CPQ Medicine (2019) 7:3
Review Article

The Cancer Diseases and Potentiality of Bee venom Toward Therapeutic Tools Antitumor

Mamdouh Ibrahim Nassar[1*] & Emad Elzayat, M.[2]

[1]*Faculty of Science, Biology-Entomology Department, Cairo University, Giza, Egypt*

[2]*Faculty of Science, Zoology Department, Cairo University, Giza, Egypt*

[*]**Correspondence to:** Dr. Mamdouh I. Nassar, Faculty of Science, Biology-Entomology Department, Cairo University, Giza, Egypt.

Copyright

Received: 08 July 2019

Published: 29 July 2019

Keywords: *Cancer Diseases; Bee Venom; Apis Mellifera; Antitumor; Antiproliferative; Antibacterial; Cytotoxic Activities*

Abstract

Cancer is a serious health problem and statistically, there are approximately 32.6 million cancer patients around the world. This Review is focusing on cytotoxic and antitumor activities of honey bee (*Apis mellifera L.*) venom. It is no doubt that cancer is one of the primary causes of death in the world. Complications connected with the use of chemotherapy and radiation therapy in cancer treatment might lower the effectiveness of such medication. So, using naturalistic yields in cancer treatment has become an important current topic. Therefore Bee venom (BV) has been suggested as an Apitherapy tool to be considered for various diseases including cancer. The bee venom has been found to have anticancer activities in different cancer cell lines involving breast, liver and prostate. However, the mechanisms action of BV and its toxicity on tumorigenic and nontumorigenic cells are poorly understood. BV is highly toxic to cancer cell lines and its mechanism action causes a cleavage of genomic DNA and inhibition of cell migration, indicating induction of apoptosis. Immunohistochemistry studies demonstrated that BV decreased the expression of Bcl-2 and P16. BV composed of many complex substances such as peptides (melittin, apamin, secapin, tertiapin, adolapin, and mast cell degranulating peptide). Melittin links to some tumor cells at a higher affinity than to healthy cells; anti- metastatic anti-invasive, and anti-angiogenesis impact which might earn

future clinical research on its anti-tumor characteristics and therapeutic effects when used as a cancer diseases treatment.

Introduction

Order Hymenoptera (ants, bees, and wasps) contains over 115,000 species worldwide, [1,2] making it the second largest order of insects. It contains the largest group of organisms on earth which possess a chemical defense which is injected directly into the victim. Venoms in general are of particular interest to natural product drug discovery due to the obvious pharmacological effects they have on their targets. According to the Global Cancer Statistics published in 2015, there are approximately 32.6 million cancer patients around the world in 2012 [3]. It is no doubt that cancer is one of the primary causes of death in the world [4]. Over the last three decades, the 5-year relative survival rate for all type of cancers has increased significantly, and this is partially due to the successful development of targeted therapy [4]. New studies have reported that bee products have a prospective effect against cancer *in vivo* and *in vitro* [4]. Whereas while using radio- and chemo- therapy to get rid of cancerous cells, also they hurt normal cells and cause un-wanted side effects that restrict the treatment and effectiveness [4]. Honeybee venom (BV) has been traditionally used with the hope to cure several diseases such as cancer, arthritis, and rheumatism [6-10]. BV composed of many complex substances such as peptides (melittin, apamin, secapin, tertiapin, adolapin, and mast cell degranulating peptide), enzymes (phospholipase A2, hyaluronidase, acid phosphomonoesterase, lysophospholipase), active amines (histamine, dopamine, norepinephrine, serotonin), and other components, which have a comprehensive pharmaceutical properties to some extent [5-10]. Recent studies have revealed that the BV increases cytoplasmic Ca2+ and reactive oxygen species (ROS) and decreases mitochondrial membrane potential, which enhance the levels of caspase-3, PARP, FAS, p53, p21 and Bax, and reduces level of Bcl-2. The effects of BV on the DNA fragmentation are due to its ability to enhance the caspase-8 and caspase-9 through promoting caspase-3 activation [11-14]. Melittin, the principal active component of BV, alone induced apoptosis in human leukemic U937 cells through reducing Bcl-2, NF-kB and increasing caspase-3, [9,10]. Other studies showed that BV inhibited cell invasion and migration by suppressing the MMP-9 activity and expression through inhibiting of NF-κB via p38 MAPK and JNK signaling pathways in PMA-induced MCF-7 cells [11], and by suppressing MMP-2 and MMP-9 activity in mouse skin fibroblast and myelogenous leukemia cell lines [12]. A recent study showed that BV and melittin substantially decelerate capability of invasion and migration of breast cancer cells via inhibiting the EGF-induced MMP-9 expression by blocking the NF-kB and PI3K/Akt/mTOR pathway [13]. The protein, Ki-67, was suppressed by BV in SMMC-7721 cells [14]. The aim of the present review was to determine therapeutic activities of Bee venom (BV) and its mechanism action when used as a cancer diseases treatment.

Cancer Causes

Cancer diseases are serious health problem where it is the cause of death mainly correlated with ageing and lifestyle [4]. Interestingly, many observations have guided Researches to a novel hypothesis for cancer mechanism. We should bear in mind, though, that in the majority of cancer cases we cannot attribute the disease to a single cause. Cancer is caused by accumulated damage to genes. Such changes may be due to chance or to exposure to a cancer causing substance. The substances that cause cancer are called carcinogens. A carcinogen may be a chemical substance, such as certain molecules in tobacco smoke. The cause of cancer

may be environmental agents, viral or genetic factors. From cancer causing factors related to work and living environments include: asbestos fibers, tar and pitch, polynuclear hydrocarbons (e.g. benzopyrene), some metal compounds and ome plastic chemicals (e.g. Vinyl chloride). Bacteria and viruses can cause cancer: Helicobacter pylori (H. pylori, which causes gastritis), HBV, HCV (hepatitis viruses that cause hepatitis), HPV (human papilloma virus, papilloma virus, which causes changes eg. Cervical cells) and EBV (Epstein-Barr virus, the herpes virus that causes inflammation of the throat lymphoid). Radiation can cause cancer: ionising radiation (e.g. X-ray radiation, soil radon) and non-ionised radiation (the sun's ultraviolet radiation) [15].

The chief, that cancer occurs just on those multicellular creatures whose have complex wound-healing abilities. The activation of oncogene occurs in normal physiology and non- cancer pathology ways and not just in cancer. Wounds stimulate oncogenes of some cells and the other release cytokines to induct stem cells to cure the wounds [16].

Bee Venom Therapy

Bee venom and is naturalistic toxin could be helpful as an anti-tumor factor through the overexpression of DR3 and inactivation of NF-κB for the treating of lung tumor cells and drug resistant tumor cells [17]. [18] reported that BV inhibits proliferation of melanoma K1735M2 cells *in vitro*, as well as B16 melanoma, a transplantable solid melanoma in C57BL/6 mice, invivo. The proliferation of K1735M2 cells *in vitro* was inhibited by BV in a concentration- and time-dependent manner. The inhibition was indicated by the arrest of the cell cycle at the G1 stage, as detected by flow cytometric measurements. Bee venom induced apoptosis-like cell death as identified by histological observations and by DNA fragmentation. In the *in vivo* study, BV was injected intraperitoneally into the mice 24 h after they had been inoculated with B16 cells and inhibition of the solid tumor was observed. Treatment with BV at concentrations of 1 or 5 mg/ml decreased the viability of human lymphoma cell line HL-60 and human lymphocytes after 24h [19]. BV induced cell membrane lysis in HL-60 cells probably due to PLA2 present in the venom. BV induced DNA fragmentation and micronuclei in HL-60 cells and also increased the expression of phosphate and tensin homolog (PTEN), a tumor suppression protein, inducing cell cycle arrest in S phase, inhibiting the proliferation of these cells. The molecular mechanisms of apoptosis induced by BV in human breast cancer MCF-7 cells [20]. BV induced morphological changes and inhibited proliferation in a dose- and time-dependent way in MCF-7 cells. Besides, BV induced reactive oxygen species (ROS) production and dysfunction of mitochondria membrane potential, releasing cytochrome c, as well as an increase in the levels of caspase-9 e Poly (ADP-ribose) polymerase (PARP), leading cells to apoptotic death. Furthermore, it has been shown that BV induces DNA damage in these cells, as verified by the comet assay. The apoptotic mechanism generated by BV on human cervical cancer Ca Ski cells. BV induced morphological changes and decreased the percentage of viable Ca Ski cells in a dose- and timedependent manner. Flow cytometric analysis demonstrated that BV induced the production of ROS, increased the level of cytoplasmic Ca^2, reduced mitochondrial membrane potential which lead to cytochrome c release, and promoted the activation of caspase-3 followed by DNA fragmentation, leading to apoptosis. A decrease in the level of Bcl-2 (B-cell lymphoma 2) and an increase in the levels of Fas, p53, p21 and Bax (Bcl-2-associated X protein) were also observed. As demonstrated by [22] for MCF-7 cells, the also showed that BV promotes apoptosis of Ca Ski cells through the mitochondrial pathway. BV therapy induces both caspase-dependent and caspase-

independent apoptotic cell death through the stimulation of intracellular Ca (2+)- modulated intrinsic death pathway in human bladder tumor cells [15].

Active Components of Bee Venom

BV is known for being composed of a complex mixture of active peptides, (melittin, apamin, secapin, tertiapin, adolapin, and mast cell degranulating peptide) enzymes and amines [15,21]. It encourages membrane lysis and prevents tumor cell proliferation, and enhances cancer cell apoptosis by a rise in reactive oxygen species (ROS) and an increase in intracellular Ca2+. Melittin is effective against many cancer types involving leukemia ,liver ,lung ,renal, prostate ,bladder and mammary cancer. It has many important functions such as: calmodulin inhibitor [6], potent pore-forming factor, hyperactivates PLA2 in ras oncogene- transformed cells [22], produces cell membrane lysis and apoptosis [23], acts in different cell signaling pathways [24]. Besides the antitumoral effect bee venom based drugs like peptides and melittin and it can be used to fight cancer bee propolis. Propolis is a resinous material and one of the products of honeybees. It has been shown that propolis has many actions, including anti-inflammatory, antibacterial, antiviral, imunommodulatory and antiproliferative effects. A compound called caffeic acid phenethyl ester (CAPE), which is present in propolis, has anti-cancer and antioxidant properties [25].

Application Strategies for Cancer Treatment

In an *in vivo* study, [7] reported that, while intravenously injected, bee venom importantly frustrated mammary carcinoma metastasis significantly in murine injected also intravenously with this sort of cancer, when contrasted to control murine [7]. Whist, no variations in metastasis formulation were noticed when the venom was subcutaneously administered. Also, the tumor reduced in size while the venom was administered intratumorally, and murine survived longer than control, suggesting that the *in vivo* venom action counts on how the venom is injected. Bee venom shows a cytostatic impact in a dose- and time-dependent manner, prevents proliferation and produces apoptosis of SMMC-7721 human hepatoma cells [23]. Three strategies have been designed to reduce the adverse effects of melittin in not targeted tissues (1: coupling of melittin to an antibody or a targeting component; (2: development of shielded pro-cytolytic melittin systems; and (3: synthesis of melittin-transporting carriers [12]. Although melittin is the most studied and common bee venom peptide, its development for clinical applications stays at most in preclinical phases. Furthermore, flow cytometric analyses showed an accumulation of cells in the sub G1 phase of cell cycle in treated cells compared to control. It was also demonstrated that BV treatment resulted in an increase in the expression of Bax, a pro-apoptotic protein, and a decrease in the expression of Bcl-2, a protein that heterodimerizes with Bax, suppressing cell death. Besides that, treated cells showed an upregulation of caspase-3 activity, a protein that plays a role in the apoptotic pathway. The expression of COX-2 in NCI-H1299was lowcompared to the control, and it is known that COX-2 is frequently up-regulated in tumors [26], so that selective downregulation of COX-2 is an important strategy in the development of anti-tumor agents. In the study, administration of an immunoconjugate containing a melittin-like peptide (peptide 101), improved the survival of immune-deficient mice bearing subcutaneous human prostate carcinoma xenografts. The specific antibodypeptide101 conjugate also significantly inhibited tumor growth compared to the controls: unconjugated antibody or peptide alone. These new strategies can be used to decrease the non-specificity of some toxins and also to increase the action potential, since the immunoconjugates showed a greater

anti-cancer potential than the peptide alone. In the latest years, PLA2 isolated from BV has become of great interest due to its great anti-cancer potential [26]. The adjuvant treatment with bee venom-sPLA2 and phosphatidylinositol-(3,4)-bisphosphate (PtdIns(3,4)P2) was more effective than any of the single components in the blocking of tumor cell growth [25]. This adjuvant treatment had a synergistic effect together with potent cell lysis. The authors suggest that the observed cytotoxicity is due to the disruption of the membrane integrity, the abrogation of signal transduction and the generation of cytotoxic lyso-PtdIns 3,4 P2. They further demonstrated a reduction in the proliferation of the human cell kidney carcinoma cell line (A498) employing the adjuvant treatment with sPLA2 and PtdIns 3,4 P2, associated with a complete downregulation of PKB/Akt phosphorylation. The PI3-kinase/PKB/Akt pathway represents a central survival-related signal transduction pathway and its activation enhances cell survival and promotes tumor invasion [27]. Furthermore, treated cells exhibited a decrease of the epidermal growth factor receptor (EGFr). The tumor lysates formed after treating the cells with bv-sPLA2 and PtdIns (3,4) P2 enhanced the maturation of immunostimulatory human monocyte-derived dendritic cells. Such tumor lysates, which represent complex mixtures of tumor antigens showing potent adjuvant properties, meet all the requirements of a tumor vaccine for application in cancer immunotherapy [28].

Antitumor Bee Venom Melittin

Considering the venoms produced by arthropods, bee venom (BV) is the most studied regarding its anti-cancer activities, due mainly to two substances that have been isolated and characterized: melittin and phospholipase A2 (PLA2). Although melittin and PLA2 are the two major components in the venom of the species Apis mellifera and many studies have been published describing their antitumoral effects [28], BV is a very complex mixture of components that may cause other physiological effects. The first study was published by Havas in 1950 and, after 30 years, other groups started to carry on interesting studies about the cytotoxicity of bee venom upon tumor cells. Due to the promising effects found, publications have been constantly growing, showing not only the effects of BV in tumor cell lines, but also characterizing the signaling pathways through which the venom inhibits cellular proliferation, besides many interesting *in vivo* studies. Besides melittin and PLA2, other important components are histamines, catecholamines and polyamines. Melittin is by far the peptide with the greatest antitumor activity isolated from BV, acting in different ways upon the physiology of cancer cells. Melittin is a small and amphiphilic peptide containing 26 amino acid residues and is the principal toxin derived from the venom of the bee, Apis mellifera. The sequence of melittin is Gly-Ile-Gly-Ala- Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln [29]. Melittin exhibits anti-microbial activities and pro-inflammatory effects [30], besides inducing perturbations in the cell membrane and damage to enzyme systems [31,32]. Several cancer cells, including leukemia, renal, lung, liver, prostate, bladder, and mammary cancer cells, can be targets of melittin [25]. [22] has shown that melittin is capable of binding calmodulin, which has a role in cellular proliferation. [33] also showed that melittin is one of the most powerful inhibitors of calmodulin activity and, as such, is an inhibitor of cell growth and clonogenicity of human and murine leukemic cells [29,33,34]. Melittin inhibits the melanotropin receptor in M2R melanoma cell membranes [35]. Other studies suggest that melittin acts in the same manner as poreforming agents, killing malignant cells [36,37]. Most recent studies have shown that melittin kills tumor cells by apoptosis through several cancer cell death mechanisms, including the activation of caspase and matrix metalloproteinases (MMP) [38,39]. Besides the above-mentioned effects, melittin also leads to cell death by other means. [40], Considering the venoms

produced by arthropods, bee venom (BV) is the most studied regarding its anti-cancer activities, due mainly to two substances that have been isolated and characterized: melittin and phospholipase A2 (PLA2). Although melittin and PLA2 are the two major components in the venom of the species Apis mellifera and many studies have been published describing their antitumoral effects [28], BV is a very complex mixture of components that may cause other physiological effects. Melittin is a small and amphiphilic peptide containing 26 amino acid residues and is the principal toxin derived from the venom of the bee, Apis mellifera. [41] also reported that melittin, as a PLA2 activator, increased the calpain activity and cell necrosis in the hepatocellular carcinoma cell lines N1S1 and McA-RH7777 [41]. Melittin can be effective against leukemic cells, KG1a, CEM, and CEM/VLB100, which are relatively resistant to tumor necrosis factor a (TNF-a), a cytokine that activates cell death [42]. This is because melittin can activate low levels of cPLA2 activity in the KG1a cell line. Furthermore, melittinmediated cytolysis of U937 human monocytic leukemia cells is associated with the transient activation of endogenous phospholipase-D, which has been suggested to participate in an uncharacterized signal transduction pathway involved in the permeabilization of cancer cell membranes [43]. Melittin and a fragment of a melittin-conjugated hormone receptor (e.g., hecate) were shown to have an anti-tumor effect in ovarian and testicular tumors. The melittin fragment (hecate) conjugated to a 15-amino acid beta-chain of human chorionic gonadotropin (hCG) shown that the conjugates selectively destroys ovarian cancer cells (OVCAR-3) *in vitro* and OVCAR-3 cells engrafted in nude mice models *in vivo* [44]. In a group of animals treated by hecate-hCG-ß, tumor volume expressed as a percentage of increase was 199 _ 18.57% when compared with control animals (263.0 _ 21.72%). *In vitro* and *in vivo* studies with the melittin/avidin conjugate [38]. *In vitro* study, the conjugate presented a great cytolytic activity in DU 145 prostate cancer cells and SK-OV ovarian cancer cells that exhibited high MMP-2 activity. Besides, the conjugate showed low cytotoxicity in normal cells with low MMP-2 activity *in vitro*. *In vivo*, the tumors injected with the complex melittin/avidin were maintained with a smaller size comparing to non-treated tumors, indicating the great potential of this treatment in the fight against cancer. A significant antineoplastic effect was detected on the transplanted tumor in nude mice after an intratumoral injection of Ad-rAFP-Mel. An AdrAFP-Mel infection markedly induces cellular apoptosis, and Fas expression on Bel-7402 cells. They suggested this to be a possible molecular mechanism for the antitumorigenecity of AdrAFP-Mel even though more studies will be needed [45]. The effects of BV in NCI-H1299 lung cancer cells and verified that cells treated with 10mg/ml of venom for 24 h exhibited morphological changes typical of apoptotic cells, which was confirmed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, DAPI staining assay and DNA fragmentation detected via agarose electrophoresis [46]. A solution to increase melittin efficiency against tumors [47]. Tumor-specific antibodies can be used to target melittin to tumor cells. Melittin, at concentrations above 0.075mM, increased the intracellular Ca2 via L-type Ca2 channels, without evoking Ca2 release from stores, in MG63 human osteossarcoma cells in a concentrationdependent manner [48]. At concentrations of 0.5 and 1 mM, melittin killed 33% and 45% of the cells, respectively, through apoptosis. The cytotoxic effect of 1 mMmelittinwas completely reversed by pre-chelating cytosolic Ca2 with BAPTA (1,2-bis(o-aminophenoxy)ethane-N,N,N0,N0-tetraacetic acid), suggesting that apoptosis was due to an increase in intracellular Ca2. The combination of melittin with TRAIL may be a promising therapeutic approach in the treatment of TRAIL-resistant human cancer. Screened for HCC cell lines (hepatocellular carcinoma) with high expression levels of Rac1 (Rasrelated C3 botulinum toxin substrate 1) to study the relationship between the inhibitory effect of melittin on HCC metastasis and the Rac1-mediated signaling pathway both *in vitro* and *in vivo* [49] and found that Rac1 plays a crucial role in the control of HCC cell motility and metastasis. Melittin prevents HCC metastasis via

inhibition of Rac1. Melittin inhibited cell motility accompanied by a decrease in Rac1, ERK (extracellular signal-regulated kinase), and JNK (c-Jun N-terminal kinases) activity, suggesting that melittin acts through the suppression of Rac1-dependent pathways. In addition, the lung metastasis rate was significantly decreased in the melittin-treated nude mouse model LCID20. However, the authors showed that administration of high doses of melittin *in vivo* has its side effects, particularly liver injury and hemolysis. Considering that HCC T.E. 502 usually develops in a background of chronic liver injury and impaired liver function, caution will be required in the clinical application of melittin [50]. Finally, the authors commented that a mutation of Val 5 to Arg, Ala15 to Arg, and deletion of Leu15 in melittin significantly reduces its adverse side effect of hemolysis, but retains its antibacterial effect [49], showing that there are ways to overcome the toxic effects of melittin in the organism in order to perform future clinical trials. The effect of melittin in human leukemic U937 cells and the underlying intracellular signal transduction pathways involved in regulating apoptosis [51]. Melittin induced a dose-dependent inhibition of the proliferation in U937 cells. After 48 h of treatment with more than 2mg/ml melittin, U937 cells exhibited morphological characteristics of apoptosis, including cell shrinkage and nuclear condensation. These results suggest that melittin-induced apoptosis contributes to the decreased proliferation of U937 cells. This apoptotic response was associated with the upregulation of Bax and caspase-3 activation and downregulation of Bcl-2 and IAP (inhibitor of apoptosis) family members. Moreover, the inactivation of Akt displayed by cells treated with melittin also has an important role in the apoptosis process observed in these cells. In contrast, [52] showed that melittin-induced apoptotic death in human melanoma A2058 cells was by a caspase-independent manner, through generation of ROS and subsequent disruption of mitochondrial membrane potential transition, followed by the release of AIF (Apoptosis Inducing Factor) and EndoG (Endonuclease G) into the nucleus. Besides that, the role of Ca2 in cell death promoted by melittin was well established, once incubation of cells under calcium-free conditions effectively diminished BV-induced apoptosis. And treatment of cells with ouabain (a Na/K ATPase inhibitor) significantly blocked BV-induced cell death in human melanoma A2058 cells. Another mechanism of the melittin anti-tumor action was recently proposed. Melittin inhibited the enzymatic activity of matrix metalloproteinase-9 secreted by PMAinduced Caki-1 (renal carcinoma) cells. MMP-9 plays an important role in the invasion and metastasis of cancer cells, and melittin inhibited the levels of phospho-ERK and phospho-JNK, affecting the levels of AP-1 and NF-kB (nuclear factor-kB), which, in turn, led to suppression of MMP-9 expression [53]. Some recent studies have shown the anti-cancer potential of melittin using nanocarriers to deliver this cytolytic peptide specifically to tumor cells. Incorporated the nonspecific peptide melittin into the outer lipid monolayer of a perfluorocarbon nanoparticle which revealed that a dramatic reduction of tumor growth without any apparent signs of toxicity in mice [27].

Bee Venom Based Drugs

New peptides have been purified from bee venom and tested in tumor cells, exhibiting promising activities in the treatment of cancer. Some of these interesting molecules are the lasioglossins isolated from the venomof the eusocial bee Lasioglossum laticeps, which exhibited potent anti-microbial activity against both Gram-positive and Gram-negative bacteria, low hemolytic and mast cell degranulation activity, and a potency to kill various cancer cells *in vitro* [50]. A breakthrough in use of venom-peptide came to light after the development of captopril, an anti-hypertensive drug [54]. Captopril, an analogue of dipeptide Ala-Pro from snake *Bothrops jararaca* effectively bind to the active site of Angiotensin- converting enzyme (ACE).

ACE a key enzyme of renin- angiotensin system that converts angiotensin I to an active vasoconstrictor angiotensin II which regulates the volume of fluids in blood. Captopril an active ACE inhibitor is used in the treatment of hypertension. Following captopril footsteps, a tri-peptide Phe-Ala-Pro analogue enalapril was also developed [54]. Captopril was approved for its use in 1981, and since then many venom-peptide or venom-peptide analogues have been tested for various disease with few success (Table 1) [55]. Table 1 depicts various venom-based drug brands in the market today and its application against various disease and its application against various disease conditions along with mechanism of actions. Many technical advances during last decade have exemplified the importance of venom-peptide in drug discovery. Today, using advanced proteomics and genomics approach it is possible to isolate and characterize the potential anti- cancer peptides from venom pool. Further, structural analysis of isolated peptides and its interactions with protein or target molecule has revealed specific amino-acid domains that exhibit anti-proliferative effects, for example- importance of RGD domains in peptide including disintegrins family. In harmony RGD sequence presents in most of the disintegrins isolated from snake species and provides a structural scaffold for interactions with transmembrane receptor integrins (importance of disintegrins in anti- cancer therapy is discussed later in this review). Structural modifications of such domain lead to increase stability and elimination of liable peptide bonds that may comprise a peptide to enzymatic degradation. Venom-based peptides being small and easily modified, the prospect of using them or their analogues in cancer therapy is promising. [56] investigated the possible growth-inhibiting effects of bee venom applied alone or in combination with a cytotoxic drug, bleomycin, on HeLa and V79 cells *in vitro*. The adjuvant treatment caused a dose-dependent decrease in cell survival due to DNA damage, suggesting that BV might find a therapeutic use in enhancing cytotoxicity of the antitumor agent bleomycin.

Table 1: *Mechanism of action of the currently available venom based drugs*

Generic name (BRAND NAME)	Mechanism of action	Indication (Diseases
Captopril (CAPOTEN®)	Angiotensin-converting enzyme inhibitor Hypertension, Cardiac failure	
Enalapril (VASOTEC®)		
Exenatide (BYETTA®) (BY-DUREON®)	Glucagon-like peptide-1 receptor agonist Type 2 diabetes mellitus	
Ziconotide (PRIALT®)	Ca 2.2 channel antagonist	Management of severe chronic pain
Bivalirudin (ANGIOMAX®)	Reversible direct thrombin inhibitor	Anticoagulant inpercutaneous coronary intervention
Lepirudin (REFLUDAN®)	Binds irreversibly to thrombin	Anticoagulation in heparin- associated thrombocytopenia; Thromboembolic disease
Desirudin (IPRIVASK®)		
Tirofiban (AGGRASTAT®)	Prevents binding of fibrinogen, von Willebrand factor, and other adhesive	Acute coronary syndrome;

Eptifibatide (INTEGRILIN®)	ligands to GPIIb/IIIa	Percutaneous coronary intervention
Batroxobin (DEFIBRASE®)	Cleaves Aα-chain of fibrinogen	Acute cerebral infarction; unspecific angina pectoris; Sudden deafness; Gelification of blood for topical applications
Platelet gel (PLATELTEX-ACT®)		
Hemocoagulase (REPTILASE®)	Fibrinogenase	Prophylaxis and treatment or hemorrhage in surgery
Fibrin sealant (VIVOSTAT®)	Cleaves Aα-chain of fibrinogen; factor X and/or prothrombin activation	Autologous fibrin sealant in surg

Bee Venom and Immune Modulation

Immune reaction represents the primary defense system against non-self components including cancer cells in an organism. In brief, the initial response to non-self components is mediated by innate immune response cells such as natural killer cells (NK) and antigen presenting cells (APC) followed by adaptive immune response mediated by T cells and B cells. Snake, bee, and wasp venoms are known to enhance both innate and adaptive immune response in parallel to inducing a cytotoxic effect within the cells [57]. In harmony some studies, bee venom is known to the by-pass innate immune system and directly influences T cell activity or adaptive immune system [57]. Venom peptides such as cobra venom factor, cobra toxin, PLA2, melittin from snake *Naja naja atra* were observed to enhance NK cells activity by increasing the production of cell stress signaling protein interferon-g in immune-suppressed mice (IFN-g) [58]. In the same study, authors have also observed suppression of T cell proliferation especially CD8 T cells through inhibition of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) [58]. In contrast, bee venom PLA2 and melittin are reported to increase T cell response by increasing synthesis of cytokines IL-1 and TNF-a on monocytes [8].

Mechanism of Cancer Invasion

Compared to normal cells, cancer cells have the ability to circumvent the cell cycle checkpoint, responsible for maintaining intracellular balance *in vivo* [59]. Hallmarks of cancer include sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replication immortality, inducing angiogenesis, and resisting cell death. Besides, there is the introduction of two emerging hallmarks including deregulating cellular energetics and avoiding immune destruction [60]. When normal cells acquire the sustaining proliferative signaling, they will enable to get other hallmarks to become tumorigenic. So an ideal anti-cancer drug would be able to inhibit and/or block any one or some of the hallmarks.

Although the multistep process of cancer development is divided into three physiological stages, i.e., initiation, promotion, and progression of cancer, the distinction between the three stages in the dimension of time is artifactual. In a leading edge review on cancer by Hanahan and Weinberg, authors discuss six important hallmarks of cancer that provides a logical framework for understanding the chronic process of cancer [60]. Metastasis of cancer cells is the leading cause of increased mortality in cancer patients. As the

tumor grows slowly (usually less than $2mm^3$), the tumor cells are in a resting non-metastatic state at this time. In a sustained hypoxic environment, tumor cells need to get more nutrients and oxygen to survive and proliferate. Then tumor cells increase cellular hypoxia inducible factor (HIF) transcription, thereby increasing blood vessel secrete growth factors (such as VEGF-A, VEGF-C) and chemokines (such as TNFα) to active endothelial cells. These growth factors stimulate blood vessels and lymphatic vessels to up-regulate the expression of specific integrins, such as αvβ3, α1β1, and α5β1, etc. Then these integrins have the ability to recruit some matrix metalloproteinases (MMPs, such as MMP2, MMP9) to degrade the basement membrane of vessels, thereby promoting endothelial cell migration and remodeling to form new blood vessels and lymphatic vessels. The new blood vessels not only provide nutrition to the tumor cells to continue to grow but also transfer the metabolic waste. Meanwhile, the blood vessels and lymphatic vessels provide a path for the local and distant metastasis of cancer cells [16]. However, only about 0.01% of cancer cells are capable of entering the circulation of metastasis to spread to distant sites, but this process is fatal for most cancer patients [61]. Initiation of metastasis begins with migration and invasion of cancer cells from the primary tumor into the surrounding tissues. To invade tissues, cancer cells undergo a pathophysiological transformation involving changes in the membrane characteristics, the process known as epithelial-mesenchymal transition (EMT) [62]. This process is followed by migration and invasion of cancer cells into the lymphatic system, and into the secondary tissue. To successfully produce a secondary tumor, cancer cells again transform back into the epithelial cell by mesenchymal-epithelial transition (MET). MET is required for anchoring of cancer cells to the surrounding tissues. Thus, the process of cancer cell metastasis is governed by many factors such as growth factors (basic fibroblast cell growth factor (bFGF), vascular epithelial growth factors (VEGF), membrane ion channels, cytokines, cell adhesion molecules, and extracellular matrix (ECM) [62].

Antitumor and Future Prospects

Initial interaction of venom peptides with the target molecule is the first and foremost step which plays a key role in venom-peptide induce anti-cancer activity. Many of the examples used in this review emphasize the importance of this initial step. This interaction is guided either by the polarity of a molecule or by specific pharmacophore domain. Followed by initial interactions, peptides tend to exhibit their effects mostly by membrane interactions, although many other mechanisms such as intracellular peptide-protein interaction, peptide-DNA interactions are still existing. Peptide based targeted therapy has gained momentum in the last two decades. Smaller size and tumor penetrating ability makes peptides an ideal choice for targeting cancer cells. Among many, peptide based anticancer drugs in market three peptides Leuprolide, Octreotide and Goserelin have reached a global sale of 1 billion US dollar per year [71]. Today, a wide range of synthetic peptides have been tested for their anticancer abilities among which some are in clinical trials. Synthetic peptides such as Cilengitide, IM862, ATN 161, and angiotensin- [63], are being tested against various types of cancers. Synthetic peptides with cancer cell specificity can also be used to deliver a cytotoxic drug, or as hormone antagonist, or even as a vaccine in reducing or stimulating immune reactions within the system. Recent studies on molecular aspects of cancer development and progression have also offered prospects in both identifying potential targets and designing potential ligands [64]. Many of potential anti-cancer peptides such as stimuvax, primovax, melanotan are in clinical trials for elucidating efficiency, bioavailability, and metabolism [63, 65]. Therefore, Venom based peptides have a wide range of application in modern biology from diagnostic to the treatment of the disease [5]. For example, Chlorotoxin analog drug BLZ-

100 tagged with a fluorescence dye is able to lights up only cancer cells in brain tumor so that it can be precisely excised from the brain [5]. BLZ-100 is also known as "Tumor paint" is currently in phase 1b clinical study. Furthermore, advancement in proteomics and genomics approach has made possible to isolate and characterize the potential anticancer peptides from venom pool. High throughput screening using mass spectrometry is very useful to venom characterization as it can read low concentration peptides along with generating mass datasets that could be analyzed further. Similar sequencing RNA isolates from venom glands will provide generate the pool of expressed proteins and peptide database. Peptidomimetic approach promotes future use of peptide- based drugs by (i) increasing the stability of peptides against chemical degradation and enzymatic degradations leading to increasing lifetime within the biological system; (ii) decreasing the size of peptides making the molecule smaller and easily accessible for interaction; and (iii) by managing the electrostatic charge distribution and polarity of peptide that is important for peptide interactions and to make cancer treatment affordable to patients in order to create new design and discovery of anti- cancer drugs [3,66-68].

Conclusions

Bee venom (BV) is the most investigated venom among the other arthropod venoms because of its anti-proliferative potential. In fact, melittin, a major component of BV has been shown to possess greater antitumor activity. However, as reported earlier other components of the BV may be responsible for the biological activities of it. The anti-proliferative activity of BV involves in apoptosis because BV induced DNA fragmentation in cells, so it is indeed, the antiproliferative activity of BV involves induction of apoptosis and suggested that BV displays its pharmacological effects via induction of apoptotic pathway rather than necrosis. This suggests that the BV suppresses cell proliferation using another pathway apart from the suppression of DNA topoisomerase I. Immunohistochemical staining of the BV treated cell showed increased P16 levels indicating confirming its antiproliferative activity. BV caused decreased expression of Bcl-2, Ki-67, Cyclin D1, and P53 in HeLa and HT29 cells, indicating the apoptotic and apoptosis-promoting effects of BV.

Interaction of venom peptides with the target molecule is the first and foremost step which plays a key role in venom-peptide induced anti-cancer activity. Many of the examples used in this review emphasize the importance of this initial step. This interaction is guided either by the polarity of a molecule or by specific pharmacophore domain. Followed by initial interactions, peptides tend to exhibit their effects mostly by membrane interactions, although many other mechanisms such as intracellular peptide-protein interaction, peptide-DNA interactions are still exist. The targeted therapy drug with the specificity on certain molecule determines the limitation of its use, for example trastuzumab can only be used for HER2-positive breast cancer which occupies about 20% of breast cancer patients. Drugs derived from venom are no exception. Currently, venom-based drugs such as chlorotoxin and integrin αvβ3 drugs are used mainly in brain tumor and cancer with overexpressed αvβ3, respectively. Understanding mechanism of action of venom-peptides helps to curate a "staple peptide" with increased specificity in various types of cancer cells. As the molecular interaction of each venom peptide may vary, each peptide needs to be evaluated for its therapeutic potential. This review on venom peptides in cancer therapy fortifies our current understanding of their molecular mechanism of action and paves the way for better utilization of venom-based drugs.

Bibliography

1. Piek, T. (1986). Venoms of the Hymenoptera: Biochemical, pharmacological and behavioural aspects, Academic: London, 1986.

2. Elzinga, R. J. (2004). Fundamentals of entomology (6th edn), Pearson/Prentice Hall: Upper Saddle River, NJ, 2004.

3. Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J. & Jemal, A. (2012). *Global cancer statistics, 2012. CA Cancer J Clin., 65*(2), 87-108.

4. Siegel, R. L., Miller, K. D. & Jemal, A. (2017). Cancer Statistics. *CA Cancer J Clin., 67*(1), 7-30.

5. Hashim, D., Boffetta, P., La Vecchia, C., Rota, M., Bertuccio, P., Malvezzi, M. & Negri, E. (2016). The global decrease in cancer mortality: trends and disparities. *Ann Oncol., 27*(5), 926-933.

6. Pongsathon Premratanachai & Chanpen Chanchao (2014). Review of the anticancer activities of bee products. *Asian Pac J Trop Biomed., 4*(5), 337-344.

7. Son, D., Lee, J., Lee, Y., Song, H., Lee, C. & Hong, J. (2007). Therapeutic application of anti-arthritis, pain- releasing, and anti-cancer effects of bee venom and its constituent compounds. *Pharmacology & Therapeutics, 115*(2), 246-270.

8. Hider, R. C. (1988). Honeybee venom: A rich source of pharmacologically active peptides. *Endeavour, 12*(2), 60-65.

9. Garaj-Vrhovac, V. & Gajski, G. (2009). Evaluation of the cytogenetic status of human lymphocytes after exposure to a high concentration of bee venom *in vitro*. *Arh Hig Rada Toksikol., 60*(1), 27-34.

10. Gajski, G. & Garaj-Vrhovac, V. (2011). Bee venom induced cytogenetic damage and decreased cell viability in human white blood cells after treatment *in vitro*: a multi-biomarker approach. *Environ Toxicol. Pharmacol., 32*(2), 201-211.

11. Cherniack, E. P. (2010). Bugs as drugs, Part 1: Insects: the "new" alternative medicine for the 21st century? *Altern Med Rev., 15*(2), 124-135.

12. Orsolic, N. (2012). Bee venom in cancer therapy. *Cancer Metastasis Rev., 31*(1-2), 173-194.

13. Hong, S. J., Rim, G. S., Yang, H. I., Yin, C. S., Koh, H. G., Jang, M. H., *et al.* (2005). Bee venom induces apoptosis through caspase-3 activation in synovial fibroblasts of patients with rheumatoid arthritis. *Toxicon, 46*(1), 39-45.

14. Ip, S. W., Chu, Y. L., Yu, C. S., Chen, P. Y., Ho, H. C., Yang, J. S., *et al.* (2012). Bee venom induces apoptosis through intracellular Ca2+ -modulated intrinsic death pathway in human bladder cancer cells. *Int J Urol., 19,* 61-70.

15. Ip, S. W., Liao, S. S., Lin, S. Y., Lin, J. P., Yang, J. S., Lin, M. L., *et al.* (2008). The role of mitochondria in bee venom-induced apoptosis in human breast cancer MCF7 cells. *In Vivo, 22*(2), 237-345.

16. Joensuu, H., Hohenberger, P. & Corless, C. L. (2013). Gastrointestinal stromal tumour. *Lancet, 382*(9896), 973-983.

17. Meng, X., Zhong, J., Liu, S., Murray, M. & Gonzalez-Angulo, A. (2011). A new hypothesis for the cancer mechanism. *Cancer and Metastasis Reviews, 31*(1-2), 247-268.

18. Choi, K., Hwang, C., Gu, S., Park, M., Kim, J., Park, J., *et al.* (2014). Cancer Cell Growth Inhibitory Effect of Bee Venom via Increase of Death Receptor 3 Expression and Inactivation of NF-kappa B in NSCLC Cells. *Toxins, 6*(8), 2210-2228.

19. Xing Liu, Dawei Chen, Liping Xie & Rongqing Zhang (2002). Effect of honey bee venom on proliferation of K1735M2 mouse melanoma cells in-vitro and growth of murine B16 melanomas *in-vivo*. *Journal of Pharmacy and Pharmacology, 54*(8), 1083-1089.

20. Lee, Y. J., Kang, S. J., Kim, B. M., Kim, Y. J., Woo, H. D. & Chung, H. W. (2007). Cytotoxicity of honeybee (Apis mellifera) venom in normal human lymphocytes and HL-60 cells. *Chem Biol Interact., 169*(3), 189-197.

21. Dotimas, E. M., Hamid, K. R., Hider, R. C. & Ragnarsson, U. (1987). Isolation and structure analysis of bee venom mast cell degranulating peptide. *Biochim Biophys Acta., 911*(3), 285-293.

22. Habermann, E. (1972). Bee andwaspvenoms. *Science, 177*(4046), 314-322.

23. Moreno, M. & Giralt, E. (2015). Three Valuable Peptides from Bee and Wasp Venoms for Therapeutic and Biotechnological Use: Melittin, Apamin and Mastoparan. *Toxins, 7*(4), 1126-1150.

24. Hu, H., Chen, D., Li, Y. & Zhang, X. (2006). Effect of polypeptides in bee venom on growth inhibition and apoptosis induction of the human hepatoma cell line SMMC-7721 in-vitro and Balb/c nude mice *in-vivo. J Pharm Pharmacol., 58*(1), 83-89.

25. Heinen, T. & Gorini da Veiga, A. (2011). Arthropod venoms and cancer. *Toxicon, 57*(4), 497-511.

26. Dunn, R. D., Weston, K. M., Longhurst, T. J., Lilley, G. G., Rivett, D. E. & Hudson, P. J. (1996). Antigen binding and cytotoxic properties of a recombinant immunotoxin incorporating the lytic peptide, melittin. *Immunotechnology, 2*(3), 229-240.

27. Putz, T., Ramoner, R., Gander, H., Rahm, A., Bartsch, G. & Thurnher, M. (2006). Antitumor action and immune activation through cooperation of bee venom secretory phospholipase A2 and phosphatidylinositol-(3,4)- bisphosphate. Cancer Immunol. *Immunother., 55*(11), 1374-1383.

28. Putz,T., Ramoner, R., Gander, H., Rahm, A., Bartsch, G., Bernardo, K., Ramsay, S. & Thurnher, M. (2007). Bee venom secretory phospholipase A2 and phosphatidylinositol homologues cooperatively disrupt membrane integrity, abrogate signal transduction and inhibit proliferation of renal cancer cells. *Cancer Immunol. Immunother., 56*(5), 627-640.

29. Coffer, P. J., Jin, J., Woodgett, J. R. (1998). Protein kinase B (c- Akt): a multifunctional mediator of phosphatidylinositol 3- kinase activation. *Biochem. J., 335*(Pt-1), 1-13.

30. Ownby, C. L., Powell, J. R., Jiang, M. S. & Fletcher, J. E. (1997). Melittin and phospholipase A2 from bee (Apis mellifera) venom cause necrosis of murine skeletal muscle *in vivo. Toxicon, 35*(1), 67-80.

31. Gevod, V. S. & Birdi, K. S. (1984). Melittin and the 8-26 fragment. Differences in ionophoric properties as measured by monolayer method. *Biophys. J., 45*(6), 1079-1083.

32. Sumikura, H., Andersen, O. K., Drewes, A. M. & Arendt-Nielsen, L. (2003). A comparison of hyperalgesia and neurogenic inflammation induced by melittin and capsaicin in humans. *Neurosci. Lett., 337*(3), 147-150.

33. Wade, D., Boman, A., Wåhlin, B., Drain, C. M., Andreu, D., Boman, H. G. & Merrifield, R. B. (1990). All-D amino acid-containing channel forming antibiotic peptides. *Proc. Natl. Acad. Sci. U.S.A., 87*(12), 4761-4765.

34. Chueng, W. Y. (1982). Calmodulin: an overview. *Fed. Proc., 41,* 2253-2257.

35. Hait, W. N., Cadman, E., Benz, C., Cole, J. & Weiss, B. (1983). Inhibition of growth of L1210 cyclic leukemic cells by inhibitors of nucleotide phosphodiesterase and calmodulin. *Cancer Res., 2,* 5-9.

36. Hait, W. N., Grais, L., Benz, C. & Cadman, E. C. (1985). Inhibition of growth of leukemic cells by inhibitors of calmodulin: phenothiazines and melittin. *Cancer Chemother. Pharmacol., 14*(3), 202-205.

37. Lee, G. L. & Hait, W. N. (1985). Growth inhibition of C6 astrocytoma cells by inhibitions of calmodulin. *Life Sci., 36,* 347-354.

38. Gest, J. E. & Salomon, Y. (1987). Inhibition by melittin and fluphenazine of melanotropin receptor function and adenylate cyclase in M2R melanoma cell membranes. *Endocrinology, 121*(5), 1766-1772.

39. Duke, R. C., Witter, R. Z., Nash, P. B., Young, J. D. E. & Ojcius, D. M. (1994). Cytolysis mediated by ionophores and pore-forming agents: role of intracellular calcium in apoptosis. *FASEB J., 8*(2), 237-246.

40. Shaposhnikova, V. V., Egorova, M. V., Kudryavtsev, A. A., Levitman, M. Kh. & Korystov, Y. N. (1997). The effect of melittin on proliferation and death of thymocytes. *FEBS Lett., 410*(2-3), 285-288.

41. Holle, L., Song, W., Holle, E., Wei, Y., Wagner, T. & Yu, X. (2003). A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells *in vitro* and *in vivo. Int. J. Oncol., 22*(1), 93-98.

42. Moon, D. O., Park, S. Y., Heo, M. S., Kim, K. C., Park, C., Ko, W. S., *et al.* (2006). Key regulators in bee venom-induced apoptosis are Bcl-2 and caspase-3 in human leukemic U937 cells through downregulation of ERK and Akt. *Int. Immunopharmacol., 6*(12), 1796-1807.

43. Veiseh, O., Sun, C., Fang, C., Bhattarai, N., Gunn, J., Kievit, F., *et al.* (2009). Specific targeting of brain tumors with an optical/ magnetic resonance imaging nanoprobe across the blood- brain barrier. *Cancer Res., 69*(15), 6200-6207.

44. Arora, A. S., de Groen, P. C., Croall, D. E., Emori, Y. & Gores, G. J. (1996). Hepatocellular carcinoma cells resist necrosis during anoxia by preventing phospholipase-mediated calpain activation. *J. Cell Physiol., 167*(3), 434-442.

45. Wu, Y. L., Jiang, X. R., Newland, A. C. & Kelsey, S. M. (1998). Failure to activate cytosolic phospholipase A2 causes TNF resistance in human leukemic cells. *J. Immunol., 160,* 5929-5935.

46. Saini S. S., Allore, B., *et al.* (1999). Exceptionally long CDR3H region with multiple cysteine residues in functional bovine IgM antibodies. *Eur. J. Immunol., 29*(8), 2420-2426.

47. Gawronska, B., Leuschner, C., Enright, F. M. & Hansel, W. (2002). Effects of a lytic peptide conjugated to beta HCG on ovarian cancer: studies *in vitro* and *in vivo. Gynecol. Oncol., 85*(1), 45-52.

48. Li, B., Gu, W., Zhang, C., Huang, X. Q., Han, K. Q. & Ling, C. Q. (2006). Growth arrest and apoptosis of the human hepatocellular carcinoma cell line BEL-7402 induced by melittin. *Onkologie, 29*(8-9), 367-371.

49. Jang, M. H., Shin, M. C., Lim, S., Han, S. M., Park, H. J., Shin, I., *et al.* (2003). Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. *J. Pharmacol. Sci., 91*(2), 95-104.

50. Russell, P. J., Hewish, D., Carter, T., Sterling-Levis, K., Ow, K., Hattarki, M., *et al.* (2004). Cytotoxic properties of immunoconjugates containing melittin like peptide 101 against prostate cancer: *in vitro* and *in vivo* studies. *Cancer Immunol. Immunother., 53*(5), 411-421.

51. Chu, S. T., Cheng, H. H., Huang, C. J., Chang, H. C., Chi, C. C., Su, H. H., *et al.* (2007). Phospholipase A2-independent Ca2| entry and subsequent apoptosis induced by melittin in human MG63 osteosarcoma cells. *Life Sci., 80*(4), 364-369.

52. Liu, S., Yu, M., He, Y., Xiao, L., Wang, F., Song, C., *et al.* (2008). Melittin prevents liver cancer cell metastasis through inhibition of the Rac1-dependent pathway. *Hepatology, 47*(6), 1964-1973.

53. Moon, D. O., Park, S. Y., Choi, Y. H., Kim, N. D., Lee, C. & Kim, G. Y. (2008). Melittin induces Bcl-2 and caspase-3-dependent apoptosis through downregulation of Akt phosphorylation in human leukemic U937 cells. *Toxicon, 51*(1), 112-120.

54. Tu, W. C., Wu, C. C., Hsieh, H. L., Chen, C. Y. & Hsu, S. L. (2008). Honeybee venom induces calcium-dependent but caspase-independent apoptotic cell death in human melanoma A2058 cells. *Toxicon, 52*(2), 318-329.

55. Park, J. H., Jeong, Y. J., Park, K. K., Cho, H. J., Chung, I. K., Min, K. S., *et al.* (2010). Melittin suppresses PMA-induced tumor cell invasion by inhibiting NF-κB and AP-1-dependent MMP-9 expression. *Molecules and Cells, 29*(2), 209-215.

56. Saghar Soman, Sajith Nair, Aneesh Issac D. S. Arathy K. P. Niyas M. Anoop E. Sreekumar (2009). Immunomodulation by duck defensin, Apl_AvBD2: *In vitro* dendritic cell immunoreceptor (DCIR) mRNA suppression, and B- and T-lymphocyte chemotaxis. *Molecular Immunology, 46*(15), 3070-3075.

57. Cerovský, V., Budesínský, M., Hovorka, O., Cvacka, J., Voburka, Z., Slaninová, J., *et al.* (2009). Lasioglossins: three novel antimicrobial peptides from the venom of the eusocial bee Lasioglossum laticeps (Hymenoptera:Halictidae). *Chembiochem, 10*(12), 2089-2099.

58. Russell, P. J., Hewish, D., Carter, T., Sterling-Levis, K., Ow, K., Hattarki, M., *et al.* (2004). Cytotoxic properties of immunoconjugates containing melittin-like peptide 101 against prostate cancer: *In vitro* and *in vivo* studies. *Cancer Immunol. Immunother., 53*(5), 411-421.

59. Orsolic, N. (2009). Potentiation of bleomycin lethality in HeLa and V-79 cells by bee venom. *Arh. Hig. Rada. Toksikol., 60*(3), 317-326.

60. Bailey, H. H., Levy, D., Harris, L. S., Schink, J. C., Foss, F., Beatty, P. & Wadler, S. (2002). Gynecol. *Oncol., 85*(3), 464-468.

61. Liu, G., Oettel, K., Bailey, H., Ummersen, L. V., Tutsch, K., Staab, M. J., *et al.* (2003). Phase II trial of perillyl alcohol (NSC 641066) administered daily in patients with metastatic androgen independent prostate cancer. *Invest. New Drugs, 21*(3), 367-372.

62. Meadows, S. M., Mulkerin, D., Berlin, J., Bailey, H., Kolesar, J., Warren, D. & Thomas, J. P. (2002). Phase II trial of perillyl alcohol in patients with metastatic colorectal cancer. *Int. J. Gastrointest. Cancer, 32*(2-3), 125-128.

63. Terra, R. M., Guimaraes, J. A. & Verli, H. (2007). Structural and functional behavior of biologically active monomeric melittin. *J. Mol. Gr. Model., 25*(6), 767-772.

64. Hanahan, D. & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell, 144,* 646-674.

65. Lee, W. R., Kim, K. H., An, H. J., Kim, J. Y., Lee, S. J., Han, S. M., *et al.* (2014). Apamin inhibits hepatic fibrosis through suppression of transforming growth factor beta1-induced hepatocyte epithelial-mesenchymal transition. *Biochem. Biophys. Res. Commun., 450*(1), 195-201.

66. Wu, J. M., Jan, P. S., Yu, H. C., Haung, H. Y., Fang, H. J., Chang, Y. I., Cheng, J. W. & Chen, H. M. (2009). Structure and function of a custom anticancer peptide, CB1a. *Peptides, 30*(5), 839-848.

67. Koehn, F. E. (2008). High impact technologies for natural products screening. *Prog. Drug. Res.*, *65*(175), 177-210.

68. Yaşar Gülmez, Ali Aydın, İlyas Can, Şaban Tekin & Ercan Cacan (2017). Cellular toxicity and biological activities of honey bee (Apis mellifera L.) venom. *Marmara Pharmaceutical Journal, 21/2,* 251-260.