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Therapeutic Efficacy of Bee Venom Conjugated Silver Nanoparticles against Breast Cancer

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ABSTRACT

Background: The Asian honey bee, *Apis cerana*, is a crucial species for honey production and pollination across Asia. Bee venom, produced by specialized glands and delivered via stingers, has demonstrated therapeutic potential due to its bioactive components. This study evaluates the cytotoxic effects of bee venom-conjugated silver nanoparticles (BVNPs) on the MCF-7 human breast cancer cell line.

Methods: BVNPs were synthesized through a green bio-reduction method, where *Apis cerana* venom was mixed with a silver nitrate ($AgNO_3$) solution. Dynamic Light Scattering (DLS) analysis revealed a Z-average mean diameter of 171.3 nm with a polydispersity index (PdI) of 0.493, while Transmission Electron Microscopy (TEM) images confirmed that the nanoparticles were well-dispersed and predominantly spherical. To assess the cytotoxicity of BVNPs, MCF-7 cells were treated with varying concentrations (2, 4, 6 and 8 µg/mL) of BVNPs for 48 hours. Apoptosis and necrosis were quantified using annexin V-FITC/PI staining and flow cytometry.

Result: Our findings indicated a concentration-dependent increase in apoptotic cell death, highlighting BVNPs' efficacy in inducing apoptosis without significantly affecting healthy cells. This study suggests that bee venom-conjugated silver nanoparticles offer a promising, biocompatible therapeutic strategy for breast cancer treatment. The findings underscore the need for further *in vivo* studies to validate the safety and efficacy of BVNPs and to explore their potential integration into current oncological treatment regimens.

Key words: *Apis cerana*, Cancer cell line, Nanotechnology, Venom.

INTRODUCTION

Asian honey bee, *Apis cerana*, is a widespread species of honey bee that is mainly related to honey production and pollination services in most parts of Asia (Koetz, 2013; Islam *et al.*, 2023). Bee venom, also known as apitoxin, is produced by the venom glands of bees in the abdominal cavity and is injected into target animals or humans through their stingers. It has the potential to trigger an immune response and cause localized inflammation (Varol *et al.*, 2022). Bee venom mostly consists of mast cell degranulating peptides, enzymes (*e.g.*, hyaluronidase and phosphatase A2), low-molecular-weight active amines, apamin andamphipathic polycationic peptides (*e.g.*, amines and melittins). Apitherapy, an injection of analgesic and anti-inflammatory medication, is one use of bee venom; others include immunotherapy, the treatment of Parkinson's disease andacupuncture. Multiple sclerosis and rheumatoid arthritis are among discussable human ailments that involve the use of bee venom for treatment. Additionally, bee venom possesses radioprotective and antimutagenic characteristics, among its many possible applications in the fight against cancer (Erkoc *et al.*, 2022; Sanjay *et al.*, 2024). Human malignancies like ovarian cancer, prostate cancer, breast cancer andhepatocellular carcinoma have shown prompt therapeutic responses to bee venom and melittin by triggering cell death and blocking cell cycle progression in a way that has little to no effect on healthy cells. A growing body of evidence from animal studies confirms that venom levels shown to be effective *in vitro* are also safe for use in humans (Moga *et al.*, 2018; Badawi, 2021; Kumar Vinoth *et al.*, 2025). Nevertheless, integrative research is necessary because cell lines, vehicles andoutcomes differ.

Breast cancer is among the most prevalent malignancies affecting women worldwide (Noreen *et al.*, 2015). About 2.3 million new cases of breast cancer were

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reported in 2020, with 685,000 deaths attributable to the disease. This placed it as the sixth greatest cause of cancer mortality globally, according to the American Cancer Society (Siegel *et al.*, 2021). Among the most common malignancies in the US, female breast cancer has the third-highest 5-year relative survival rate (including all stages) at 90% (The American Cancer Society Medical and Editorial Content Team, 2021). The survival rate, however, drops sharply as the stage advances. Breast cancer's exact cause is still a mystery, although established risk factors include heredity predisposition, the environment, sociobiological factors andthe physiology of patients (Noreen *et al.*, 2015; Waks, 2019; Siegel *et al.*, 2021).

The presence of multiple molecular markers provides the basis for subtyping breast cancer into three distinct subtypes: hormone receptor-positive/ERBB2 negative, ERBB2 positive andtriple-negative. Based on these subgroups, treatment approaches including hormone therapy, chemotherapy, surgery, radiation therapy, or a mix of these are chosen (Ridner, 2013). Medications that either reduce estrogen levels in the blood by blocking its conversion to androgens or that competitively inhibit estrogen's binding to its receptors are the mainstays of conventional endocrine treatment. These drugs can cause a variety of adverse effects, such as hot flashes, osteoporosis, arthralgia, myalgia anduterine cancer.

By interfering with mitosis and DNA replication, chemotherapy is a vital component of treatment plans for recurrence prevention in some cancer types. Asthenia, edema, myalgia andleukemia are some of the symptoms that patients experiencing this therapy report. Surgery for breast cancer can range from removing just the affected area to removing the whole breast along with the axillary lymph nodes, depending on how far the disease has spread (Ridner, 2013; Ducic *et al.*, 2014). When the lymphatic drainage system is disrupted or nerves are injured during surgery, it can result in lymphedema. Absolute survival benefit and risk of local recurrence are both improved by radiation therapy, especially radiation therapy administered after a mastectomy (Lyons and Sherertz, 2014). Still, research that followed patients for ten years found loco-regional recurrence and confirmed the presence of arm lymphedema, along with significant symptoms (Mignot *et al.*, 2022). Patients with cancer often turn to complementary and alternative medicine to lessen the impact of conventional treatment on their bodies. Many patients have found relief using natural remedies derived from plants and animals (Gajski *et al.*, 2017). Oncological diseases have provided a clinical setting for the evaluation of toxins that have evolved to harm other forms of life (Shapira and Benhar, 2010). In cancer radiotherapy, for example, botulinum toxin acts as an anesthetic while simultaneously inhibiting tumor development and inducing cell death in cancer cells (Grenda *et al.*, 2022).

The production of silver-based nanomaterials is expanding globally by around 830 tons per year, while the current production range is about 340-480 tons annually (Patel and Joshi, 2023). Research on the improved potential of biogenic AgNPs is need of hour thus, it was predicted that the formation of these nanoparticles using bee venom may be used as a cost-effective agent against alternative drugs.

MATERIALS AND METHODS

Honeybee venom nanoparticles preparation

Apis cerana venom was obtained from henan kaixiang biological technology Co., China andstored at 4°C in dark glass vials for further use. Five milligrams (5 mg) of bee venom were dissolved by vortexing in 1 ml of normal saline (NaCl 0.9%). Green silver nanoparticles were synthesized by bio-reduction of Ag^+ using a fresh suspension of bee venom. 5 mL of the bee venom was added drop by drop to an aqueous solution of $AgNO_3$ (50 mL, 0.1 mM/mL) and was stirred at 45-50°C for 30 min. The ultrasonication was applied to the mixed solution for 3 hours. Silver nitrate solution color was changed from colorless to deep brown, indicating the formation of honey bee venom conjugated AgNPs. The residual $AgNO_3$ was removed by dialysis against deionized water at 4°C. A study involving BALB/c mice injected with AgNPs of different sizes (20 nm and 50 nm) estimated the LD_{50} to be approximately 169 mg/kg and 354 mg/kg, respectively. Smaller nanoparticles (20 nm) exhibited higher toxicity than larger ones (50 nm).

Characterization of nanoparticles

The synthesis of bee venom nanoparticles (BVNPs) was confirmed by UV-Vis spectro-photometer in the range of 200-1000 nm wavelength. The absorption spectra were recorded with a Perkin-Elmer Lambda 40 B double-beam spectrophotometer using 1 cm matched quartz cells. Dynamic Light Scattering (DLS) is one of the famous techniques that rely on light scattering principles to measure the particle size and stability in the solution. The size of formed BVNPs was analyzed by zeta sizer (ZEN 3600, Malvern, UK). Structural characterization of BVNPs was done using transmission electron microscopy (TEM) (JEM-1011, JEOL, Akishima, Japan).

Cell culture

Cells of the human breast cancer cell line named michigan cancer foundation-7 (MCF-7) were cultured in 75 cm^2 tissue culture flasks containing RPMI 1640 media (Sigma) supplemented with 10% (v/v) fetal bovine serum (FBS). Cell culture flasks were incubated at 37°C, in a humidified (90%) and CO_2 (5%) containing atmosphere. Cells were routinely observed under a microscope, trypsinized andpassaged in a laminar flow hood, every second day in a proportion of 1:4. For passaging, firstly, the cell culture medium was removed andcells were washed with 15 ml of phosphate buffer saline (PBS). After washing, 4 ml of trypsin was added to the flask and spread evenly all over the bottom. After the removal of excessive trypsin, the flask was incubated for 2 minutes. Detachment of cells from the

bottom of the flasks was assured under the microscope andthe cells were suspended in RPMI 1640 media. Cells were also cultured in 6-well plates at a density of 1×10^6 cells/ml. Culture flasks and plates were placed in an incubator at 37°C with 5% CO_2 for 48 hours.

Apoptosis and necrosis assay

After 2 days, the cells were checked under a microscope andthe Annexin V-FITC/PI staining method was employed to assess and quantify apoptotic and/or necrotic cell death induced by BVNPs. Cells at a concentration of 1×10^6 cells/ml were treated with varying concentrations of BVNPs for 48 hours. Cells were treated with 2,4,6 and 8 µg/ml solution of BVNPs. Control cells, without any treatment, were also included for comparison. After 48 hours, the cells were washed with PBS and re-suspended in binding buffer (10 mM Hepes/NaOH pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$). FITC-conjugated Annexin V (Pharmingen, Becton Dickinson Co., San Diego, CA, USA) was added to the cell suspension andthe cells were incubated for 15 minutes in the dark at room temperature. After washing, the fluorescence intensity of FITC/PI was measured using a flow cytometer. Each experiment was done in triplicate.

RESULTS AND DISCUSSION

Synthesis and characterization of BVNPs

The Asian honey bee plays a significant role in honey production and pollination services across the globe (Koetz, 2013). The use of bee venom for immunotherapy, the treatment of Parkinson's disease, acupuncture, multiple sclerosis, rheumatoid arthritis andcancers has already been reported (Erkoc *et al.*, 2022). The safe use of bee venom as a therapeutic agent against some human cancers is usually brought about by triggering apoptosis and blocking the progression of the cell cycle (Moga *et al.*, 2018; Badawi, 2021). The use of biogenically synthesized silver nanoparticles against human malignancies is gaining the attention of researchers globally. In this study conjugation of silver nanoparticles with bee venom was proposed to have a significant role in the therapeutic management of breast cancer, which is the most prevalent cancer among women (Noreen *et al.*, 2015). Bee venom Nanoparticles (BVNPs) were successfully synthesized using a green synthesis approach. The color of the bee venom and aqueous solution of $AgNO_3$ was changed from colorless to deep brown, indicating the formation of BVNPs. DLS determined the particle size and stability of BVNPs in the solution. The Z-average mean diameter was 171.3 nm andthe polydispersity index (PdI) of formed nanoparticles was 0.493, as shown in Fig 1. TEM was done to identify the resulting nanoparticles' size, shape andmorphology. It reveals that the bee venom nanoparticles are well dispersed and mostly spherical, as shown in Fig 2. Bee venom's impact on normal cells varies across studies. For instance, in the A549 lung cancer cell line, bee venom inhibited cell proliferation with an IC_{50} value of 2 µg/mL, whereas normal lung cells (LL24) showed no significant growth inhibition, highlighting the selective cytotoxicity of bee venom. However, it's important to note that bee venom can induce cytogenotoxicity and oxidative stress in human peripheral blood lymphocytes at high concentrations, leading to DNA damage and cellular instability.

Apoptosis and necrosis assay

The DLS approach determined the particle size and stability of BVNPs in the solution. The Z-average mean diameter was 171.3 nm andthe polydispersity index (PdI) of formed nanoparticles was 0.493. TEM was done to identify the resulting nanoparticles' size, shape andmorphology. It reveals that the bee venom nanoparticles are well dispersed and mostly spherical. The extent of apoptosis was quantified by determining the percentage of Annexin V-positive cells. This measurement provides insights into the apoptotic response induced by different concentrations of BVNPs (Fig 3). This methodological approach provides a systematic means to evaluate the impact of Bee Venom on apoptosis and necrosis in the

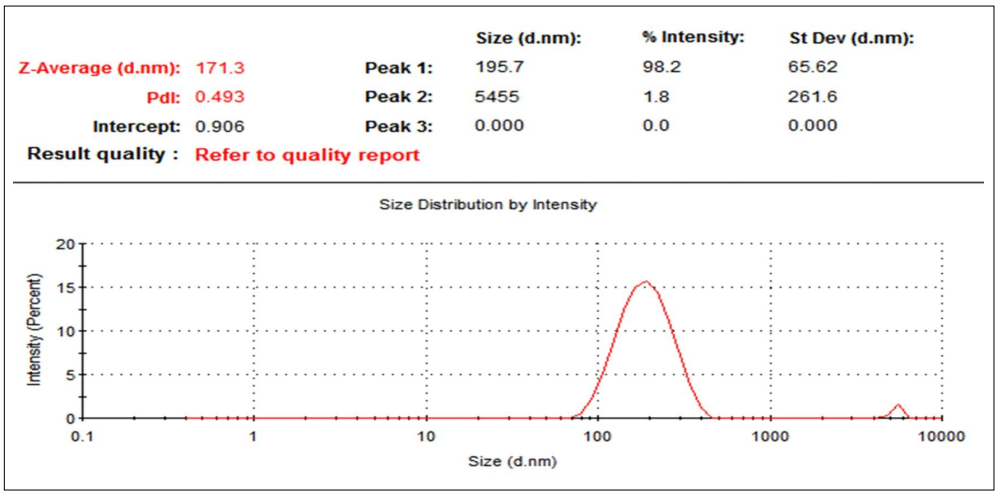

Fig 1: DLS analysis of synthesized Bee venom nanoparticle.

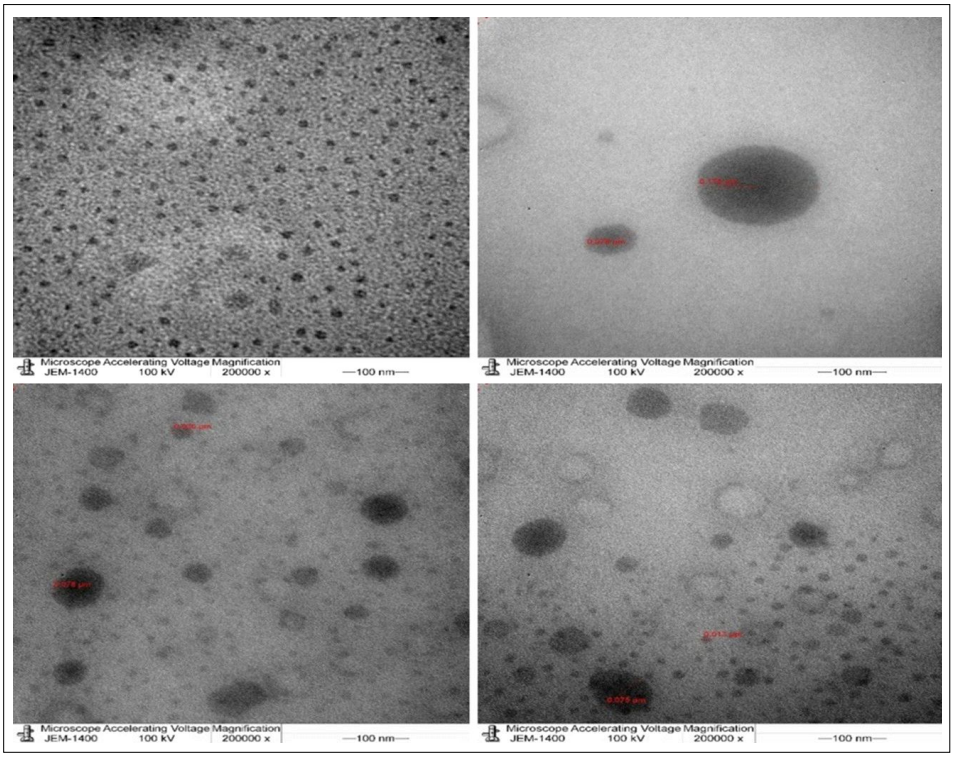

Fig 2: TEM images of Bee venom nanoparticles.

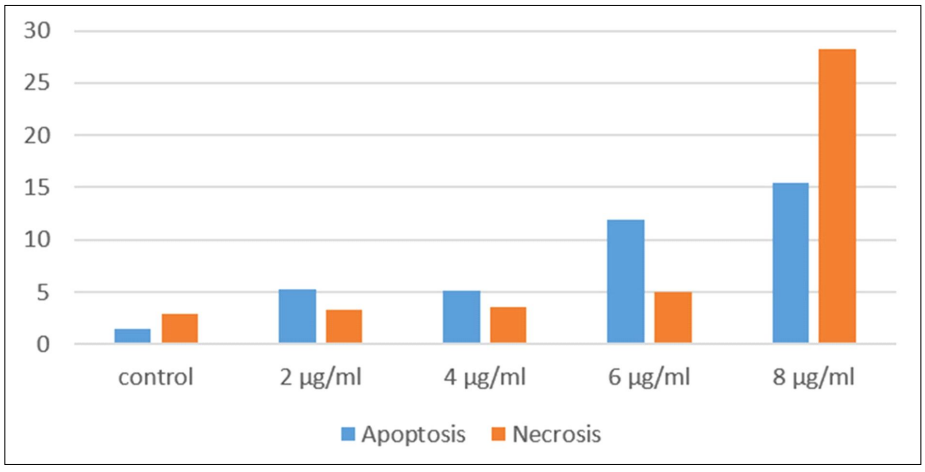

Fig 3: Apoptosis and Necrosis of MCF-7 cells after treatment with bee venom nanoparticles.

tested cell population, utilizing the Annexin V-FITC/PI staining method and flow cytometry analysis. Yes, the aggregation and intracellular accumulation of nanoparticles can pose significant risks to cellular health. While nanoparticles offer promising applications in drug delivery, imaging andtherapy, their behavior within biological systems, especially when aggregated, can lead to unintended toxicological effects.

CONCLUSION

The data obtained will contribute to understanding the apoptotic effects of BV and may have implications for future research and therapeutic applications.

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Data availability statement

This published article includes all the datasets generated or analyzed during this study.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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