REVIEW ARTICLE

In-vitro Cytotoxic Anticancer Effects of Honeybee Venom Fractions on Different Cell Lines

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ABSTRACT

Background: Honey bee venom (HBV) has been used from ancient history in traditional medicine, but recently with the progression of medical and biological science, some HBV fractions were found to had specific therapeutic use. Honey bee venom fractions such as melittin were noticed to have had *in-vivo and in-vitro* anti-tumor effects. Honey bee venom has about twenty different biological fractions. Melittin was the dominant fraction extracted from crude HBV.

The aim is to study the in-vitro anti-cancer effect of one HBV fractions extracted by reverse-phase high-performance liquid chromatography (RP-.HPLC) against four cancer and one none tumorigenic normal cell line.HBV was collected by using the electric collector and dried by lyophilization and separated by RP-HPLC into sixteen different weights yield uncharacterized fractions. For detection of the anti-cancer effect, all the 16 HPLC peaks were tested separately on four cancer cell lines and one none tumorigenic cell line.

Materials and methods: Four cancer cell lines, B16, MCF-7, Hep-G2, HCT-116, and MDCK normal canine kidney cell line was cultured and maintained on RBMI-1640 media with 10% fetal bovine serum. The HBV 16 fractions was tested on the above cell lines separately by using MTT cytotoxicity assay protocol. The viability percent was obtained versus five concentrations of HBV (50, 25, 12.5, 6.25, 3.125 Ug). The half-maximal inhibitory concentration (IC50) was calculated and plotted using sigma plot software, it was found as 5.6 for B16, 21.2 for MCF-7.14 for Hep-G2, 21.4 for HCT116, and 45 for MDCK cell line subsequently.

Cell line subsequently from the result we found that only fraction number 8 (HBv-F8), has had cytotoxic effects on the cancer cell lines and the normal canine kidney cell line while the other fifteen HPLC fraction had no any cytotoxic effects.

Conclusion: We conclude that HBV-F8 has a cytotoxic effect on cancer and normal cell lines with different IC 50 for each cell line. The best cytotoxic effect was found on B16 melanoma cell line, and the least effect was found on HCT-116 cell line. At the same time, it is also toxic on MDCK normal cell line at 45.13 ug half-maximal inhibitory concentration.

Keywords: Cytotoxicity, MDCK. HBV. MTT, RP-HPLC.

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INTRODUCTION

Honey bee venom was used as complementary medicine along thousand years ago from history. It was used in traditional orient medicine to treat pain and chronic inflammatory condition such as joint arthritis.[1] Apis mellifera is the endemic honey bee subspecies present in the Middle East (Lebanon, Syria, Jordan, Palestine, Iraq.[2]

Honey bee venom is composed of a complex mixture of biologically active peptides, including melittin a major component of BV, apamin, adolapin, mast-cell-degranulating (MCD) peptide; enzymes phospholipase A2, and hyaluronidase, and non-peptide components (histamine, dopamine, and norepinephrine), which have a variety of pharmaceutical properties.[3] Several studies have shown that melittin has a broad spectrum of biological, pharmacological, and toxicological activities including anti-bacterial, anti-viral, anti-inflammatory, and anti-tumor properties, together with hemolytic properties.[4]

Moreover, recent reports indicate that BV is also able to inhibit tumor growth and exhibit anti-tumor activity *in vitro*

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and *in vivo*.[5] Several recent studies have reported that some natural bee products inhibit tumor cell growth and metastasis and induce apoptosis of cancer cells, suggesting the potential application of these natural compounds (or their active components) as part of an alternative medical treatment of human tumors.[6]

Because, most human deaths resulting from one or a few bee stings are due to allergic reactions, heart failure, or suffocation from swelling around the neck or the mouth, as compared with other human diseases, accidents and other unusual cases, deaths dues to bee stings are rare, indicating that BV is very safe for treating diseases in humans.[7]

Bee venom, also known as apitoxin, is a complex fluid secreted by the bee venom gland located in the abdominal cavity and injected into victims by a stinger, causing local inflammation, anticoagulant effect, and immune response. Honey bee venom contains antigenic proteins causing allergic reactions, which presently limits its clinical use. Administration of HBV without a full understanding of its allergic responses, especially anaphylactic shock, puts practitioners and patients at great risk

Collection purification and separation of Honey bee venom

HBV was collected using an electric collector; the dry Weight was collected by scraping and dissolved in deionized distilled water and spent at 4000 rpm for precipitation of any none soluble artifact. Then the supernatant was filtered by 0.45 um and 0.22 syringe filters. The sample was dried by lyophilizer and sent for separation and fractionation by RP-HPLC into sixteen none identified main peaks. The fraction has been dried by lyophilization with different yields. The product of each peak was dissolved in deionized distilled water (DDW) as a stock solution in a concentration of 2000 ug/mL and stored at -20 during the experiment period. Melittin (C131H229N39O31) is the main component in honey bee venom, which accounts for 40-50% of the dried venom.[9]

MATERIALS

Cell culture materials were purchased from local suppliers of sigma and gibco groups, including RPM-1640 culture media, trypsin, phosphate buffer saline. Antibiotics, flask and microtiter plate, MTT stain, DMSO, and all the other reagents and materials.

Cell lines culture

Cancer cell lines (B16, Mcf-7, Hct-116, Hep-G2) and none tumorigenic cell line (MDCK) were offered by Babylon medical college cell culture unit. It was cultured and maintained on RPM-1640 medium, plus L.glutamine and 10 % fetal bovine serum and 50 mg gentamycin/L. The cell culture was maintained according to the routine cell culture protocols.

Cytotoxicity assay

The cell line was cultured and maintained at 37°C and 5% CO_2 subculture on 96 well microtiter plates at 90% confluence for determination of cytotoxic effects of HBV fractions on each cell line. All the 16 HPLC fractions were tested on the above cell lines in concentrations of (50, 25, 12.5, 6.25, 3.125, 1.562) ug /ml in triplicate wells for each concentration. According to Mtt assay protocol.[10] By Using the Humans microtiter plate reader, the absorbance of each well was read for determination of viability % and calculation of the halve maximal inhibition concentration fifty (IC50) of HBV fractions on each cell line,

Viability % = (OD.t – OD.b)/(OD.c – OD.b) x 100%.

T = test, b = blank, c = control.

The IC50 was calculated for each test by Sigma plot 12 software.

RESULT

A cytotoxicity pilot test was performed for all the sixteen honey bee fractions, which was obtained by the RP- HPLC with a fraction collector. We found that only one honey bee venom peak fraction number 8 (HBV-F8) has significant inhibitory effect on the cancer cell lines and none tumorigenic cell line at

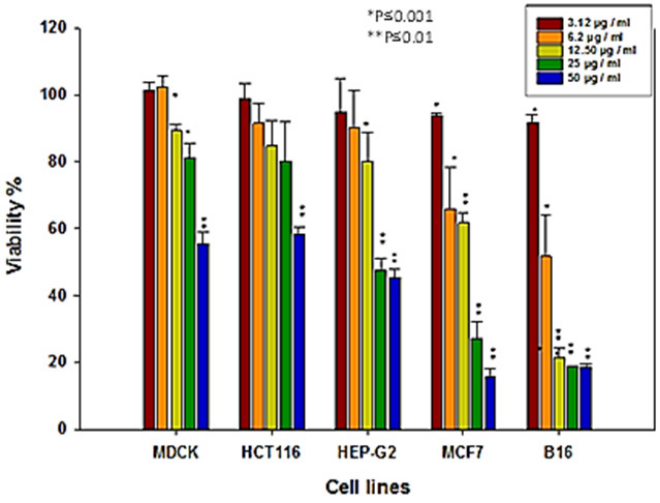

Figure 1: Viability % of serial concentrations of HBV-F8 on different cancer cell lines and none tumorigenic cell line for 48 hours.

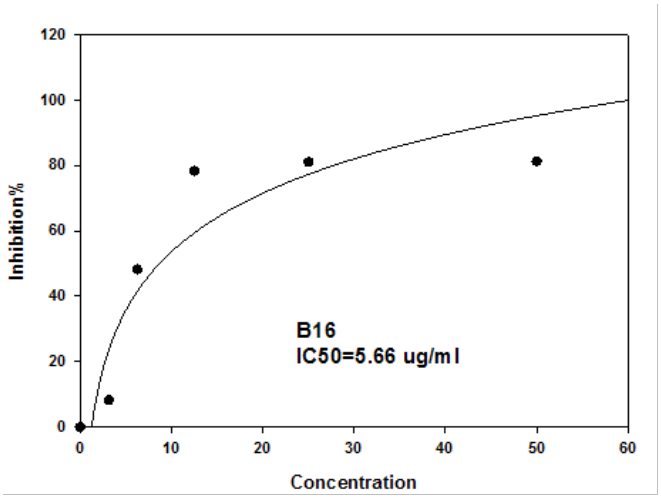

Figure 2: IC50 of the cytotoxic effect of different concentrations of HBV-F8 on B16 Melanoma cell line at 48 hours of exposure = 5.66 ug/mL.

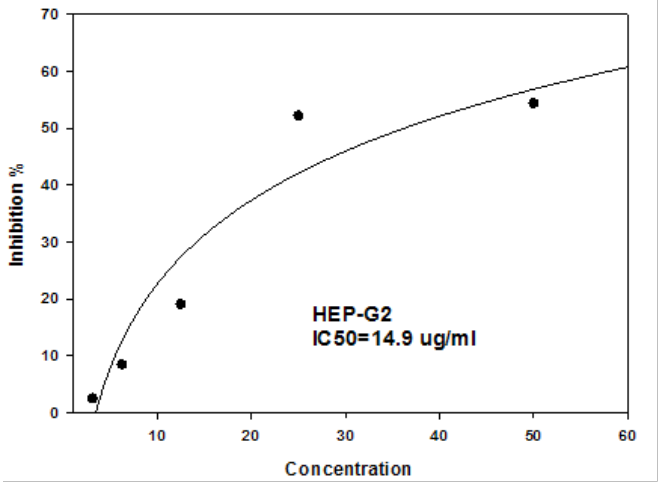

Figure 3: IC50 of the cytotoxic effect of HBV-F8 different concentrations on Hep-G2 Hepatoma cell line at 48 hours of exposure = 14.9 ug/mL.

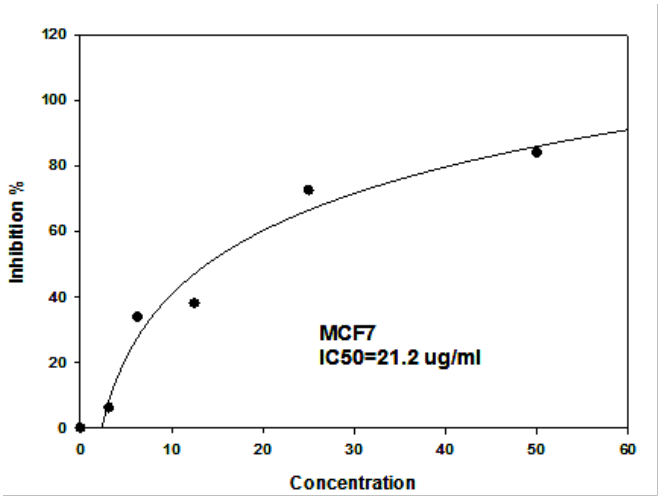

Figure 4: IC50 of the cytotoxic effect of different concentrations of HBV-F8 on MCF7 breast adenocarcinoma cell line at 48 hours of exposure = 21.2 ug/mL.

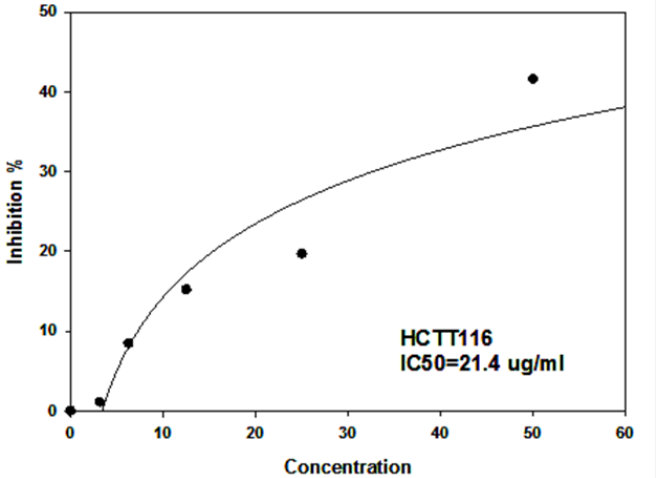

Figure 5: IC50 of the cytotoxic effect of HBV-F8 different concentrations on HCT116, Colon adenocarcinoma cell line at 48 hours of exposure = 21.4 ug/mL.

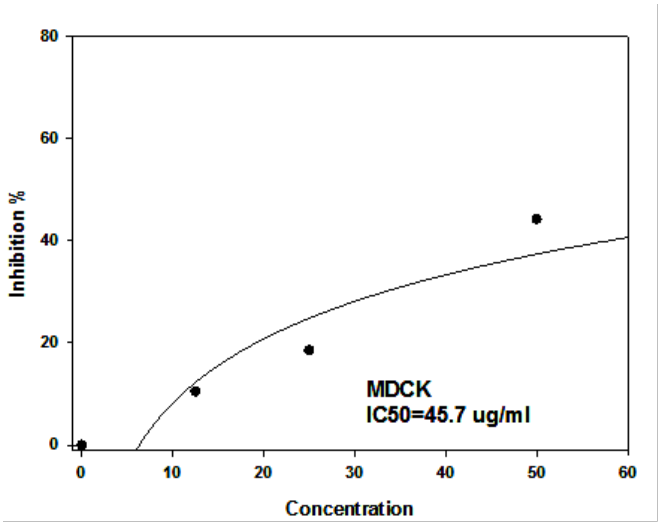

Figure 6: IC50 of the cytotoxic effect of HBV-F8 different concentrations on MDCK, none tumorigenic canine kidney cell line at 48 hours of exposure.

least for higher concentrations (50, 25, 12.5 ug/mL), while all the other fraction was found to had an insignificant cytotoxic effect on any of the above cell lines, so it was not included in this study. The viability assay was performed and the (IC50) was calculated for the effect of HBf-F8 on each cancer cell lines and the none tumorigenic MDCK cell line. The IC 50 was 5.6 for B16, 21.2 for MCF-7.14 for Hep-G2, 21.4 for HCT116, and 45. for MDCK cell line subsequently.

DISCUSSION

According to the previous *in-vitro* and *in-vivo* researches, "HBV from honeybees (Apis mellifera) is a mixture of substances with bioactive properties including anti-cancer, anti-inflammatory, and antioxidant effects."[11]

From the present study, we found only one fraction (HBV-F8) from sixteen main rp-HPLC as a dominant uncharacterized fraction of HBV, has significant dose dependent reduction of cell viability of cancer cell lines, especially B16 melanoma cell line at IC50 (5.66 ug/mL for B16, 14.9 ug/mL for Hep-G2, 21.2 ug/mL for MCF7, and 21.4 ug/mL for HCT11). This reduction of cell viability could be due to direct or indirect inhibition of cell growth or could be due to apoptotic cell death in comparison to the control untreated cells.

Jang Mh *et al*., mention that" most of the reports on the mechanism of action of bee products in inhibiting tumor growth *in vitro* and *in vivo* suggest it is mediated via apoptosis, necrosis, and lysis of the tumor cells".[12] Previous studies mentioned another explanation have shown that "The amphipathic property of melittin makes it soluble in water in a monomeric form which allows melittin to be easily inserted into membranes by disrupting both natural and synthetic phospholipid bilayers disrupting cell membranes either by transient or stable pores formation".[13] So the cell membrane becomes permeable to relatively large molecules, such as glucose.[14]

We also found that HBV-F8 approximately has the cytotoxic effect on none tumorigenic normal cell line (MDCK) at IC50 = 45.7ug/mL. Grrisotto declares that acute renal failure results either from the direct toxic effect on proximal tubules of Mellittin or secondary to vasoconstrictive effect".[15]

The same result was mention by Gulmez *et al*., who proposed that, " that BV has a significant anti-proliferative and cytotoxic effect on Vero normal cell line suggest that BV may not be a good candidate as an anti-cancer agent."[16]

Raghuraman and Chattopadhyay reported that "BV induces apoptosis in Human Breast Cancer MCF7 Cells either by caspase-9 and-3 activation or through the release of EndoG and AIF from mitochondria".[17]

Regarding the anti-cancer effect of bee venom on colon cancer, Jie Zheng *et al*., mention "it result by activation of death receptors and inhibition of nuclear factor kappa B".[18]

CONCLUSIONS

From this study, we conclude that HBV has none specific biological cytotoxic effects; it has cytotoxic effects on the cancer cell line and noncarcinogenic (normal) cell line. In other words, it is not a specific treatment for cancer cells.

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REFERENCES

1. Lee JH, Kown YB, Han HJ, Mar WC, Lee HJ, Yang IS, *et al*. Bee venom pretreatment has both an antinociceptive and anti-inflammatory effect on carrageenan-induced inflammation. J Vet Med Sci. 2001;63(3):251-259.
2. Zaitoun, S.T.; Al-Ghzawi, A.M.; Shannag, H.K. Population dynamics of the Syrian Honeybee, Apis mellifera syriaca, under semi-arid Mediterranean conditions. Zool. Middle East. 2000;21:129–132.
3. R. C. Hider, "Honeybee venom: a rich source of pharmacologically active peptides," Endeavour. 1988;12(2):60–65.
4. Chen, J.; Guan, S.M.; Sun, W.; Fu, H. Melittin, the Major Pain-Producing Substance of Bee Venom. Neuroscience. 2016;32:265–272. [CrossRef] [PubMed]
5. Orsolic N, Sver L, Verstovsek S, Terzic S, Basic Inhibition of mammary carcinoma cell proliferation *in vitro* and tumor growth *in vivo* by bee venom. Toxicon. 2003;41(7):861-870.
6. Tamura T, Fuji A, Kuboyama N. Effects of royal jelly onexperimental transplantable tumours. In: Proceeding of the XXXth International Congress on Apiculture; Nagoya, Japan. Bucharest: Apimondia Publishing House. 1985, p. 474-477.
7. Abdu Al-Samie., MA Studies on bee venom and its medical uses. IJOART. 2012;1(2):1–15.
8. Korean Pharmacopuncture Institute. [Pharmacopuncturology: principles and clinical application] Elsevier Korea LLC; Seoul: 2012. pp. 147–179.
9. WY. Attia, M.S. Gabry, K.A. El-Shaikh, G.A. Othman, Melittin an active ingredient of honeybee venom (Apis Mellifera), as a potent inhibitor of tumor growth in mice through stimulation of the immune response, Egy. J. Nat. Toxins. 2009;6:33-58.
10. Morgan DML. Tetrazolium (MTT) assay (for cellular viability and activity. Methods Mol Biol. 1998;79:179-184.
11. Jang MH, Shin MC, Lim S, Han SM, Park HJ, Shin I, Lee JS, Kim KA, Kim EH, Kim CJ. Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. Journal of pharmacological sciences. 2003;91(2):95-104.
12. Pino-Angeles A, Lazaridis T. Effects of peptide charge, orientation, and concentration on melittin transmembrane pores. Biophysj. J. 2018;114:2865–2874. DOI: 10.1016/j.bpj.2018.05.006. [PMC free article] [PubMed]
13. Lee MT, Sun TL, Hung WC, Huang HW. Process of inducing pores in membranes by melittin. Proceedings of the National Academy of Sciences. 2013 Aug 27;110(35):14243-14248.[CrossRef]
14. Grisotto LS, Menels GE, Castro I, Baptista MA, Alves VA, Yu L *et al*. Mechanism of bee venominduced acute renal failure. Toxicon 2006; 48:44-54
15. Gülmez Y, Aydın A, Can I, Tekin S, Cacan E. Cellular toxicity and biological activities of honey bee (Apis mellifera L.) venom. Marmara Pharm. J. 2017;21:51–260.
16. Raghuraman H, Chattopadhyay A. Melittin: a membrane-active peptide with diverse functions. Biosci. Rep. 2007;27:189–223. DOI: 10.1007/s10540-006-9030-z
17. Zheng J, Lee HL, Ham YW, Song HS, Song MJ, Hong JT. Anti-cancer effect of bee venom on colon cancer cell growth by activation of death receptors and inhibition of nuclear factor-kappa B. Oncotarget. 2015;6:44437–44451. DOI: 10.18632/oncotarget.6295.