ABSTRACT

Cancer is a multifactorial disease in which cells grow abnormally or uncontrollably that can originate in almost any organ or tissue of the body and may spread to adjoining tissues, organs or distal parts of the body. It can progress via a large number of pathways and involve multiple genetic factors and signaling pathways or mediators. Cancer is ranked as the second most leading cause of worldwide death and is estimated to soon surpass cardiovascular diseases by the end of this century. The Global Cancer Observatory (GLOBOCAN) has estimated that among the different types of cancer, lung cancer has the highest incidence and mortality worldwide, whereas, breast cancer has the second highest incidence and mortality. In India, however, breast cancer is responsible for the highest incidence and mortality whereas, lung cancer ranks fourth in incidence and mortality in 2022. Although progress in medical science has helped patients to overcome cancer and increase their life span, however, challenges still exists which needs to be addressed, such as, treatment associated side-effects, resistance to chemotherapeutic drugs, and recurrence of cancer which may significantly impact the health and quality of life of the cancer patient.

Among a large number of alternatives, many synthetic drugs are being explored to treat cancer patients. Snake venom, which is a natural source of highly specific biomolecules, have exhibited promising outcomes in numerous anticancer experiments. Snake venom is often called as an "advanced biological weapon" which has evolved to assist snakes in predation, defense and digestion. To subdue the prey, snake venom target various vital systems like the central and peripheral nervous system, blood coagulations cascade, cardiovascular system and neuromuscular system. Studies on structure-function relationship of various snake venom proteins has previously led to the development of anti-hypertensive drug Captopril® from *Bothrops jararaca* venom, and anti-platelet drugs Aggrastat® and Integrillin® from the venom of *Echis carinatus* and *Sistrurus miliarius barbouri* respectively. These venom proteins are either enzymatic or non-enzymatic components and hence it is often looked at as "mini drug library".

Therefore, the core objective of this thesis is to explore the venom of Indian Monocled cobra (*Naja kaouthia*) for therapeutic molecule particularly against cancer. The venom of *N. kaouthia* is known to be neurotoxic and results in blistering and necrosis at the local bite site in envenomated victims due to the presence of three finger toxin family.

Three-finger toxins (3FTxs) which are one of the major groups of snake venom protein family found in Elapids are responsible for neurotoxic and cytotoxic activity. They are known to induce cytotoxicity in various cells types such as lymphocytes, cardiac cells, spleen cells, red blood cells (RBCs), tumors and cancer cells. Cytotoxins from various *Naja* sp. such as *N. kaouthia*, *N. oxiana*, and *N. haje* have demonstrated cytotoxicity against various human cancer cell lines, such as, lung cancer, breast cancer and erythroleukemia and also exhibited antimetastatic activity is various studies.

In this study, the cytotoxicity of *N. kaouthia* venom from North-East India origin has been explored to identify their cytotoxic potential against lung and breast cancer cells. The following six chapters highlight the various phases of this study and are briefly summarized below:

Chapter 1: In this chapter, the global burden of cancer, its types, causes, risk factors and hallmarks are introduced. Moreover, other pertinent topics associated with this study, such as, anti-cancer drug development from natural sources, and snake venom as potential source of drugs were also briefly discussed. This was followed by identifying the need of the proposed study and defining the research hypothesis. Finally, the aims and objectives of the study were set along with the proposed work flow.

Chapter 2**:** In this chapter, extensive literatures on various aspects of the study was reviewed. This includes important topics such as cancer progression, anti-cancer drugs and mechanisms of cancer cell death, metastasis and its regulation, recent advances in anti-cancer drug development. This was followed by further review of natural products and animal venom which are explored as source of anti-cancer leads, snake venom families and their anti-cancer effects, proteomics of *N. kaouthia* venom, and finally the toxicological and pharmacological effects of the venom.

Chapter 3**:** This chapter deals with the partial biochemical characterization of the crude venom of *N. kaouthia* followed by determination of its cytotoxic activity against cancer cell lines**.** Firstly, SDS-PAGE profiling of the crude venom was performed which was followed by determination of the *in vitro* enzymatic activities such as phospholipase A_2 activity, anti-coagulant activity (recalcification time, prothrombin time and activated partial thromboplastin time) and hemolytic activity. This was followed by determination of cytotoxicity by calculating the half-maximal inhibitory concentration (IC_{50}) of the venom using MTT assay against lung and breast cancer cell lines.

***Chapter 4*:** In this chapter, isolation and identification of cytotoxic fraction of *N. kaouthia* venom is discussed. Crude venom was separated using RP-HPLC and the fractions were screened for their cytotoxicity against the lung and breast cancer cells. The fraction with highest cytotoxicity (P9) was purified by re-chromatography and identified using ESI-LC-MS/MS as Cytotoxin 10. This was followed multiple sequence alignment and phylogenetic tree analysis with other known cytotoxins from *N. kaouthia*, and Cytotoxin 10 from other *Naja* sp. venoms. Further, *in silico* characterization of Cytotoxin 10 was performed through tertiary structure prediction, followed by validation using the Ramachandran plot. Finally, the dose-dependent cytotoxicity of Cytotoxin 10 against lung and breast cancer cells was studied.

***Chapter 5*:** In this chapter, the mechanism of cytotoxicity of Cytotoxin 10 in lung and breast cancer cells was deciphered. Initially the time and dose-dependent morphological changes in the cancer cells were observed followed by AO/EtBr staining to check the induction of apoptosis. The findings were validated using flow cytometry where early apoptosis was observed at a low dose (1.2 μg/ml), whereas, late apoptosis was observed in higher doses (2.4 and 4.8 μg/ml). Moreover, the expression of apoptotic proteins (Bax, Bcl-2, Caspase-7, PARP and p53) was also validated using Western blotting. Further, the antimetastatic activity of Cytotoxin 10 was deciphered using *in vitro* migration and adhesion assays. Finally, the interaction of Cytotoxin 10 with the apoptotic proteins was also investigated using *in silico* approach.

***Chapter 6*:** In the final chapter, the findings to this study are summarized as well as a brief outline of the future prospects of this study is proposed. Further, a critical discussion regarding the challenges and possible solutions of this field are also discussed.