HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 3 (2022)

Effect of Taurine on the Proliferation of *Leishmania tarentolae* Cells in Culture

Srihas Rao[1] and Majorie Jones[#]

[1] Adlai E. Stevenson High School, Lincolnshire, IL, USA
[#]Advisor

ABSTRACT

Taurine (Tu), an amino acid which has antioxidant and wound healing effects, is being used to treat the wounds of individuals infected with *Leishmania*. *Leishmania* are parasitic protozoans that cause the leishmaniasis disease. The purpose of this experiment is to determine if taurine can be used to directly reduce the proliferation of *Leishmania* cells. Treatment of leishmaniasis is getting complicated due to multiple side effects and drug resistance to first-line drugs. The MTT viability assay was used to determine the effect of taurine on *Leishmania* tarentolae cells in culture. *Leishmania* cells were incubated with 20µM taurine, 200µM taurine, 500 µM taurine or 200µM Vanadium which is a known inhibitor of acid phosphatase activity. Control cells did not have any additions. Incubations with and without additions were carried out for 48 hours. This study showed the ability to improve cell proliferation, although taurine had no direct anti-leishmaniasis effect. Further studies are needed to determine if higher amounts of taurine are effective for treatment of *Leishmania* cells.

Introduction

Annually, 700,000 to 1,000,000 new cases of leishmaniasis occurs around the world. Currently having no cure, this skin and intestinal infection is caused by one of the 20 species of *Leishmania*, a parasitic protozoan transmitted by the bite of a sand fly. This disease is still prevalent today because it infects people without proper homes, food, or money to protect themselves. Thus, it is very common in developing countries (Leishmaniasis, 2021).

Treatment of leishmaniasis is difficult because of side effects and drug resistance to mainstream drugs. Taurine (Tu) is an amino acid found in the brain, retina, heart, and blood cells and has some function in blood clotting, initiated by platelets in blood. It is found in many foods such as energy drinks, meat, or dairy products. In the body, it also has an anti-inflammatory and anti-apoptotic effect, and it reduces inflammation and unnecessary cell death. Because of this, rapid re-epithelization, or regrowing of damaged epithelial tissue, is increased by Tu. Thus, it plays a significant role in wound healing because regrowing the epithelial tissue can speed up the healing process (Ashkani-Esfahani et al., 2014). Additionally, it can benefit people with diabetes because it can lower blood glucose levels which can make it easier for a person to manage symptoms. People susceptible to heart diseases are also benefited by taking taurine because it reduces risk factors, such as blood pressure and glucose. Finally, taurine aids muscles in exercise by burning more fat, thus damaging the muscle less and reducing fatigue. Thus, taurine has many health benefits, and daily supplements of 500–3,000 mg of Taurine are considered to be safe, inexpensive, and effective at improving physical and mental cognitive performance (Turner et al., 2012).

Although humans produce taurine, some people do not get enough from their diet or supplements, which can have complications. In order to treat these complications, doctors give more supplements of taurine. For example, doctors use taurine to treat hepatitis and congestive heart failure. It is also commonly used to legally enhance athletic performance because it can increase an athlete's energy while also not having harmful

side effects, but there is no scientific evidence that it can boost energy. Taurine also plays a significant role in the nervous and cardiovascular system. By supporting nerve growth, it allows the nervous system to function properly. It also benefits people by reducing the risks of heart attacks by decreasing blood pressure thereby helping to prevent heart failure.

The three types of Leishmaniasis, which spreads via sandflies, are Mucocutaneous, Cutaneous, and Visceral leishmaniasis. The most serious type of leishmaniasis is Visceral. Visceral Leishmaniasis causes severe fever, liver enlargement, weight loss, and, if left untreated, death.

Visceral Leishmaniasis affects people who have poor nutrition, housing, or weakened immune system. Thus, it commonly affects poor people in underdeveloped countries. Even if visceral Leishmaniasis is successfully treated, a skin condition called post-kala-azar dermal leishmaniasis, or PKDL, can appear years after. Even though PKDL is not a life-threatening condition, PKDL can disfigure a person's face or limbs. Like other diseases, people with PKDL can still transmit visceral leishmaniasis, which makes it difficult to eliminate the disease. People in North and South America have tried to use various types of chemotherapy for treatment and to reduce how infectious the disease is for a person. However, high failure rates to treat a person have caused a surge in people trying to find a cure for the disease (Vargas et al., 2019).

Cutaneous leishmaniasis (CL) is the most prevalent disease caused by *Leishmania* parasites. In 2019, more than 87% of new CL cases occurred in just ten countries: Algeria, Afghanistan, Pakistan, Colombia, Tunisia, Iraq, Libya, Brazil, the Syrian Arab Republic, and Iran. Thus, this disease is mostly occurring in developing countries without proper sanitation and healthcare systems. It can result in lesions on skin around where a sand fly bites a person, which leads to permanent scars and disfigurement. Initial damage caused by the infection causes collagen, the protein building block of the skin, to be damaged, resulting in an atrophic scar that is permanent. For the past few years, researchers have been looking into possible ways to eradicate *Leishmania* and also find how to fight the symptoms that affect people for their entire lives. Pentostam and Glucantime are two drugs that are commonly used for treatment right now. However, they cannot reduce the significant scarring and they can be harmful because of the life-threatening complications. Depending on the dose, the drugs can cause damage to organs such as the heart, liver, or pancreas. Thus, studies in order to find new treatments that have better results in improving the wound healing process and decrease scar formation with fewer side effects are being conducted (Ashkani-Esfahani et al., 2014).

Precautionary Steps to Prevent Leishmaniasis: Currently, no vaccine is available for leishmaniasis, so the only way to combat the disease is by avoiding sandflies and not getting bitten (Cafasso, n.d.).

- Reduce travel to developing countries where there are cases of any form of leishmaniasis in the population.
- When outside, wear clothes that cover as much skin as possible such as long pants, jackets, and long socks.
- If any skin is exposed, spray it with an insect repellent such as DEET, or N, N-Diethyl-meta-toluamide.
- Sleeping in the upper levels of a building can reduce the risk of encountering sand flies and thus leishmaniasis
- Avoid the outdoors between dawn and dusk because sandflies are most active during that time.
- Sandflies are much smaller than mosquitos, so using a bed net tucked in a mattress can help.

Mucocutaneous leishmaniasis leads to complete or incomplete damage of mucous membranes in the body, specifically in the orthonasal cavity. More than ninety percent of mucocutaneous Leishmaniasis cases occur in just four countries: Peru, Brazil, Bolivia, and Ethiopia (Leishmaniasis, 2021).

For this experiment, the species of *Leishmania* cells used were the *Leishmania* tarentolae that only infect reptiles to ensure our safety. This species still has all of the same impacts on reptiles as other species have on humans but is not capable of infecting humans. In a previous study, it was found that when mice infected with leishmaniasis were treated with taurine, they were able to heal their wounds faster (Ashkani-Esfahani et al., 2014). However, this study did not determine whether taurine was directly inhibiting *Leishmania* cells or if

it is speeding up the wound healing process. Thus, this experiment can help clarify the results of a previous study.

This experiment was undertaken to evaluate the effect of taurine on *Leishmania* tarentolae cells by using the MTT viability assay. The MTT assay was used to measure cellular metabolic activity as an indicator of cell viability and proliferation. Sodium orthovanadate (Na_3VO_4) was used as a positive control because of previous research that showed that it inhibited the proliferation of *Leishmania* cells. Na_3VO_4 is a common inhibitor for protein phosphotyrosyl another phosphatases (PTPs). Thus, Na_3VO_4 can inhibit cell to cell interactions because PTPs are used for cell signaling. Because of this, vanadium compounds have been used to treat a wide array of diseases, such as diabetes, cancer, and now Leishmaniasis (Turner et al., 2012).

Materials & Equipment

Reagents:
Phosphate Buffered Saline (PBS)
Cell culture of L. tarentolae: grown in sterile Brain Heart Infusion medium (BHI) and heme groups
96-well plate(s), tissue culture grade, flat bottomed
5 mL tubes
Serological pipettes
Pipette tips (1 - 200 µL)
Equipment:
Inverted microscope
Multichannel pipettor
Cell culture facilities including a laminar flow hood and a cell incubator
Absorbance Plate reader with 595 nm filters
MTT Assay kit (Cell Proliferation) (ab211091) abcam

Procedure

The experiment was performed by dividing the cells into 5 groups: Control cells, +20 µM taurine, 200 µM taurine, 500 µM taurine or 200µM vanadium. The blank is flask with medium only (no added cells).

MTT Assay Protocol: Steps for the Assessment of Cell Viability by the MTT Viability
Take cells that come with nutrients and add 50 mL to a flask. Then add the concentration of taurine or vanadium required and label the flask with tape. Place flasks in an incubator at room temperature. Transfer 450 µL of cell suspension into vials while ensuring no contamination is occurring with gloves and sterile techniques. Take vials into the adjacent fume hood. Place 96 well plates on a flat surface. Add 100µL of each suspension into 4 wells; Label so that E, F, G, and H for each number is one group. Add 10µL of MTT reagent (3 mg/mL water) into each well containing sample; cover and incubate at room temperature. After one hour, add 100 µL of stopping reagent (isopropanol, triton X-100 detergent with HCl) into each well and mix. Evaluate absorbance of the formazan solution spectrophotometrically at 595 nm; the blank is the cell medium only. Monitor cell culture for viability at 0.5hrs, 2hrs, and 48hrs, after addition of taurine or vanadium. Determine the average values from readings and subtract the average value for the blank from the samples with cells. . Plot absorbance on the y-axis versus treatment on the x-axis
Beer Lambert Law:

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm

MTT Assay: MTT assay is a laboratory test and standard colorimetric assay for measuring cellular proliferation (cell viability). The test is based on the reduction of yellow tetrazolium salt (MTT) to purple formazan crystals by metabolically active cells; viable cells contain NADH-dependent oxidoreductase enzymes that reduce the MTT to formazan. The insoluble crystals are dissolved using a solubilization solution, in which the darker the color of the resulting solution, the greater the number of metabolically active cells. MTT Assay is used to determine cell viability and can quantitatively and qualitatively obtain data on the number of viable cells. Reductase enzymes in the *Leishmania* cells react with the MTT reagent and reduce the MTT, turning it blue. A deeper blue correlate to more viable cells due to the Beer-Lambert law, where Absorbance=Molar absorptivity x Concentration x Path length. Because path length and absorptivity are constants, absorbance and concentration are proportional, so a deeper blue (higher concentration and absorbance) result from a larger number of viable cells. The absorbance at 590 nm was measured for each well on an absorbance plate reader at 0.5 hour, 2 hours, and 48 hours. The molar absorptivity is a measure of how well the species absorbs the particular wavelength of radiation that is being shined on it (Wenzel, 2018). Because the higher the molar absorptivity, the higher the absorbance, the molar absorptivity is directly proportional to the absorbance. Net absorbance used for data analysis was calculated by subtracting cell sample medium data absorbance - BHI medium absorbance. Calculated absorbance rate was determined by using average values from readings and subtracting the average value for the blank and dividing by number of hours of incubation. The formula to calculate absorbance rate = 100 * (Sample data medium absorbance - BHI medium absorbance) /(number of hours). This shows the rate at which the cells are growing, because an increased absorbance is caused by having more cells in the medium.

Results

To investigate the effect of taurine on Leishmaniasis, cultured cells were exposed to Control, 20μM taurine, 200μM taurine, 500 μM taurine, or 200 μM vanadium. The Tables 1, 2, and 4 below show the corrected MTT viability data at zero time, 2 hours, and 48 hours after additions. In each Table, the mean for 4 repliaces is also shown for each of the 6 flasks. Tables 3 and 5 indicate the calculated rates of activity (an assessment of cell viability) as a function of the time and additions.

Inhibition of Cell Proliferation by Taurine

Table 1. MTT absorption results for all 4 replicate samples when data collection was completed before adding substances. These values refer to the amount of light that is absorbed by a sample when exposed to 595 nm (blue) light Mean values was calculated for each flask.

MTT Absorbance at 2 hours						
Replicate	**BHI**	**Control cells**	**20µM Tu**	**200µM Tu**	**500µM Tu**	**200 µM Na_3VO_4**
1	0.142	0.286	0.302	0.284	0.319	0.250
2	0.138	0.296	0.284	0.290	0.285	0.244
3	0.129	0.307	0.291	0.304	0.296	0.279
4	0.164	0.313	0.294	0.292	0.290	0.246
Mean	0.143	0.301	0.293	0.293	0.298	0.255
STDEV	±0.015	±0.012	±0.007	±0.008	±0.015	±0.016

MTT Absorbance at 0 hours						
Replicate	**BHI**	**Control cells**	**20µM Tu**	**200µM Tu**	**500µM Tu**	**200 µM Na_3VO_4**
1	0.043	0.109	0.109	0.109	0.109	0.109
2	0.027	0.109	0.109	0.109	0.109	0.109
3	0.039	0.109	0.109	0.109	0.109	0.109
4	0.034	0.109	0.109	0.109	0.109	0.109
Mean	0.036	0.109	0.109	0.109	0.109	0.109

Table 2. MTT absorption results for all 6 samples when data collection was completed 2 hours after adding substances.

Table 3. Average and Standard deviation are calculated for each concentration after 2 hours. Rate of change calculated by subtracting BHI reading from average values of each concentration and dividing by hours. Rate = 100 * (Sample data - BHI) /(2)

	Average Absorbance	STDEV	Average Net Absorbance	Rate
BHI	0.143	±0.015	--	--
Control	0.301	±0.012	0.157	0.079/hour
20µM Tu	0.293	±0.007	0.150	0.075/hour
200µM Tu	0.293	±0.008	0.149	0.075/hour
500µM Tu	0.298	±0.015	0.154	0.077/hour
200 µM Na_3VO_4	0.255	±0.016	0.112	0.056/hour

Table 4. MTT absorption results for all 6 samples when data collection was completed 48 hours after adding substances.

MTT Absorbance at 48 hours						
Replicate	**BHI**	**Control cells**	**20µM Tu**	**200µM Tu**	**500µM Tu**	**200 µM Na_3VO_4**
1	0.278	0.555	0.608	0.434	0.572	0.408
2	0.257	0.514	0.692	0.435	0.503	0.426
3	0.216	0.589	0.575	0.444	0.477	0.444
4	0.172	0.486	0.533	0.447	0.441	0.451
Mean	0.231	0.536	0.602	0.440	0.498	0.432
STDEV	±0.047	±0.045	±0.067	±0.006	±0.055	±0.019

Table 5. Average and Standard deviation are calculated for each concentration after 48 hours. Rate of change calculated by subtracting BHI reading from average values of each concentration and dividing by hours. Rate = 100 * (Sample data - BHI) /48

	Average Absorbance	STDEV	Average Net Absorbance	Rate
BHI	0.231	±0.047	--	--
Control	0.536	±0.045	0.305	0.636/hour
20µM Tu	0.602	±0.067	0.371	0.773/hour
200µM Tu	0.440	±0.006	0.209	0.436/hour
500µM Tu	0.498	±0.055	0.268	0.557/hour
200 µM Na_3VO_4	0.432	±0.019	0.202	0.420/hour

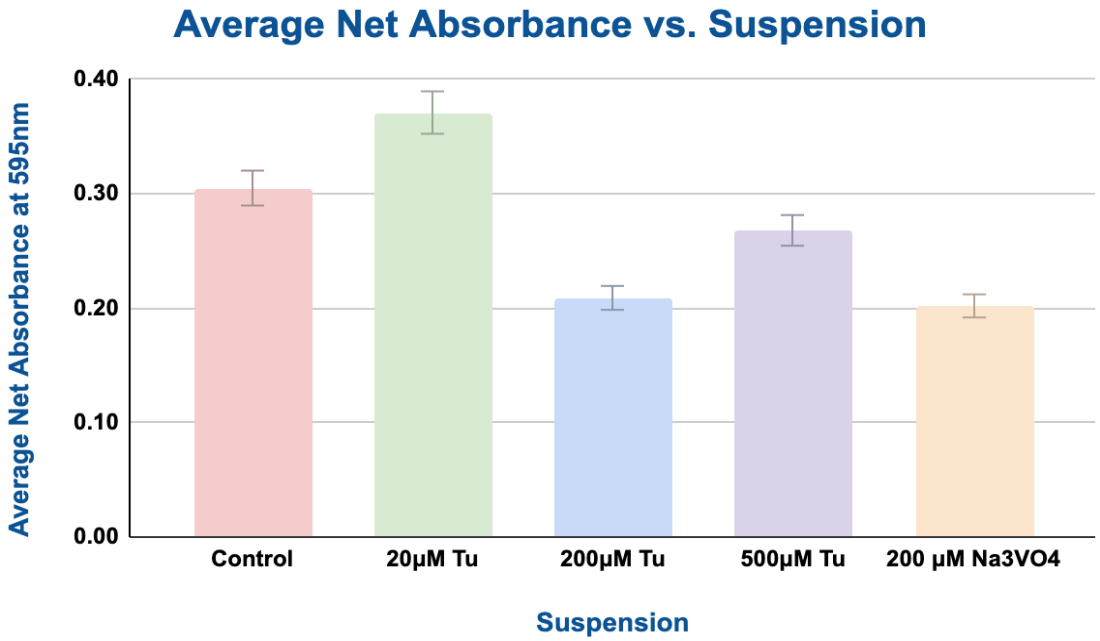

Figure 1. Is a comparison of the corrected cell viability with and without additions. As shown, the major inhibition shown when vanadium was added to the flask and is inhibited by about 20%. With the addition of the 500 µM taurine, the cell culture viability was only inhibited by about 8%.

The effects of taurine were analyzed by plotting net absorbance on the y-axis versus treatment on the x-axis. As shown **in Figure 1**, 20 µM of Taurine showed a modest increase in absorbance compared to the control group. However, the additions of 200 µM and 500 µM of taurine showed a lower absorbance than the control but higher than 200 µM of vanadium. Thus, we conclude that taurine has an inhibitory effect on the proliferation of *Leishmania* cells, but not as strong as Na_3VO_4. Error bars on the graph are not overlapping, showing that these data are statistically significant. As shown in Table 3 and Table 5, the rate of change in absorbance of the five samples after 2 hours and 48 hours of duration also show similar effects.

Based on this bar graph, 200 µM taurine had the most inhibitory effect on the parasite compared to other additions since the 500 µM also showed an inhibitory, but weaker, effect, whereas 20 µM taurine actually showed an increase in viable parasitic cells. This experiment should be repeated to confirm these results by increasing the sample size.

Statistical Analysis of the Data

All the experiments were performed in quadruplicates. The data were collected, analyzed, and reported as an average and standard deviation (mean ± SD). Statistical analysis was evaluated by using a T- test in Excel and error bars on the graph. In this analysis, $p < 0.05$ is considered statistically significant, showing that there is only a very small probability that the result occurred by chance.

Conclusion

Drawing upon previous studies that showed vanadium to be a known inhibitor for *Leishmania* activity, I compared net absorbance data results collected during the study using MTT assay. Based on the results of the study, taurine may show modest ability to inhibit *Leishmania* activity depending on the concentration. Specifically, from our results, 200 μM Taurine had the most inhibitory effect on the parasite compared to other concentrations. This shows that taurine can potentially play a substantial role in fighting against these severe skin infections, compared with only wound healing. To confirm these results, future studies should repeat and expand upon this experiment so as to further analyze the different effects of Taurine on *Leishmania* cells.

Limitations

Through our research, we establish baseline changes in *Leishmania* activity following taurine treatment, which will be an important reference point for future studies. However, changes in *Leishmania* activity were limited by the short study duration. Future studies should therefore investigate the effects of longer-term Taurine treatment.

Acknowledgments

In the successful completion of this project, I would like to thank Dr. Marjorie Jones from the Illinois Summer Research Academy (ISRA), for being an excellent mentor and a help in preparing and editing this paper. I would like to thank my family for their support and encouragement throughout my project.

References

1. Ashkani-Esfahani, S., Zarifi, F., Asgari, Q., Samadnejad, A. Z., Rafiee, S., & Noorafshan, A. (2014, October 7). Taurine improves the wound healing process in cutaneous Leishmaniasis in mice model, based on stereological parameters. Advanced biomedical research. Retrieved December 1, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4202507/.
2. Cafasso, J. (n.d.). Leishmaniasis: Causes, diagnosis, & treatment. Healthline. https://www.healthline.com/health/Leishmaniasis#prevention
3. Leishmaniasis. [online] Available at: <https://www.who.int/news-room/fact-sheets/detail/Leishmaniasis> [Accessed 13 September 2021].
4. Mawer, R. (2018, November 27). What Is Taurine? Benefits, Side Effects and More. Retrieved from https://www.healthline.com/nutrition/what-is-taurine#what-it-is
5. Reece, J. B., & Campbell, N. A. (2011). Campbell biology. Boston: Benjamin Cummings / Pearson.
6. Taurine: Uses, Side Effects, Interactions, Dosage, and Warning. (2019). Webmd.com. https://www.webmd.com/vitamins/ai/ingredientmono-1024/taurine
7. Vargas, D. A., Prieto, M. D., Martínez-Valencia, A. J., Cossio, A., Burgess, K. E. V., Burchmore, R. J. S., & Gómez, M. A. (2019). Pharmacometabolomics of Meglumine Antimoniate in Patients With Cutaneous Leishmaniasis. Frontiers in Pharmacology, 10. https://doi.org/10.3389/fphar.2019.00657
8. Wenzel, T. (2018, October 20). 1.2: Beer's Law. Chemistry LibreTexts. https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Molecular_and_Atomic_Spectroscopy_(Wenzel)/1%3A_General_Background_on_Molecular_Spectroscopy/1.2%3A_Beers_Law