

Spasmodic Response and Neurogenic Mechanism of Water Extract of *Vernonia cinerea* (L.) Less on Rat Duodenum

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ABSTRACT

Use of different agonists and antagonists for elucidating the mechanism of aqueous extract of *Vernonia cinerea* (L.) Less. on duodenal smooth muscle of female albino rat suggested the presence of an active ingredient in plant having spasmogenic response that has neurogenic mechanism on rat duodenal smooth muscle. The responses of extract were dose dependent and fully reversible on wash. The findings suggested that the extract responses mediate through agonistic action without blockade of any receptor enzyme. Extract possibly stimulates presynaptic cholinergic nerve endings to produce spasmodic response in rat duodenal smooth muscle.

INTRODUCTION

In continuation to our studies on essential oils and extracts of medicinal plants and their pharmacological study (Prakash *et al*, 2006), we investigated spasmodic response and neurogenic mechanism of action of water extract of *Vernonia cinerea* (L.) Less on contractility of rat duodenal smooth muscle. The whole plant of *V.cinerea* has been documented to exhibit significant diuretic activity (Herrera *et al*, 1998), anti-inflammatory

Activity (Mazumder *et al*, 2003), antipyretic potential and antibacterial activity (Malaya *et al*, 2003b, a). Leaves showed diuretic and anti-diuretic activity (Adeboye *et al*, 1997) analgesic, antipyretic and anti-inflammatory activities (Iwalewa *et al*, 2003). Plant *Vernonia cinerea* (Sahadevi) belonging to genera *Vernonia*, is used as a folk medicine by the people of Nepal (Hu *et al*, 2004). The plant is found in tropical Asia, Africa, Australia and New Zealand. In India it is found throughout the country, ascending up to 2400 m in the Himalaya, Khasi hills and hills of peninsular India and is very common weed of the roadsides, garden lands and open forest.

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MATERIAL AND METHODS

Plant material

Fresh plants of *V. cinerea* were collected locally from Tarai region of Himalaya, Pantnagar (Uttarakhand), India. The specimen has been deposited in the Department of Chemistry, G.B. Pant University of Agriculture & Technology, Pantnagar, India.

Animals

The Pharmacological study was conducted on adult female albino rats (175-225g), purchased from Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. The rats were maintained in Polypropylene cages and housed in the animal sheds of Department of Pharmacology and Toxicology College of Veterinary & Animal Sciences, G.B. Pant University of Agriculture & Technology, Pantnagar Uttarakhand, India under good managemental conditions. Animals were fed standard ration *ad libitum* and had free access to clean drinking water.

Isolation of water extract

Fresh plants of *V. cinerea* (5 kg) were collected from its natural habitat in the month of May and were shade dried for about 30 days. Shade dried plant material was powdered. For the purpose of extraction, about 1 kg powdered plant material was dipped in triple distilled water for 20 days and stirring was done after every 4 to 5 days. The infusion was filtered through muslin cloth. The crude aqueous extract of plant was obtained after evaporation of water under reduced pressure.

Tissue preparation and mounting

Female albino rats weighing between 175 -225 g body wt. were anaesthetized with pentobarbitone sodium (40 mg/kg, IP) and sacrificed by cervical dislocation and exsanguination. A small piece (3 cm long) of duodenum 4 cm apart stomach was removed, cleaned and mounted on an organ bath containing Tyrode solution (composition in mM: NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 0.2, NaHCO₃ 11.9, NaHPO₄ 0.4 and glucose 5.5, pH 7.4). The bathing fluid was continuously bubbled with air and maintained at constant temperature (37±0.5°C). The tissue was allowed to equilibrate for a period of 30 min under a resting tension of 0.5 g. During this period the tissue was washed every 15 min.

After equilibration, drugs were administered in bath fluid to record their effect on physiograph (Bio-devices, India). Every time responses of 2 successive doses of acetylcholine (ACh), at an interval of 10 min were recorded to confirm optimum responsiveness of the tissues.

Drugs

Serial solutions of water extract of *V. Cinerea* whole plant (VWE) were prepared directly in Tyrode solution and mixed properly with the help of an Ultra-Sonicator (Soni prep 150, UK). The solutions were sonicated before use.

2.6 Effect of VWE on basal tone of rat duodenum

Effect of cumulative doses (0.25 to 25 mg/ml) of VWE on rat duodenal smooth muscle was recorded. To elucidate the mechanism of action of VWE, different agonists and antagonists were used as per the following steps-

1. Each tissue preparation was exposed to only one agonist or one combination.
2. Dose dependent contractions induced by VWE (0.25 to 25 mg/ml) were recorded.
3. Atropine sulphate (1 µg/ml) was added to the bath fluid 15 min before recording the response of VWE (10 mg/ml) and ACh (2 ng/ml) to antagonise their effects.
4. The independent and combined effects of VWE (10 mg/ml) & ACh (2 ng/ml) were observed.
5. The response of ACh (2 ng/ml) was recorded in presence of low dose VWE (1 mg/ml, 15 min.), which produced fible contractile response in rat duodenum.
6. The response of VWE (10 mg/ml) and ACh (2 ng/ml) were taken before and after incubating the tissue with lignocaine hydrochloride (50 µg/ml) for 5 min.
7. The ACh (2 ng/ml) and VWE (10 mg/ml)-induced contractions were recorded in absence and presence of fresh rat serum (40 µl/ml, 5 min.).
8. Experiments were also conducted on the preparations that had been started at low temperature of 4°C for 96 h for enervation, but were later maintained at 37°C bath temperature during experiment.

Statistical analysis

The results are presented as mean ± S.E. of *n* observations. Values were analyzed using a student's t-test or ANOVA an appropriate and were considered to differ significantly when $p \leq 0.05$.

RESULTS AND DISCUSSION

VWE produced dose dependent contractile responses on rat isolated duodenum (Table I & II, Fig. 1&2). This response indicated that the plant contained an active ingredient, which has spasmogenic response on rat duodenal smooth muscle. The responses of VWE been fully reversible on wash. This finding suggests that VWE's responses were mediated through an agonistic action without blockade of any receptor or enzyme. Acetylcholine (2 ng/ml) and VWE (10 mg/ml) produced contractile response on isolated rat duodenum by 1.178 ± 0.16 and 1.086 ± 0.08 g, respectively. In presence of atropine (1 µg/ml), the contractile effects of ACh (2 ng/ml) and VWE (10 mg/ml) were blocked completely (Fig. 3). Atropine is a muscarinic cholinergic receptor blocker. Hence, inhibition of contractile responses of ACh and VWE by atropine indicates that the responses of these two agonists were mediated through muscarinic receptors. There seems to be one or more than one of the three possible mechanisms responsible for this contractile action of VWE- (i) stimulation of ACh release from the cholinergic nerve endings, (ii)

inhibition of acetylcholinesterase enzyme at the neuroeffector junction, or (iii) direct activation of the muscarinic receptors of rat duodenal smooth muscle (Hore *et al*, 1999). The combination of ACh and an anticholinesterase agent produces a potentiated effect (Kela *et al*, 1995), because the fraction of ACh molecules, which is normally destroyed by acetylcholinesterase enzyme in absence of anticholinesterase, becomes available to act on the receptors to produce extra effect.

Table. 1 Dose dependent contractile responses (mm) of VWE on rat duodenum (n=6).

Dose of VWE (mg/ml)	Contractions in mm						Mean \pm SE (mm)
	1	2	3	4	5	6	
0.25	3	4	2	3	3	2	2.83 \pm 0.31
0.75	7	12	4	6	8	4	6.83 \pm 1.22
2.50	9	15	13	9	15	11	12.00 \pm 1.13
7.50	17	22	18	13	24	16	18.33 \pm 1.65
25.00	33	29	23	18	29	22	25.67 \pm 2.28

Table. 2 Dose dependent contractile responses (g) of VWE on rat duodenum (n=6).

Dose of VWE (mg/ml)	Contractions in g.						Mean \pm SE (g)
	1	2	3	4	5	6	
0.25	0.15	0.20	0.10	0.15	0.15	0.15	0.14 \pm 0.02
0.75	0.35	0.60	0.20	0.30	0.40	0.20	0.34 \pm 0.06
2.50	0.45	0.75	0.65	0.45	0.75	0.55	0.60 \pm 0.06
7.50	0.85	1.10	0.90	0.65	1.20	0.80	0.92 \pm 0.08
25.00	1.65	1.45	1.15	0.90	1.45	1.10	1.28 \pm 0.11

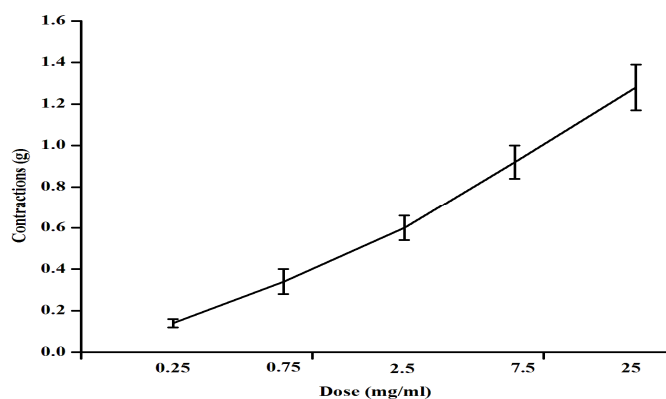


Fig.1 : Cumulative dose- dependent Contractile responses of aqueous extract of *Vernonia cinerea* whole plant on rat duodenum(n=6).

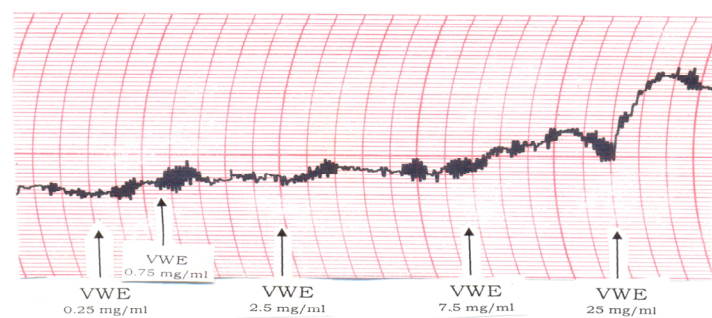


Fig. 2: Cumulative dose dependent responses of VWE on isolated rat duodenal smooth muscles.

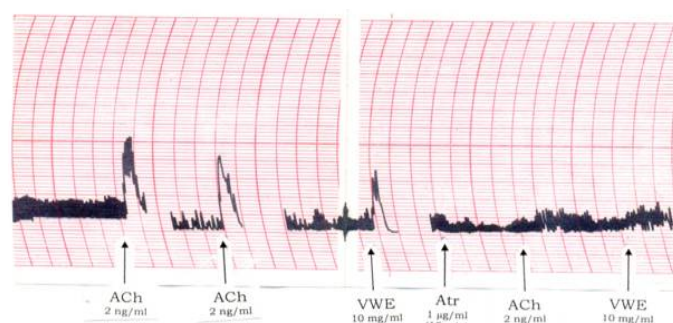


Fig. 3: Effect of atropine (atr) on ACh and VWE-induced contraction in rat duodenum.

This finding supports the neurogenic mechanism of VWE-mediated response in rat duodenum. Here, VWE stimulated the presynaptic cholinergic nerve endings to release ACh, which was responsible for the contractile response. The cholinesterase enzymes of serum or plasma hydrolysed the ACh and thereby abolished the response of VWE. The non-responsiveness of VWE in presence of serum or plasma (AChE) also suggests that VWE did not directly stimulate the cholinergic receptors. In the present study, VWE did not potentiate the responses of ACh (Fig. 4). The combination of ACh (2 ng/ml) and VWE (10mg/ml) produced additive response (2.00 ± 0.159 g). The contractile response of ACh (2 ng/ml) remained unaltered in tissue preparations previously incubated with low dose of VWE (1 mg/ml, 15 min) (Fig. 5).

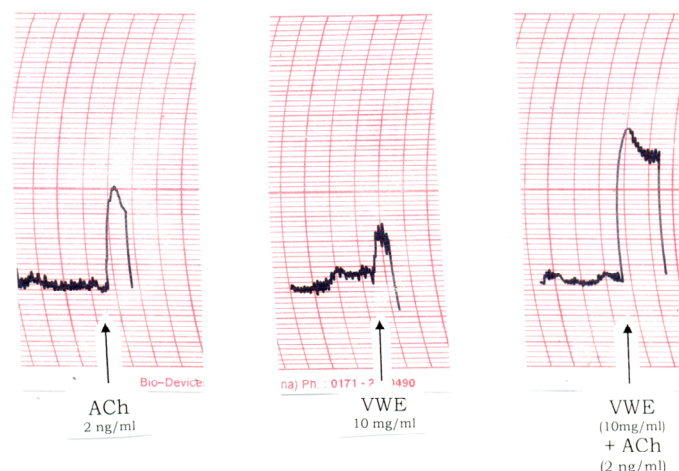


Fig. 4: Independent and combined effects of Ach and VWE on basal tone of rat duodenal smooth muscles.

Hence, this can be assumed that VWE did not inhibit the acetylcholinesterase enzyme. These findings thereby nullify the probable involvement of acetylcholinesterase enzyme in VWE-induced contraction in rat duodenum. Lignocaine, a membrane stabilizer, blocks the axonal conduction and thereby neurotransmitter release in smooth muscles at a concentration of 50 μ g/ml (Mishra and Raviprakash,1980). In our study, the same dose of lignocaine blocked the contractile response of VWE (10 mg/ml). However, the ACh (2 ng/ml)-induced contraction

remained unaltered in presence of lignocaine (Fig. 6). Therefore, it is an indirect evidence that there was possible stimulation of ACh release at the prejunctional site by VWE. This view is further strengthened by the finding that the contractile response of VWE (10 mg/ml), but not ACh (2 ng/ml) was reduced in cooled (4°C for 96 h) rat duodenal preparations (Fig. 7). The methods of cooling provide a means of obtaining smooth muscle preparation free from the neuronal influences. Hence, the VWE-mediated contraction in present study was seemed to be a neurogenic response.

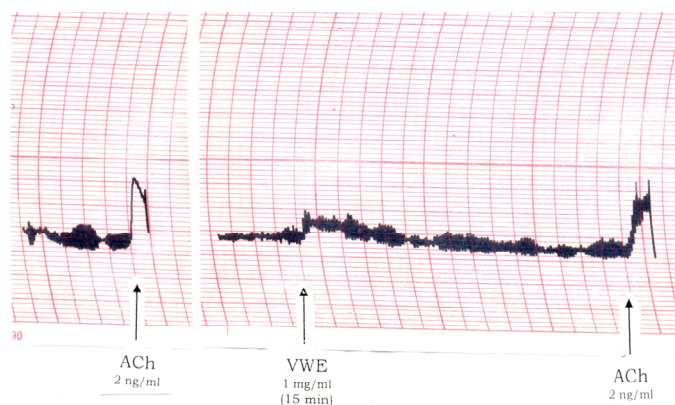


Fig. 5: Response of ACh in absence and presence of extract on rat duodenum.

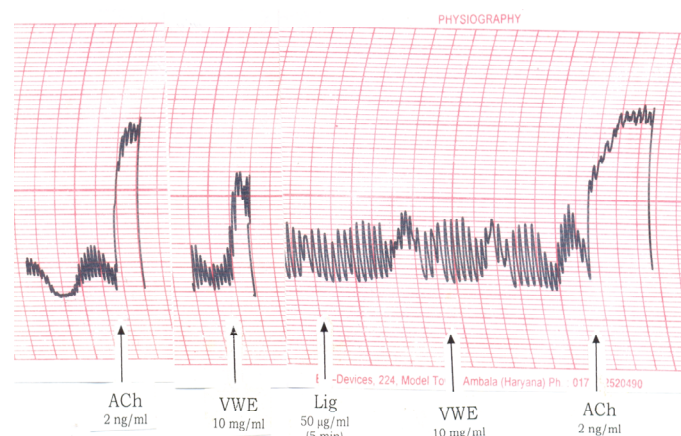


Fig. 6: Responses of VWE and ACh on rat duodena smooth muscle before and after incubating the tissue with lignocaine hydrochloride (Lig.).

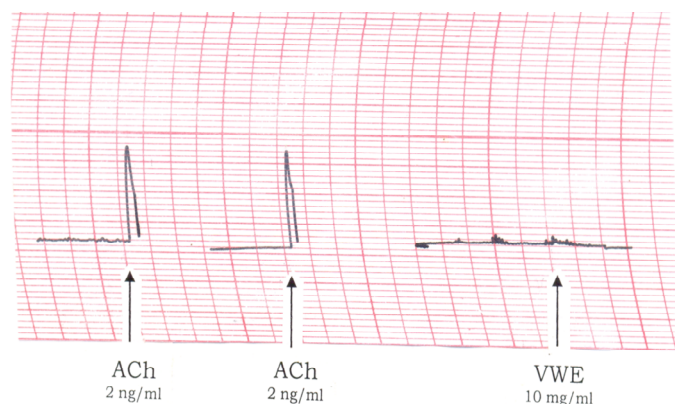


Fig. 7: Responses of ACh and VWE in rat duodenum that had been stored at low temperature of 40°C for 96 h for denervation but later maintained at 37°C bath temperature during experiment.

Rat serum or plasma, which contains cholinesterase enzymes responsible for hydrolysing ACh, abolished VWE (10 mg/ml) and ACh (2 ng/ml)-induced contractions in rat duodenum (fig. 8 & 9).

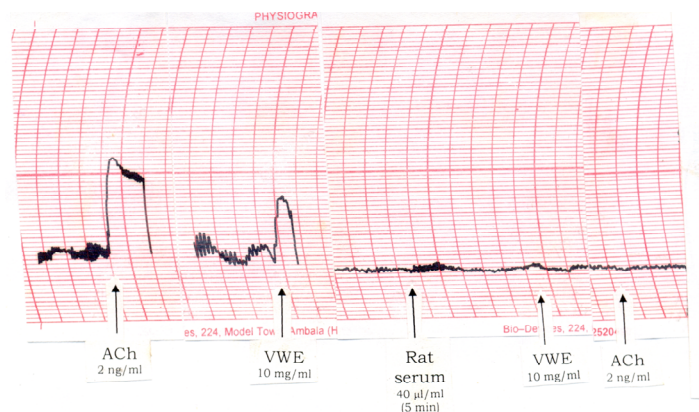


Fig. 8: ACh and VWE-induced contractions of rat duodenal smooth muscle in absence and presence of rat serum.

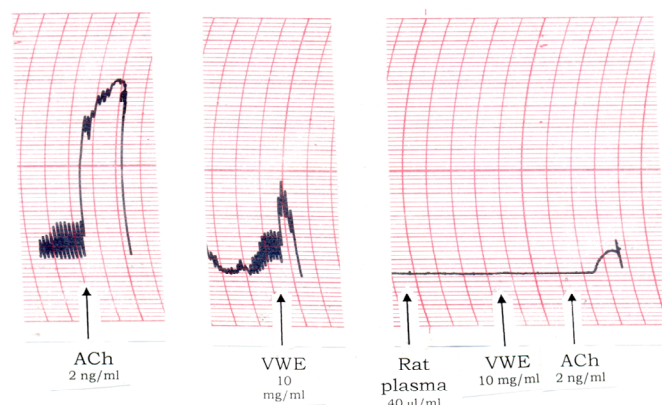


Fig. 9: ACh and VWE-induced contractions of rat duodenum in absence and presence of rat plasma.

In conclusions, it is suggested that VWE possibly stimulates presynaptic cholinergic nerve endings to produce spasmodic response in rat duodenal smooth muscle.

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