Pre-Clinical Assessment of Olive Leaf and White Tea with Hodgkin's Lymphoma

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Importance of the Project

About 2 in 5 Canadians will develop a type of cancer in their lifetime, and about 1 in 4 will die from it. In the past year alone, over 200,000 new cases of cancers were discovered in Canada, and over 75,000 Canadians died from this disease. Approximately one Canadian is diagnosed with a blood cancer (leukemia, lymphoma, or myeloma) every twenty eight minutes. Leukemia and lymphoma alone account for almost half of all the cancer cases of children ages 0 – 14.

In normal, healthy organisms, cells must frequently die to make room for new ones. Cells may also induce apoptosis (programmed cell death) in response to stressful events, such as damage to their DNA, to prevent these faults from replicating. However, cancer cells do not induce apoptosis, and thus are able to keep increasing and spreading. Cancer cells avoid cell death by either overexpressing anti-apoptotic proteins, reducing pro-apoptotic proteins, or by altering normal cellular processes, such as autophagy and necrosis.

There are different types of cancer treatments available, but many of them have negative side effects. For example, radiation treatment uses high-energy radiation to reduce the size of cancerous tumors and kill the cancer cells by damaging their DNA so they can no longer replicate. However, radiation treatment does not differentiate between healthy and cancerous cells, and also damages normal cells. This in turn can cause many short and long term side effects depending on what area of the body is treated. Such side effects include skin irritation, hair loss at the areas treated, fatigue, nausea and vomiting, fibrosis, memory loss, infertility and even a secondary cancer from radiation exposure.

There are many natural alternatives to these cancer treatments that are being developed. Natural alternatives may reduce efficacy of cancer cells and stop the spread of cancer without the side effects of current treatments. Many plants, such as white tea and olive leaf, have been shown to promote health and treat illnesses, and some possess anti-cancer effects. Some natural remedies include raw herbs that are boiled to drink, or ointment for the skin. Although these herbs may show anti-cancer effects, there is no conclusive evidence that supports a cure for cancer. More research should be done on the anti-cancer properties that these natural alternatives possess.

Existing State of Knowledge

Previous research has shown that white tea has a similar chemical make-up and health effects to that of green tea. Analysis has shown how white tea has been able to improve cardiovascular function, as well as reduce cholesterol and decrease blood pressure. It is also known for both its pro- and anti-oxidative properties.

Olive leaves were first used in Ancient Egypt for medicinal purposes. Modern research has shown that the olive leaf has been able to modulate blood pressure and increase overall energy. It has also been shown to benefit both the cardiovascular system, as well as the immune system.
Unfortunately, very little research has been done on the effects of white tea and olive leaves on cancer, and almost no research has been done on their effects on lymphoma. The purpose of this research is to show the effect these extracts have in vitro and in vivo models of lymphoma.

**Research Question**

This project aims to evaluate the chronology of biological events that occur following treatment with olive leaf and white tea in lymphoma cells as a way to determine if these extracts have anti-cancer properties.

**Methodology**

Both white tea and olive leaf were extracted in cold water, hot water and 100% ethanol. These extracts were passed through a series of filters before being dried and made into stock concentrations.

A WST-1 assay will be used to screen each extract to measure the effect varying concentrations of each extract has on metabolic activities. Actively metabolizing cells are able to metabolize the WST-1 dye into a product that can be distinguished with absorbance spectrometry. A low amount of absorbance will correspond to low amounts of viable cells whereas a high absorbance will correspond to a higher amount of viable cells. U-937 will be treated with each extract at increasing concentrations to determine efficacy and effective concentrations.

Following these screenings, the results gained will be used to determine the concentrations and correlating time points that show the most effect on cell viability. Fluorescence microscopy and image based cytometry will be used to determine the induction of programmed cell death. Specifically Annexin - V will be used to determine the induction of apoptosis, propidium iodide for necrosis, and monodansylcadaverine for autophagy. Fluorescence microscopy and image based cytometry in conjunction with western blot analysis will be used to assess the biochemical events that occur before the induction of programmed cell death.

In the future, animal models will be used to test the toxicity and efficacy of these extracts. This will hopefully bring us one step closer to testing on human models.

**Your Findings**

White tea extracts were efficient in reducing the viability of the U-937 cells. However, it was apparent that the water extracts were more efficient in decreasing the cell’s sustainability than the ethanolic extracts in a dose and time-dependent manner.
Olive leaf extracts do not seem to be as effective in reducing the viability of the U-937 cells. The ethanolic extracts showed better efficacy than the water extracts.

These results have provided us with the effective concentrations and time points needed to induce cytotoxicity in U-937 cells. Future studies will be carried out on these doses and time points to further assess the anti-cancer potential of these extracts. The results from this project may be beneficial as it will promote the development of non-toxic alternatives to cancer therapy.