: The rabbit was killed as previously described. A segment of the ileum was excised and placed in a Petri dish containing physiological salt solution according to the method of Finkleman (1930).

Drug used was Acetylcholine bromide Sigma, St Louis, MO, USA and the extract as previously described.

RESULTS

In vitro study

Effect of extract on ACH-induced contraction in RSS: Dose-effect relationship was established using acetylcholine (ACH) concentrations ranging from (3.3×10⁻⁸-13.2×10⁻⁸ M), after which the strip was equilibrated in extract (1.25 mg mL⁻¹) for 3 min. The tissue was again stimulated with same doses of Ach; similarly this was repeated in extract 2.5 mg mL⁻¹ for 3 min (Fig. 1).

In some experiments time effect study on the extract was examined on the muscle strip at 3, 10 and 30 min interval in the rat stomach strip (Fig. 2). In this experiment methanolic extract inhibited the Ach-induced contractile response of RSS (Fig. 1, 2), indicating antispasmodic like action and the inhibition was non-competitive due to suppression of maximum responses and a shift of the curve to the right.

Effect of extract (1.25 mg mL⁻¹) on the pendular movement of rabbit jejunum: A regular spontaneous contractions (pendular movement) were recorded thereafter Adrenaline 10⁻⁶ M inhibited contractions (tracing nor shown) out the effect of adrenaline, the extract 1.25 mg mL⁻¹ was introduced into the physiological solution. The extract caused inhibition of pendular movement, similar to Adrenaline effect. This effect was prolonged even after washing out the extract from the bath, in some experiments prolonged cessation of pendular movement was observed (Fig. 3).

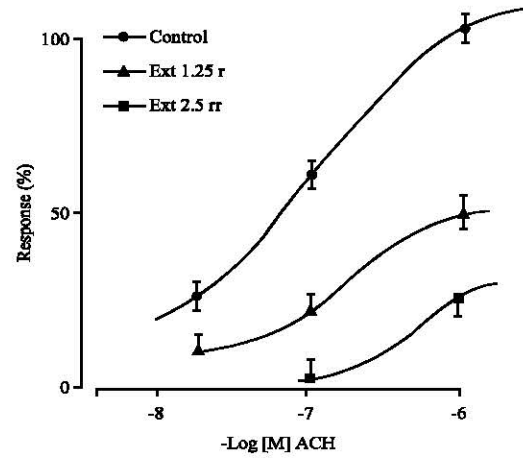


Fig. 1: Non competitive effect of *Ledebouria ovaltifolia* extract on dose response curve to acetylcholine

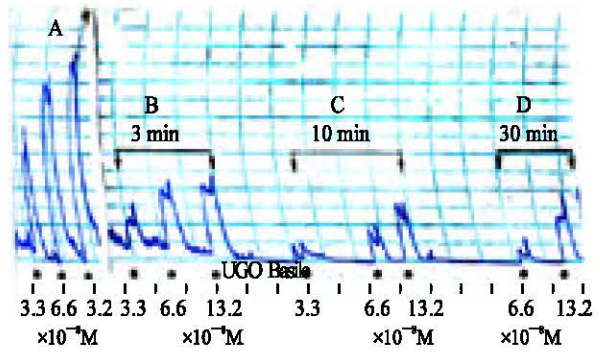


Fig. 2: Typical tracing showing time effect actions of extract *Ledebouria ovaltifolia* (1.25 mg mL⁻¹) in (A) control Extract (B) 3 min, (C) 10 min and (D) 30 min of introducing extract: vertical bar (time) horizontal bar tension 0.5 g

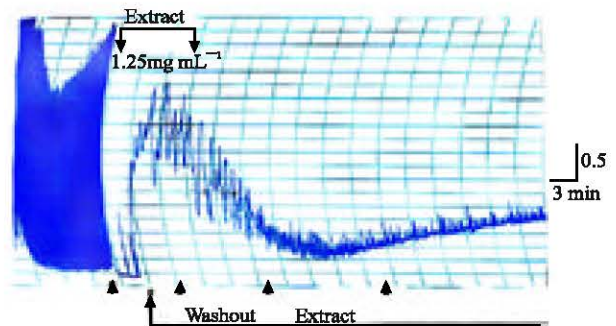


Fig 3: Relaxation caused by extract *Ledebouria ovaltifolia* (1.25 mg mL⁻¹) on isolated rabbit jejunum preparation arrow, (indicate regular PDM in the absence of extract, (●) point of extract addition. (⤴ indicate point of washing out the extract: horizontal bar 0.5 g tension vertical bar time in min

DISCUSSION

The present experiments indicate that in smooth muscle isolated from the rat stomach strip and the rabbit jejunum pendular movement, *Ledebouria ovaltifolia* significantly inhibited contractions produced by either acetylcholine or pendular movement in a concentration dependent manner in RSS. The cellular mechanism of contraction involved in the responses of smooth muscle is mainly by receptor mediated supply of Ca^{2+} (Bolton, 1979; Karaki *et al.*, 1997). Muscle contractions is largely dependent on free cytosolic Ca^{2+} which activate the contractile protein through various mechanisms (Suzuki and Chen, 1990). The actions of the extract on Acetylcholine (a muscarinic agonist) by depressing maximum contractile heights responses and right ward shift of the dose effect curve (Fig. 1) is indicative of non-competitive interactions, which exclude the possibility of the extract interacting with muscurinic receptors in this study.

Varying effects, of the extract on Ach-induced contractions, showed a shift of logdose between the concentrations of extracts (1.25 and 2.5 mg mL⁻¹) while the time effect showed increased inhibitory action of the extract in this muscle. The present study has shown that the inhibitory action of the extract on the rabbit pendular movement mimicked Adrenaline like effect, monoamines are generally known to inhibit-gastro intestinal motility. This effect may be due to either the elevated cyclic-GMP in vascular or smooth muscle cells, causing relaxation or inhibition of smooth muscle due to reduction of cytosolic Ca^{2+} through activation of Ca^{2+} ATPase distributed in the membrane of internal stores (Karaki *et al.*, 1997). Hyperpolarisation of the membrane is also one of the important factor in inhibitory or relaxatory effect in muscle contraction. From the following, the action of the extract exhibited similar actions to Adrenaline indicating the presence of monoamine like activity in the extract. Furthermore, the spontaneous activity of the rabbit jejunum was first used to study the actions of drugs acting at adrenergic site (Finkleman, 1930). However, the prolonged cessation of pendular movements in this study remains unclear. The medium of preparation of the extract in this study was not significantly different from the aqueous medium used in previous studies Aziba (2007). The phytochemical screening which indicated positive tests for tannin, saponins, glycosides and flavonoids

(Trease and Evans, 1983), these constituents are known to be bioactive agents which could have significant effect on the observed actions of the extract. Thus the analysis of tissue responses to unknown agents may help to elucidate their pharmacological properties.

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