"تقييم مزيج الصيام ومستخلص الأشواجاندا المائي كعلاج للتغلب على مقاومة السيسبلاتين في سرطان الثدي: دراسة خارج وداخل الجسم""

"Evaluation of Fasting and Ashwagandha Water Extract as a Combination Therapy to Overcome Cisplatin Resistance in Breast Cancer: An *in Vitro* and *in Vivo* Study

Abstract

Breast cancer is considered a universal public health dilemma and is currently the most common cancer-affecting women. It is a leading cause of cancer death in women in the globe. So far, chemotherapy, radiation and surgery are used to eradicate cancer, although they have been linked with adverse effects in addition to the development of multi drug resistance (MDR). Alternatively, medicinal plants have been used to fight cancer and chemo-resistance owning to the fact that natural products are safe with low cost and they have many components with anticancer effect such as Ashwagandha *(Withania somnifera,*WS*)*. In addition, food restriction programs, such as intermittent fasting (IF), have been engaged to the newly developing treatment approaches because of their benefit in fighting cancer. These two ways in combination with chemotherapy provide possible solution to evade cancer, multi drug resistance (MDR) and reduce side effects of chemotherapy, while maintaining the efficacy.

In this study, WS root (WSR), IF, cisplatin and their combinations were tested to evaluate their impact on cisplatin-sensitive (EMT-6/P) and cisplatin-resistant (EMT-6/C) mouse epithelial breast cancer cells. Phytochemical content of WSR water extract was analyzed using LC-MS analysis. Antiproliferative and apoptotic effects of WSR water extract, cisplatin and their combination were assessed *in vitro* using MTT and caspase-3 assays, respectively. *In vivo* study was used to assess the effect of the WSR water extract, IF, cisplatin and their combination in mice inculcated with EMT-6/P and EMT-6/C cells. In addition, safety profile was investigated using ALT, AST and creatine assays.

The results of *in vitro* showed that combination of WSR water extract and cisplatin had a synergistic effect in both cell lines with IC_{50} values of 0.54 ± 0.011 mg/mL WSR water extract and 10 μM cisplatin in EMT-6/P compared to 0.66 ± 0.05 mg/mL WSR water extract with 27 μM cisplatin in EMT-6/C. Furthermore, the same combination at the resulted IC_{50} induced apoptotic effect higher than the single treatment in both cell lines. *In vivo*, several combinations of WSR water extract (100 mg/kg/d), IF (18 h daily) or cisplatin (5 mg/kg/week) were used and caused significant tumor size reduction and improved cure rate in mice implanted with EMT-6/P and EMT-6/C cell lines. IF-treated groups showed significant reduction in serum glucose and β-hydroxybutyrate (BHB) levels. In safety profile, WSR water extract, IF and their combinations with or without cisplatin were safe, however, cisplatin showed a degree of toxicity in liver and kidney as indicated by the levels of liver enzymes and creatinine.

In conclusion, WSR water extract and IF combination and WSR water extract, IF and cisplatin combination provide a promising solution for breast cancer treatment by reducing proliferation of cancer cells through induction of apoptosis. In addition, these combinations reduce tumor size efficiently and minimise the toxicity of the cisplatin to liver and kidney. To optimize the doses and determine the exact mechanism of action exhibited by the novel combinations, further investigations are required.

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