**ABSTRACT**

Many dietary bioactive agents have been found to induce apoptosis through the intrinsic mitochondrial-mediated pathway in cancer cells, relying on the alteration in Mitochondrial Permeability Transition (MPT) and the consequent release of cytochrome c. The efficacy of *Momordica charantia* (MC) against certain cancers has been linked to its ability to induce apoptosis, however, the underlying mechanism of the induction is still unknown. This study was designed to evaluate the effects of leaf extract of MC on the opening of MPT in normal rat liver mitochondria, and on selected human cancer cell lines.

Leaves of MC obtained from the Botanical Garden, OAU, Ile-Ife Campus were authenticated with the voucher number FPL-1783 at the Herbarium, Faculty of Pharmacognosy, OAU and cold-extracted in distilled water to obtain the Crude Water-Soluble Extract (CWSE). Butanol (D2), ethyl acetate (D3), n-hexane (D4), dichloromethane (D5) and the residual aqueous (D6) fractions were obtained from the CWSE via solvent partitioning. Bioassay-guided fractionation of D3 on preparative HPLC/MS yielded flavonoid-rich fractions. Using D1-D6 and the flavonoids fractions (75-125 µg/mL), the induction of MPT pore opening in isolated rat liver mitochondria was assayed spectrophotometrically. Mechanism of MC-induced cell death in selected cancer cell lines [MDA-MB-231 and MDA-MB-436 (breast cancer), HeLa (CCL-2) (cervical cancer) and A549 (lung cancer)] was investigated using the MTT, DAPI (4,6-diamidino-2-phenylindole dihydrochloride) and Luciferin-Luciferase assays for cell viability, membrane integrity, chromatin condensation and ATP levels’ assessments respectively. Cytochrome c release and caspase-3 activation were determined by Western blotting while mitochondrial potential depolarization and Reactive Oxygen Species (ROS) generation were assayed using flow cytometry. Data were analysed using ANOVA at α 0.05.

Concentration-dependent (125>100>75 µg/mL) MPT pore opening induction was observed for fractions of MC with the highest induction of 23 and 21 folds seen in D2 and flavonoids, respectively relative to control. Also, D3, D4, and D5 showed concentration-dependent cytotoxicities; with D3 being the most cytotoxic for MDA-MB 231, 436, A549 and HeLa cells at 125 µg/mL (33.6%, 34.7%, 31.6% and 38.6%, respectively) while D2 and D6 showed no significant toxicities. For the sub-fractions, flavonoids exhibited the highest cytotoxicities at 13.0% and 20.9% for MDA-MB 436 and A549 cells, respectively relative to control. At 125 µg/mL, the mitochondrial potential of D3-treated MDA-MB 436 and A549 cells were most significantly depolarized relative to control i.e. (43.1±2.3 vs 175.8±6.1) and (24.8±2.5 vs 131.7±11.2) respectively, same as flavonoids-treated A549 cells at 146.9±4.5 vs 267.4±11.0. Cells treated with D3 at 125 µg/mL showed significant decrease in intracellular ATP levels (80.6±4.7 vs 106.7±5.7) for MDA-MB 436 and (43.7±0.1 vs 98.6±0.7) for A549 cells; and flavonoids-treated A549 cells at 81.2±1.2 vs 105.0±2.8 relative to control. The ROS generation significantly increased in D3-treated MDA-MB 436 (332.1±3.2 vs 100.0±2.2) and A549 (305.0±16.0 vs 42.0±4.5) cells, as well as in flavonoids-treated A549 cells (571.9±45.5 vs 58.2±1.4) at 125 µg/mL relative to control.

The bioactive agents of *Momordica charantia* induced a mitochondrial-dependent cell death in the selected cancer cell lines.

**Keywords:** Cancer cell lines, Mitochondrial Permeability Transition,Mitochondrial potential,

*Momordica charantia.*

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