The Effect of *Lomatium Californicum* on Mouse Embryonic Cell Line NIH-3T3

Margaret Johnson  
Faculty Mentor: Dr. Mary McCarthy-Hintz

**Abstract**

The plant species *Lomatium Californicum* has authentic spiritual and therapeutic aspects that are well-known in the Native American communities of California, such as the Chumash, Karuk, Yuki, and Kawaiisu. Historically, the customary name for *L. Californicum* is oshá, whose root is used to make chuchupate. Chuchupate is a sacred medicine used in medical treatments for various ailments, including colds, fever, and upper respiratory conditions. In stronger doses, it has been used in women’s medicine as an abortifacient. To better understand the medicinal properties of *L. Californicum*, scientists have studied it from a Western perspective. The purpose of this research is to understand the pharmaceutical uses and safety concerns of this alternative medicine and to convey awareness of it to non-Traditional health practitioners who work with Native American communities. In previous research, an ethanolic extract of *L. Californicum* roots showed cytotoxicity toward breast cancer cells and normal human peripheral blood mononuclear cells. Because of its use as an abortifacient, it was hypothesized that it would also have cytotoxic effects on embryonic cells. Therefore, a dilution series of an *L. Californicum* root extract was tested on a mouse embryonic cell line, NIH-3T3. The results were inconclusive due to the low number of cells that were used in the assay. Therefore, future experiments will be conducted using a higher quantity of cells.

**Introduction**

There is a growing interest in medicinal botanicals as part of therapeutic alternatives in the United States (Borchers et al. 2000). In particular, pharmaceutical companies are becoming more aware of the use of herbs by Native American societies. In addition, there is a high demand from consumers seeking alternative medicines that are natural, rather than using prescription medicines. Many anthropologists have studied California Indian communities, to try to understand how Native Americans have used medicinal plants (Timbrook 1987). One must understand traditional practices to understand their context in the society and culture (Strike 1994). Native Americans believe that everything has a spirit: earth, animals, trees, roots, and rocks, as well as elements such as lightning and wind (Martin 1981; Zigmond 1977).
Hallberstein (2005) states that, during pre-contact times, medicinal plant products were valuable for dealing with an array of health problems. The use of plants as medicine is still common in Native American communities (Moerman 1998; Adams and García 2005). The pharmacological properties of these medicinal plant species have led to abundant interest by Western herbalists. However, without proper guidance from a traditional healer, misuse of medicines leads to safety concerns. These concerns led to the investigation of the cytotoxicity of some powerful medicines used by California Indians (Castro 2009; Jackson 2013; Lai 2009; Somaweera, 2011; Somaweera et al. 2013).

The medicinal plants called oshá or oshála are important in the cultures and traditions of many peoples. Historically, oshá roots were used by Native Americans for both their spiritual and medicinal properties (Timbrook 1987). Throughout western North and Central America, Native American communities utilized species of oshá to make the medicine called chuchupate, which is used to treat various health ailments, such as colds, fever, and upper respiratory ailments. The Chumash, Karok, Paiute, Kawaiisu, and Pomo of California use chuchupate made from the roots of Lomatium Californicum (Baker 1991; Timbrook 1990; Zigmond 1981)

Besides its traditional uses, L. Californicum exhibits antibacterial activity (Chou et al. 2006), phytotoxicity, and antifungal activity (Meepagala et al. 2005). These activities have been attributed to a single chemical compound, (Z)–ligustilide (Meepagala et al. 2005). Many medicinal plants contain a curative bioactive chemical ingredient, so they can serve as supplemental therapies when medically supervised (Hallberstein 2005).

The goal of this research is to develop an understanding of L. Californicum from a Western perspective in order to inform non-Native peoples of possible safety concerns. Therefore, this study examines the cytotoxic effects of L. Californicum on mouse embryonic cells. The research proposed to test an ethanol extract of the roots of the plant species L. Californicum against the mouse embryonic cell line NIH-3T3.

**Methodology**

**Extract**

An ethanolic extract of L. Californicum roots was obtained from a previous McNair researcher (Jackson 2013).

**Starting Cells**

NIH-3T3 mouse embryonic cells (American Type Culture Collection) were retrieved from storage in liquid nitrogen. The cells were thawed for 1 min in 37° C water bath. Cells were rinsed twice in 5 ml of growth media, which consisted of Improved Minimal Essential Media (Zinc Option, Richter’s Modification, with 2 mg/L-glutamine, 2 mg/L-proline, 50.0 g/mL L-gentamicin sulfate; Invitrogen Corporation) containing 50 IU/mL penicillin, 50 g/mL streptomycin, and 10 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid, and 10% (v/v) fetal bovine serum (Axenia Biologix). Cells were resuspended in 5 ml growth media and placed in a T-25 tissue culture flask (Corning CoStar), then maintained at 37° C in 5% CO2 atmosphere in a humidified incubator.

**Culturing Cells**

Proper maintenance plays an important factor in the culturing of NIH 3T3 cells. Todaro and Green (1963) established that it is essential that healthy cells are used in any experiment so that they remain alive throughout the experiment. It is necessary to maintain cells in a rising (logarithmic) growth rate, rather than allowing their growth to become arrested in culture (Green and Kehinde 1976). If they are not maintained in this manner, 3T3 fibroblasts differentiate into adipose cells. Therefore, a researcher must use a microscope often to monitor the cells, and “split” or dilute the cells as often as necessary to maintain this state.

Trypsin was used as a cell dissociation agent to lift the 3T3 cells from the bottom of the T-25 flask. The cells were rinsed with 5 ml media, then re-suspended into new media. Cells were transferred to a T-75 tissue culture flask. Then, the cells were back into the 37° C incubator.

**Cytotoxicity Assay**

NIH 3T3 cells were plated (5000 cells per well) in a 96 well plate, and then were incubated at 37° C in a 5% CO2 atmosphere for 2 days. A dilution series of ethanolic oshá extract was mixed with media and applied to the cells. After 2 days of incubation, the relative number of cells in each well was determined by adding CellTiter, incubating 2 hours, and reading the absorbance in a plate reader (spectrophotometer). The absorbance at 490 nm was recorded and plotted vs. concentration of the plant extract. Absorbance is an indicator of the intensity of the color; intensity is an indicator of the number of cells.

**Results and Discussion**

After analyzing the data from the microplate reader (Figure 1), the results are inconclusive because all of the samples absorbed very little light. A low absorbance means a low number of cells. A low amount of cells might occur for two specific reasons, 1) during the plating process, an insufficient amount cells are transferred into the wells, or 2) sometimes chemical contaminants (like excessive ethanol) are introduced into the media, and these contaminants can kill the cells. To determine whether the plating process or contaminants in the media were the key factors leading to the low absorbance, the plate was observed underneath the
microscope. Healthy cells were detected in the wells. The presence of healthy cells shows that chemical contaminants did not contribute to the low number of cells. It supports the hypothesis of a plating error, resulting in low number of cells in each well.

Figure 1. Effect of Lomatium Californicum on 3T3 cells

Conclusion

The purpose of this research was to understand the pharmaceutical uses and safety concerns of this alternative medicine and to convey awareness of it to non-Traditional health practitioners who work in Native American communities. The pharmacological properties of oshá give rise to investigate its cytotoxicity. According to Adams and Garcia (2005), many Californian plants should be investigated to find and test the active compounds in them.

Understanding the medicinal plant called oshá is important in understanding the ethnomedicine of cultures and traditions of the Native American communities who use it. Because of its use as an abortifacient, it was hypothesized that it would have cytotoxic effects on embryonic cells. Therefore, a dilution series of the L. Californicum root extract was analyzed on a mouse embryonic cell line, NIH-3T3. The results were inconclusive due to the low number of cells that were used in the assay. However, future research experiments will be conducted by repeating this experiment and using a larger quantity of cells to increase the probability of better results.

References


Alejandra Montelongo
Faculty Mentor: Dr. Robert Kraemer

Abstract

The goal of this study was to determine Latino parents’ perceptions regarding their experiences during the Individualized Education Plan (IEP) process. Legislation such as the Individuals with Disabilities Education Act (IDEA) of 2004 stresses that the parents must be active members of the IEP team and have the right to be an active participant in their child’s education (Office of Special Education and Rehabilitative Services 2006). However, cultural differences and language barriers may affect a parent’s level of involvement during the IEP meeting. The investigator conducted qualitative in-depth interviews of five Latino parents seeking to determine: 1) how well the parents understood the highly technical information given to them, 2) how well-represented they felt by the professionals during the IEP process, and 3) how much they felt they could advocate for their children throughout the process. The findings show that Latino parents had little knowledge of speech and language impairments prior to the IEP and saw many errors in the translated documents they were given that made it hard for them to understand the information. Participants felt the information was given to them quickly, and was difficult to understand even with an interpreter or translated documents. Most of the participants stated that they did not feel supported by the professionals involved in their child’s IEP. All five participants demonstrated a need to help their child receive the services despite the lack of support from the school. Participants’ expectations of primary language maintenance, as well as expectations of professionals will also be discussed. These findings can help professionals provide services to families from Latino backgrounds.

Keywords: IEP, IDEA, Cultural and Language barriers, Latino parents

Introduction

Since the passing of PL 94-142 “Education of All Handicapped Children Act” by Congress in 1975, people with disabilities and their families have advocated to have the same rights as those given to other members of the community. After being amended in 1990, PL 94-142 became known as IDEA (Rogers, Rogers, and Yell 1998), and was enacted to ensure parents’ rights in public education and guarantee a free and appropriate public education for every child regardless of race, language, culture and/or intellectual disability. IDEA emphasizes a “team