[6]-Shogaol induces apoptosis of murine bladder cancer cells

Diana Gabriela Nina Nina [1,†], Thaiane Alcarde Robeldo [2,†], James Almada da Silva [3], Vitória Shiévila dos Santos Gonçalves [3], Ricardo Carneiro Borra [2] and Fernanda de Freitas Anibal [1,*]

[1] Laboratory of Inflammation and Infectious Diseases, Department of Morphology and Pathology, Federal University of São Carlos (UFSCar), CEP 13565-905 São Carlos-SP, Brazil.

[2] Laboratory of Applied Immunology, Department of Genetics and Evolution, Federal University of São Carlos (UFSCar), CEP 13565-905 São Carlos-SP, Brazil.

[3] Laboratory of Bioactive Natural Products, Department of Pharmacy, Federal University of Sergipe (UFS), CEP 49400-000, Av. Gov. Marcelo Deda, 330-São José, Lagarto-SE, Brazil.

[†] These authors contributed equally to this work and share the first authorship.

*Corresponding Author

Prof. Dr. Fernanda de Freitas Anibal and MSc. Diana Gabriela Nina Nina

Laboratory of Inflammation and Infectious Diseases.

Department of Morphology and Pathology.

Federal University of São Carlos.

Rod. Washington Luís, Km 235.

São Carlos – SP – CEP. 13565-905 – Mailbox 676 – Brazil.

Phone: +55 16 99137-6422 / +591 65548560.

E-Mail: ffanibal@ufscar.br / dianagabrielaninanina@yahoo.com

Extraction and isolation of [6]-shogaol

The fresh ginger rizhomes (SIsGEn Registration No. AF12053), purchased in different local markets (Lagarto, SE, Brazil), were cut into thin slices, dried at 40°C and ground into powders. Eight hundred and sixty grams of dry ginger were extracted using series Soxhlet apparatus (60 grams of ginger in each) and 6.8 L of absolute ethanol (0.48 L in each system). The systems were subjected to solvent reflux at a temperature of 90 °C (± 2 °C) for 12 hours (1) with the flasks submerged in a water bath (Limatec, model LTBMU20). After the extraction, the solvent was concentrated under reduced pressure at 40°C and its concentration adjusted to contain 70% ethanol. The isolation and purification of [6]-shogaol was performed according to the methodology of Silva et al (2). Briefly, the liquid ginger extract was submitted to liquid–liquid partition with n-hexane and dichloromethane. The dichloromethane fraction (12.0 g) was subjected to SiO_2 column (37 × 3.6 cm i.d.) (MACHEREY-NAGEL) eluted with n-hexane/ethyl acetate (1:1, v/v) in isocratic mode. Thirty subfractions (30 mL each) were collected and monitored by thin layer chromatography (TLC), with the same mobile phase, and developed with UV light (254 nm) and sulfuric vanillin, to identify the fractions that contained [6]-shogaol, using the standard [6]-shogaol (Rf=0.9) previously isolated in the laboratory (2). Analytical and semi-preparative HPLC were carried out using a Shimadzu Proeminence 20A system with an SPD-M20A UV–vis detector (254 and 282 nm) and LC-20AR pump. The [6]-shogaol-enriched fraction (ShEF) was submitted to [6]-shogaol isolation (t_r = 11,3 min) by semi-preparative HPLC (Shim-Pack C18 column, 250 mm × 21.2 mm i.d., 10 µm), mobile phase MeOH/H_2O 75:25 (v/v), flow rate at 19.0 mL/min and volume injection 1000 µL. Previous injections of ShEF and standard [6]-shogaol (t_r = 12,7 min) were performed in analytical HPLC (Shim-Pack C-8 column, 250 × 4.6 mm i.d., 10 µm) to analyze the chromatographic separation, using mobile phase MeOH/H_2O 75:25 (v/v), flow rate at 1.0 mL/min and volume injection 20 µL. Standard [6]-shogaol previously isolated and identified (2) was used for HPLC analysis. After HPLC analysis with diode array detector, the degree of purity determined was greater than 97%.

References

1. Ok S, Jeong W-S. Optimization of Extraction Conditions for the 6-Shogaol-rich Extract from Ginger (Zingiber officinale Roscoe). Preventive nutrition and food science. 2012;17(2):166-71.

2. da Silva JA, dos Santos CY, Mohammadi M, Fernandes JB, das Graças Fernandes da Silva MF, Vieira PC. Cathepsin K Inhibitors Isolated from Ginger Rhizome. Revista Brasileira de Farmacognosia. 2021;31(6):859-64.