Cytotoxic Insights of Underexplored *Apis cerana indica* Bee Venom: An In-Vitro and In-Silico Study on MCF-7 Breast Cancer Cells

Abstract

Bee venom (BV) or the apitoxin produced and released by a gland in the abdominal cavity of worker bees are studied extensively for their biological and therapeutic properties. The current study uses in vitro and in-silico methods to examine the cytotoxic activity of *A. cerana indica* bee venom against MCF-7 human breast cancer cells. The MTT assay showed potent cytotoxicity toward MCF-7 cells, with an IC_{50} value reflecting the effective reduction in cell viability. Additionally, molecular docking studies of melittin and apamin with cancer-associated proteins such as MUC16, Cathepsin D and Trefoil Factor 1 (TFF1) revealed strong binding affinities, supporting the cytotoxic effects observed in vitro. The key findings suggest that bee venom has ccytotoxic effects on breast oncogenic cells. These results reveal the potential therapeutic use of bee venom in cancer.

Keywords: *Apis cerana indica*, Bee venom (BV), MCF-7, Molecular Docking.

1. INTRODUCTION

Honeybees remain vital pollinators that contribute significantly to global food production, either directly or indirectly, with approximately one-third of our food supply reliant on their activities [1&2]. Beyond their role in agriculture, honeybee products, particularly bee venom, have garnered interest in medicinal science due to their diverse therapeutic properties. Apitherapy, the use of bee products for healing purposes, encompasses various techniques that leverage the health benefits of substances like honey, royal jelly, propolis and venom [3&4]. Despite the historical utilization of these products in ancient civilizations for medicinal purposes, modern Western medicine has seen a decline in their application, prompting a renewed exploration into their potential benefits [5] and because of the growing issue of medication resistance, which has made it necessary to look for new pharmacologically active compounds [6&7] .

Bee venom (BV), a colorless and acidic substance with a bitter taste [8] has gained wide focus necessitated by its varied pharmacological characteristics including antioxidant, antibacterial, anticancer and other immune modulatory properties. The existence of bioactive metabolites such as peptides, enzymes and amines, collectively play a role in its pharmacological effects [9]. The most important components of bee venom are melittin, is known for its membrane disrupting effects and other constituents such as phospholipase A2, apamin and adolapin have been shown to induce anti-inflammatory responses and reduce pro inflammatory cytokine production [10].

The most vital application of bee venom is its cytotoxic effect on cancer cells. Research has shown that bee venom can induce apoptosis and limit the propagation of cancer cell lines include MCF-7 breast cancer cell [11,12]. Studies on breast cancer cells have shown that bee venom disrupts cell membrane structure and promotes cell death [13]. The ability of bee venom to spare normal cells and selectively target cancer cells highlights the bee venom potential as a natural anticancer agent that could complement existing therapies.

In addition to invitro research, molecular docking was used to examine how bee venom peptides, melittin and apamin, interacted with proteins linked to cancer, including mucin, trefoil factor and cathepsin.

Apamin and Melittin are two of the most widely studied components of bee venom because of their potential for therapeutic use. The venom is a complex mixture of bioactive peptides and

enzymes consisting 18 amino acids, apamin is a small neurotoxic peptide that selectively blocks small-conductance Ca^{2+} activated K^{+} (SK) channels [14&15]. Melittin, a 26-amino-acid amphipathic peptide, has been shown to cause apoptosis, stop tumor growth and make cancer cells more sensitive to chemotherapy. It also exhibits potent cytolytic activity [9&16].

In cancer biology, proteins such as MUC16 (also known as CA125), Cathepsin D and Trefoil Factor 1 (TFF1) are critical targets. MUC16 is a high molecular weight glycoprotein overexpressed in ovarian and pancreatic cancers, playing a role in immune evasion and metastasis [17], Cathepsin D is a lysosomal aspartic protease involved in tumor progression through extracellular matrix remodeling and angiogenesis [18]. TFF1, classified within the trefoil factor family, contributes to mucosal healing and is frequently dysregulated in gastrointestinal and hormone-responsive cancers [19]. The interaction of bee venom peptides with these cancer-related proteins remains under explored and presents a promising avenue for therapeutic development. According to recent research, bee venom may also have anti-inflammatory and anticancer effects by modifying the pathways involved in cell migration and apoptosis.

The goal of the research was to explore the potential therapeutic characteristics of honey bee venom isolated from Indian bee *Apis cerana indica* which is reared largely in India when compared to other species. The research work is intended to establish the pharmacological benefits of bee venom, including its anticancer roles through in vitro assay. Additionally, in-silico molecular docking was carried out to evaluate the binding potential of melittin and apamin with breast cancer–associated targets, thereby supporting the observed cytotoxic effects mechanistically.

2. METHODOLOGY

2.1 Identification of Bee species

The honeybee species used to study the bee venom was identified and authenticated by Dr. V.R. Swaminathan from the Tamil Nadu Agricultural University (TNAU), based on its distinct morphological features. The honeybee was authenticated as *A. cerana indica* was used to obtain the bee venom.

2.2 Collection of Bee Venom

Bee venom was collected from *A. cerana indica* colonies using an electric venom collector (Model: SB-BVC, Hi-tech Natural Products (India) Ltd.), as per the method reported by Graaf et al. (2020). The venom was obtained by electrical stimulation at 12 V for 30 min per hive, ensuring minimal harm to the bees [21]. The venom was subsequently lyophilized and preserved at -20 °C for further use.

2.3 Anti-Cancer Activity

The bee venom induced cytotoxicity on MCF-7 breast carcinoma cells was assessed using the MTT assay, based on the method by Mosmann (1983). MCF-7 cells were cultured in DMEM containing 10% FBS and antibiotics. The cells were added in a 96-well plate at a density of 1×10^4 cells per well cultured at 37 °C for 24 h. Bee venom was prepared in PBS and serially diluted to final concentrations of 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5and 10 µg/mL, including experimental wells and four control groups a blank control (media without cells), a vehicle control (DMSO), a negative control (cells with media)and a positive control (3 Sodium Dodecyl Sulphate, SDS). The plate was returned to the CO_2 incubator and left for a fresh 3 hours at 37 °C, during which metabolically active cells converted the MTT into grandiloquent formazan chargers.

After incubation, 10 µl of MTT reagent (5 mg/mL) was introduced to each well and incubated for 3 h at 37 °C. The medium was carefully removed and the resulting formazan crystals were solubilized by adding 100 µl of DMSO. Absorbed value was measured at 570 nm using a plate reader. Cell viability and cytotoxicity were evaluated using the following formulas:

$$Cellviability = (Absorbanceoftreatedcells / Absorbanceofcontrolcells) \times 100 \quad (1)$$

$$Cellcytotoxicity = (AbsorbanceofcontrolcellsAbsorbanceoftreatedcells / Absorbanceofcontrolcells) \times 100 \quad (2)$$

Morphological changes indicative of apoptosis was observed under a light microscope, including chromatin condensation, cell shrinkage and membrane blistering.

2.4 In-silico Molecular Docking Analysis

2.4.1 Retrieval and Preparation of Protein and Ligand Structures

The 3D conformations of the targeted proteins such as MUC16 SEA domain (PDB ID: 6UVA) [23], Cathepsin D (PDB ID: 1LYB) [24] and Trefoil Factor 1 (TFF1) (PDB ID: 1HI7) [25] were taken from the RCSB Protein Data Bank (https://www.rcsb.org). The selected receptor structures were cleaned by removing water molecules, heteroatoms, co-crystallized ligands and polar compounds were added using BIOVIA Discovery Studio Visualizer 2025 [26]. The ligand structures of Apamin and Melittin were retrieved from the pubchem database (https://pubmed.ncbi.nlm.nih.gov/static-page/down_bethesda.htmL) [27] in SDF format and converted to PDBQT using Open Babel integrated within PyRx 0.8 [28]. The ligands were minimized prior to docking.

2.4.2 Molecular Docking

Molecular docking was carried out with AutoDock Vina implemented in PyRx 0.8. For each protein-ligand pair, a grid box was configured to enclose the known or predicted active site based on literature or co-crystallized ligands. Exhaustiveness was set to 8 and default docking parameters were used. The best binding pose was selected based on the lowest binding energy score (in kcal/mol).

2.4.3 Visualization and Interaction Analysis

The docked complexes were visualized using BIOVIA Discovery Studio to assess receptor-ligand interactions. Hydrogen bonds, hydrophobic contacts and electrostatic interactions were analyzed and recorded. 2D interaction diagrams and 3D surface views were generated to illustrate the molecular orientation and bonding behavior of Apamin and Melittin with each target protein.

3. RESULTS AND DISCUSSION

3.1 Anti-Cancer Activity

3.1.1 MTT Assay on MCF-7 Cells

The cytotoxic effects of bee venom on MCF-7 breast carcinoma cells were assessed using the MTT assay, which measures cell viability by quantifying mitochondrial activity. This assay focuses on the principle that viable cells reduce MTT, a yellow tetrazole to purple formazan crystals due to

their active mitochondria [29]. Thus, higher absorbance readings indicate greater cell viability, while lower readings suggest increased cytotoxicity. Cell viability and cytotoxicity were quantified using equations (1) and (2), respectively, to evaluate the venom's impact on cancer cell survival.

As presented in fig. 1 & fig. 2, bee venom exhibited a concentration-dependent reduction in cell viability, paralleled by a rise in cytotoxicity. At the highest concentration of 10 µg/mL, bee venom significantly reduced cell viability to 17.74 ± SE ($p < 0.05$), suggesting a potent cytotoxic effect. At intermediate concentrations, 5 µg/mL and 2.5 µg/mL, cell viability was also notably lowered, registering 15.86 ± SE and 15.78 ± SE, respectively. These results confirm bee venom's strong cytotoxic activity at higher doses.

In contrast, at the lowest concentrations (0.156 µg/mL and 0.078 µg/mL), cell viability remained relatively higher, around 65.33 ± SE ($p < 0.05$), indicating reduced cytotoxic impact. Cytotoxicity results aligned with this trend, reaching a maximum inhibition of 84.21 ± SE ($p < 0.05$) at 10 µg/mL, with comparable levels at 5 µg/mL (84.13 ± SE) and 2.5 µg/mL (82.25 ± SE), affirming bee venom's increasing cytotoxicity with dose escalation. Hence the IC_{50} value for cytotoxicity was found to cause 50% toxicity to the cancer cells at 0.365 µg of bee venom composition.

Significant morphological alterations were observed under a microscope in MCF-7 cells treated with bee venom. These changes included cell rounding, shrinkage and detachment, suggesting cell damage and potential induction of apoptosis. At lower concentrations, the cells maintained their typical epithelial morphology with minimal signs of damage, indicating lower cytotoxicity. Additionally, cell morphology observations in venom-treated cells revealed shrinkage, irregular shapes and severe membrane damage (fig. 5 - fig. 8), effects absent in the control cells (fig. 3 - fig. 4). These morphological changes further underscore bee venom's concentration-dependent cytotoxic impact on MCF-7 cells.

Assay controls further validated these outcomes hence the positive control (3% SDS) exhibited maximum cytotoxicity, with cell viability plummeting to 11.03 ± SE, as expected, while the untreated negative control retained 100% viability, affirming that healthy cells were unaffected. The vehicle control (DMSO) recorded a viability of 77.95 ± SE, confirming that the solvent had minimal impact on cell health.

These findings are consistent with previous studies, such as Kwon et al. (2022), who demonstrated that melittin, a primary component of bee venom, induces cell death in breast carcinoma cells by disrupting cell membranes and inhibiting critical pathways involved in cancer progression. Moreover, the apoptosis observed in microscopic analysis corroborates that bee venom induces cell death through both membrane disruption and apoptotic mechanisms, aligning with research by Hassani et al. (2021).

The findings of this study demonstrate that honeybee venom sourced from the *A. cerana indica* species exhibits concentration-dependent anticancer effects on MCF-7 cells. Higher concentrations of bee venom cause significant cell damage and induce apoptosis, supporting its potential as an anticancer agent. While further studies are necessary to determine selectivity and mitigate potential adverse effects, this research underscores bee venom's shows potential as a therapeutic option in cancer treatment.

3.2 Molecular Docking Analysis of Bee Venom Peptides Against Breast Cancer-Associated Targets

To further support the cytotoxic effect of bee venom observed in vitro, molecular docking studies were carried out using melittin and apamin. The two major peptides present in bee venom. These peptides were docked against cancer-associated proteins such as MUC16 (Mucin), Trefoil Factor 1 (TFF1) and Cathepsin D to evaluate their possible interaction and binding affinity.

The docking results showed that apamin exhibited the highest binding affinity with MUC16 (-8.0 kcal/mol), followed by Cathepsin D (-7.5 kcal/mol) and TFF1 (-7.0 kcal/mol). In the Apamin - MUC16 complex, apamin was localized in the SEA (Seaurchin Entrokinase agrin domain family) domain binding pocket and formed stable hydrogen bonds and electrostatic interactions with residues critical to MUC16's structural integrity (Fig. 9). In the Cathepsin D interaction (Fig. 10), Apamin was observed to bind within the active pocket of Cathepsin D, forming hydrogen bonds and polar contacts with surrounding residues, suggesting its potential to interfere with the protease's catalytic function. Apamin's docking with TFF1 revealed interaction across the homodimer's flexible linker, forming hydrogen bonds that may disrupt its role in epithelial healing and cancer cell migration (Fig. 11).

In comparison, melittin showed moderate interaction with MUC16 (-7.2 kcal/mol) and lower affinities with Cathepsin D and TFF1 (6.0 kcal/mol each). These values suggest that apamin has stronger and more stable interactions with breast cancer-associated proteins than melittin. In the Melittin-MUC16 complex (Fig. 12), melittin inserted into the SEA domain groove, forming multiple hydrophobic contacts and a few hydrogen bonds, though with less spatial complementarity than apamin. Cathepsin D docking (Fig. 13) showed that melittin interacted at the edge of the protease cleft, indicating weaker or partial inhibition. With TFF1 (Fig. 14), melittin engaged primarily with surface-accessible residues and formed limited bonding interactions, suggesting a lower binding stability.

Among the targets, Mucin and Trefoil Factor are known to play crucial roles in cancer cell survival, proliferation and immune evasion, while Cathepsin is associated with metastasis and tissue invasion. The stronger binding of apamin with these proteins indicates its potential role in interfering with these cancer-supporting pathways.

These in-silico results align with the MTT assay data, where bee venom exhibited significant cytotoxicity against MCF-7 cells. The molecular docking helps establish a possible mechanism behind the observed cell death and thus suggesting that the venom peptides might exert their anticancer activity by binding to and disrupting the function of these key proteins involved in breast cancer progression.

4. CONCLUSION

To conclude, the present study demonstrated the potential of *Apis cerana indica* bee venom as a natural therapeutic agent, exhibiting notable cytotoxic properties *in vitro*, this study proved that the cytotoxic effects on cancer cells could be a promising alternative in cancer therapeutics. In addition, molecular docking of melittin and apamin with cancer-associated proteins such as Mucin, Trefoil Factor and Cathepsin revealed strong binding affinities, especially between apamin and Mucin (-8.0 kcal/mol), supporting the observed cytotoxic effect and providing a molecular basis for its anticancer potential. However, as the effects of bee venom is dose dependent, suitable physiological conditions are also can be a deciding factor of bee venom performance *in-vivo* as well as *in vitro*, additional studies are required to better understand its complete therapeutic potential.

Competing Interest: The authors declare that they have no competing interests.

6. REFERENCES

1. Bava R, et al. In vitro evaluation of acute toxicity of five *Citrus* spp. essential oils towards the parasitic mite *Varroa destructor*. *Pathog.* 2021; 10: 1182. https://doi.org/10.3390/pathogens10091182.

2. Castagna F, et al. Green veterinary pharmacology for honey bee welfare and health: *Origanum heracleoticum* L. (Lamiaceae) essential oil for the control of the *Apis mellifera* varroatosis. *Vet Sci.* 2022; 9: 124. https://doi.org/10.3390/vetsci9030124.

3. Abdela N, Jilo K. Bee venom and its therapeutic values: a review. *Adv Life Sci Technol.* 2016; 44: 18-22.

4. Weis WA et al. An overview about apitherapy and its clinical applications. *Phytomedicine Plus.* 2022; 2: 100239. https://doi.org/10.1016/j.phyplu.2022.100239.

5. Münstedt K, Bogdanov S. Bee products and their potential use in modern medicine. *J ApiProd ApiMed Sci.* 2009; 1: 57-63. https://doi.org/10.3896/ibra.4.01.3.01.

6. Boukraa L. Bee products: the rediscovered antibiotics. *Anti-Infect Agents.* 2015; 13: 36-41. https://doi.org/10.2174/2211352513666150318233855.

7. Szweda P, Kot B. Bee products and essential oils as alternative agents for treatment of infections caused by *S. aureus. InTech eBooks.* 2017. https://doi.org/10.5772/65978.

8. Vahidinia Z et al. Bee venom as a promising therapeutic strategy in central nervous system diseases. *Neuropeptides.* 2024; 107:102451. https://doi.org/10.1016/j.npep.2024.102451

9. Oršolić N. Bee venom in cancer therapy. *Cancer Metastasis Rev.* 2011; 31:173-94. https://doi.org/10.1007/s10555-011-9339-3.

10. Son D et al. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. *Pharmacol Ther.* 2007; 115:246-70. https://doi.org/10.1016/j.pharmthera.2007.04.004.

11. Heinen TE et al. Arthropod venoms and cancer. *Toxicon.* 2011; 57:497-511. https://doi.org/10.1016/j.toxicon.2011.01.002

12. Liu CC et al. Application of bee venom and its main constituent melittin for cancer treatment. *Cancer Chemother Pharmacol.* 2016; 78:1131-1130. https://doi.org/10.1007/s00280-016-3160-1

13. Bindlish A, Sawal A. Bee sting venom as a viable therapy for breast cancer: a review article. *Cureus.* 2024; 16: e55454. https://doi.org/10.7759/cureus.54855.

14. Habermann E. Bee and wasp venoms. *Science.* 1972; 177: 314-322. https://doi.org/10.1126/science.177.4046.314.

15. Gu H et al. Therapeutic effects of apamin as a bee venom component for non-neoplastic disease. *Toxins.* 2020; 12: 195. https://doi.org/10.3390/toxins12030195.

16. Raghuraman H, Chattopadhyay A. Melittin: a membrane-active peptide with diverse functions. *Biosci Rep.* 2006; 27: 189-223. https://doi.org/10.1007/s10540-006-9030-z.

17. Felder M et al. MUC16 (CA125): tumor biomarker to cancer therapy, a work in progress. *Mol Cancer.* 2014; 13: 129. https://doi.org/10.1186/1476-4598-13-129.

18. Fusek M, Větvička V. Dual role of cathepsin D: ligand and protease. *Biomed Pap.* 2005; 149: 43-50. https://doi.org/10.5507/bp.2005.003.

19. Thim L. Trefoil peptides: from structure to function. *Cell Mol Life Sci.* 1997; 53: 888-903. https://doi.org/10.1007/s000180050108.

20. De Graaf D C et al. Standard methods for *Apis mellifera* venom research. *J Apic Res.* 2020; 60: 1-31. https://doi.org/10.1080/00218839.2020.1801073.

21. Bogdanov S. Bee venom: Composition, health, medicine-A review. *Bee Prod Sci.* 2012; 1-20.

22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983; 65: 55-63. https://doi.org/10.1016/0022-1759(83)90303-4.

23. White B, Bräutigam C, Newman D, Wilson I A. Crystal structure of a human MUC16 SEA domain reveals insight into the nature of the CA125 tumor marker. *Proteins Struct Funct Bioinf.* 2022; 90: 1210-1218. https://doi.org/10.1002/prot.26303.

24. Baldwin E T, Bhat T N, Gulnik S, Hosur M V, Sowder R C, Cachau R E, Collins J, Silva A M, Erickson J W. Crystal structures of native and inhibited forms of human cathepsin D: implications for lysosomal targeting and drug design. *Proc Natl Acad Sci USA.* 1993; 90: 6796-6800. https://doi.org/10.1073/pnas.90.14.6796

25. Williams M A, Westrop G D, Ellis I R, Bandari P, Loughlin J, Fazal F, McLellan A, Cowan A, Odell E, Ganesan A, Parkinson E K, Prime S S, Lord J M. The solution structure of the disulphide-linked homodimer of the human trefoil protein TFF1. *FEBS Lett.* 2001; 493: 70-4.https://doi.org/10.1016/s0014-5793(01)02276-1

UNDER PEER REVIEW

26. BIOVIA Discovery Studio Visualizer v20.1. Dassault Systèmes, San Diego, USA. 2020.

27. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker B A, Thiessen P A, Yu B, Zaslavsky L, Zhang J, Bolton E E. PubChem in 2021: new data content and improved web interfaces. *Nucleic Acids Res.* 2021; 49: D1388-D1395. https://doi.org/10.1093/nar/gkaa971.

28. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. *Methods Mol Biol.* 2014;243-50. https://doi.org/10.1007/978-1-4939-2269-7_19.

29. Ghasemi M et al. The MTT assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *Int J Mol Sci.* 2021;22:12827. https://doi.org/10.3390/ijms222312827.

30. Kwon N Y et al. Anticancer activity of bee venom components against breast cancer. *Toxins.* 2022;14:460. https://doi.org/10.3390/toxins14070460.

31. Hassani Z M et al. Melittin inhibits the expression of key genes involved in tumor microenvironment formation by suppressing HIF-1α signaling in breast cancer cells. *Med Oncol.* 2021;38:7. https://doi.org/10.1007/s12032-021-01526-6.

32. Vijayakumar K K et al. in-silico characterization of melittin from *Apis cerana indica* and evaluation of melittin intron for transgene expression in mammalian cells. *J Appl Biol Biotechnol.* 2022;10:1. https://doi.org/10.7324/jabb.2023.101771.

33. El-Wahed A A A et al. Bee venom composition: From chemistry to biological activity. *Stud Nat Prod Chem.* 2018;59:459-484. https://doi.org/10.1016/b978-0-444-64181-6.00013-9.

34. Ferreira R S et al. Africanized honey bee (*Apis mellifera*) venom profiling: Seasonal variation of melittin and phospholipase A2 levels. *Toxicon.* 2010;56:355-362. https://doi.org/10.1016/j.toxicon.2010.03.023.

7. TABLES AND FIGURES:

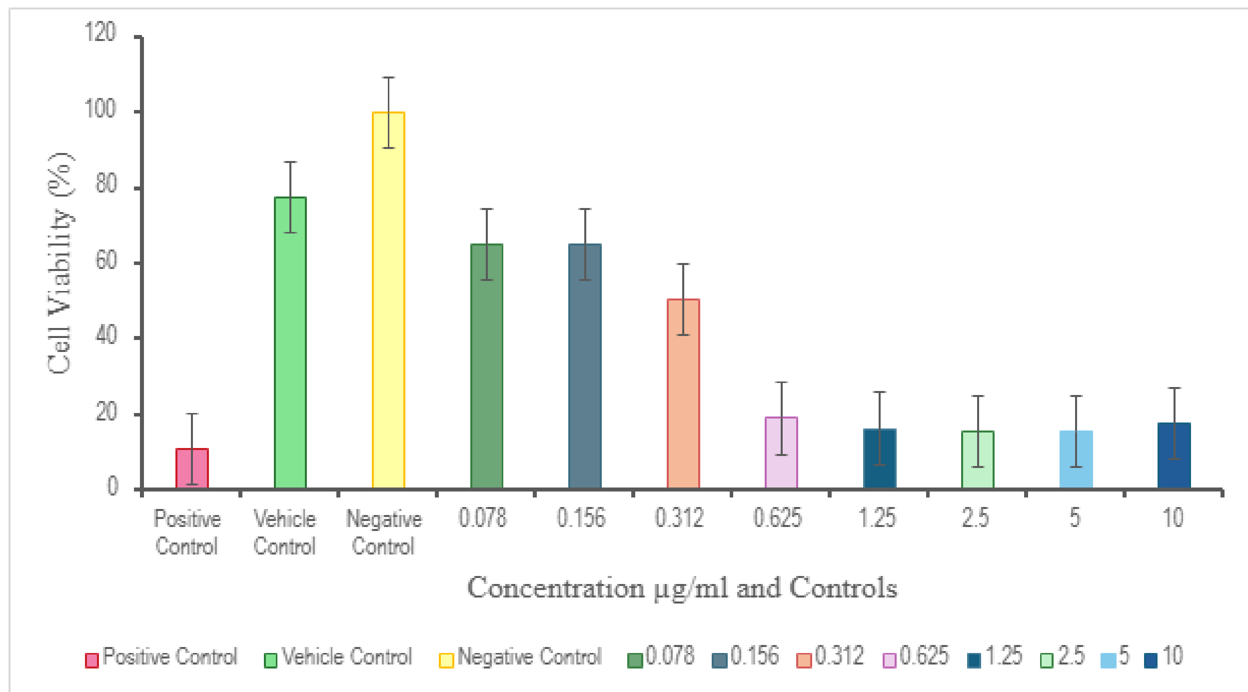

Fig. 1: The graphical representation of cell viability of MCF-7 breast cancer cells treated with bee venom at varying concentrations (0.078-10 µg/mL), measured using the MTT assay. Values are presented as mean ± standard deviation (n = 3), showing a concentration-dependent decrease in cell viability.

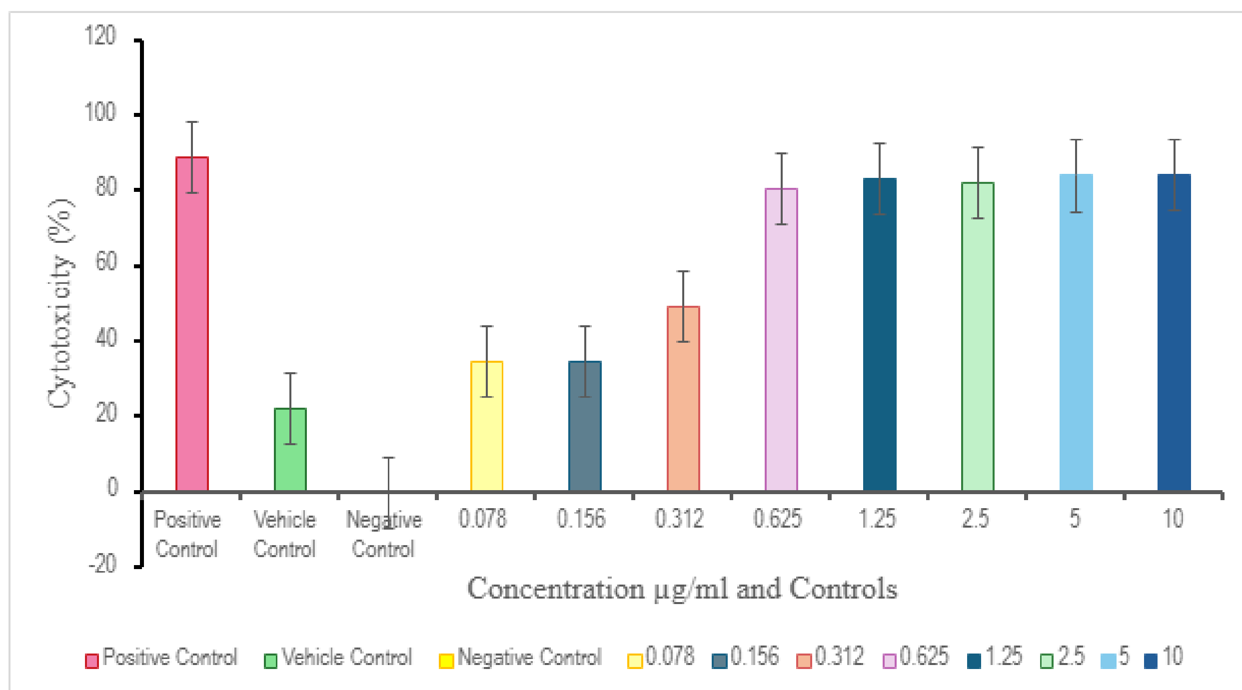

Fig. 2: The cytotoxic effect of bee venom on MCF-7 breast cancer cells measured by the MTT assay at various concentrations (0.078-10 µg/mL). Values are presented as mean ± standard deviation (n = 3), demonstrating a concentration-dependent increase in cytotoxicity.

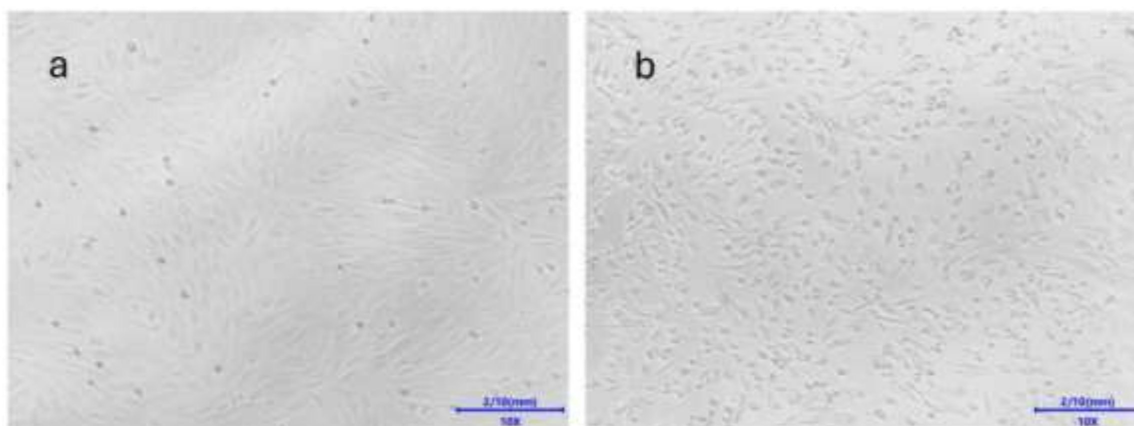

Fig. 3: Microscopic images of MCF-7 breast carcinoma cells as controls. (a) Negative control showing typical cell morphology with intact structure, (b) Vehicle control treated with DMSO, displaying minimal morphological changes.

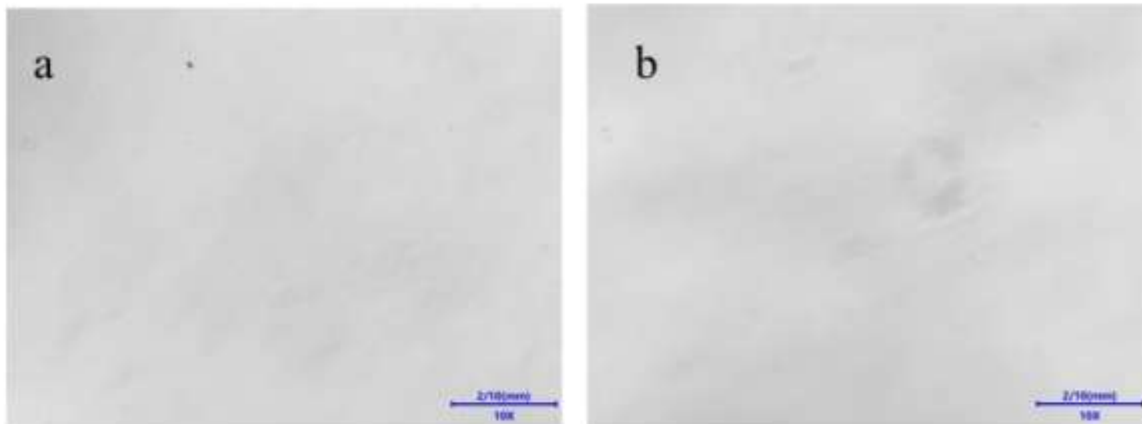

Fig. 4: Microscopic images of MCF-7 cells for positive and blank controls. (a) The positive control (3% SDS) exhibited maximum cytotoxicity (b) Blank control showing a media without cells.

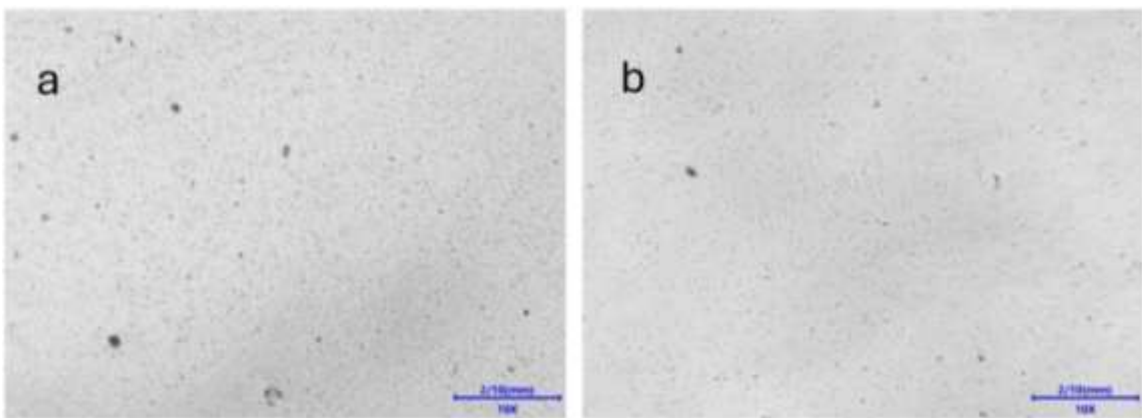

Fig. 5: Microscopic images of MCF-7 cells subjected with high doses of bee venom. (a) 10 µg/mL bee venom treatment showing severe morphological changes, including cell rounding, shrinkageand detachment. (b) 5 µg/mL bee venom treatment with moderate signs of cell damage.

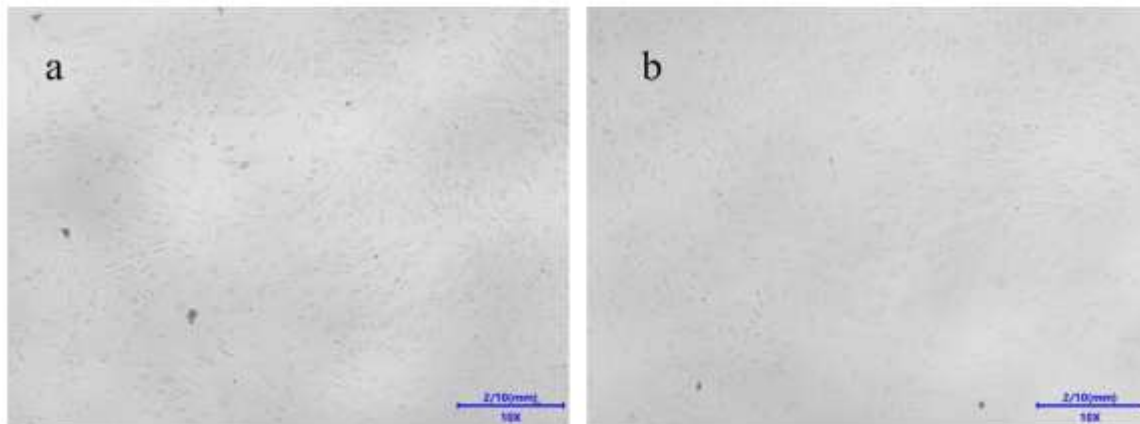

Fig. 6: Microscopic images of MCF-7 cells subjected with mid-range doses of bee venom. (a) 2.5 µg/mL and (b) 1.25 µg/mL treatments, showing initial signs of cell rounding and detachment with moderate cell viability.

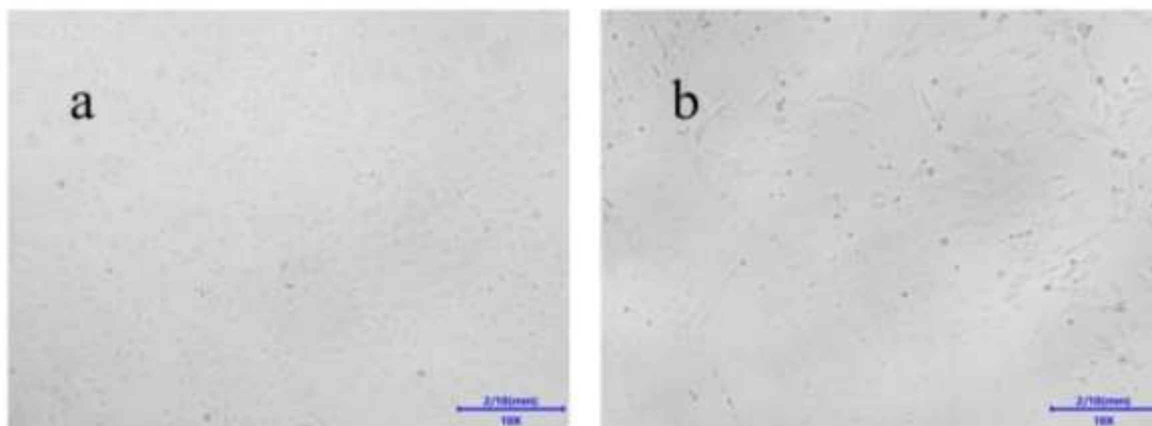

Fig. 7: Microscopic images of MCF-7 cells subjected with lower doses of bee venom. (a) 0.625 µg/mL and (b) 0.312 µg/mL, where cell morphology remains largely intact with minimal cytotoxic effects observed.

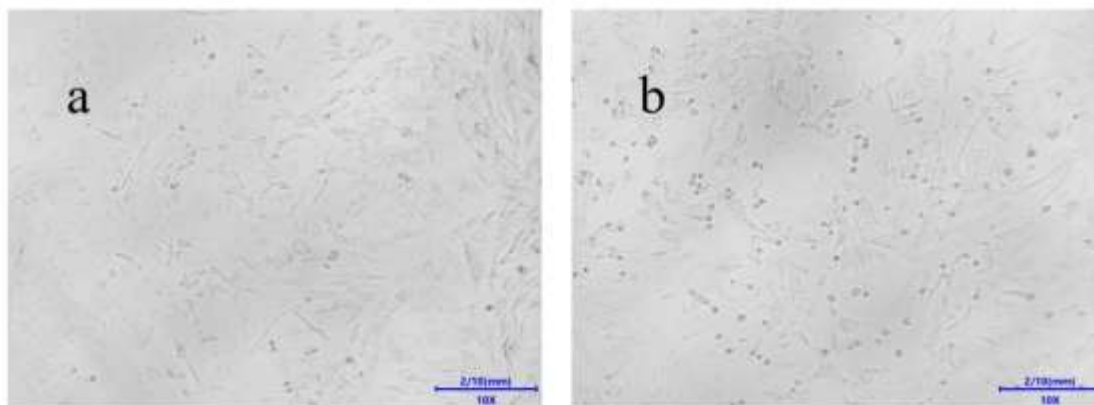

Fig. 8: Microscopic images of MCF-7 cells subjected with minimal doses of bee venom. (a) 0.156 µg/mL and (b) 0.078 µg/mL, showing typical cell morphology with minimum observable cytotoxic effects.

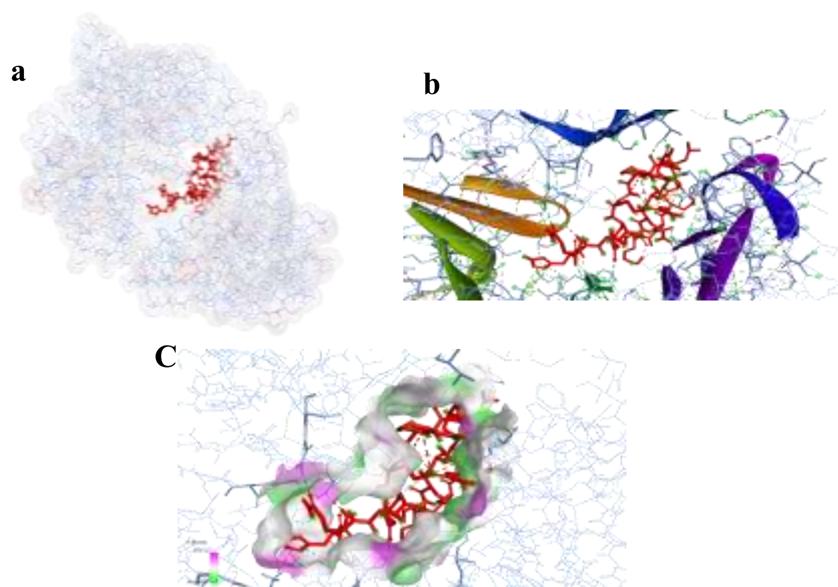

Fig. 9: Molecular docking visualization of Apamin, a bioactive peptide from bee venom, with the MUC16 receptor's SEA domain (PDB ID: 6UVA), which is essential for the growth and spread of tumors. (a) Molecular docking complex of apamin (a bee venom peptide) with the SEA domain of the MUC16 cancer antigen receptor (PDB ID: 6UVA), visualized in surface and cartoon representation, (b) Ligand-receptor interaction diagram showing key residues of MUC16 interacting with apamin and (c) Hydrogen bonding network formed between apamin and the active site residues of MUC16 SEA domain.

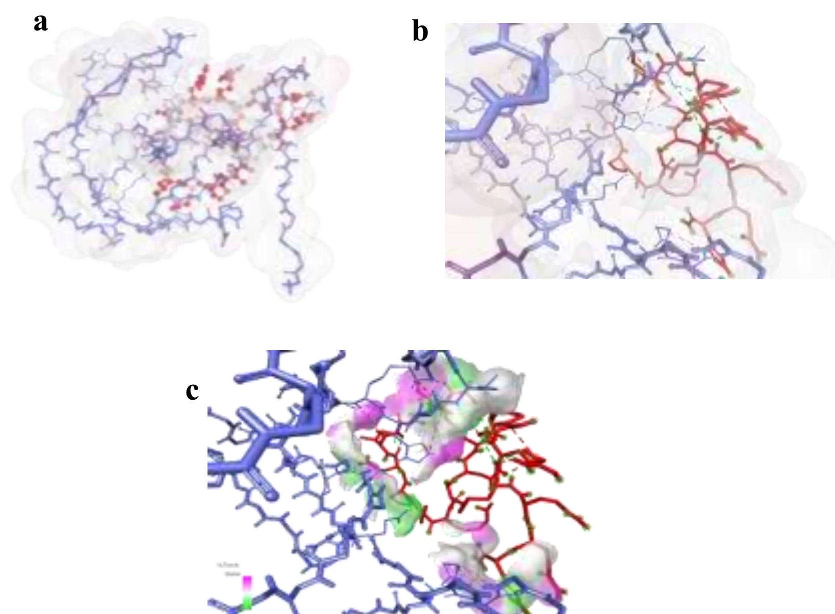

Fig. 10: Molecular docking visualization of Apamin with the lysosomal protease Cathepsin D (PDB ID: 1LYB), involved in cancer progression and protein degradation.(a) Molecular docking complex of apamin, a bee venom neurotoxin peptide, with the active site of Cathepsin D (PDB ID: 1LYB), visualized in surface and cartoon representation, (b) Ligand-receptor interaction diagram showing key binding residues of Cathepsin D interacting with apamin through hydrophobic and polar contacts and (c) Hydrogen bonding network formed between apamin and the active site residues of Cathepsin D, contributing to the stability and specificity of the interaction.

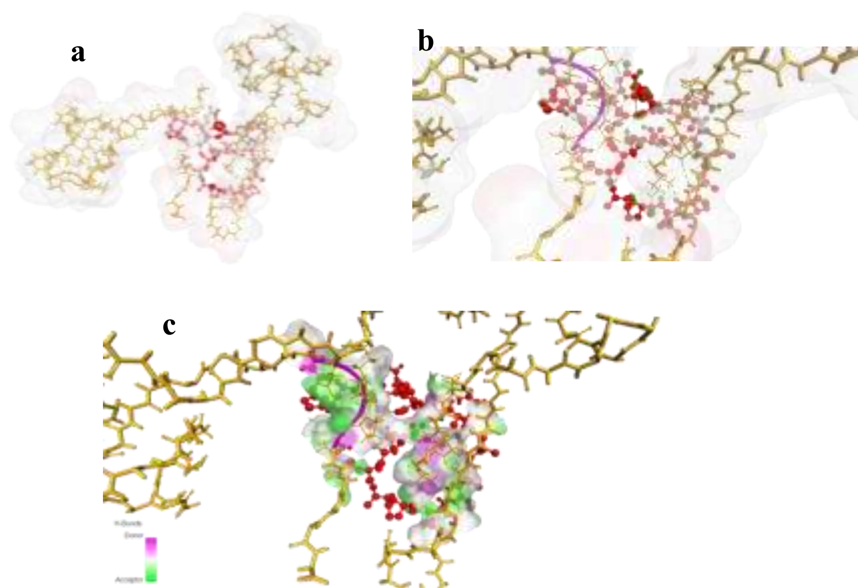

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Fig. 11: Molecular docking visualization of Apamin, with the Trefoil Factor Family protein TFF1 (PDB ID: 1HI7), involved in mucosal healing and cancer progression.(a) Molecular docking complex of apamin with the TFF1 homodimer, visualized in surface and cartoon representation, (b) Ligand-receptor interaction diagram illustrating key amino acid residues of TFF1 interacting with apamin and (c) Hydrogen bonding network formed between apamin and the functional domains of TFF1, suggesting potential bioactivity of apamin in modulating mucosal repair or epithelial regeneration pathways.

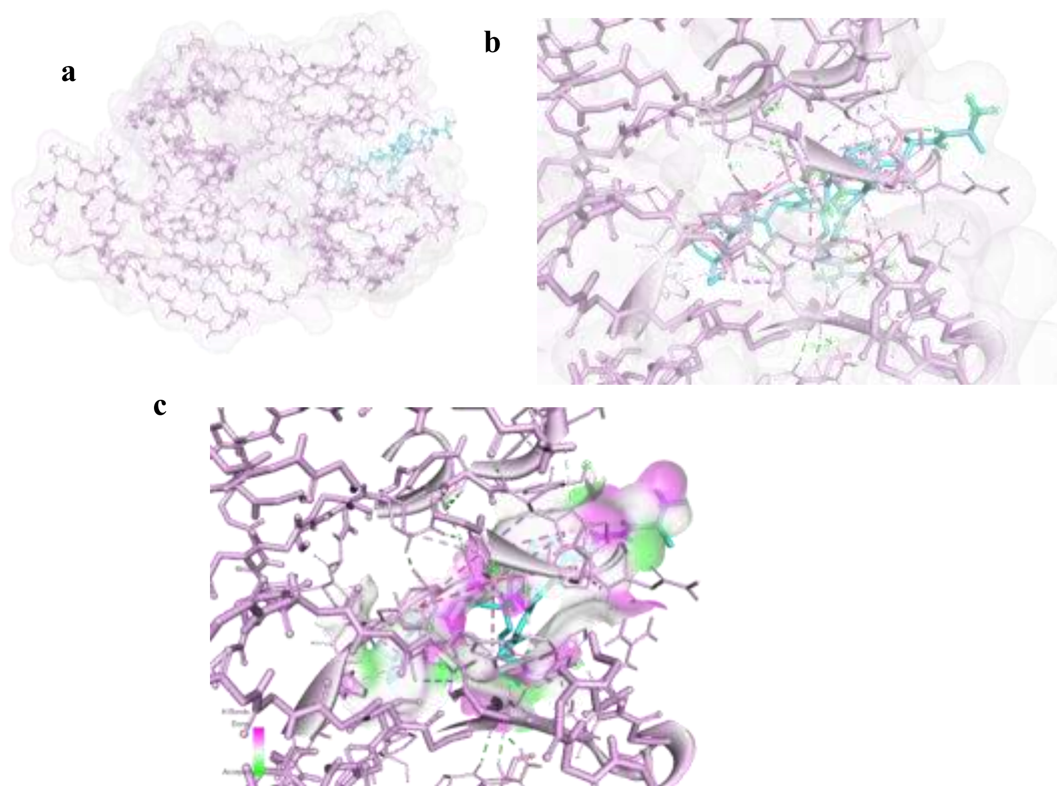

Fig. 12: Molecular docking visualization of Melittin, a principal component of bee venom, with the SEA domain of the MUC16 receptor (PDB ID: 6UVA), a tumor-associated glycoprotein.(a) Docked complex of melittin with the SEA domain of MUC16 shown in surface and cartoon representation, (b) Ligand-receptor interaction diagram depicting essential amino acid residues of MUC16 involved in binding with melittin and (c) Hydrogen bond interactions formed between melittin and the active site residues of the SEA domain, indicating potential binding affinity relevant to anticancer mechanisms.

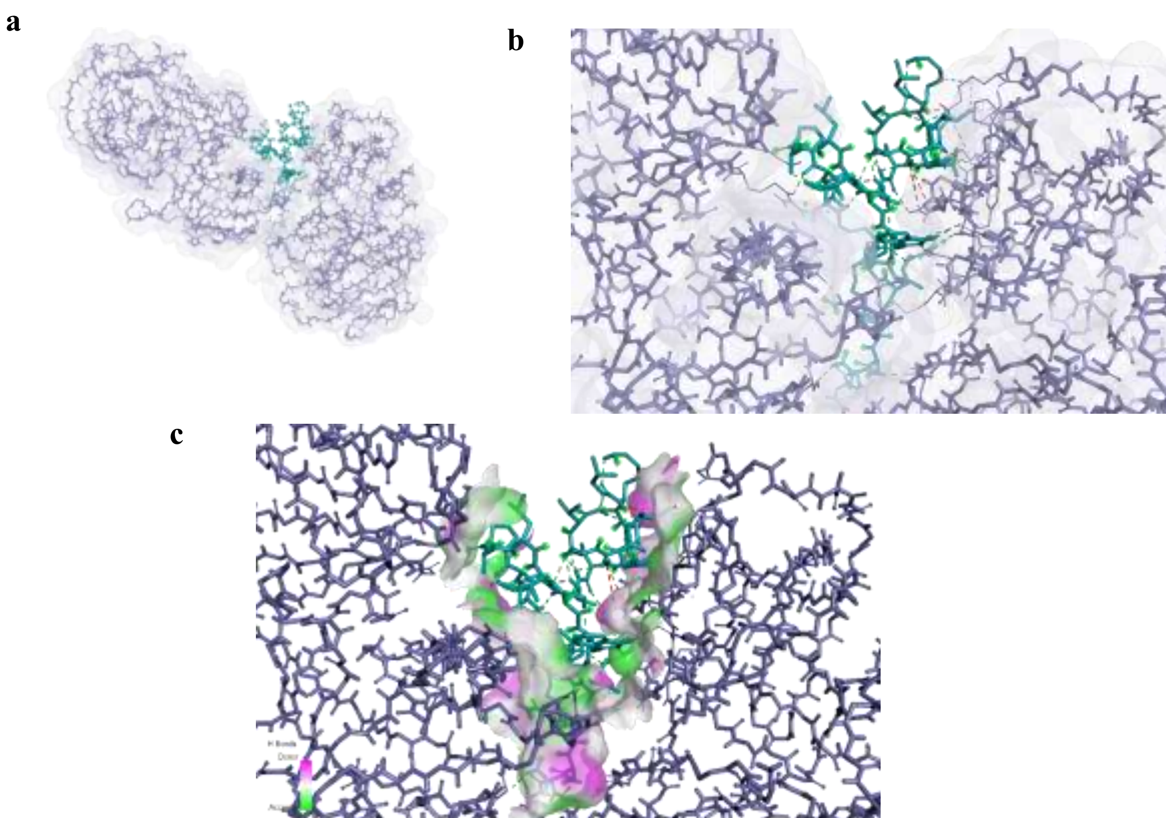

Fig. 13: Molecular docking visualization of Melittin, a potent lytic peptide from bee venom, with Cathepsin D (PDB ID: 1LYB), a lysosomal protease implicated in cancer metastasis and protein degradation. (a) Docked complex of melittin and Cathepsin D illustrated in surface and cartoon view, (b) Ligand-receptor interaction diagram highlighting key residues of Cathepsin D involved in melittin binding and (c) Hydrogen bonding network formed between melittin and the active site residues of Cathepsin D, demonstrating the potential for inhibitory or regulatory effects on proteolytic activity.

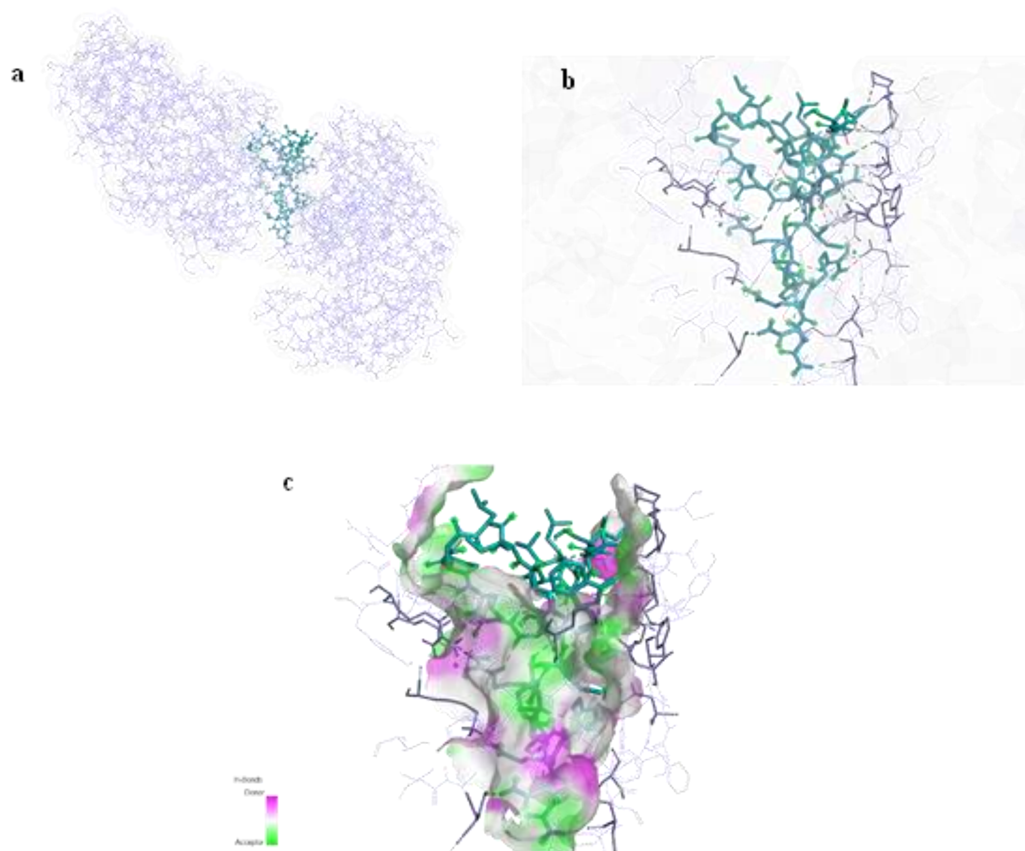

Fig. 14: Molecular docking visualization of Melittin, with the Trefoil Factor 1 (TFF1) homodimer (PDB ID: 1HI7), a protein involved in mucosal healing and epithelial protection. (a) Surface and cartoon representation of the melittin-TFF1 docking complex, showing the flexible linker and separated binding domains of the TFF1 dimer, (b) Ligand-receptor interaction diagram detailing the key TFF1 residues involved in the binding interface with melittin and (c) Hydrogen bonding interactions indicating potential binding affinity and structural complementarity between melittin and the TFF1 homodimer.