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Calcium Ions Influences Smooth Muscle Relaxant Response to Aqueous Extract of *Portulaca oleracea*

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Aqueous extract of the leaf and stem of *Portulaca oleracea* (AEPO) was tested on isolated Guinea Pig Ileum (GPI) preparation. The extract was also tested on ileal smooth muscles of guinea pig precontracted with 10^{-5} g mL^{-1} carbachol and *in vivo* motility assessment in mice was investigated. The study showed the following order of sensitivity of GPI preparation to the drugs; AEPO> histamine> acetylcholine. Both atropine and promethazine abolished the contractile responses of GPI to AEPO only at low doses; 1×10^{-5} to 10^{-3} g mL^{-1} while nifedipine completely inhibited ileal muscle contractions to AEPO. The extract also caused relaxation to carbachol-sustained contractions. *In vivo* study demonstrated dose dependent reduction in peristaltic index in mice. From the results of these studies, the mechanism of action of the extract on relaxant response of GPI to AEPO may be dependent on Ca^{2+} antagonism.

Key words: *Portulaca oleracea*, calcium ions, guinea pig ileum, intestinal motility, relaxant effect

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INTRODUCTION

Portulaca oleracea (family: Portulaceae) have been used in traditional medicine by the people of West Africa and in Nigeria as concoctions in the treatment of various ailments such as swellings, bruises, boils, abscesses, ear and toothaches and the relief of muscular aches and pains (Dalziel, 1937). It is commonly called purslane in english language, ebe ehofen in edo language and esan omode or papasan in yoruba language, all within Nigeria (Burkill, 1997).

Aqueous extract of *Portulaca oleracea* was reported to induce an initial increase in twitch tension before muscle relaxation on the electrically stimulated rat hemidiaphragm muscle preparation *in vitro* (Okwuasaba *et al*., 1986; Parry *et al*., 1987, 1993). Simultaneous addition of the extracts (aqueous, dialyzable- and methanol-) and dantrolene resulted in an increase in the rate of twitch tension inhibition and a decrease in the time to maximum relaxation of twitch amplitude (Okwuasaba *et al*., 1987). In another study, there was a positive correlation between the concentration of K^+ ions in the extract and the effects of potassium chloride of similar molarity in the relaxant effect observed (Parry *et al*., 1993).

Aqueous extract of *Portulaca oleracea* leaves and stems produced a dose - dependent relaxation of guinea pig fundus, taenia coli and rabbit jejunum, also a dose-dependent contraction of the rabbit aorta (Parry *et al*., 1988). The LD_{50} of the aqueous extract in mice had been reported as 1040 mg kg^{-1} (Parry *et al*., 1987).

Earlier studies had confirmed that the aqueous extract showed muscle relaxant effect in skeletal muscle and some selected smooth muscles, however no work has been carried out on the guinea-pig ileum, *in vivo* motility in mice and the likely mechanism(s) of action. This study was undertaken to investigate the effects of the aqueous extract of *Portulaca oleracea* on GPI preparation, its in-vivo activity and further mechanism(s) of action.

MATERIALS AND METHODS

Extract preparation: Fresh specimens of *Portulaca oleracea* were collected from the Botanical garden of the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria between July and August 2003. Mr. T.K. Odewo of FRIN authenticated them. Voucher specimens of *P. oleracea* were deposited at FRIN herbarium.

The leaves and stems were air-dried for six weeks and later ground thoroughly into powder form using, laboratory mortar and pestle. A fairly large quantity (380 g) of the ground material was soxhlet - extracted with distilled water at 100°C for 6-8 h. The starting sample gave a mean yield of 9.2%. The extract was reconstituted in distilled water to make up required concentration and was stored at 4°C until use.

Isolated guinea pig ileum strips experiments: Adult guinea pigs of either sex weighing between 300 and 450 g were used for the study. Each animal was killed by a sharp blow to the head and bled by cutting through the neck region. The abdomen was opened by a midline incision to locate the region of terminal ileum with the caecum. A fairly long strip of the ileum excluding a portion of about 10 cm distal to the ileo - caecal junctions was removed. Suitable length of between 2-3 cm ileum was cut, mesenteric attachments dissected free. The lumen of the tissue was then flushed with Tyrode's solution and suspended in a 5 mL organ bath maintained at 37°C and aerated with 95% O_2 and 5% CO_2. A resting tension of 1 g was applied to each tissue. Values of responses induced by test materials and standard drugs are expressed as a percentage of the maximum. The Tyrode's solution used has the following composition (mM L^{-1}): Na^+ 149.2, K^+ 2.7, Ca^{2+} 3.6, Mg^{2+} 2.1, Cl^- 145.3, $H_2PO_4^-$ 0.4, HCO_3^- 11.9 and glucose 10. All contractions were monitored with a force displacement transducer (FT.03) connected to a grass polygraph model 7.PD.

***In vivo* intestinal transit measurements:** Seventy two albino mice of either sex weighing between 18 and 25 g were used for the study. These animals were divided into nine groups of eight mice per group. The animals were fasted overnight but allowed to have free access to water. The first group was given 5.5 mL kg^{-1} normal saline and served as control. The next six groups (AEP0 1- AEPO 6) received different doses of aqueous extract of *Portulaca oleracea* (AEPO). Another group received 1 mg kg^{-1} of carbachol while the last group-received 10 mg kg^{-1} of atropine. All drugs were given intraperitoneally (i.p) and volume administered never exceeded 0.2 mL. Ten min after the injection 0.5 mL of 5% w/v charcoal (BDH, England) in tragacanth (May and Baker) mucilage was orally administered to each mouse. The charcoal was to provide an opaque intestinal medium for easy measurement of the distance transited by the meal. Twenty minutes post - charcoal meal, the mice were killed by an overdose of chloroform. The abdomens were opened and the entire length of the small intestines were carefully cut and brought out. Length of distance moved by the charcoal meal towards the caecum from the pyloric junction of the stomach was measured and expressed as percentage of the total length of the small intestine. This represents the peristaltic index .

DRUGS USED

Reference drugs and chemical used were acetylcholine (Ach; sigma), atroprine sulphate (sigma), carbachol chloride (sigma), histamine hydrochloride (sigma), promethazine and nifedipine. Stock solutions were prepared in distilled water.

STATISTICAL ANALYSIS

All results were expressed as Mean±SEM. Differences between means of control versus test were analyzed using student t-test, while p-value of 0.05 was taken as being statistically significant.

RESULTS

Effects of AEPO, acetylcholine and histamine on guinea pig ileum (GPI) preparation: Concentration - response curves for AEPO, acetylcholine and histamine gave EC_{50} values of 4.47×10^{-5}, 6.31×10^{-5} and 5.31×10^{-5} g mL^{-1}, respectively (Fig. 1). The ileal smooth muscle strips were more sensitive to AEPO than histamine and the latter than acetylcholine (Fig. 1). Cumulative doses of AEPO induced contractions which were concentration-dependent (Fig. 2).

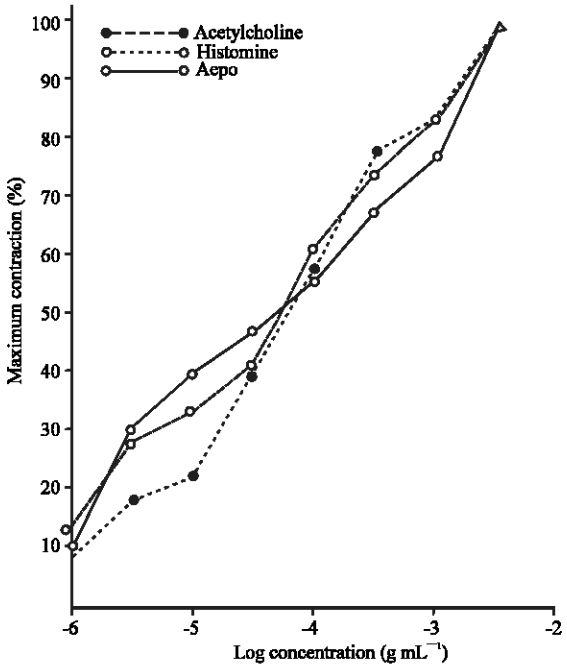

Fig. 1: Concentration response curves of acetylcholine, histamine and AEPO on isolated guinea-pigileum preparations using cumulative doses (Each point represents the Mean±SEM of six experiments)

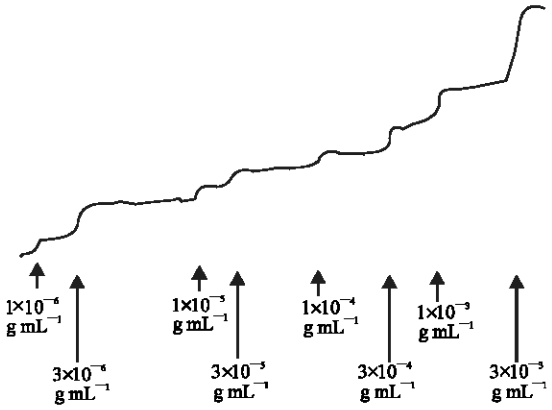

Fig. 2: Typical trace showing the effect of cumulative doses of AEPO on isolated guinea-pig ileum

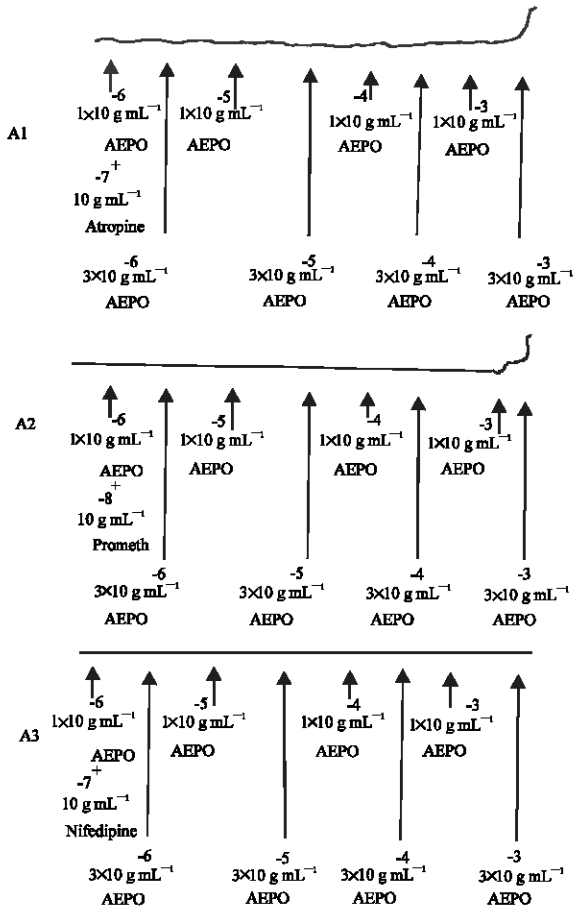

Fig. 3: Atropine, a competitive receptor antagonist of muscarinic receptor (A1), promethazine, a selective H_1-receptor blocker (A2) and nifedipinea calcium blocker were used to preincubate GPI preparations. A1 and A2 abolished extract-induced contractions up to 1×10^{-3} and 3×10^{-4} g mL^{-1} AEPO, respectively, A3 however completely abolished the contractile responses

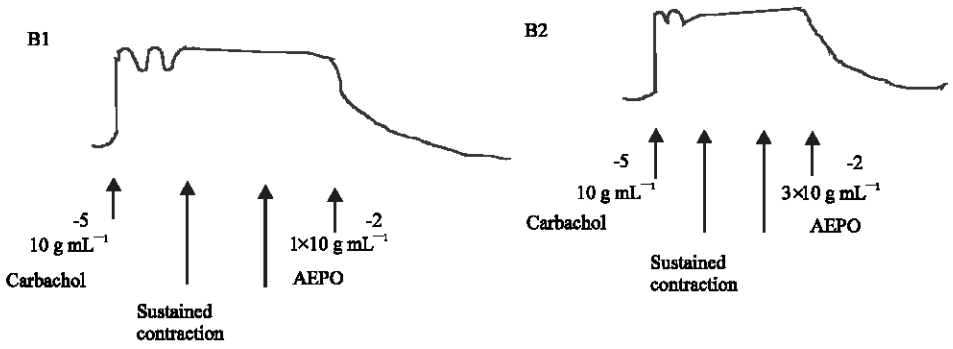

Fig. 4: Carbachol (10^{-5}g mL^{-1}) evoked sustained contraction of guinea-pig ileum preparation. Bland B2 showed relaxant responses to AEPO in a concentration dependent manner

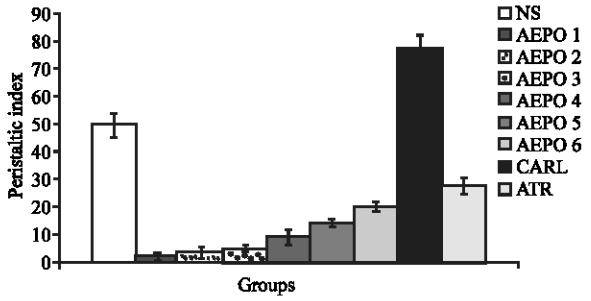

Fig. 5: Effect of *Portulaca oleracea* extract on intestinal transit in mice

Effect of antagonists on AEPO-induced contractions: Atropine (10^{-7} g mL^{-1}) abolished contractile activity of guinea pig ileal strips at concentrations of AEPO between 1×10^{-6} and 1×10^{-3} g mL^{-1}, but did not significantly affect contractile response to 3×10^{-3} g mL^{-1} AEPO. Similarly, promethazine inhibited contractile activity of ileal muscles to AEPO up to a concentration of 3×10^{-3} g mL^{-1}. Nifedipine however, showed inhibition to various concentrations of AEPO used (Fig. 3).

Effect of AEPO on carbachol sustained contraction: AEPO produced relaxant response to carbachol-sustained contractions at concentrations 1×10^{-2} and 3×10^{-2} g mL^{-1} (Fig. 4).

Effect of AEPO on intestinal transit time in mice: With respect to control, different doses of AEPO produced significant increase in transit time, that is, decrease peristaltic index ($p<0.05$). This response is dose dependent (Fig. 5).

DISCUSSION

Guinea pig ileal strips pre-incubated with atropine and promethazine inhibited the stimulatory effect of AEPO at low doses (Fig. 3). However, higher cumulative doses of the extract caused muscle contractions (Fig. 3). Quite unlike atropine and promethazine, nifedipine completely abolished contraction of Guinea Pig Ileum (GPI) preparation to various doses of AEPO used. Atropine and promethazine are known to inhibit smooth muscle contraction via muscarinic and H_1- receptor interference, respectively. The slight contractile effect of AEPO at high cumulative doses may not necessarily depend on muscarinic and H_1-receptor activation. Nifedipine, a Ca^{2+} antagonist fully abolished contractile activity of GPI to AEPO. This implies that, the primary mode of action of AEPO is related to Ca^{2+} mobilization.

Studies with the carbachol pre-contracted guinea pig ileal strips showed that, the extract had relaxant effect on the smooth muscle (Fig. 4). The relaxant effect induced by AEPO on carbachol pre-contracted GPI preparation is similar to the report on skeletal muscle that aqueous extract of *Portulaca oleracea* was a more effective muscle relaxant agent, since the extract caused reduction in muscle tone of maximally-contracted gastrocnemius and quadriceps muscle (Parry *et al*., 1987).

The aqueous extract of *Portulaca oleracea* was found to increase intestinal transit time in this study (Fig. 5). This showed a significant dose-dependent reduction in peristaltic index (intestinal motility) in mice treated with different doses of AEPO compared with the control ($p<0.05$). This effect appears to be a direct interference in the excitation - contraction coupling of the intestinal smooth muscle. This action thus correlated with our study on pre-contracted ileal smooth muscle strips (Fig. 4) and that which proposed the interference with Ca^{2+} release process as being associated with *Portulaca oleracea* induced relaxation (Okwuasaba *et al*., 1987). Despite this mode of action, other mechanisms had been put forward such as increase content of K^+ ions in the extract reported with skeletal muscle (Okwuasaba *et al*., 1987; Parry *et al*., 1993).

REFERENCES

Burkill, H.M., 1997. The Useful Plants of West Tropical Africa. The Whitefriars Press Ltd. Tonbridge Kent TN9 Great Britain, Vol. 4.

Dalziel, J.M., 1937. The Useful Plants of West Tropical Africa. Crown Agents for the Colonies London.

Okwuasaba, F., C. Ejike and O. Parry, 1986. Skeletal muscle relaxant properties of the aqueous extract of *Portulaca oleracea.* J. Ethnopharmacol., 17: 139-160.

Okwuasaba, F., C. Ejike and O. Parry, 1987. Effects of extracts of Portulaca oleracea on skeletal muscle *in vitro.* J. Ethnophamacol., 21: 55-63.

Parry, O., F. Okwuasaba and C. Ejike, 1987. Skeletal muscle relaxant action of an aqueous extract of *Portulaca oleracea* in the rat. J. Ethnopharmacol., 19: 247-253.

Parry, O., F. Okwuasaba and C. Ejike, 1988. Effect of an aqueous extract of Portulaca oleracea leaves on smooth muscle and rat blood pressure. J. Ethnopharmacol., 22: 33-44.

Parry, O., J.A. Marks and F.K. Okwuasaba, 1993. The skeletal muscle relaxant action of *Portulaca oleracea*: Role of potassium ions. J. Ethnopharmacol., 40: 187-194.