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Creating Unique Undergraduate Research Projects for Nursing Majors that Investigate the Anti-proliferative Effects of Heavy Metal Compounds on MCF-7, A375, and HFF Cells

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Abstract Heavy metals, such as thallium and barium, are known for their toxicity. The focus of this project was to create research techniques for undergraduate nursing majors who had only two semesters of chemistry laboratory experience. This study investigated the antiproliferative effects of thallium and barium salts on breast cancer cells (MCF-7), skin cancer cells (A375), and normal Human Foreskin Fibroblasts (HFF). The project was designed to provide a unique research opportunity for nursing majors having only two semesters of chemistry laboratory experience. Critical thinking in chemistry and its application to real life situations were key learning objectives in investigating the toxic effects of various metals compounds on biological cells. Thallium, barium, and potassium possess very similar atomic radii, resulting in the ability of Tl^+ and Ba^{2+} to easily enter the cell via the Na^+/K^+ pump. Cytotoxicity was monitored utilizing a Sulforhodamine B (SRB) assay. Results indicated cell death for MCF-7 (2 μM $TlCH_3COO$, 75 μM $TlNO_3$), A375 (100 μM $TlCH_3COO$, 75 μM $TlNO_3$), and HFF (100 μM $TlCH_3COO$, 100 μM $TlNO_3$). Remarkably, these cells tolerated 800 μM $Ba(NO_3)_2$. The procedure and techniques were designed to limit exposure to toxins and enhance the education of Walsh University's nursing majors.

Keywords: *A375 cells, barium, cancer, chemical education, HFF cells, MCF-7 cells, thallium, undergraduate research*

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1. Introduction

Walsh University is a small catholic university located in North Canton, Ohio. Undergraduate research is one of our primary goals that aligns with Walsh's mission as we prepare our students to become responsible scientists. Research in chemistry is time intensive and, therefore, presents many challenges for nursing majors due to their program requirements including both clinical experiences and evening courses. The focus was to create safe research techniques and protocols for undergraduate nursing majors who had only two semesters of chemistry laboratory experience. One challenge was to design techniques that limit exposure to toxins and designing a protocol that would fit into nursing students' schedules without compromising on the effectiveness and productivity of the project. The main goal was to enhance the education of Walsh University's nursing majors by guiding them through undergraduate research, CHEM 411-412.

Heavy metals, such as thallium and barium, are known for their toxicity. Thallium, specifically, has been found to be a toxin in both chemical and forensic sciences. Arsenic has also been studied in recent years and its possible application as a toxin to kill cancer cells. For more than twenty-five centuries, the Chinese have been using compounds containing this poisonous metal for medical interventions. Arsenic trioxide, As_2O_3, has been found to be effective for patients having acute promyelocytic leukemia (APL). [1] After extensive study, it was concluded that this compound is a successful treatment for APL. From their work, it was concluded that Arsenic trioxide is considered a good option because it is safe and effective for those patients with leukemia. Studies also indicate its effectiveness for patients with other malignancies [1]. Further work showed that anti-carcinogenic effects of arsenic trioxide might be associated with the initiation of apoptosis [2].

This study was conducted to investigate the antiproliferative effects of thallium and barium salts on breast cancer cells (MCF-7), skin cancer cells (A375), and normal human foreskin fibroblasts (HFF). Consequently, this work provided a greater understanding to the sensitivity of each cell line and the comparison of results of cancerous cells to noncancerous cells under the same

conditions. MCF-7 cells are the most widely studied estrogen dependent cell line in breast cancer research. Because thallium and potassium possess very similar atomic radii, we predict that this can result in the ability of Tl^+ to easily enter the cell via the Na^+/K^+ pump. Once inside the cell, Tl^+ binds to riboflavin and could potentially interfere with oxidative phosphorylation, causing disruption of the mitochondrial membrane potential. [3] This disruption is predicted to result in mitochondrial membrane dysfunction and the release of Cytochrome C, an early initiating step of the intrinsic apoptotic pathway. Apoptosis, otherwise known as programmed cell death, was a focus of this work in order to understand more about the sensitivity of these cells. One main goal was to see if thallium's toxicity could be used in another way, to fight and destroy cancer cells. This could lead to further studies to evaluate the heavy metal's effectiveness in cell death and its potential for a new cancer treatment.

Thallium-201 is used as one FDA-approved diagnostic metalloradiopharmaceutical for diagnostic imaging for the heart and thyroid gland, but traditionally thallium has been considered a forensic poison. [4,5,6,7] In recent years, thallium has proved to be a concern due to its ease of exposure including through the skin. [8,9,10] Thallium poisoning can cause a wide range of symptoms including flu-like symptoms, hair loss, dystrophy of nails, tachycardia, hypotension, gastroenteritis, and polyneuropathy. [11,12] During pregnancy, thallium poses a huge concern because it can pass the placental barrier and can be responsible for birth defects and/or fetal toxicity. [13] The most widely observed side effects upon exposure to thallium include gastroenteritis, polyneuropathy, and hair loss and, therefore, have become the main indicators of thallium poisoning. [14] Thallium exposure can present itself through contaminated food, drink, soil, and air as its related to cases of industrial accidents or occupational exposure throughout the world. [15,16,17,18,19] Many studies have been conducted to analyze contaminated soil and its impact on the environment. [20,21,22,23] In addition, there have been reports of chronic poisoning, too. The symptoms in these cases are similar to those listed, but they are found to be milder compared to cases involving acute intoxication. [24]

With this respect to this challenge, our work was designed to help students understand the toxicity of these heavy metals and observe the impact on biological cells. Kortz and coworkers are investigating thallium's antibacterial abilities and so this project is both relevant and current for undergraduate students. [25] This study gave nursing students, having only two semesters of chemical laboratory experience, the unique opportunity to not only reinforce content from other courses, but to apply their knowledge to real-life applications in chemistry. This work demonstrated the antiproliferative effects of thallium and barium on cancer cells, *in vitro*.

2. Experimental

2.1. Cell Culture and Reagents

This work was conducted in the university's tissue culture research laboratory. The students received several weeks of training sessions regarding safety and how to complete manipulations without compromising the sterile technique. As required under our protocol, the tissue culture hood was exposed to UV light for 15 min. prior to manipulations and further treatment with 70% ethanol ensured a sterile environment. Experimental procedures and manipulations were conducted under sterile conditions and cells were allowed to grow in the following media to optimize growth: Dulbecco's Modified Eagle's Medium (DMEM, cellgro/meditech catalog #10-013-CV), 50 mL of 10% Fetal Bovine Serum (FBS), 5 mL of 1% PSF antibiotic/antimycotic (cellgro/mediatech), 10 mL of 50X GlutaGro (L-alanyl-L-glutamine), and 1 mL of 500X Plasmocin (InVivoGen). Cells were grown for several days in a humidified cell incubator held constant at an atmosphere of 5% CO_2 at 37°C. Ranin pipettes (L10, L20, L200) were used in this work. The cells were counted using a disposable hemocytometer (incyto, C-chip Neubauer improved). Cells were treated with metal ions and the effects were monitored using SRB assays. Each assay took about three weeks to complete depending on the rate of cellular growth. Walsh University supported the ethics of this study to improve the advancement in the area of science as we upheld high standards for our students and their results. This study was completed without any limitations.

2.2. SRB Assay and Project Design

Healthy cells, grown under sterile conditions, were counted using a hemocytometer, and transferred into a 96-well plate (1500 cells per well). Drug treatments were conducted for several days and the cytotoxicity was monitored via the Sulforhodamine B assay also known as SRB assay. There were several advantages for using the SRB assay including: 1) the reagents were safe and inexpensive, 2) the step-by-step protocol became ideal for nursing students who also were registered for hospital clinical courses that required long shifts, 3) the several start/stop points allowed for flexibility so work could be done between classes or exams, 4) SRB assays demonstrated nice visuals for the students to monitor progress (color changes), 5) our procedures were compatible with our current equipment at Walsh University.

For safety, the undergraduate students did not conduct any of the drug treatments (Thallium acetate, Thallium nitrate, and Barium nitrate) and, therefore, eliminated the potential exposure to toxic reagents. This project provided students with the unique opportunity to acquire knowledge in biological applications of inorganic compounds, tissue culture procedures, and sterile techniques.

In 1973, Herbert Soule and co-workers discovered this stable and viable breast cancer cell line, MCF-7. [26] MCF-7 stands for Michigan Cancer Foundation-7, the center in Detroit where they conducted these initial studies. MCF-7 cells are adherent epithelial cells of the mammary gland, a human adenocarcinoma cell line. Studies with this cell line initially began in 1970, originating from Frances Mallon, a 69 year-old female. [26,27] This research was pivotal to the advancement of cancer research due to limited knowledge of this disease and its complications. Before the exploration of MCF-7 cells, challenges arose due to the instability of breast cancer cells. It was

impossible for research groups to acquire a mammary gland cell line that was able to survive longer than a few months. [27,28] Therefore, the discovery of the MCF-7 cell line has been critical to the advancement in breast cancer research. [26,27] Figure 1 shows a photograph of the MCF-7 cells used in this work.

There are many general risk factors for developing melanoma including: excessive exposure to the sun including blistering sunburns, fair and or freckled skin that easily burns, family history of skin cancers, previous melanoma, previous nonmelanoma skin cancer (squamous cell carcinoma and/or basal cell carcinoma), and a significant quantity of moles and abnormal moles on the skin. [29] A375 cells, as shown in Figure 2, are adherent malignant melanoma cells from the surface of the skin that originated from a 54 year-old female. [29]

HFF cells are adherent cells that originated from a male newborn's foreskin following circumcision. These cells are an optimal choice because they can serve as an experimental control in research projects as well as to aid in comparisons due to their noncancerous characteristics [30,31]. In 2003, Hovatta and coworkers conducted a study using human foreskin fibroblasts as feeder cells allowing for the production of human embryonic stem cells. [30,31] Current research is continuing in this area to further understand how these cells may benefit the advancement of cancer research. A photograph of HFF cells used in this work is as shown in Figure 3.

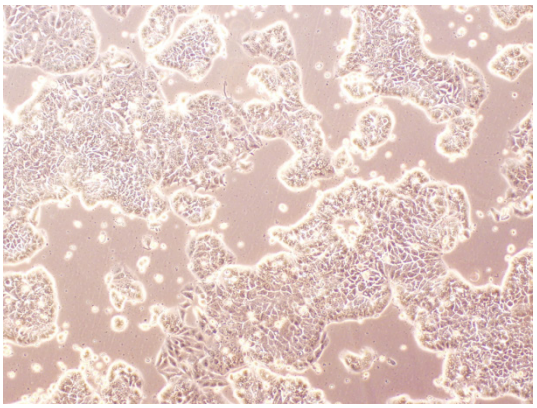

Figure 1. MCF-7 cells

Figure 2. A375 cells

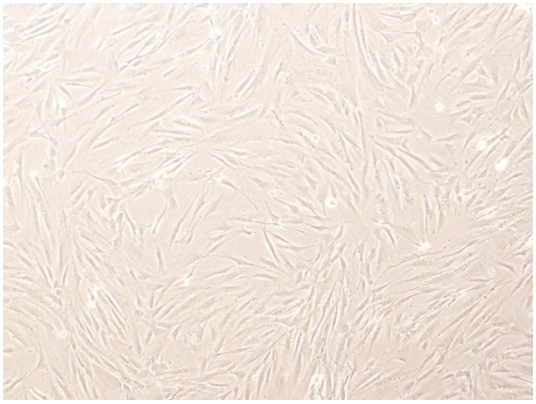

Figure 3. HFF cells

3. Results

3.1. SRB Assay Utilizing Thallium Acetate

The first study involved treating the cells with thallium (I) ions using 1-100 µM $TlCH_3COO$. The cells were treated with the compound until the cells in each well were 80% confluent. Complete cell death was apparent at the following concentrations: 2 µM $TlCH_3COO$ (MCF-7 cells), 100 µM $TlCH_3COO$ (A375 cells), and 100 µM $TlCH_3COO$ (HFF cells). The antiproliferative effects of $TlCH_3COO$ on these cells lines is illustrated in Figure 4 – Figure 6.

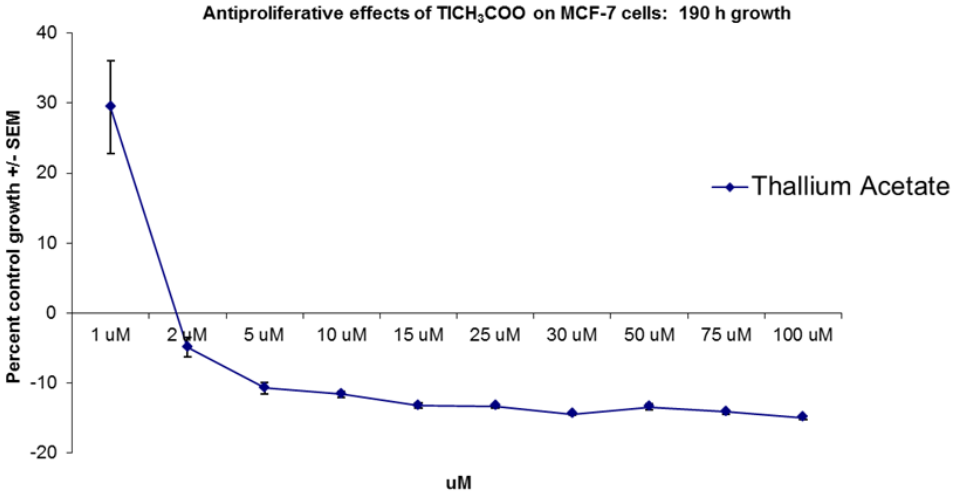

Figure 4. MCF-7 cells & Thallium acetate

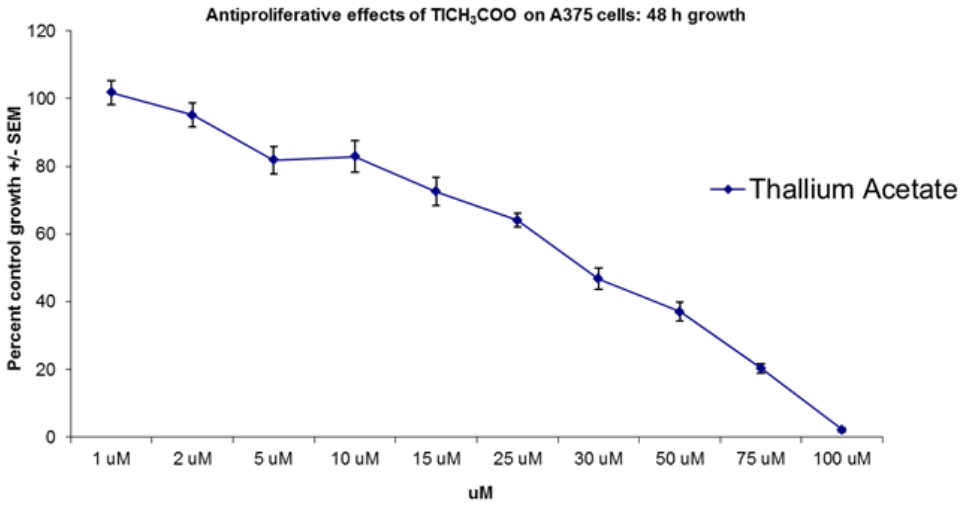

Figure 5. A375 Cells & Thallium acetate

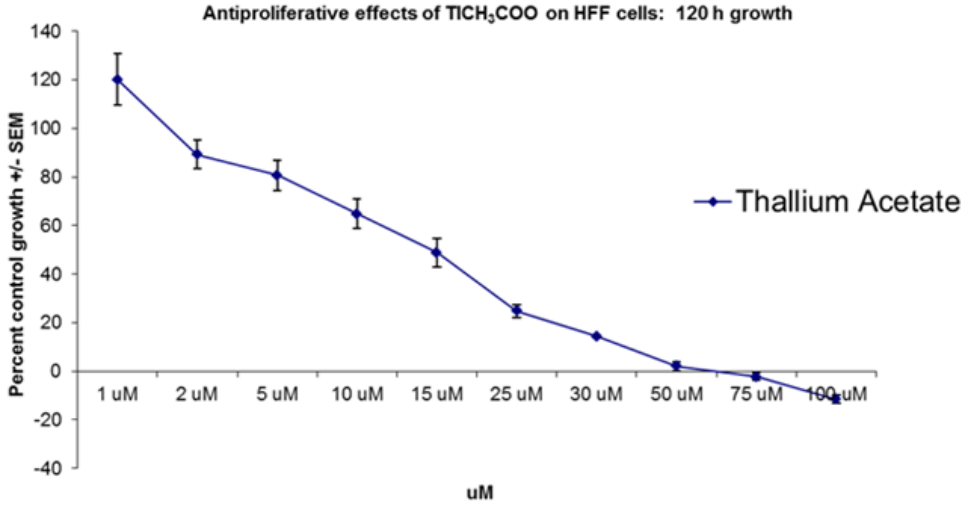

Figure 6. HFF cells & Thallium acetate

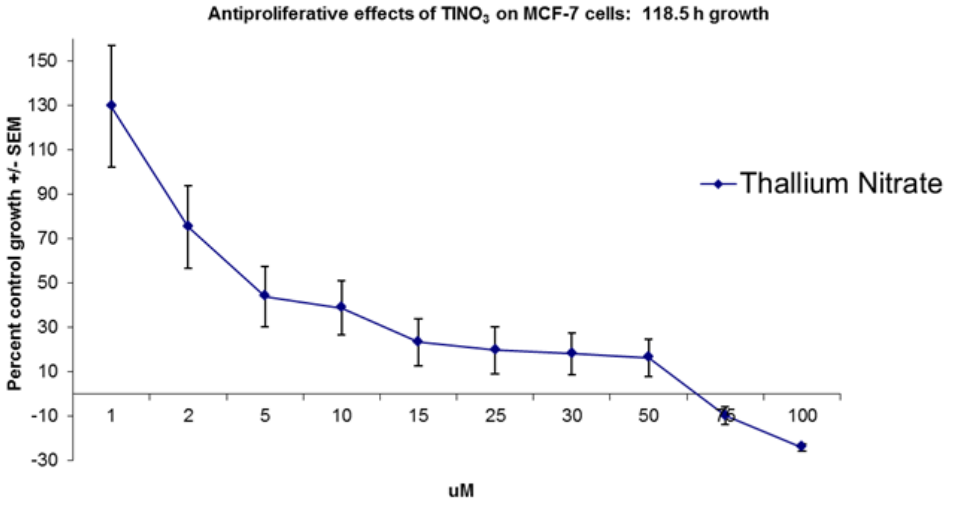

Figure 7. MCF-7 cells & Thallium nitrate

3.2. SRB Assay Utilizing Thallium Nitrate

The second study involved treatments with 1-100 µM $TlNO_3$. The cells were treated with this compound until the cells in each well were 80% confluent. Complete cell death was apparent at the following concentrations: 75 µM $TlNO_3$ (MCF-7 cells), 75 µM $TlNO_3$ (A375 cells), and 100 µM $TlNO_3$ (HFF cells). The antiproliferative effects of $TlNO_3$ on these cells lines is illustrated in Figure 7-Figure 9.

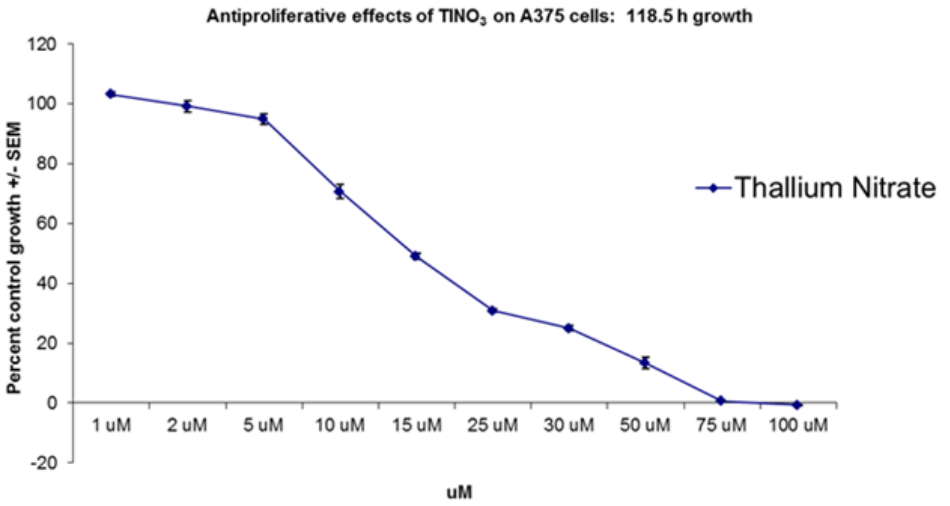

Figure 8. A375 cells & Thallium nitrate

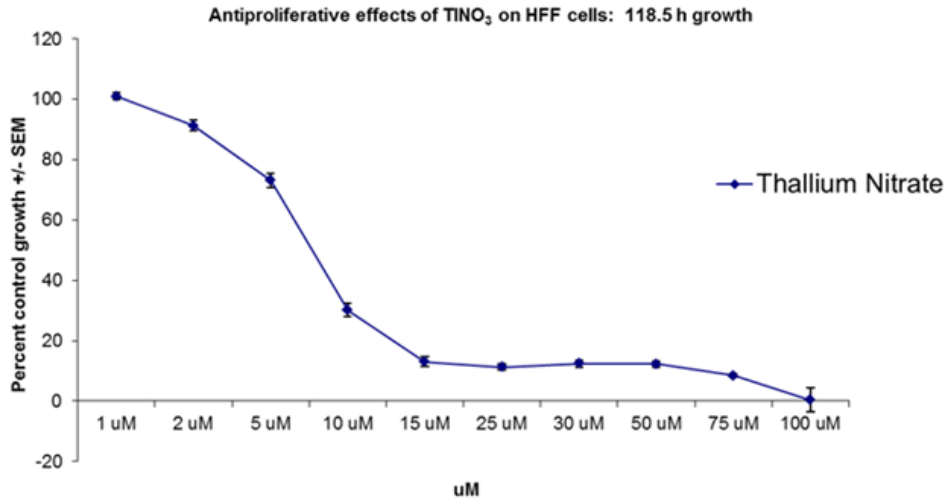

Figure 9. HFF cells & Thallium nitrate

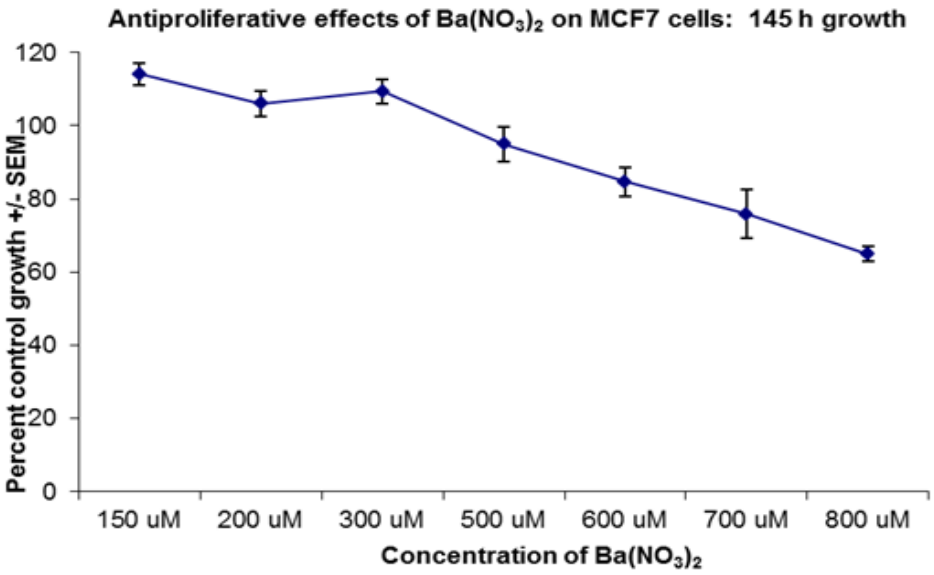

Figure 10. MCF-7 cells & Barium nitrate

3.3. SRB Assay Utilizing Barium Nitrate

The third study involved treating the cells with barium ions with 150-800 μM $Ba(NO_3)_2$. The cells were treated with the compound until the cells in each well were 80% confluent. Remarkably, these cells tolerated 800 μM $Ba(NO_3)_2$. The antiproliferative effects of $Ba(NO_3)_2$ on these cells lines is illustrated in Figure 10 - Figure 12.

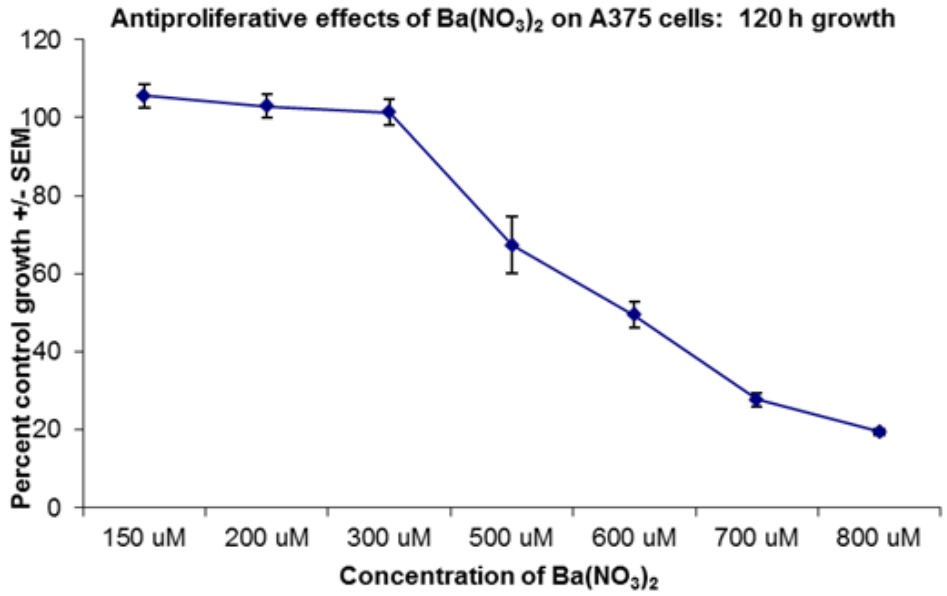

Figure 11. A375 cells & Barium nitrate

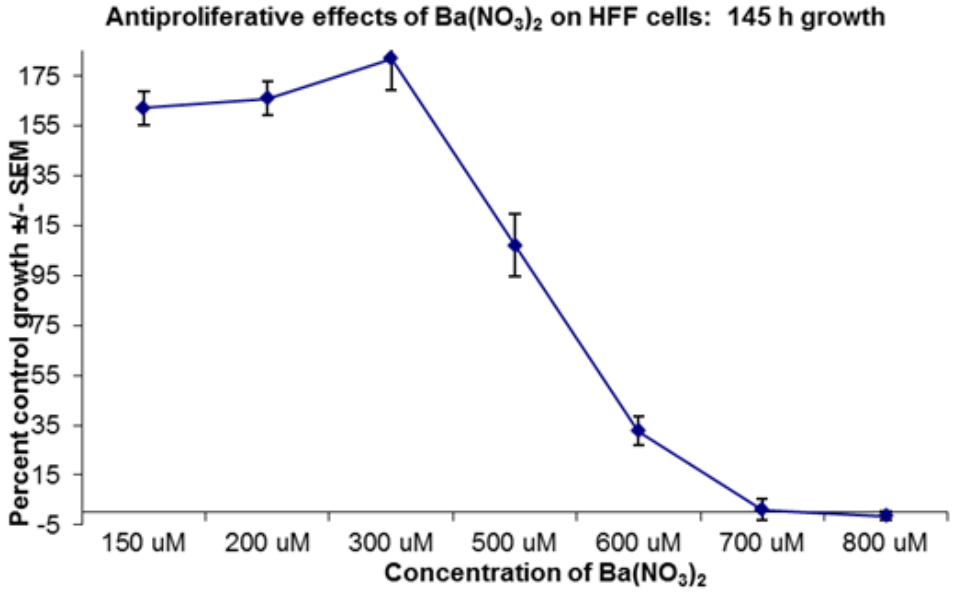

Figure 12. HFF cells & Barium nitrate

4. Discussion

The results of these experiments show that 2 uM $TlCH_3COO$ killed breast cancer cells while leaving the human foreskin fibroblasts relatively unchanged, meaning very little cell death was observed at this concentration. In comparison, complete cell death was found at 100 uM $TlCH_3COO$ for A375 cells and HFF cells. This was an interesting result for the students to see as a first study and they became increasingly curious about further outcomes. In 2016, Kortz and coworkers reported how thallium has the ability to serve as an antibacterial agent effective against Gram-positive bacteria, specifically *Bacillus aquimaris* and *Bacillus subtilis*. [25,32] Our results further support that this toxic metal can also serve a beneficial role, to kill cancer cells.

For the second study, it can be concluded that the MCF-7 cells and A375 cells are the most sensitive to $TlNO_3$. Complete cell death was observed for both breast cancer cells and skin cancer cells at 75 µM $TlNO_3$, whereas the HFF cells showed cell death at 100 µM $TlNO_3$. As shown in the last study, the HFF cells were the most sensitive to $Ba(NO_3)_2$. Remarkably, the cancerous cells tolerated 800 µM $Ba(NO_3)_2$. Cell death was not observed at this concentration leading to the fact that changing the metal cation, not the anion, made the most impact. This is what one would predict considering if, in fact, the metal ion is entering the cell through the sodium-potassium pump and responsible for cellular apoptosis. The SRB assays in this project demonstrated the antiproliferative effects of thallium ions on MCF-7, A375, and HFF cells, *in vitro*.

One primary goal for this undergraduate experience was to be sure the students could understand its importance to the field of chemistry and positively contribute and compliment current advances in chemistry. This project accomplished this goal. Our results help to provide greater insight into thallium and its antiproliferative

effects on these cell lines and, thereby, supports the work done by other research groups seeking to understand thallium. Some of these current topics include thallium's beneficial role as a radioisotope, its role as an antibacterial agent, and its impact on the environment. [4,7,8,25] Upon the completion of this project, students have gained more real-life applications in inorganic chemistry.

5. Conclusions

Due to the success of this project and positive feedback from the students, this work should continue with future undergraduate students. It has proved to be a valuable learning experience for any science or nursing major. A future project could involve investigating the antiproliferative effects of other heavy metal compounds on these cells in order to kill cancerous cells, but leaving the HFF cells unchanged, the primary focus of this work.

An honors project could be realized for either a nursing student or science major. This work can include investigation of higher concentrations of $Ba(NO_3)_2$. A Western blot analysis could be used to further understand the mechanism of cell death of these cells. Other metals, having similar atomic radii, could be explored and compared to the results shown here. Another possible project could be to use different cancer cell lines or different noncancerous cell lines to provide a greater insight into the effectiveness of these compounds. Another interesting comparison could include varying the treatment times in order to see how that may give useful trends or information regarding these cells. Due to the success of the project, it would be best to continue using fetal calf serum to ensure optimal growth and continue utilizing sterile techniques to avoid contamination.

New students joining the research team can provide new advantages for the continuing students that include student mentoring and leadership opportunities. The main goal should be to help inspire students to be curious about the world around them and to reinforce material from other courses. In addition, this work will help students with a special opportunity to see the scientific applications to real-life situations, enhance their critical thinking in chemistry, and increase student self-efficacy in chemistry.

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