CHEMICAL COPAIBA OF ESSENTIAL OIL AND STUDY OF VIABILITY AND CYTOTOXICITY CELLULAR

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Abstract

We used two techniques to characterize copaiba oil, gas chromatography coupled mass spectrometry and electrospray ionization coupled with a mass spectrometer Q-TOF. To verify cell viability and cytotoxicity oil used the MTT and Neutral Red test. It was noted that the oils used were positive on cell proliferation in human glioma line (U251).

Key words:

Copaiba Oil, cytotoxicity and cell viability, human glioma..

Introduction

The genus *Copaifera* sp (*Leguminosae* – *Caesalpinioideae*), also known as "Copaiba", "Copaibeiras", "Pau d'óleo"¹, that naturally grows in Africa (4 spp.), Central America (4 spp) and South America (about 37 spp)² presents important pharmacological properties. Even with the already proved scientific applications, like the diuretic action, antiseptic action of urinal system, healing and anti-inflammatory results, and a tumor inhibitor, there are still contradictions related to the variation of physico-chemical characteristics of the Copaiba Oil. In this context, it was analysed, using comparative effects, the chemical characteristics of 3 types of resin-oils and their respective antineoplastic effects against the cell line of the human glioma (U251).

Results and Discussion

The analysis of the composition of copaiba oils 1 (sample of Pernambuco); 2 (sample of Manaus) and 3 (sample of Manaus) was analyzed using two assays: the first by a gas chromatography system coupled to mass spectrometry Agilent (HP6890), equipped with HP5MS column and the second assay infusion right, in negative mode, and electrospray ionization (ESI [-] - MS) using an automatic injector UHPLC chromatograph coupled with a mass spectrometer Q-TOF (LC-MS-6550 iFunnel Agilent Technology).

The spectrum obtained were based on review studies³ that bring together the main types of compounds found in Copaiba oil. Through the EASI-Orbitrap were found in 15 types of oil components, including the 3-clerodeno-15,18-dioic acid, 13-clerodeno-15, copalic acid and 11- acetoxycopalic.

The U251 cells treated with *Copaifera* oils sp in the concentration of 0,1ug / mL had become intense nuclear pleomorphism, little cell cohesiveness, pyknosis and sometimes, with scant cytoplasm, dense chromatin, nuclear atrophy and cell death, as observed in photomicrographs. Regarding the cytotoxicity assay, evaluated the cell viability of human glioma line U251 exposed to copaiba oils from different regions. One method used was the MTT second Mossmann (1983)⁴, which is based on reducing Tetrazolium salt ([3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazoliumbromide - Sigma M2128) yellow, by succinic hydrogenase enzyme present in the mitochondria of the tumor cell, which acquires a purplish color which was measured by spectrophotometrically at 570nm. The test Neutral Red

(NR)⁵ was also used to assess cell viability based on the ability of viable cells to incorporate the dye inside of the lysosomes. The resulting neutral red staining of this test was evaluated by spectrophotometry at 540 nm. The result was expressed as a percentage of viability compared to the control. It was used in concentrations of 1 to 0,0001ug / mL for each type of oil.

The positive control was the doxorubicin hydrochloride. In the MTT assay, the three copaiba oils from the Pernambuco region (1), Manaus (2) and another from Manaus region (3) were cytotoxic in U251 cells (IC₅₀ = 6,171.10⁻² ug / mL, 8,344.10⁻²ug / ml and 1,385.10⁻⁴ug / mL, respectively). In the Neutral Red assay, all three types of oils also showed cytotoxic effect, with values of IC₅₀=0,24ug /ml (1), 0,037ug/ml (2) and 0,046ug/ml (3). There was a strong correlation between the MTT assay and Neutral Red for the three types of oils, with p <0.0001, and r = 0.99 using the Pearson correlation. The results showed small differences between the IC₅₀ of the NR test and MTT test for the oils tested. It has been suggested that oils more interfered about the metabolic activity in the lysosomal membrane integrity at the concentrations tested.

Conclusions

The three types of resin oils had positive effect on cell proliferation in vitro assays, with relationship between concentration and effect.

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