UNIVERSITI TEKNOLOGI MARA

EFFECT OF EUKARION-207 AND ASIATIC ACID 'ANTI PROLIFERATIVE AND ANTI CANCER STUDIES'

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of the requirements for the degree of
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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic or non-academic institution for any other degree or qualification.

I, hereby acknowledge that I have been supplied with the Academic Rules and Regulations for Postgraduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Chemotherapeutic drug resistance occurs when tumors develop resistance to chemotherapeutic drugs. It is a major problem faced by current cancer treatment modality. Therefore, there is an urgent need to find a new anti cancer drug which can effectively kill cancer cells. In this study, the anti proliferative effect of Eukarion-207 (EUK-207) and asiatic acid (AA) were screened against human hepatocellular carcinoma (HepG2), colorectal carcinoma (HCT116) and oral squamous cell carcinoma (OSCC). Compound that showed best anti proliferative effect were chosen for downstream analysis and its protective effect in orthotopic cancer xenograft model has been evaluated. Anti proliferative activity of EUK-207 and AA was determined by MTS assay. Expression of genes that account for apoptosis of cells namely Bax and Bcl-2 were determined by quantitative RT-PCR with AA (30 and 38 μM). To determine protective effect of AA against OSCC *in vivo*, AA (1, 5 and 10 mg/kg, *i.p.*) and normal saline were given daily for three weeks prior to tumor inoculation to tongue of severely compromised immunodeficiency (SCID) mice. Treatment continued for three weeks following tumor inoculation. Blood and organs of mice were harvested after euthanization for blood biochemistry and histopathology analysis, respectively. Results reveal that EUK-207 showed best anti proliferative effect against HCT116 with IC_{50} value of 31 ± 3.61 μM while AA exhibit better anti proliferative effect against OSCC with best IC_{50} value of 12 ± 3.5 μM following 72 h incubation time. Therefore, AA was selected for downstream analysis. The results also demonstrated that the best IC_{50} value of AA was not drastically different from IC_{50} of cisplatin against OSCC of 10 ± 8.5 μM. Anti proliferative effect of AA was highly selective as selectivity index of AA against OSCC (IC_{50}: 12 ± 3.5 μM) versus human gingival fibroblasts (IC_{50}: 82 ± 2.8 μM) was 6.8. This shows that AA was selective at inhibiting the proliferation of cancer cells yet was not detrimental to normal cells. AA initiated apoptosis by down regulation of Bcl-2 genes by 59% and 68% and upregulated Bax genes by 2.35 and 2.05 folds in OSCC treated with 30 μM and 38 μM of AA, respectively. AA did not possess protective effect in orthotopic xenograft model of oral cancer as tumors still grow despite presence of AA in the mice, however, the tumors that were formed were smaller in their areas of invasion. Treatment with 1, 5 and 10 mg/kg of AA significantly reduced the area of tumor invasion by 74%, 61% and 83%, respectively. Body weight of AA-treated mice were significantly different from cancer bearing mice but not significantly different from normal control mice. Blood biochemistry results demonstrated that lung, liver and kidney of mice administered with high dose of AA were normal.. Similarly, histology results showed no signs of toxicity or metastatic cancer in the liver, lung and kidney of AA-treated cancer-bearing mice. In conclusion, AA showed greater anti proliferative effect than EUK-207 against oral squamous cell carcinoma *in vitro* and showed inhibitory effect on tumor invasion *in vivo*.

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