UNIVERSITI TEKNOLOGI MARA

ANTITUMOUR ACTIVITY OF *Myrmecodia platytyrea* METHANOLIC TUBER EXTRACT ON HEPATOCELLULAR CARCINOMA

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ABSTRACT

Liver cancer is a common deadly malignancy that globally have caused over 700,00 deaths each year. In Malaysia, liver cancer is one of the ten most common malignancies diagnosed. Tubers of *Myrmecodia platytyrea* (Rubiaceae) or well known as Ant-plant have been used traditionally as in treating swelling, headaches, diarrhea, as well as cancer and other inflammatory-related diseases. Nevertheless, there is only one study that has been reported for the last 10 years to prove these folkloric claims apart from the current research team. Hence, there is a need to decipher the mechanism of action of MPMTE in deterring the proliferation of the hepatocellular carcinoma (HCC). In this study, antiproliferative activity of MPMTE against HepG2 cells has been carried out using MTS assay. Doxorubicin (1 μM) was chosen as a chemotherapeutic drug reference. To further affirm the mode of cell death triggered by MPMTE, cell cycle and apoptosis analysis were conducted using flow cytometry. Next, morphological analysis using light microscope, fluorescent microscopy via Acridine Orange/ Propidium Iodide staining and Transmission Electron Microscope (TEM) were performed. Then, the expression of apoptotic genes was determined by quantitative RT-PCR. Lastly, to confirm the expression of the proteins, western blot analysis was carried out. In the *in vivo* study, the xenograft of Severe Combined Immunodeficiency (SCID) mice model was employed. The HCC-induced SCID mice were treated twice daily with MPMTE (100, 200 and 400 mg/kg; p.o.) and doxorubicin (positive control, 10 mg/kg). Behaviour analysis, mortality rate, body weight parameter, food and water intake changes, tumour development analysis were performed apart from other tests such as blood haematology, blood biochemistry, gross necroscopy, and organ-to-fasted body weight ratio. Western blot analysis was done on the liver lysate to determine the effect of MPMTE on the apoptotic-related genes. Histological analyses of the liver, lungs, kidneys, heart, spleen, and the tumour of the SCID mice were also executed to study the microscopic anatomy of the organs after MPMTE treatment. Results have demonstrated that MPMTE extract was found to be most potent in inhibiting the growth of HepG2 cells (IC_{50}=5.76 μg/mL) without affecting normal cells, showing high selectivity (SI=25.92). The morphological study using an inverted light microscope observation and AO/PI staining. Cell cycle analysis showed that the percentage of cells in G_0/G_1 phase gradually decreased in a dose-dependent manner following treatment with MPMTE. While in apoptosis analysis, MPMTE at a concentration of 6 μg/mL inhibited the proliferation of HepG2 cells at 50% concentration. In morphologic analyses, HepG2 cells treated with MPMTE were found to produce typical apoptotic characteristics in a dose-dependent manner. As for the expression of apoptotic genes, MPMTE triggered apoptosis in the HepG2 cells. CDK2 and CDK5 genes were significantly overexpressed in HepG2 treated cells. Furthermore, increased levels of Bax and caspase-3 protein expression were observed in the HepG2 cell lines. For the in vivo studies, oral administration of MPMTE did not cause any toxic effect, physical nor behavioural changes. MPMTE managed to delay the tumour outgrowth and improve the levels of NF-κB and caspase-3 protein expression in HCC-induced mice. This suggests that MPMTE induced apoptosis in hepatocarcinoma cells to inhibit the proliferation of the cancerous cells, in vitro and in vivo. Besides, MPMTE did not affect normal cell line or worsen the condition of the tumour-bearing mice. In conclusion, MPMTE has notable antitumour activity that may be due to its phytochemicals such as stigmasterol, morindolide, and flavonoid and can be developed as a potential anticancer adjuvant therapy.

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