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Research Article

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Investigating the effect of bee venom on human colon cancer cells (HT-29) and hepatic cells (HepG2) in comparison to L929 cells

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ABSTRACT

Nowadays as the result of environmental conditions like fast foods and stress, colon cancer is becoming common. Different treatments are presented for cancer disease. Chemotherapy and radiotherapy are the main parts of cancer therapy and different drugs are also used for increasing immune system and specifically targeting cancer cells. Natural methods for treating human disease are becoming popular because of their low side effects and good results. Bee products are probably one of the first things used for treating human disease. The anti-proliferative properties of bee venom investigated against different types of cells. In this study the effect of Honey bee venom investigated against colon cancer cells HT-29 and compared with normal cells L929.

Key words: cancer cells, honey bee venom,

INTRODUCTION

Honey bee venom is a colorless liquid and contained mixture of proteins, peptides and low molecular components which causes local inflammation and acts as an anticoagulant [1]. Melittin, apamin, adolapin, mast cell degranulating peptide are the most important peptides of BV [2]. Ancient civilizations know the healing properties of painful bee stings. Bee stings were used in ancient civilizations of China, Egypt, Babylon and Greece for threating different illness like arthritis [3]. Hippocrates, the ancient Greek doctor, used bee venom for its therapeutic properties [4]. Charlemagne used bee stings for therapy against gout, and Monfat used it to improve the flow of urine and studied its use against kidney stones [5]. Nowadays, different therapeutics of bee venom are clear and it is used for threating different disease like Multiple Sclerosis (MS)[6, 7], Alzheimer[8, 9], Parkinson [10], HIV [4], different cancers [11, 12]and skin and eye diseases. BV is effective against various types of cancer like renal, lung, liver, prostate, melanoma and bladder [2]. Melittinis one of BV peptides which is known for its high anticancer properties. It was also used to control neuropathy caused by cancer chemotherapy [12]. It is a cationic, amphiphilic α helical peptide of 26 amino acid residues which cause membrane perturbation in both eukaryotic and prokaryotic cells (93).The cytotoxic effect of lasioglossin, a bee venom peptide, against various types of cancer are exhibited [13]. Stimulation of the local cellular immune responses in lymph nodes by BV leads to inhibition of carcinoma cells proliferation and tumor growth[14-16] .Apoptosis, necrosis and lysis of the tumor cells are involved in fighting BV with cancer cells [15, 16]. It is demonstrated that bee venom induces apoptosis in leukemic cells via induction of Bcl-2 and caspase-3 expression by down regulation of mitogen activated signal pathways [17]. Another way for induction of apoptosis by HBV is the activation of caspase-3 in synovial fibroblasts [18] and inhibition of cyclooxygenases-2 expression in human lung cells [15]. Choiet all showed that natural toxin BV could be useful as

an anticancer agent through the overexpression of DR3 and inactivation of NF-kB. They showed that BV is a good choice for treatment of lung cancer[19].The study of SIU and his coworkers showed that bee venom induced cell cycle arrest and apoptosis in human cervical epidermoid carcinoma cells [20]. Colorectal cancer or colon cancer is the development of cancer in colon or rectum. It is because of abnormal growth of cells that have ability to invade or spread to other parts of body. Lifestyle factors like diet, obesity, smoking and not enough physical activity are the main cause of colorectal cancer[21]. In this study the effect of HBV against human colon cancer cells to find the best concentration that does not have inhibitory effect against normal cells but cause death of cancer cells.

EXPERIMENTAL SECTION

Hepatic cells, (HepG2), Colon cancer cells (HT-29), squamous cell carcinoma cells (KB) and normal mouse fibroblast cells (L929) purchased from pasture institute of Iran. All four cell lines cultured in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum, 100 Unit/ml penicillin and 100 µg/ml streptomycin and incubated in 5% CO2 incubator at 37 Co. Cell viability determined using Trypan Blue dye.

MTT assay

Cytotoxicity of honey bee venom was assessed in HepG2 cells, KB cells, HT-29 cells as well as L929 cells by measuring the amount of insoluble formazan formed in live cells based on the reduction of 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) salt (Roche Diagnostics GmbH, Germany) according to the manufacturer's protocol. This method is based on activity of enzyme succinate dehydrogenase that convert the yellow dye of MTT to unsoluble crystals of furmazan. 200 µl cell suspension with density of 5×104 cell/ ml seededin 96-well plates. The cells were treated with different concentrations of HBV (0.1, 0.2, 0.4, 0.8, 1, 2, 4, 6, 8, 10, 12, 24, 48, 96, 292, 384 µg/ml) and 0.2 % (v/v) DMSO (Merck, Germany) as a negative control. The cells incubated for 24, 48 and 72 hours in 5% CO2 incubator. After treatment 20µl MTT added to each weel. The plates were incubated at 37°C in a humidified atmosphere with 5% CO2 for 4 hours. Thereafter, 100 µl of the solubilization solution (DMSO) was added to each well. Absorbance was ultimately read using an ELISA plate reader at wavelength of 570 nm.

Statistical analysis

The results were analyzed with SPSS software. Analysis of variance and comparing the averages was done with Toki method and Spearman T test. Kolomogorov-Smirnov test was used for insuring that data distributed normally. Assumption of normality of the data ($p < 0.05$) was confirmed. On the other hand, the assumption of homogeneity of variance between groups ($p < 0.05$) was approved.

RESULTS AND DISCUSSION

The statistical analysis in times 24 and 48 hours showed that the amount of cytotoxicity in HT-29 cells and L929 cells enhanced by increase in HBV concentration. Generally there is a significant correlation between concentration of HBV and cytotoxicity in HT-29 cells and L929 cells ($p \leq 0.05$), but from concentration 0.6 µg/ml up to 384 µg/ml the cell toxicity in the case of HT-29 cells significantly decreased more than L929 cells. At concentrations 2 and 4 µg/ml the percentage of living cells of HT-29 reached to its minimum amount while in the case of L929 cells the amount of living cells at concentration 12 µg/ml reached to the minimum amount. The cytotoxicity of HBV against HT-29 and L929 cells at 72 hours is greatly more than hours 24 and 48. In general, there is a significant correlation between increases in concentration of HBV and cell cytotoxicity, and in concentration 2 µg/ml cell cytotoxicity of HBV against HT-29 is more than L929, and in concentration 10 µg/ml the cell cytotoxicity of HBV against L929 reached to its minimum amount. Liu and his coworkers in 2002 studied the effect of honey bee venom against melanoma cells in vivo and in vitro, and showed that there is a significant relation between concentration of BV, time and inhibitory concentration. It is demonstrated that BV caused the arrest of cell cycle in G1 phase and finally lead to cell death [14]. In another experiment performed by Moon et all in 2006, the effect of BV against leukemia cell line investigated and their results showed that BV cause apoptosis by increasing in caspase 3 and FAS/FASL and inhibiting Bcl-2 , Htert, cox-2 and Akt/ERK [22]. Nabiuni studied the effect of Bv against pro myelocyte cell line HL-60 in concentrations 0.5 , 7.5 and 10 µg/ml and showed that low concentration of HB is able to inhibit cell growth of HL-60and in concentration 2.5 µg/ml the inhibitory effect is greatly high [23]. Many studies on the effect of BV against different types of cancer cells has been done and various mechanisms are described. Jang et all in 2003 investigated the effect of BV on COX2 and induction of apoptosis in NCL- H1299 cell line [15]. Also the results of different studies on HBV showed that mechanisms like inhibiting the core factor Kappa-β, and reduction

in expression of COX-2, apoptosis proteins like Bcl-x1 and Bcl-2 are involve in cell death by HBV (13). In another study the inhibitory effect of HBV on polycystic ovarian syndrome showed by Pouyanmanesh[24]. Mahmoodzadeh et all in 2013 studied the effect of Melitin against growth of human gastric carcinoma and showed that this compound greatly inhibit the growth of cancer cells [25]. Our study clearly showed the inhibitory effect of HBV on HT-29 and L929. It showed that concentrations below 6 µg/ml lead to decrease in number of living cancer cells but does not significantly decrease the number of normal cells.

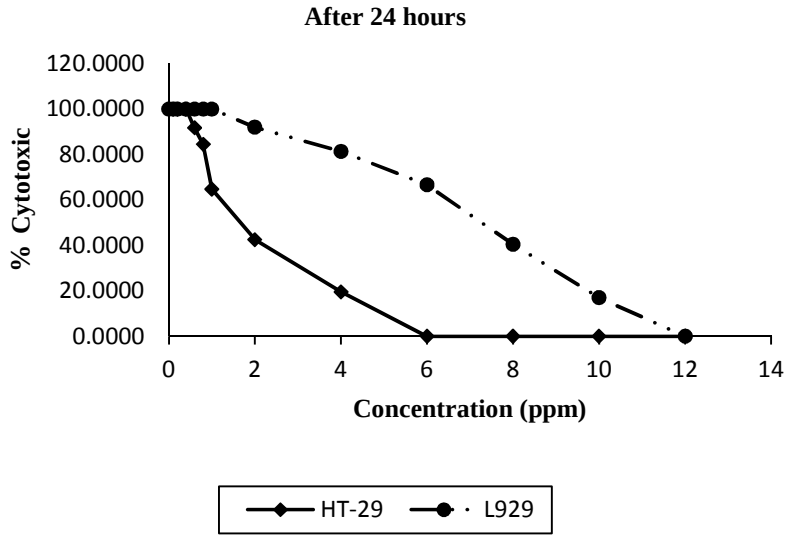

Figure1: the effect of honey bee venom against HT-29 and L929 cells after 24 hours incubation

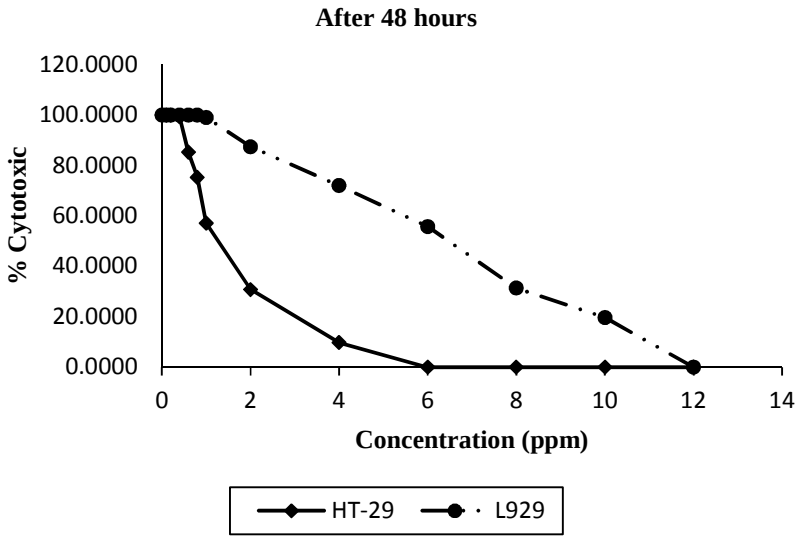

Figure 2: the effect of honey bee venom against HT-29 and L929 cells after 48 hours incubation

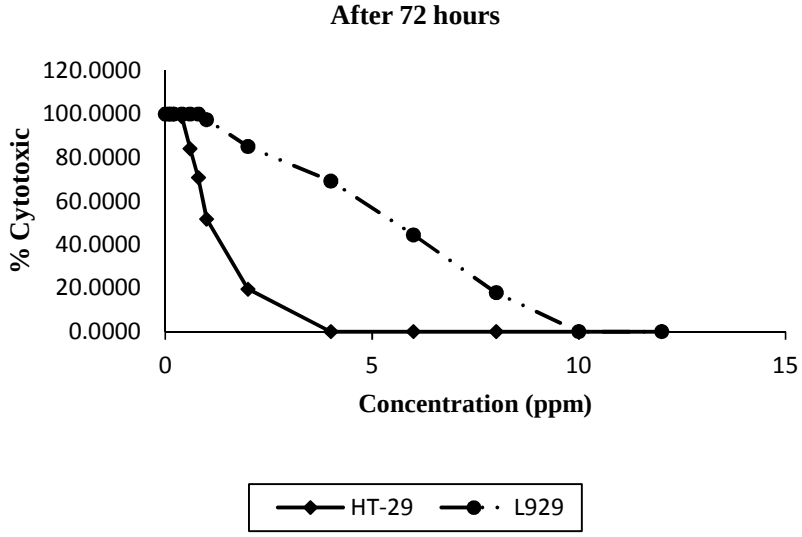

Figure 3: the effect of honey bee venom against HT-29 and L929 cells after 24 hours incubation

CONCLUSION

In conclusion, our results showed that BV induced cell death in both HT-29 and L929 cell types, but in concentrations below 4.5 µg/ml the inhibitory effect against cancer cells is greatly more than normal one and this is very important in finding a target specific drug for treating cancer.

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