PHYTOTHERAPY RESEARCH, VOL. 10, 322–324 (1996)

SHORT COMMUNICATION

Spasmolytic Activity of *Cissampelous mucronata* Leaf Extract

V. N. Offiah,* P. A. Akah and A. O. Isizoh
Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria

The aqueous leaf extract of *Cissampelous mucronata* (Menispermaceae) was studied for spasmolytic properties. The extract inhibited the responses of histamine, acetylcholine and nicotine on the guinea-pig ileum in a dose-related manner. The extract completely abolished the spontaneous pendular movement of the rabbit jejunum reversibly, and decreased gastrointestinal motility in mice. It did not modify the effect of Ca^{2+} on the guinea-pig ileum. Acute toxicity tests in mice established the i.p. LD_{50} value of the extract to be 1698.24±77 mg/kg.

Keywords: Cissampelous mucronata; aqueous leaf extract; spasmolytic; guinea-pig ileum; rabbit jejunum.

INTRODUCTION

Cissampelous mucronata (Menispermaceae) is a climbing shrub which is widespread in dry parts of tropical Africa (Hutchinson and Dalziel, 1954). In Nigeria the aqueous leaf extract of the plant is popular among traditional healers as an antispasmodic remedy. Little information is reported in the literature about *C. mucronata* (Oliver, 1959), but the root of a related specie *C. owariensis* has been reported to possess diuretic, antiperiodic and antidiarrhoeal activities (Oliver, 1959), The claim that the antispasmodic activity of *C. mucronata* resides in the leaves is speculative and not yet documented. In this study we have attempted to investigate the antispasmodic activities of *C. mucronata* extract.

MATERIALS AND METHODS

Collection. Fresh leaves of *C. mucronata* were collected from plants growing in Onitsha, Anambra State, Nigeria. Botanical identity was confirmed by Mr A. Ozioko, Department of Botany, of the University of Nigeria, Nsukka (UNN). A specimen of the plant is deposited in the University Herbarium.

Preparation of the aqueuous extract. Fresh leaves of the plant were thoroughly washed with water, air dried and milled to a coarse powder. About 1 kg of the powder was soaked in 1 L of distilled water. The mixture was allowed to stand for 24 h with occasional shaking. The materials were filtered and the filtrate lyophilized to obtain the extract as a solid residue (59 mg/mL).

Phytochemical tests. The freshly prepared extract was chemically tested for the presence of chemical constituents employing standard methods (Trease and Evans, 1983).

Acute toxicity test. The LD_{50} of the extract was determined in mice i.p. using the graphical method of Miller and Tainter (1944).

* Author to whom correspondence should be addressed.

Animals. White albino mice (20–25 g), guinea-pigs (250–500 g) and rabbits (1.5–2 kg) bred in the Department of Veterinary Medicine, UNN were used in these studies. The animals were kept in the Experimental Animal House of the Department of Pharmacology and Toxicology, UNN for 7 days with free access to food and water before the beginning of the experiments.

***In vitro* pharmacological activity on guinea-pig ileum.** An isolated tissue study was performed to determine the antispasmodic effect of the extract on isolated guinea-pig ileum. Segments of ileum (2 cm long) obtained from a freshly killed guinea-pig were suspended in a 20 mL organ bath containing aerated Tyrode solution at 37 °C. The composition of the Tyrode solution was (mM/L) NaCl, 137; $CaCl_2$ 1.0; $NaHCO_3$ 12; NaH_2PO_4 0.2; KCl 0.7; $MgCl_2$ 1.0 and glucose 5.6. The preparations were set up under a tension of 0.5 g and responses were recorded on a smoked kymograph paper through an isotonic frontal writing stylus (magnification × 7). After 60 min equilibration period, the effect of the extract on the responses induced by known spasmogens — histamine, acetylcholine and nicotine was investigated and compared with that of standard antagonists. Each agonist was used on a separate preparation. Four separate determinations were made for each agonist.

The effect of the extract on Ca^{2+}-induced responses of the guinea-pig ileum was studied using depolarized tissues. For this, the Tyrode solution was replaced with a low-sodium high-potassium solution of the following composition (mM/L): KCl 157; $NaHCO_3$ 12; $MgCl_2$ 1.0 and glucose 5.5 (Van Dan Broucke and Lemli, 1980). In this medium, the responses of the ileum to Ca^{2+} in the absence and presence of the extract were determined after 60 min equilibration period.

Test for gastrointestinal motility

Test on isolated rabbit jejunum. The preparation was set up as described by Staff of the Department of Pharmacology, University of Edinburgh (1970). The spontaneous pendular movements of the tissue were recorded isotonically using an

CCC 0951–418X/96/040322–03

Accepted (reviseed) 16 March 1995

isotonic transducer, 7006, (Ugo Basile, Italy) connected to a 2-channel recorder 'Gemini' 7070. The effect of the extract on the tone and motility of the intestine was studied.

Charcoal meal test in mice. Fifteen adult albino mice of either sex (20–25 g) were randomly divided into three groups of five mice each. The animals were starved for 24 h prior to the experiments, but were allowed access to water. One group of animals was given 50 mg/kg of the extract of *C. mucronata* while the other two groups received p.o. 20 mL/kg of normal saline and 10 mg/kg of atropine respectively. Five minutes after drug administration, 0.5 mL of a 5% charcoal suspension in 10% aqueous solution of tragacanth powder was administered to each animal orally. The animals were killed 30 min later and the abdomen opened. The percentage distance of the small intestine (from the pylorus to the caecum) travelled by the charcoal plug in both the extracts and the normal saline-treated groups was determined (Akah, 1989). The results were analysed using Student's *t*-test.

RESULTS AND DISCUSSION

The fresh extract of *C. mucronata* gave positive reactions for alkaloids, saponins, tannins, glycosides, flavonoids and steroids. In the preliminary acute toxicity test in mice the i.p. LD_{50} of the extract was 1698.24±77 mg/kg.

The extract was devoid of contractile effects on the guinea-pig ileum. The extract inhibited the histamine-evoked response of ileal segment and shifted the dose-response curve to the right in a dose-related manner (Fig. 1). The ID_{50} (dose causing 50% inhibition) of the extract for histamine was 1.10±0.05 mg. The inhibition appeared competitive. Contractions evoked by acetylcholine and nicotine were similarly inhibited by the extract with ID_{50} values of 10.10±2.6 mg and 11.48±2.07 mg respectively (Fig. 2 and 3). The blockade was dose-related. The extract did not modify the contractile effect of Ca^{2+} on the guinea-pig ileum (data not shown).

On isolated rabbit jejunum, the extract completely abolished the spontaneous pendular movements (Fig. 4). The inhibition was reversible since the contractions returned after washing off the extract.

The results of the charcoal meal test show that the extract of *C. mucronata* caused a significant ($p<0.05$) decrease in gut motility when compared with normal saline. The average percentage distance travelled by the charcoal plug in the three groups is shown in Table 1.

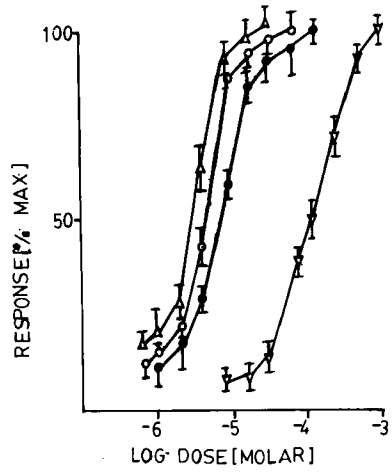

Figure 2. Effect of the extract and atropine on acetylcholine induced contractions of the guinea-pig ileum. △ Control, ○ extract 5 mg; ● extract 10 mg; ▽ atropine 3.5×10^{-8} M.

The results of this study confirmed that the aqueous leaf extract of *C. mucronata* contains pharmacologically active principle(s) with spasmolytic properties. The contractions evoked by various spasmogens — histamine, acetylcholine and nicotine — with different pharmacological mechanisms of causing contraction were reduced or inhibited. This suggests a non-specific antagonism.

Paradoxically, the extract did not modify Ca^{2+} induced responses of the ileum. Inhibition of Ca^{2+}-induced contractions of K^+-depolarized tissues is commonly accepted as a test for agents that act non-specifically by inhibiting Ca^{2+} participation in excitation-contraction-coupling process (Godfraind and Kaba, 1969; Northover, 1977; Quintana, 1978). Since Ca^{2+} induced contractions of K^+-depolarized smooth muscle seem to be produced as a result of Ca^{2+} flux, it suggests that the spasmolytic effect of the extract may not involve a decrease in Ca^{2+} availability.

The inhibition of the spontaneous pendular movements of the jejunum by the extract was rapid and remarkable, and most importantly reversible — a desirable quality of a potent and novel spasmolytic agent.

The charcoal meal test, which allows for comparative evaluation of the degree of inhibition/stimulation of

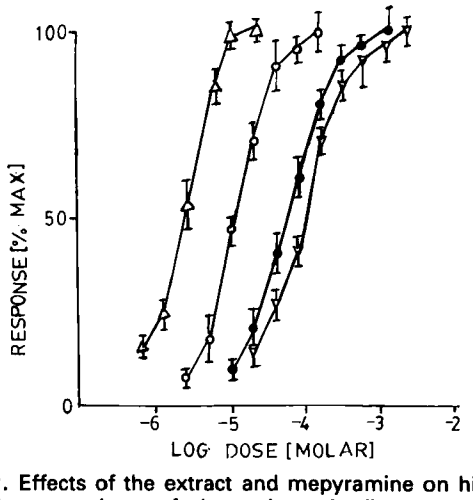

Figure 1. Effects of the extract and mepyramine on histamine induced contractions of the guinea-pig ileum. △ Control, ○ extract 5 mg; ● extract 10 mg; ▽ mepyramine 2.5×10^{-8} M.

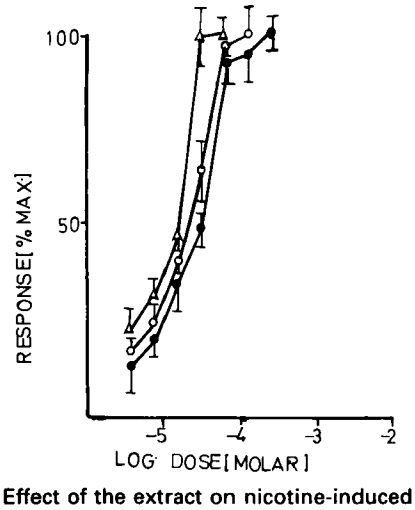

Figure 3. Effect of the extract on nicotine-induced contractions of the guinea-pig ileum. △ Control, ○ extract 5 mg; ● extract 10 mg.

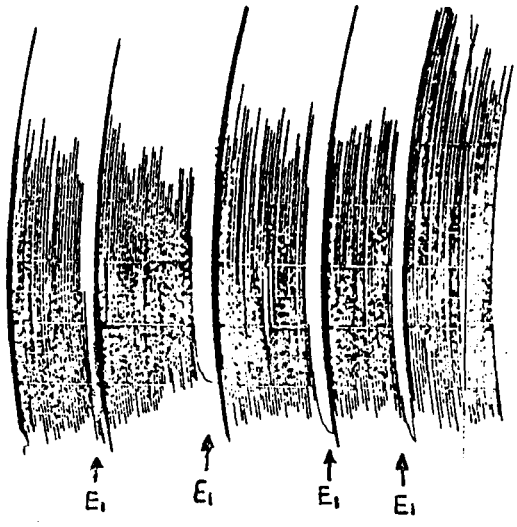

Figure 4. Inhibition of spontaneous pendular movement of the rabbit jejunum by the extract, E.

gastrointestinal motility showed that *C. mucronata* decreased peristaltic movement. This result correlates those of isolated tissue studies. Inhibition of smooth muscle contraction can result from two pathways. A neurotropic pathway, an inhibition of neurotransmitter release from nerve terminal or blockade of neurotransmitter release from nerve terminal or blockade of specific membrane receptors sites (Van Dan Broucke and Lemli, 1980). A musculotropic

Table 1. Percentage distance travelled by the charcoal plug in the treatment groups

Drug	Dose	Distance travelled (%)
Normal saline	20 mL/kg	71.2±5.3
Extract	50 mg/kg	40.8±5.6
Atropine	10 mg/kg	32.5±6.5

pathway which may involve: (a) stabilization of the muscle membrane; (b) interference with the availability of Ca^{2+} at the step(s) in excitation-contraction-coupling; (c) interference with the normal function of the regulatory proteins involved in contraction and relaxation; (d) inhibition of actomyosin — ATP-ase and subsequent inhibition of chemomechanical transduction (Malagodi and Chiou, 1974). The mechanism of action of the extract seems to be both neurotropic and musculotropic. Blockade of nicotine responses shows neurotropic (indirect) action. Nicotine in a small concentration stimulates autonomic ganglia, and causes contraction by preganglionic acetylcholine release, followed by postganglionic parasympathetic nerve action potential with concomitant release of acetylcholine at the nerve terminals (Lefkowitz *et al.*, 1990). On the other hand, a direct effect is suggested by inhibition of acetylcholine and histamine contractions. The extract contains several biologically active groups. At present, it is not yet known which of the groups of phytochemicals are responsible for the observed spasmolytic effect.

REFERENCES

Akah, P. A. (1989). Purgative potentials of *Euphorbia heterophylla*. *Fitoterapia* **60**, 45–48.

Godfraind, T., and Kaba, A. (1969). Blockade or reversal of the contraction induced by calcium ions and adrenaline in depolarised arterial muscle. *Br. J. Pharmacol.* **36**, 548–560.

Hutchinson, J., and Dalziel, J. M. (1954). *Flora of West Tropical Africa*, p. 25. Crown Agents for Overseas Government and Administration, London.

Lefkowitz, R. J., Hoffman, B. B., and Taylor, P. (1990). Neurohumoral transmission: The autonomic and somatic motor nervous system. In, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edn, ed. by A. G. Gilman, T. W. Rall, A. S. Nies and P. Taylor, pp. 84–101. Pergamon Press, New York.

Malagodi, H., and Chiou, C. Y. (1974). Pharmacological evaluation of a new Ca^{2+} antagonist 8-(N,N-dimethylamino)-octyl-3, 4,5-trimethoxybenzoate hydrochloride (TMB-8): Studies on smooth muscles. *Eur. J. Pharmacol.* **27**, 25–33.

Miller, L. C., and Tainter, M. L. (1944). Estimation of ED_{50} and its error by means of logarithmic probit graph paper. *Proc. Soc. Exp. Biol. NY* **57**, 261–264.

Northover, B. J. (1977). Indomethacin, a calcium antagonist. *Gen. Pharmacol.* **8**, 293–296.

Oliver, B. (1959). *Medicinal Plants in Nigeria*. College of Arts, Science and Technology, Ibadan, Nigeria.

Quintana, A. (1978). Effect of primozide on the responses of smooth muscle to non-dopamine agonist and calcium. *Eur. J. Pharmacol.* **53**, 113–116.

Staff of the Department of Pharmacology, University of Edinburgh (1970). *Pharmacological Experiments on Isolated Preparations*, 2nd edn, pp. 60–61. Churchill Livingstone, London.

Trease, G. E., and Evans, W. C. (1983). *Textbook of Pharmacognosy*, 12th edn, pp. 383–385. Bailliére Tindall, London.

Van dan Broucke, C. O., and Lemli, J. A. (1980). Antispasmodic activity of *Origanum compactum*. *Planta Med.* **38**, 317–331.