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Inhibitory effects of *Ledebouria ovaltifolia* (hyacithaceae) aqueous root extract on contractile responses of Rat Vas deferens to K and adrenaline, pendular movement of isolated rabbit jejunum and acetic acid induced pain in Mice.

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ABSTRACT

To investigate the pharmacological effects of aqueous root extract of *Ledebouria ovaltifolia* on Potassium and Adrenaline-induced contractions of the rat vas deferens, the spontaneous pendular movement of Isolated rabbit jejunum, and acetic acid induce pain in mice. Isolated epididymal end of the adult male rat {200-350g}, and rabbit jejunum in separate experiment were mounted in Ugo Basil organ bath containing Tyrode solution aerated with 95% O₂ and 5% CO₂, and responses monitored through isometric transducer connected to a 2-channel dynamometer recorder. The effect of extract [27.4mg/ml] was examined on submaximal dose of K & ,adrenaline, and rabbit pendular movement , similarly the extract was examined on acetic acid induced pain in mice. The extract in concentration used, produce a significant inhibition on K and Adrenaline induced contractions [height] up to 75 and 68% respectively. In most of the experiments and this effect was more marked on K than Adrenailne contractions. The extract showed initial potentiation of PDM, followed by a stepwise relaxation leading to a prolonged cessation of pendular movement thus showing dual actions., the extract prolonged the reaction time of 75% [p>0.05] over 20min observation time with no overt signs of toxicity. These results suggest the presence of spasmolytic and analgesic activities in the plant extract.

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Keywords: Ledebouria ovaltifolia, rat vas deferens, rabbit jejunum, KCl, adrenaline,

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INTRODUCTION

In Swaziland, a very tiny country in Southern Africa, herbal medicine is very well accepted as alternative medicine because of the socio-cultural affinity of the people [Makhubu, 2002, 2003]. The very rich biodiversity of the flora in the kingdom is exploited very well in traditional practise. *Ledebouria ovaltifolia* called Umhlabhelo in Swaziland is used mainly as a pain relieving medicinal plant for bone fracture healing when taken as concoction, its gastrointestinal efficacy in cleaning bowel and post operative analgesic properties has been attested by the people. To the best of my knowledge, no pharmacological report of this plant has been reported or investigated on the smooth muscles in rat vas deferens [RVD], the isolated rabbit jejunum [IRJ] and the analgesic effects on acetic induced pain in mice. Thus the analysis of tissue response to unknown agents may help to elucidate their pharmacological properties.

MATERIALS AND METHODS

(Plant collection & Extraction)

The root of *Ledebouria ovaltifolia* {hyacinthaceae} 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 {200g} were pound using mortar and pestle and then ground using a blender (moulinex), the ground extract was soaked in water overnight and filtered using the vacuum pump, the brown filtrate was collected and preserved in the refrigerator below -200c.

Phytochemical Assay

A phytochemical assay of the aqueous extract was carried out using the method of (Trease and Evans). The results indicated the presence of steroids, saponin tannins and antraquinone.

Animals

Healthy male albino rats [200-300g], Rabbit [350-450g] and mice of both sexes [25-35g] were used in these studies. All animals were raised at room temperature with natural lights-dark cycle and maintained at a standard with standard rodent pellets and had access to water.

Experimental Procedures

Rat Vas Deferens (RVD) preparation

The epididymal end of the rat vas deferens was mounted with resting tension 0.5g in an organ bath containing [15ml] Tyrode solution and allowed to equilibrate for 60 min and the solution in the bath was continuously bubbled with 95% O₂ and 5% CO₂ with temperature maintained at 37°C, contractions were monitored through isometric force transducer model [Ugo Basil] and contractions recorded on a 2-channel dynamometer [Gemini]. Dose Responses to K and Adrenaline were recorded on the thereafter a sub maximal doses of K [60mM] and Adrenaline 2x10⁻⁷M were exposed to the physiological salt solution containing 27.4mg/ml of the extract.

Isolated Rabbit Jejunum Preparation (IRJ)

The rabbit was killed by a blow on the head and arteries cut to bleed. A segment of the jejunum was excised and removed into a physiological salt solution maintained at 37°C. Contractions were monitored as previously described for the RVD.

Acetic Acid Induced Pain in Mice

The analgesic pain was induced into mice of both sexes using the method of Whittle [1964], mice were grouped five in the cage and given oral administration of 27.4mg/kg, while the reference drug Paracetamol [1.25mg/ml] was administered to another group of mice [control] after 30 min. 0.4ml of 0.1% acid were administered i.p and placed in an observation box for count. The control animals were given saline & acid, a stop watch [Herwin precision instrument] was used for timing, counting of squirming was taken as manifestation of pain from

the following observations repeated abdominal contraction, stretching, of the trunk and hind limbs and torsion of the body at hips with inward rotation of one limb

effect was calculated from heights of contractions the maximum responses before the addition of the extract this action was more marked in K than Adrenailne induced contractions.[see fig 1)

RESULTS

Effect of *Ledebouria ovaltifolia* (27,4mg/ml on K 60mM and Adrenaline ($2 \times 10^{-7}M$) induced contractions

A dose effect relationship was established, after which sub maximal doses were examined in the physiological solution containing the extract, the extract inhibited up to 68% heights of contractions in all experiments (the% inhibitory

Effects of extract 27.4mg/ml on Isolated Rabbit jejunum

After obtaining a regular spontaneous contractions [pendular movement] adrenaline $2 \times 10^{-7}M$ inhibited contractions and the extract caused initial potentiations followed by a stepwise relaxations leading to prolonged cessation of pendular movement, even after washing out the extract from the bath. Thus the action was dual in nature, [see fig 2a)

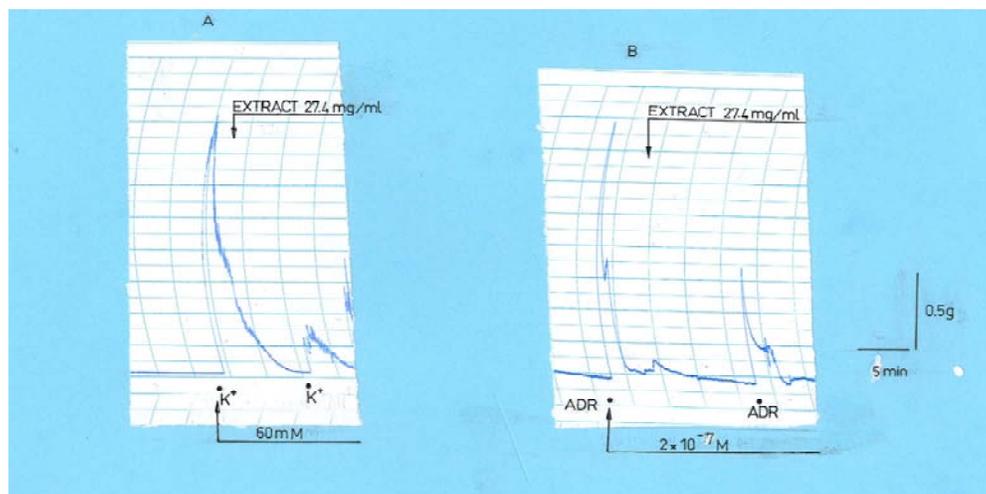


Figure 1
Effects of root extract *L. Ovaltifolia* (27.4 mg/ml) on KCL (60mM) and Adrenaline ($2 \times 10^{-7}M$) (.) point of addition of drug. Panel A (K^+). Panel B Adrenaline, vertical bar 0.5 tension and horizontal interval (min).

Table 1.
The % Inhibitory analgesic properties of *L. Ovaltifolia* and Paracetamol

Treatment	Amount Used mg/kg	Acetic H ⁺ (ml)	Significance	% Inhibition
Control	0.4ml saline	0.1	141±4.2 (n=10)	-
Extract	5.3	0.1	72.5±3.5 (n= 10)	51
	10.6	0.1	51.5±4.5 (n=10)	65
	21.2	0.1	20.5±2.5 (n=10)	86
	27.4	0.1	7.5± 1.5 (n=10)	94.6
Paracetamol	1.25	0.1	6.2±1.5 (n=10)	95.7

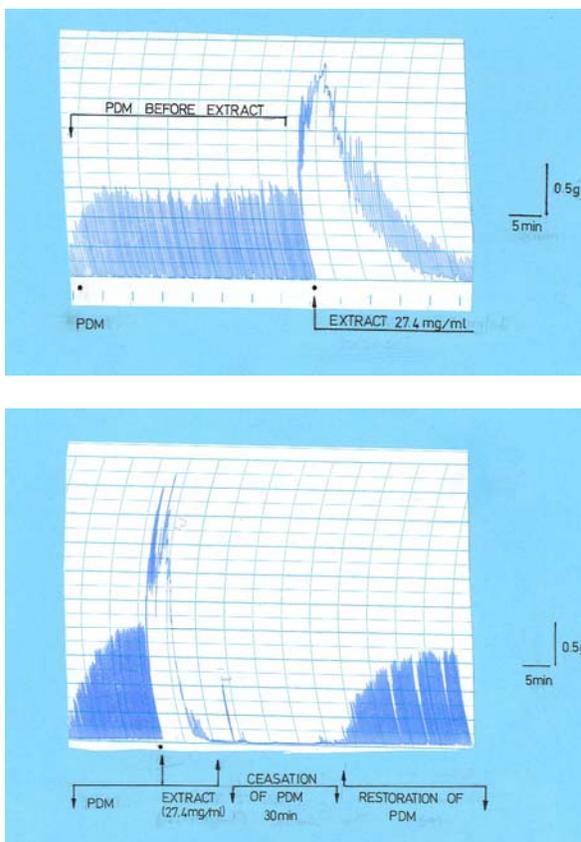


Figure 2
 (A) Effects root of extract *L. Ovaltifolia* (27.4mg/ml) on spontaneous pendular movement of rabbit jejunum. (arrows) point of drug addition; ceasatium of PDM and restoration of PDM. (B) shows regular PDM and the potentiation of PDM and stepwise relaxation of PDM. Arrows indicate point of addition in drug vertical bar tension, horizontal time interval.

Effects of Varying concentrations of extract on acetic acid induced pain

The extract significantly evoked a prolongation of reaction time of 75% [$p > 0.05$] dose dependently over 20min observation time with no overt signs of toxicity (See table 1).

DISCUSSION

The result in the present studies in smooth muscles indicated inhibitory action of the extract on the muscles used. The cellular mechanisms of contractions involved in smooth muscle responses evoked by K and Adrenailne by

different mechanisms., the former imainly by influx of of ca^{2+} through voltage ca^{2+} channel [Karaki, et al 1997] while the later is through receptor mediated calcium channel, however smooth muscle contractions is largely dependent on free cytosolic calcium which activate the contractile proteins through various mechanisms the k involves VOCC influx of calcium [Karaki, et al, 1997] involves depolarisation and subsequent influx of calcium from Vocc while Adrenaline evoked contractions is due mainly to interactions with either alpha or beta receptors, generally, stimulation of alpha receptors leads to to hydrolysis of phosphatidyl inositol into triphoshate [IP3] and diacyl glycerol [dag] causes release of intra cellular calcium from its storage site while diacyl glyceride depolarises the cell membrane leading to calcium influx [ref.] the stimulation of alpha receptor activate adeny cyclase responsible for the increase of CAMP that release calcium which leads to contraction [Suzuki and Chen, 1990; Kuriyama et al, 1998; Bulman et al, 1993; Loirand et al, 1997]. However, the inhibitory action of the extract was more on K suggest action more on VOCC calcium channel. The patern of motility inhibition and particularly the prolonged ceasation of PDM resembled inhibition elicited by adrenergic mechanisms, this may be due to hyperpolarisation of the membrane which is one of the important factors to induce relaxation [Suzuki and Chen, 1990] and also anticholinergic, since cholinergic mechanisms mediate gastrointestinal motility and the initial potentiations resembles anti cholinesterase properties. The significant inhibitory effect of the extract on acetic acid induced pain in mice suggest analgesic properties on the on the pertipheral nervous system [Aziba, 2001]; this also collaborate or support the pain relieving use of the extract in bone fracture use in Swaziland. The totality of the above results suggest the plant extract possesses anti spasmodic and analgesic effect under the concentrations used in this study.

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