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## **Bee Venom and its Potential to Treat Cancer**

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## **Abstract**

**Background:** Bee venom therapy (BVT) is a biotoxin, using bee products like honey, pollen, and bee venom to prevent or cure illness and enhance healing. It also helps to treat many diseases like arthritis, back pain, skin diseases, multiple sclerosis and recently have been found to have anticancer activities in treating tumors, including liver, lung, kidney, prostate, bladder, mammary cancer cells and leukemia cells.

**Objectives:** The aim of this report is to figure the major components, potential mechanisms and side effect of BVT in treating cancer cells.

**Results:** BVT significantly inhibited proliferation MDA-MB-231 cells in which the targets of BV peptides are melittin and phospholipase A2 (PLA2). bee venom caused concentration-dependent growth inhibition in K1735M2 cells, moreover, bee venom inhibited primary tumor growth of B16 melanoma cells on the 6 mice after intraperitoneal injection.

**Conclusion:** BVT against cancer have shown significant efficacy of inducing apoptosis, necrosis, mitochondrial disruption blocking of angiogenesis, cell cycle arrest and inhibition of cancer cell metastasis and invasion. Although, bee venom has a lot of therapeutic benefits, however, as any treatment it has many side effects such as local itching, swelling and anaphylactic shock so it must be used with a great caution.

## **Introduction**

Bee venom therapy (BVT) is a biotoxin or api-toxin synthesized and secreted from the venom gland located in the abdominal cavity. It is a very complex mixture of natural products removed from honey bee which contains various pharmaceutical properties, such as several biologically active peptides, including melittin (a major component of bee venom apamin, mast cell degranulating peptide, and enzymes: phospholipase A2, and hyaluronidase). As well as non-peptide components, such as, dopamine, histamine, and norepinephrine. Some of these peptides and non-peptides exhibit anti-inflammatory, antioxidant, and anti-apoptosis properties.<sup>(1,2)</sup> There are three forms of BVT: live bee sting, bee venom injection and bee venom acupuncture (BVA). Bee venom(BV) as a non-steroidal anti-inflammatory drug has been used traditionally for the relief of pain and the treatment of chronic inflammatory diseases, such as arthritis, rheumatism, pain, skin diseases, and multiple sclerosis, as well as in recent times BV is used in the treatment of tumors, naturalistic yields are weaker but they are much safer.<sup>(1,2,3)</sup> Several cancer cells, including liver, lung, kidney, prostate, bladder, and mammary cancer cells and leukemia cells, can be targets of BV peptides such as melittin and phospholipase A2(PLA2). The cell cytotoxic effects through the activation of PLA2 by melittin have been suggested to be the significant mechanism for the anti-cancer activity of BV, additionally by the induction of apoptotic cell death through several cancer cell death mechanisms.<sup>(1,2,3)</sup> The aim of this report is to mainly describe BV its major components, potential mechanism to treat cancer and its side effect.

## **Materials and methods**

The MDA-MB-231 human breast cancer cells were obtained. The cancer cells were cultured in Dulbecco's modified Eagle medium (DMEM). Additionally, Human peripheral blood mononuclear lymphocytes (PBMLs) were isolated from the blood of the donors to differentiate it with the cancer cells for Raman spectroscopic analysis. The cancer cells were cultured and treated with BV. Moreover, to identify the apoptotic effect of BV in MDA-MB-231 cells, the TUNEL assay was performed. However, in the second study, the K1735M2 mouse melanoma cells and B16 melanoma cells were experimented in vivo and in vitro.

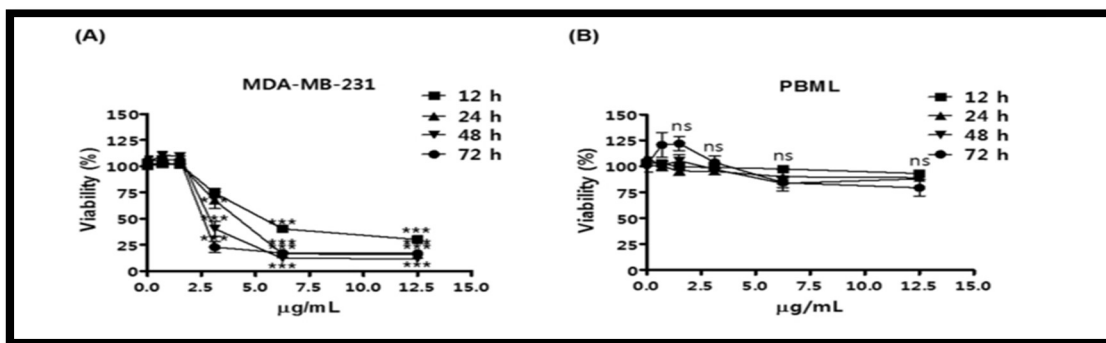
## Results

BV is a complex mixture taken from honeybee, which possesses various peptides including melittin, apamin, adolapin, PLA2, and hyaluronidase. Moreover to non-peptide components such as histamine, dopamine and norepinephrine. Some of the constituents exhibit anti-inflammatory, antioxidant, and anti-apoptosis properties the main constituents are discussed in Table 1.<sup>(3)</sup>

**Table 1:** The major components of bee venom and their biological effects.<sup>(3)</sup>

<b>Major components</b>	<b>Biological effects</b>
Melittin	-Anti-inflammatory, antibacterial, antifungal, anti-arthritis and anti-nociceptive effects -Cytotoxic effects against cancer cells -Activation of PLA2
Apamin	-Anti-inflammatory and anti- nociceptive effects -Cytotoxic effects against cancer cells
PLA2	-Anti-inflammatory effects -Cytotoxic effects against cancer cells
Hyaluronidase	-Immune response -Increase in capillary permeability
Adolapin	-Anti-inflammatory and analgesic effect -Inhibition of PLA2

BV significantly inhibited the proliferation of MDA-MB-231 cells as seen in figure A, as well as the study explored effects of BV in Peripheral Blood Mononuclear Lymphocytes(PBMLs) as seen in figure B.<sup>(5)</sup> Differences in Raman spectra between the control and BV treated cells correlated with the cellular events during apoptosis.



**Figure A:** Shows the effects of bee venom on the viability of human MDA-MB-231 breast cancer cells, Bee venom significantly inhibited the proliferation of MDA-MB-231 cells.<sup>(5)</sup>

**Figure B:** Shows the peripheral blood mononuclear lymphocytes (PBMLs). Both MDA-MB-231 and PBML were then treated with various doses of BV (0.7, 1.5, 3.0, 6.0 and 12.5 µg/mL) for 12, 24, 48, and 72 h. BV did not show any significant cytotoxic effect on PBMLs till doses of 12.5 µg/mL and 72h incubation.<sup>(5)</sup> (Adopted from Jung, G., Huh, J., Lee, H., Kim, D., Lee, G., Park, H. and Lee, J. (2020). Anti-cancer effect of bee venom on human MDA-MB-231 breast cancer cells using Raman spectroscopy.)

Additionally, to identify the apoptotic effect of BV in MDA-MB-231 cells, the TUNEL assay was performed and showed positive cells with small, dense, and fragmented morphology emitting yellow fluorescence, whereas the PI stained nucleus in the control cells exhibited round morphology and emitted red fluorescence. Moreover, to the results regarding the Raman spectrum for the cells treated with 3.0 µg/mL of BV for 48 h corresponded to the Raman bands assigned to DNA and protein, thus demonstrated a decrease in signal intensity, which was attributed to nuclear fragmentation and protein degradation. The results of Raman spectroscopy showed good agreement with tunnel assay<sup>(5)</sup> Moreover in the second study, in vitro experience when observed with a phase-contrast microscope, the K1735M2 melanoma cells formed as sheet of polygonal cells, the cells treated with the bee venom showed destruction of a sheet of polygonal cells.<sup>(6)</sup> As well as to the impact of bee venom on the growth of implanted B16 melanoma, the treatment with bee venom inhibited primary tumor growth of B16 melanoma cells on the 6 mice after intraperitoneal injection.<sup>(6)</sup>

## Discussion

Natural products such as BV and its potential mechanisms to treat cancer due to mainly two substances that have been isolated: melittin (an amphiphilic peptide) present in BV 50% -70% and phospholipase A2 (PLA2). Main activity of BV against

cancer is to inhibit tumor cell growth, metastasis and induce apoptosis.<sup>(1,2)</sup> Melittin's action was thought to involve membrane pore formation, resulting in the disruption of the membrane or both as well as is an antimicrobial and hemolytic activity. Melittin can enter the phospholipid bilayers and exhibit surfactant activity. Cell death was correlated with phospholipase A2 activation, since melittin was reported to be an activator of PLA2 both endogenously in intact cells, as well as in vitro assays. The activation of PLA2 might have a cytotoxic effect on cancer cells. The TNF- $\alpha$ -induced activation of cytosolic PLA2 is an important component of the signaling pathway leading to cell death.<sup>(1,2)</sup> Although the therapeutic advantages of BV has been established, its safety profile is a significant limiting consideration, because immune responses to bee venom therapy can extend from inconsiderable skin reactions that resolve over several days to life-threatening reactions such as anaphylaxis.<sup>(4)</sup> Venom concentration and frequency of venom administration can influence the severity and rate of incidence of adverse effects resulting from bee venom Acupuncture. <sup>(4)</sup> BV inhibited proliferation or had a cytostatic effect on K1735M2 mouse melanoma cells in-vitro. As the bee venom concentration increases, the relative percentage of inhibition of K1735M2 cells also increases. The in-vivo experiment of B16 cell line indicated that bee venom played the successful completion of primary tumor proliferation and also had a beneficial therapeutic potential for treatment of a solid tumor. The effect might be due to its cytostatic activity against tumor cells.<sup>(6)</sup>

## **Conclusion**

In conclusion, BV have been discovered to have anticancer activities in different cancer cell lines involving breast, liver and prostate. BV and its main peptide melittin, apamin and PLA2 are attractive candidates for cancer therapy. They have shown significant efficacy of inducing apoptosis of the tumor cells were found to be the possible mechanism by which the bee venom inhibited tumor growth in-vitro. It can also block the angiogenesis. The cell also enhanced necrosis, mitochondrial disruption cycle arrest, inhibition of cancer cell metastasis and invasion. Although, bee venom has a lot of therapeutic benefits, however, as any treatment it has side effects so it must be used with a great caution.

## **Future work**

Numbered findings and researches done suggested that BV can be used as a chemotherapeutic agent against tumors. Furthermore, several further researches are still needed to further improve the outcome of BV in cancer therapy. The clinical translation of BV is still a long way to be achieved, but the current results suggest optimism for the future.

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