The Effects of Royal Jelly-Derived Exosomes on the Proliferation Rate of the HepG2 Liver Cancer Cell Line

Abstract

Hepatocellular carcinoma (HCC) is one of the major global health challenges, receiving significant attention due to its high prevalence and notable mortality rate. Conventional chemotherapy methods, such as doxorubicin, are associated with severe side effects and drug resistance. In recent years, exosome-based therapies have emerged as a novel strategy due to their ability to modulate the tumor microenvironment and enhance targeted drug delivery. Royal jelly (RJ), with numerous biological properties including antioxidant, anti-inflammatory, and antitumor activities, has been investigated in this context. This study evaluates the antiproliferative effects of royal jelly-derived exosomes on the HepG2 liver cancer cell line. Exosomes were isolated from royal jelly using a precipitation method and characterized by DLS, transmission electron microscopy (TEM), and flow cytometry for the marker CD63. The protein concentration of exosomes was measured using the Bradford method. The cytotoxic effects of royal jelly-derived exosomes and doxorubicin on HepG2 cells were assessed using the MTT assay, and IC50 values were determined. Additionally, a scratch test was performed to evaluate cell migration, and Real-time PCR was conducted to measure the expression levels of Caspase-3, Bax, and BCL2 genes after treatment with IC50 concentrations of doxorubicin and exosomes. The isolated exosomes from royal jelly exhibited a spherical structure with an average size of 96 nm, and the presence of the CD63 marker confirmed their exosomal nature. Cytotoxicity analysis showed that these exosomes reduced the viability of HepG2 cells in a dose-dependent manner (IC50 = 743.6 µg/mL), while doxorubicin demonstrated stronger cytotoxicity (IC50 = 0.6374 µg/mL). The scratch test revealed that both treatments significantly reduced cell migration compared to the control group. Gene expression analysis indicated a significant increase in Caspase-3 and Bax and a decrease in BCL2, suggesting the induction of apoptotic pathways in cancer cells. Although doxorubicin had a stronger effect on inducing apoptosis, royal jelly-derived exosomes also demonstrated notable anticancer effects. The results of this study indicate that exosomes derived from royal jelly have significant anticancer effects on HepG2 cells, reducing proliferation and migration while increasing the expression of pro-apoptotic genes like Bax and Caspase-3 and decreasing BCL2 expression, thereby inducing apoptosis. While doxorubicin showed greater potency in inhibiting cell growth, royal jelly exosomes present a promising natural therapeutic option with the potential to reduce the side

effects of chemotherapy drugs and improve the efficacy of liver cancer treatments. Further in vivo and clinical studies and investigations into the precise mechanisms and clinical applications of these exosomes are recommended.

Keywords: Liver cancer, exosome, royal jelly, HepG2, apoptosis.