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Antiproliferative activity and apoptosis activated by clerodane diterpenes

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Introduction: Pre-clinical studies have indicated the antihyperlipidemic, anti-ulcer, anti-venom and cicatrizing properties of Casearia sylvestris Swartz. Objective: To assess the in vitro antiproliferative activity and cell death pattern activation of four clerodane diterpenes (casearins B, D, X and caseargrewiin F) isolated from Casearia sylvestris leaves on HL-60 cells after 24h compound exposure. Methods: The human leukemia HL-60 cell line was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin, at 37°C with 5% CO_2. The antiproliferative potential of the clerodanes was evaluated by BrdU incorporation assay. Then, HL-60-treated cells were evaluated for cellular membrane integrity, DNA fragmentation, cell cycle arrest, mitochondrial depolarization, caspases and phosphatidylserine externalization by flow cytometry (Guava EasyCyte Mine). Negative control was incubated with the vehicle used to dilute the substance (DMSO 1.6%). Doxorubicin (Dox, 0.6 µM) was used as positive control. Data were compared by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test (P<0.01). Results: The BrdU assay revealed that all compounds inhibited leukemia proliferation, since treated cells showed significant reduction in BrdU incorporation after 24h when they were assessed in the CI_{50} value: 40.0 ± 4.8, 35.0 ± 1.4, 32.7 ± 6.1, 24.5 ± 4.0 and 28.5 ± 1.3% for Cas B (2 µM), D (4 µM), F (1 µM), X (1.5 µM), respectively, in comparison with negative control (66.4 ± 1.8%) (P<0.01). Dox-treated cells showed 24.5% of incorporation. Cytometry analyzes confirmed these antiproliferative action, since all concentrations tested [1 and 2 µM (B); 2 and 4 µM (D); 0.5 and 1 µM (F); 0.7 and 1.5 µM (X)] caused dose-dependent DNA fragmentation [7.5 ± 0.6 and 22.3 ± 2.2 (B); 2.9 ± 0.4 and 33.2 ± 1.8 (D); 23.7 ± 1.1 and 44.2 ± 1.1 (F); 10.5 ± 0.5 and 25.4 ± 0.9% (X), respectively], though only Cas X (0.7 and 1.5 µM) caused cell cycle arrest in G_0/G_1 (63.1 ± 2.5 e 63.1 ± 10.6 %, respectively) when compared to negative control (17.1 ± 1.0%) (P<0.01). At the higher concentrations of each compound it occurred increases in membrane disintegration and mitochondrial depolarization (P<0.01), while caseargrewiin F lead to mitochondrial dysfunction at the both concentrations (0.5 and 1 µM, 5.4 ± 0.3 and 10.7 ± 0.9%, respectively) when compared to negative control (1.5 ± 1.2%). Cas F and X augmented the percentage of apoptotic cells in both concentrations tested (0.5 and 1 µM; 0.7 and 1.5 µM) after quantification of caspases -8 (35.1± 5.7 and 42.7 ± 9.2%; 63.1 ± 5.0 and 65.9 ± 8.4%) and -9 (46.4 ± 0.4 and 69.2 ± 19.0%; and 71.8 ± 7.5%), respectively. Caspases -3/-7 were triggered with Cas F (1 µM, 30.2 ± 3.7%) and with Cas X [0.7 µM, 31.4 ± 1.6%; 1.5 µM, 73.2 ± 8.9%) (P<0.01). Conclusion: All substances showed potent antiproliferative action and activation of cell death suggestive of apoptosis as represented via the biochemical changes analyzed by flow cytometry.