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### Neem Leaf Extracts and Apoptotic Induction: A Potential Anti-Cancer Therapy

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## **Neem Leaf Extracts and Apoptotic Induction: A Potential Anti-Cancer Therapy**

**Alessia Roma, *University of Windsor***

Natural products have played a significant role in the development of anti-cancer drugs for a number of decades. Although effective chemotherapies exist, the development of resistance to anti-cancer drugs creates a continuous need for novel, safer and nontoxic anticancer therapies. Our group analyzes the anti-cancer effects of natural health products (NHPs), in order to find effective and safer alternatives to current chemotherapies. We have previously demonstrated the efficacy of several NHPs, including dandelion root extract (DRE) and long pepper extract as potential anti-cancer agents. This research prompted further studies into other natural extracts such as Neem (*Azadirachta Indica*), which has been used in Ayurvedic medicine for centuries. Although research has been done on the anti-cancer effects of Neem leaf extracts, a precise mechanism of action has not been delineated, preventing the study of NLE from going to the clinical trial level. The objective of the study is to determine the possible anti-cancer potential of Neem leaf extracts (NLE) and to identify the specific mode of action.

Neem (*Azadirachta Indica*), is a tree of the *Meliaceae* family of plants that is native to India where it has been used in Ayurvedic medicine for over 4000 years. Each part of the tree has been used to treat various illnesses like Malaria and diabetes. Recently, Neem leaves have been shown to have anti-cancer properties in pancreatic, prostate and melanoma cancers by targeting many of the hallmarks of cancer that were outlined by Hanahan and Weinberg in 2000. These studies revealed that varying preparations of Neem leaf are able to induce apoptosis, inhibit cell proliferation and angiogenesis, modulate transcription factors involved in oncogenesis and enhance anti-tumour immune responses in these cancers. Varying preparations of Neem leaf extracts have never been tested simultaneously which could give better insight into the mechanism underlying the anti-cancer activity of Neem leaf. In an attempt to better comprehend this mechanism, this study will look at both aqueous and ethanolic extracts of Neem leaf. The objective of this study is to determine the possible anti-cancer potential of aqueous and ethanolic Neem leaf extracts and to evaluate their potential mode of action.

In order to complete this study, aqueous and ethanolic extracts will be prepared in the laboratory using powdered Neem leaf. To test their anti-cancer potential, E6-1 leukemia and HT-29 colon cancer cells will be subjected to varying concentrations of either aqueous or ethanolic Neem leaf extracts (NLE). Cellular viability will then be measured using the WST-1 cell viability assay. The type of cell death that is occurring will be determined using fluorescence microscopy. It is also important to determine whether oxidative stress plays a role as it is often heavily involved in various cell death mechanisms. Next, we will look at the possible change in levels of cell death genes in these treated cells and we will determine whether these changes are also expressed at the protein level. Finally, other studies will aim to test these extracts through in vivo mice models and also determine whether the extracts are toxic to non-cancerous cells.

Our results indicate that both ethanolic and aqueous extracts of Neem leaf were effective in inducing apoptosis in leukemia and colon cancer cells, following the destabilization of the mitochondrial membrane. Furthermore, an increase in the production of reactive oxygen species

(ROS) was observed in isolated mitochondria from leukemia cells, indicating that the mitochondria are a potential target of NLE. Additionally, preliminary results in mice showed that aqueous NLE was well tolerated. These findings suggest the potential of NLE as safer alternatives to conventional chemotherapy.