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Short Report

Bioactive oxoaporphine alkaloids from *Guatteria calva*

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Plant. *Guatteria calva* R.E. Fries (Annonaceae), dried leaves, collected (April and September) in the Amazonian forest, along the Cataniapo riverside, 8 km southeast of Puerto Ayacucho city, Venezuela and identified by Dr Anibal Castillo, Escuela de Biología, Facultad de Ciencias, Universidad Central de Venezuela. A voucher specimen is deposited in the National Herbarium of Venezuela (VEN), Botanic Garden in Caracas, Venezuela.

Uses in traditional medicine. No report.

Previously isolated constituents. Many aporphinoid alkaloids from the genus [1–4].

New-isolated constituents. Oxostephanine (0.001%) [5], oxoxylopine (0.002%) [5] and oxoputerine (0.002%) [5].

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Table 1
Mitotic index (%) of oxostephanine for HeLa, $SKVO_3$ and primary culture from mouse embryo, calculated at 100 μg/ml for 96 h

Tested material	Mitotic index (%)		
	HeLa	$SKVO_3$	Mouse embryo
Control	3.0	0.2	0.4
Oxostephanine	0.4	0.05	0.1
Camptothecin[a]	0.3	0.01	0.3

[a] Reference substance (Aldrich).

Tested material. Oxostephanine, following a bioassay-guided (brine shrimp lethality test [6,7]) isolation procedure (IC_{50} = 5.05 μg/ml). Oxoxylopine and oxoputerine showed lower cytotoxicity (IC_{50} > 20 μg/ml) on brine shrimp with respect to the most active fraction (IC_{50} = 3.25 μg/ml) containing the three alkaloids.

Used tumor cells. HeLa uterus carcinoma, $SKVO_3$ ovary carcinoma, and primary culture from mouse embryo were routinely propagated in RPMI (GIBCO) with 100 U/ml penicillin G, 100 U/ml streptomycin and supplemented with 10% fetal bovine serum (GIBCO). Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2. Cells were dissociated with 0.25% trypsin for further passage.

Studied activity. The cellular response and mitotic index against human solid tumor cells were measured [8]. The cellular response at concentrations of 0.1, 1.0, 10.0 and 100 μg/ml of the alkaloid at 24, 48, 72 and 96 h for the different cultures was observed under an optical microscope and the mitotic index was calculated in each bioassay [8]. Cell cultures without alkaloids were used as control.

Results. Report in Table 1.

The HeLa cells treated with the alkaloid at 100 μg/ml showed swelling and vacuolization of the cytoplasm. Concomitantly with the vacuolization, the cells undergo retraction of the cytoplasm, resulting in an alteration of their morphology. The cytoplasm becomes reduced, while the nucleus becomes hyperchromatic, being reduced to a small mass surrounded by contracted cytoplasm. In the case of $SKVO_3$ and the primary culture from mouse embryo, no microscopic variation from the control cells was observed at any concentration during the experimental period.

Conclusions. Oxostephanine showed a selective toxicity in HeLa cells and a marked reduction in the mitotic index for this tumoral cell. In the case of $SKVO_3$ and primary culture from mouse embryo, oxostephanine showed an appreciable reduction in the mitotic index but no microscopic changes in cell morphology were observed.

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