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Sedative, vasorelaxant, and cytotoxic effects of convolvulin from *Ipomoea tyrianthina*

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ABSTRACT

Aim of the study: *Ipomoea tyrianthina* has been used in Mexican traditional medicine as a mild purgative, for the treatment of nervous disorders, and against tumors. In this study, the effect of convolvulin (an ether-insoluble resin glycoside) from the root of *Ipomoea tyrianthina* on: Central Nervous System; as spasmolytic and vasodilator; cytotoxic against cancer cell lines is evaluated.
Materials and methods: Convolvulin isolated from the root of *Ipomoea tyrianthina* (IT-EM) was tested on pentylentetrazole induced seizures, pentobarbital-induced hypnosis, release of GABA and glutamic acid, isolated rat aorta and ileum rings, and against Caco-2 and KB cell lines.
Results: IT-EM increased the hypnotic effect induced by pentobarbital and the release of GABA in brain cortex of mice, but did not protect mice against pentylenetetrazole-induced convulsions. IT-EM produced a significant vasodilator effect in concentration- and endothelium-dependent manners on isolated rat aorta, but did not inhibit significantly contractions on rat ileum, colon, and jejune rings. IT-EM showed cytotoxic activity against nasopharyngeal carcinoma KB cell line.
Conclusions: Convolvulin (IT-EM) from *Ipomoea tyrianthina* has sedative effect, vasorelaxant effect in concentration- and endothelium-dependent manners, and cytotoxic activity against nasopharyngeal carcinoma KB cell line.

1. Introduction

The so-called resin glycosides isolated from Convolvulaceous plants are the characteristic ingredients of the crude drugs Scammony, Mexican Scammony, Jalap Root, and Rhizoma Japae Braziliens (Pereda-Miranda and Bah, 2003). Resin glycosides were classified into an ether-soluble resin called jalapin and an ether-insoluble one called convolvulin (Mannich and Schumann, 1938). Resin glycosides consist of mixtures of glycolipids. The most of the glycolipids present in jalapin contain a hydroxy-fatty acid bonded to an oligosaccharide (partially acylated by short chain acids) forming a macrolactone ring. The convolvulin group had been confined to the characterization of the component organic acids and the glycosidic acids afforded by alkaline hydrolysis of mixtures of glycolipids (Pereda-Miranda et al., 2010).

The genus *Ipomoea* includes a great number of ornamental, medicinal, and nutritional species in Mexico. *Ipomoea tyrianthina* Lindley (syn. *Ipomoea orizabensis* Pelletan, Lebed. ex Steud., Convolvulaceae) is a perennial twining herb with a large root. The root of *Ipomoea tyrianthina* has been used as a purgative, to treat epilepsy, tumors, abdominal fever, dysentery hydrocephaly, meningitis, and to alleviate abdominal pains. This root is popularly known in Mexico as "escamonea" (Mexican scammony), and is available as dried root, crude resin, and as a liquid alcoholic extract (Díaz, 1976).

The glycolipids isolated from the jalapin of *Ipomoea tyrianthina* have been characterized as macrolactones composed by a tetrasaccharide, 11-hydroxyhexadecanoic acid as the aglycon, and short chain acids ester linked to the oligosaccharide cores (Hernández-

Abbreviations: AOA, amino-oxyacetic acid; ANOVA, analysis of variance; CNS, central nervous system; DZP, diazepam; GABA, gamma amino butyric acid; HPLC, high performance liquid chromatography; IT-EM, ether-insoluble resin glycoside from *Ipomoea tytrianthina*; NA, noradrenaline; PTZ, pentylenetetrazole; TW 2.5%, 2.5% Tween 20 solution.

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Carlos et al., 1999; Pereda-Miranda and Hernández-Carlos, 2002; Mirón-López et al., 2007). Intraperitoneal administration to mice of pure glycolipids from the jalapin of *Ipomoea tyrianthina* resulted in antidepressant activity, protective effects against pentylenetetrazole-induced seizures, and relaxant effects on spontaneous contractions in isolated rat ileum (Mirón-López et al., 2007). The aim of this work was to evaluate the effects produced by convolvulin (IT-EM) from the root of *Ipomoea tyrianthina* on: central nervous system (CNS) models; isolated rat aorta, ileum, colon, and jejune rings; Caco-2 and KB cancer cell lines.

2. Materials and methods

2.1. Animals

Albino mice ICR weighing 30–36 g and male Wistar rats weighing 250–300 g were used (Harlan, Mexico City). All animals were housed 8 per cage, maintained under laboratory conditions at 25 °C, 12 h light/12 h dark cycle, with lights turned on at 07:00 a.m. and free access to water and standard food pellets (Harlan). Mice were allowed at least three weeks to adapt to the laboratory environment before experiments. Experiments for determination of activity on CNS were carried out between 8:00 a.m. and 12:00 p.m. in an adjacent special noise-free room with controlled illumination. All experimental procedures were carried out according to a protocol approved by the Institutional Research Committee in compliance with the official Mexican norm (NOM-062-ZOO-1999). The minimum number of animals and duration of observation required to obtain consistent data were employed.

2.2. Plant material

The root of *Ipomoea tyrianthina* was collected in Ahuazotepec, state of Puebla, Mexico in August 2000. Identification of the species was provided by Biol. Manuel Castro from Facultad de Ciencias, UNAM, and a voucher specimen (IMSSM-15004) was deposited in the herbarium of the Instituto Mexicano del Seguro Social, Mexico, Distrito Federal.

2.3. Drugs

Diazepam (DZP, Sigma) was used as anticonvulsant and sedative drug; pentylenetetrazole (PTZ, Sigma) was used as the convulsivant drug; sodium pentobarbital (PTB, Pfizer) was used as hypnotic drug; 2.5% Tween 20 solution (TW 2.5%, Merck) as vehicle no drug; papaverine (Sigma) was used to induce relaxation on ileum and jejune rings; noradrenaline (NA, Sigma) was used to induce contraction on aorta rat rings; carbachol (Sigma) was used as a relaxant positive control; campothecin (Sigma) was used as positive control on cytotoxic assay.

2.4. Isolation of convolvulin

The air-dried powdered ground root of *Ipomoea tyrianthina* (100 g) was successively extracted with n-hexane (400 mL × 3), ethyl acetate (400 mL × 3), and methanol (400 mL × 3) by maceration at room temperature. The solvents were separated from the residues by gravity filtration and then evaporated by vacuum. The methanol-soluble extract (20 g) was subjected to gravity column chromatography over reverse phase (C_{18}) silica gel (50 g) using gradients of CH_3OH in H_2O. A total of 20 fractions (50 mL each) were collected and combined to give several pools containing mixtures of compounds. The fractions 12–16 eluted with CH_3OH–H_2O (60:40) were pooled, after elimination of solvent a white resin (10 g) was obtained. This resin material is the convolvulin of *Ipomoea tyrianthina* (IT-EM).

2.5. Pharmacological evaluations

2.5.1. Anticonvulsant activity

Mice were divided into three groups. Group A was treated with 10, 20, 40, and 80 mg/kg, i.p. of IT-EM dissolved in 2.5% Tween solution; Group B received diazepam dissolved in 2.5% Tween solution (1.0 mg/kg, i.p.) as positive control; Group C served as the control and received i.p. the vehicle 2.5% Tween 20 solution (100 μL/10 g). Animals were pretreated with IT-EM, vehicle, or diazepam. Thirty minutes later pentylenetetrazole was administered intraperitoneally (75 mg/kg). Following the injection of PTZ, mice were placed separately into transparent Plexiglas cages (25 cm × 15 cm × 10 cm) and observed for 30 min for the occurrence of seizures. The time taken before the onset of clonic convulsions and the percentage of mortality protection were recorded (Willianson et al., 1996).

2.5.2. Sodium pentobarbital-induced hypnosis (Pbi)

Mice were divided into three groups: Group A was treated with 20, 40, and 80 mg/kg, i.p. of IT-EM dissolved in 2.5% Tween solution; Group B received diazepam dissolved in 2.5% Tween solution (1.0 mg/kg, i.p.) as positive control; Group C served as the control and received i.p. the vehicle 2.5% Tween 20 solution (100 μL/10 g). Animals were pretreated with IT-EM, vehicle, or diazepam. Thirty minutes later sodium pentobarbital was administered intraperitoneally (a sub-hypnotic dose, 30 mg/kg). The hypnotic effect was recorded as the time interval between disappearance (latency) and reappearance (duration) of the righting reflex (Willianson et al., 1996).

2.5.3. GABA and glutamic acid release

Mice were sacrificed by decapitation and the anterior brain cortex was dissected and slices (250–300 μm) were cut manually using a razor blade and a cover glass guide (Gutiérrez and Delgado-Coello, 1989). Brain slices were placed at 4 °C in 5 mL in a modified Krebs–Ringer medium (120.0 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1.0 mM Tris–HCl buffer pH 7.4), 10.0 mM glucose (pH 7.4) previously oxygenated with bubbling O_2. Amino-oxyacetic acid (AOA) 10.0 μM, was added to the medium to prevent GABA metabolism. After 5 min, slices were placed in 5 mL of Krebs–Ringer medium at 37 °C for 10 min. Next, IT-EM was added at a final concentration of 4.0 μg/mL and aliquots of 200 μL were taken out at 0.5, 1.0, 1.5, 2.0, and 3.0 min. At the end of the experiment, the content of GABA into each collected aliquot was determined by high performance liquid chromatography (HPLC), previous derivation with O-phthaldialdehyde. Protein content in brain slices was determined after its homogenization in 1 mL of water according to Lowry method (Lowry et al., 1951).

2.5.4. Spasmolytic assay

Male Wistar rats were sacrificed by exposure to diethyl ether. Ileum, colon, and jejune were dissected out and placed in Krebs–Henseleit (KH) solution, with the following composition: 119.0 mM NaCl, 4.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 20.0 mM $NaHCO_3$, and 11.4 mM glucose. Strips (2 cm long) were obtained and mounted in organ baths containing Krebs solution (pH 7.4) and gassed with a mixture of O_2/CO_2 (19:1) and continuously recorded for isometric tension (1 g) with a Grass FTO3 force–displacement transducer and registered on a Grass 7D transductor. After a stabilization time of 30 min, a 10 min control period was recorded. The separate responses for standard solutions of noradrenalina (5 μg/mL) were initially recorded using the respective tissue preparations at doses of 0.2–0.8 mL. IT-EM was dissolved in DMSO and added to the bath. After precontraction with noradrenaline, the test samples (IT-EM, vehicle, and papaverine used as the positive control) were added to the bath in a volume of 100 μL;