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ACTIVITY OF *AGERATUM CONYZOIDES* ON ISOLATED RAT PHRENIC NERVE-DIAPHRAGM AND BLOOD PRESSURE ON ANAESTHETISED RATS

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ABSTRACT

Crude root and aerial part extracts of A. conyzoides *were found to have neuromuscular blocking activity in isolated rats phrenic nerve-diaphragm. Physostigmine failed to reverse the blockade. It was also observed that the extracts caused greater fall on diastolic pressure compared with that of systolic pressure in anaesthetised rats. In addition, the plant extract has calcium channel blocking activity similar to that of verapamil.*

INTRODUCTION

Ageratum conyzoides L. (Compositae) grows abundantly in many parts of Kenya (Agnew, 1974). It is used in traditional medicine for its antiasthmatic, antispasmodic and haemostatic effect (Kokwaro, 1976). The crude extract of *A. conyzoides* has also been used as insecticide (Gonzalez, 1991), and in treatment of wounds (Adesogan and Okunde, 1979; Oliver, 1986) on *Staphylococcus aureus.* Little work has been done to examine the scientific basis of the use of traditional herbal medicine in Kenya, and other unknown pharmacological properties of those plants which may be either toxic or beneficial when administered. This is the basis of our work which examined neuromuscular blockade, calcium channel blockade and blood pressure activities of *A. conyzoides,* a traditional herbal medicine in Kenya.

Keywords: Ageratum conyzoides, Compositae, neuromuscular blocker, hypotensive, calcium channel blocker, anaesthetised rats, rabbit jejunum.

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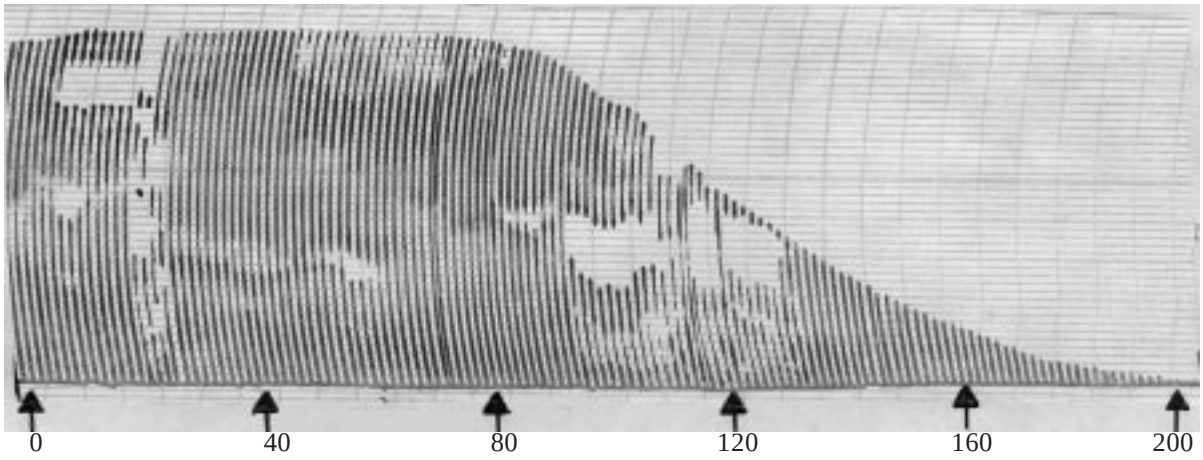
EXPERIMENTAL

Plant Materials

Ageratum conyzoides was collected from Nairobi area, Kenya around Kenyatta National Hospital in April, 1991. The plant was identified by the East African Herbarium and voucher specimens have been deposited in the above Herbarium and also in the Department of Pharmacology and Pharmacognosy, University of Nairobi, Kenya. The roots were separated from the aerial parts and the two portions were air dried separately at room conditions (25°C) for a minimum of ten days. The dried roots and the aerial parts were chopped and powdered separately. The materials (1.6 kg aerial part and 0.5 kg root portions) were separately extracted by cold percolation with 70% methanol. The resulting extracts were filtered and the filtrate reduced to solid by rotary vacuum evaporation to yield 487.1 g and 26.0 g, respectively.

Animals

New Zealand white rabbits (3–3.5 kg) and Wistar strain rats (200–250 g) of either sex were used. They were fed on normal rabbit pellets, vegetables, mice pencils and water. The animals were obtained from the National Public Health Laboratories, Nairobi, where they are bred for research purposes. The ani-



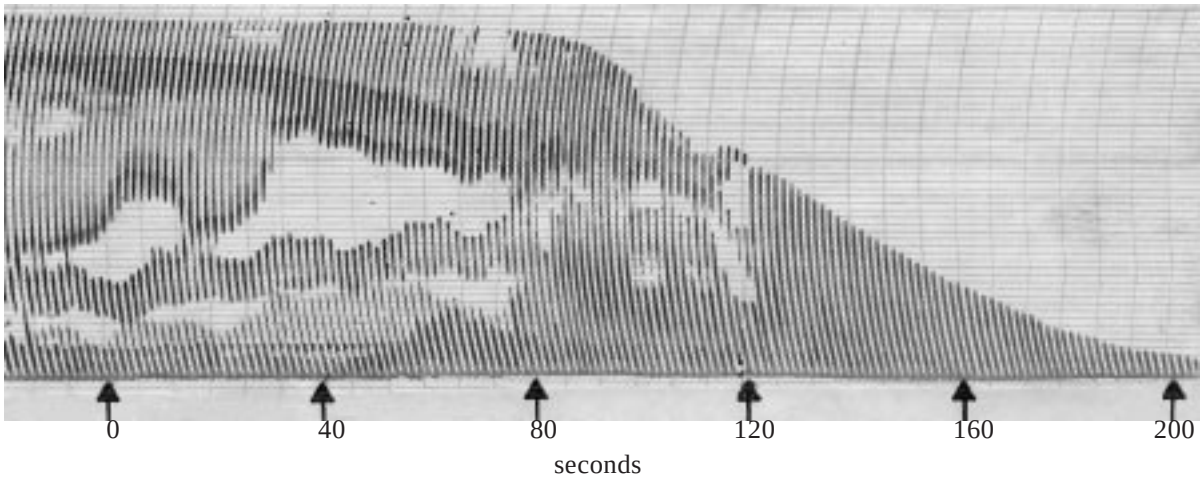


Fig. 1. Neuromuscular blockade on isolated rat phrenic nerve diaphragm by aerial part and root extracts of *A. conyzoides* (6 mg/ml bath).

mals were kept on floor with saw dust and in cages when breeding.

Standard Solutions

Stock solutions (300 mg/ml) of crude root and aerial part extracts were prepared in distilled water, physostigmine (50, 100 or 200 μg/ml), verapamil (4 mg/ml) and calcium solution (10 mg/ml).

Rat Phrenic Nerve-Diaphragm

The rats were sacrificed, the chest wall was opened to remove the phrenic nerve-diaphragm. The tissue was placed in a Petri dish containing Krebs Henseleit solution (Domer, 1971). The rat phrenic nerve-diaphragm was attached to an electrode and placed in a double walled 50 ml organ bath containing Krebs Kenseleit solution aerated with 95% O_2 and 5% CO_2 at 37°C. The tissue was allowed to stabilise for about 20 min.

The rat phrenic nerve-diaphragm was electrically stimulated at one pulse/sec at 5 volts. The concentrations of crude root or aerial part extracts, ranging from 2–6 mg/ml bath concentrations were investigated using 2 min tissue drug contact time cycle. The neuromuscular blocking activities were recorded on a 400 MD 2C Oscillograph BioScience re-

corder. The tissue was rinsed three times with Krebs Henseleit solution and the experiment was repeated seven times, for each concentration of root or aerial part extracts.

Physostigmine solutions used were prepared from stock solution and ranged from 1–4 µg/ml bath concentration, in an attempt to reverse the neuromuscular blocking activities of root and aerial part extracts of *A. conyzoides*.

Isolated Rabbit Jejunum

Rabbits starved for 24 h, were killed by a blow at the back of the head and their abdomens opened. Pieces of jejunum (2.5 cm) were mounted in a 20 ml organ bath containing Krebs solution, aerated with 95% O_2 and 5% C O_2 at 37°C. The tissues were allowed to equilibrate for about 20 min. The baseline was recorded on a kymograph. The Krebs solution was replaced with a Krebs solution lacking calcium and the tissues were challenged with 10, 30 or 50 µM calcium solution.

The experiment was repeated. The tissues were challenged with the plant extract (1 mg/ml bath), at 1 min intervals calcium solutions (30 or 50 µM) were added. Results were recorded on a kymograph.

A third experiment used the calcium channel blocker verapamil (1 µM). Calcium solutions (30 or 50 µM) were added to the tissue bath at 1 min intervals. The results were recorded on a kymograph.

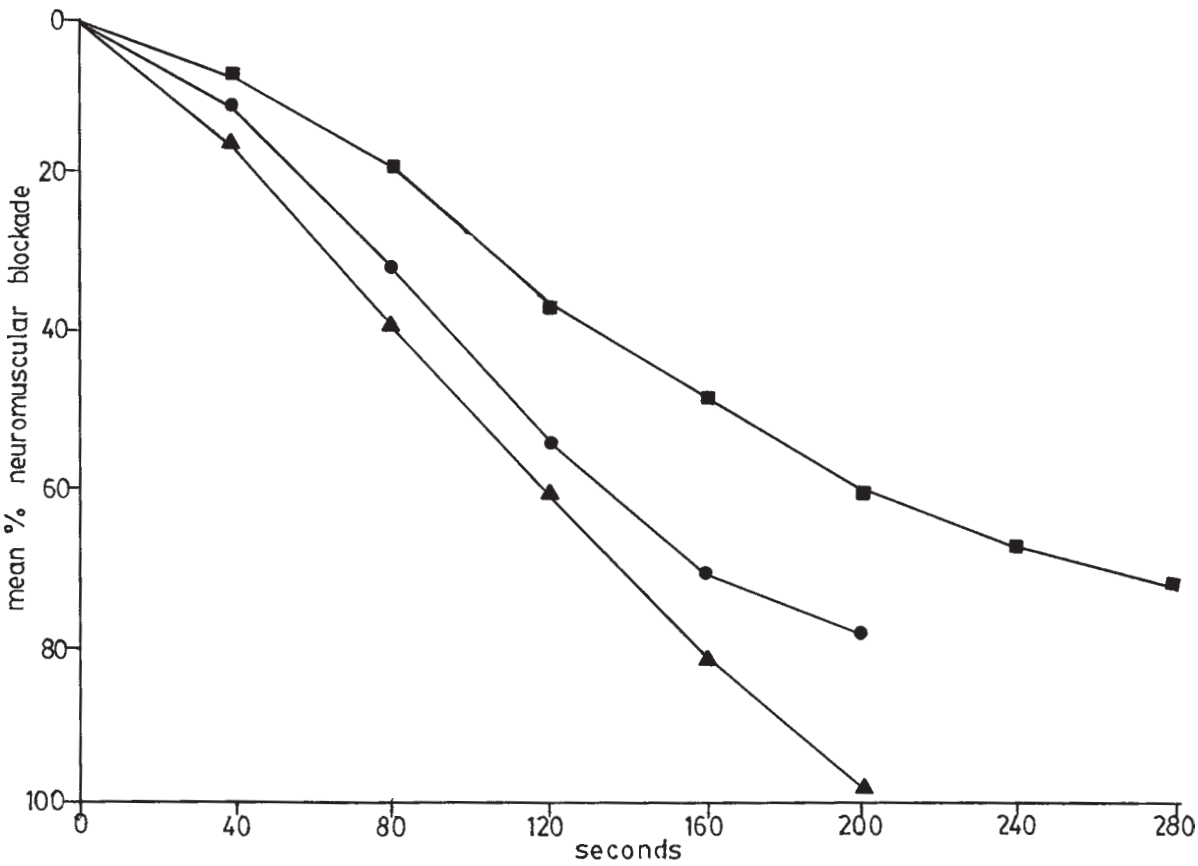


Fig. 2. Mean percentage neuromuscular blockade on isolated rat phrenic nerve diaphragm by root extract of *Ageratum conyzoides* (*n*= 7).
Legend: filled squares: 2 mg/ml bath concentration; filled circles: 4 mg/ml bath concentration; filled triangles: 6 mg/ml bath concentration.

Blood Pressure on Anaesthetised Rats

The rats were anaesthetised intraperitonially with 50% urethane (0.3 ml/100 g). The trachae was incised to insert a wind pipe. The femoral vein was cannulated for the administration of drugs, the cannulated carotid artery was attached to the Gould P23 ID transducer connected to Hellige recorder for blood pressure readings.

RESULTS AND DISCUSSION

The crude root or aerial part extracts of *A. conyzoides* (6 mg/ml bath concentration) produced complete neuromuscular blockade from which the tissue could not recover without washing. After 15 min, it recovered with three washings in Krebs Henseleit solution.

The root and aerial part of the plant extract had similar neuromuscular blocking activities (Fig. 1).

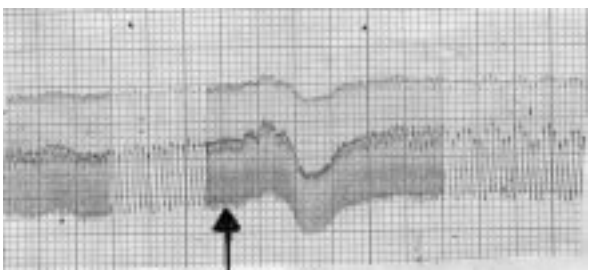
The neuromuscular blockade of the root extract of *A. conyzoides* was dose-dependent (Fig. 2). Physostigmine at various bath concentrations ranging from 1–4 μg/ml failed to reverse the neuromuscular blockade of the phrenic nerve-diaphragm by crude root or aerial part extracts of *A. conyzoides*. This suggests that neuromuscular blockade caused by the crude root and aerial part extracts of *A. conyzoides* may occur through a mechanism other than blocking acetylcholine receptors.

A 10 μg dose of a 70% methanol plant extract (aerial part and root) in anaesthetised rats lowered blood pressure (Fig. 3). A 10 μg dose of the root extract reduced systolic blood pressure by 13% and diastolic blood pressure by 41% (Fig. 4). The maximal reduction of systolic blood pressure was 37% after a 50 μg dose and diastolic blood pressure have been observed with isoprenaline, a beta agonist causing a vasodilation associated with increased cardiac output and a greater fall in diastolic pressure than systolic pressure (Katzung, 1987).

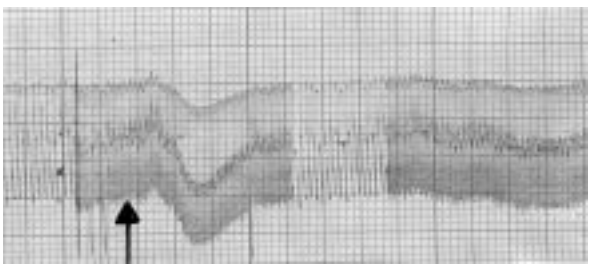
The mean heart rate of the rats was steady at 360 beats/min throughout the experiments.

We have shown that the plant extract suppressed the spontaneous movement of rabbit jejunum (Achola *et al.*, 1994). Most types of smooth muscles are dependent on transmembrane calcium influx for normal resting tone and contractile reponses. They are relaxed by calcium influx inhibitors, such relaxation has been shown for bronchial, gastrointestinal and uterine smooth muscles. Blood pressure can be reduced by calcium blockers through reduction in peripheral vascular resistance (Katzung, 1987). The inhibition of spontaneous movements of rabbit jujunum by the plant extract may be due to interference either with the depolarization process or with the calcium influx through voltage-dependent calcium channels (Gilani *et al.*, 1994).

Spontaneous contraction of rabbit jejunum were abolished when exposed to calcium free solution, but were restored when calcium was added to the organ bath (Fig. 5). Calcium failed to restore spontaneous movements when the tissues were pretreated with the plant extract or verapamil (Fig. 5), making calcium unavailable to contractile elements in cytoplasm (Gilani *et al.*, 1994). It was observed that the plant extract blocked calcium channel in a manner similar to verapamil, a standard calcium channel blocker (Fig. 5). The calcium channel blocking activity of the plant extract, may explain in part, the



Aerial part extract (10 μg)



Root extract (10 μg)

Fig. 3. A fall in blood pressure in anaesthetised rats caused by aerial part and root extracts of *A. conyzoides* (10 μg) (n = 7).

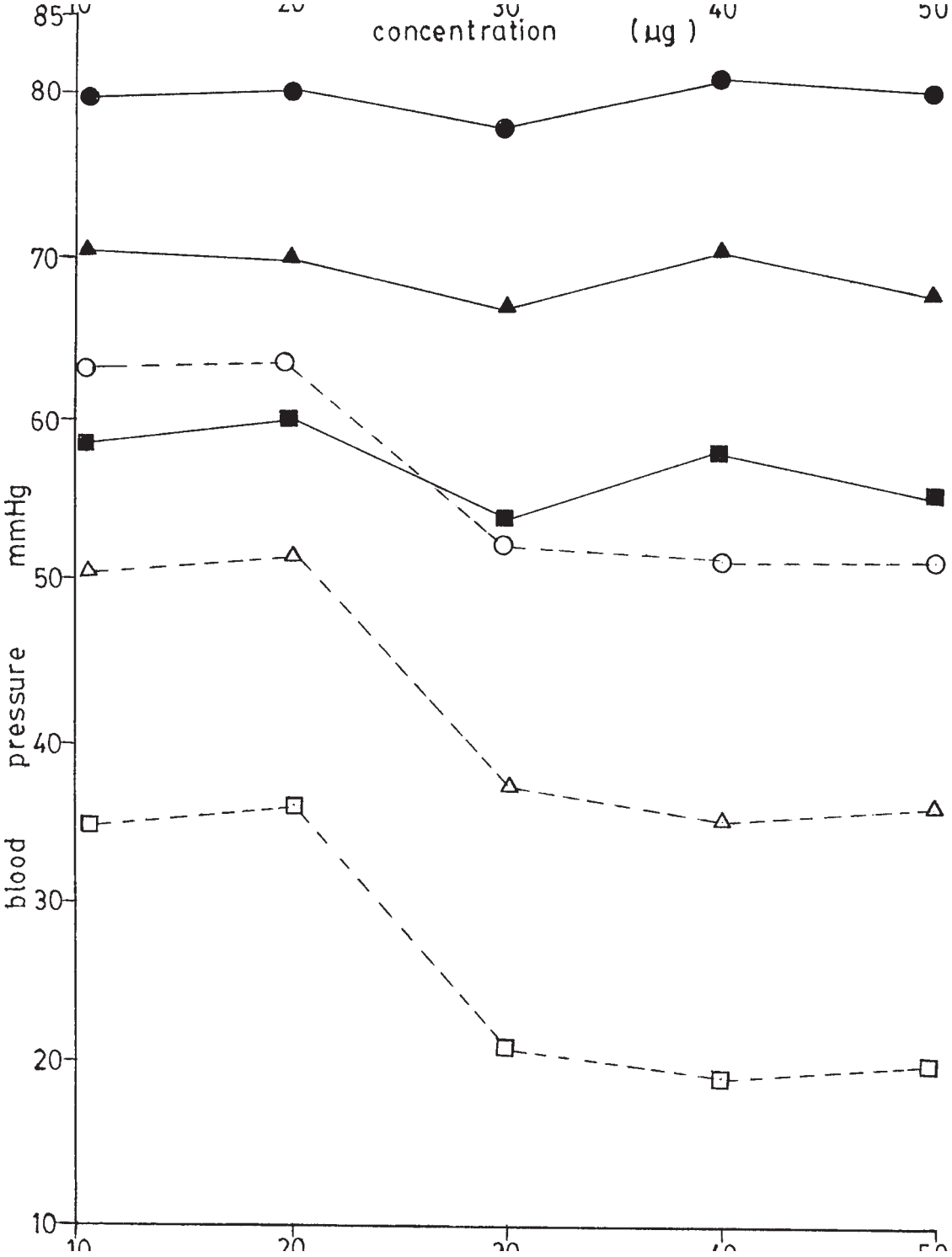


Fig. 4. Mean blood pressure in anaesthetised rats before and after administration of root extract of *Ageratum conyzoides* (n = 7).
Legend: filled circles: normal systolic blood pressure; filled triangles: normal mean blood pressure; filled squares: normal diastolic blood pressure; open circles: systolic blood pressure after plant extract; open triangles: mean blood pressure after plant extract; open squares: diastolic blood pressure after plant extract.

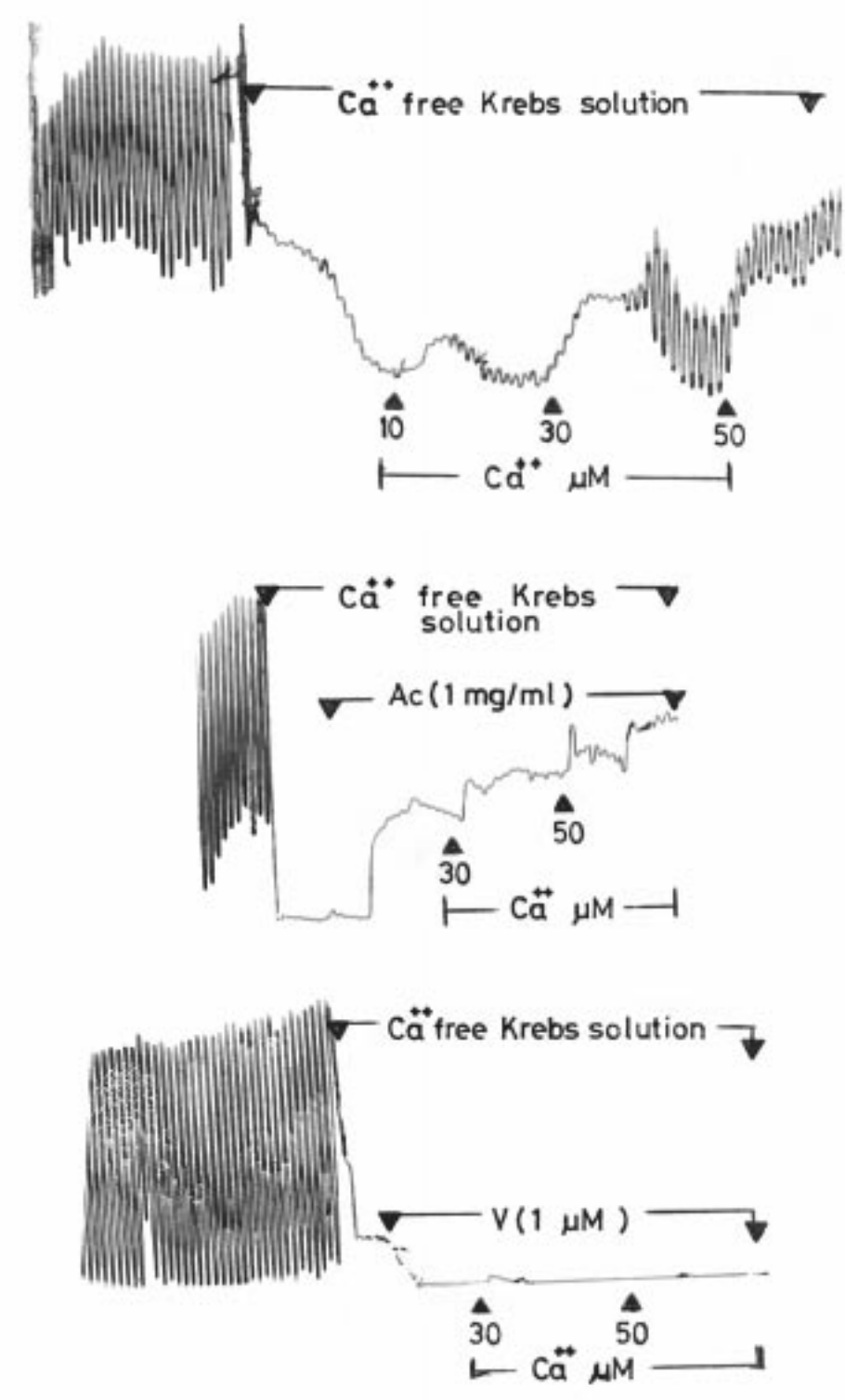


Fig. 5. Tracing showing effect of calcium on isolated rabbit jejunum in calcium free Kreb's solution. Effect of pretreatment with *Ageratum conyzoides* extract (Ac) or verapamil (V) on calcium supplementation. $n = 7$.

hypotensive, neuromuscular blocking and bronchodilating activities observed.

In conclusion, the results showed that *Ageratum conyzoides* contained neuromuscular blocking, cardioactive and calcium channel blocking agents.

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