Mission Folder: View Mission for 'Myto-critters'

State	New Mexico
Grade	9th
Mission Challenge	Food, Health and Fitness
Method	Scientific Inquiry using Scientific Practices
Students	mountainlion soccer29 zarg525

Team Collaboration

(1) Describe the plan your team used to complete your Mission Folder. Be sure to explain the role of each team member and how you shared and assigned responsibilities. Describe your team's process to ensure that assignments were completed on time and deadlines were met.

The team used a multi-layer command structure to complete the Mission Folder and adhere to deadlines. soccer29 and zarg525 reported to team leader mountainlion who in turn reported to the Team Advisor. Each individual had specialties that were combined into one final team effort.

Summer Months: Research
mountainlion and zarg525 researched cellular function, mitochondria, cell viability and dyes used to identify mitochondrial activity, while soccer29 researched antibiotics, contaminants and the reaction of the contaminants chemically to other detectors.

August-September:
mountainlion and zarg525 worked on early trials of worm cellular culturing, progressing with different cellular reactants including antibiotics, natural remedies and capsaicin for reaction. soccer29 began to collate paperwork.

October-November:
Experimentation, all team members were involved in caring for the cellular cultures and conducting experimentation for half hour trials.

December-January:
Completion of experimentation, collation of data and data analysis of all team members, conclusion drawing.

Scientific Inquiry

Problem Statement

(1) What problem in your community did your team try to solve? Why is this problem important to your community?

Antibiotics work by affecting things that bacterial cells have but human cells don't. They kill bacteria by neutralizing bacteria, tearing bacteria apart, or by disabling the bacteria. It is being determined that many antibiotics can cause mitochondrial toxicity, an affliction where the mitochondria of a body's cells become damaged or decline significantly in number, and therefore the cell ceases to function properly. This leads to a number of mystery ailments and even death. Chlorofluoroquinones (Cipro) and the stronger antibiotics meant for multi-level dosing or radical antibiotic dosing have the potential of wiping out the mitochondria, and causing a situation where the mitochondria never return. Natural antibiotics while less aggressive on bacteria, are also more gentle to the system, and in particular, the cells, breaking down and metabolizing so that the bacterium is neutralized but the other cells are not confused as bacterium. The purpose of this project is to compare and contrast natural vs. synthetic antibiotics both strong and commonly prescribed to determine their impact in causing mitochondrial toxicity or dysfunction. This is a vital issue for our community who primarily have well water access, and are finding themselves exposed to bioaccumulative antibiotics and other prescription drugs. This not only demonstrates the impact these antibiotics are having on mitochondria and illness, but also demonstrates an alternative to using these antibiotics by using natural remedies that degrade and metabolize in water. The worms are also being used as a viable alternative to human/vertebrate testing.

(2) List at least 10 resources you used to complete your research (e.g., websites, professional journals, periodicals, subject matter experts).

See attached.

(3) Describe what you learned in your research.

Our research initially brought us through the entire cell cycle, and a deeper meaning to mitochondria. We studied news reports about how certain antibiotics impacted mitochondrial function producing mitochondrial toxicity, which is the major malfunction of the mitochondrial path. This can lead to the ultimate failure of cellular function which in turn leads to oxidation of the cell, and disease of the tissues and entire body. Originally mitochondrial toxicity was believed to be related to just HIV/AIDS patients, but has since been determined in people who are not HIV positive. Antibiotics work to deactivate bacteria, but many of the cell's inner workings are similar to bacteria, particularly the mitochondria, and therefore the antibiotics do not stop at the bacteria, and can reprogram the cell in its normal function. Natural remedies can be used as antibiotics, and are antibacterial and do not impact the function and structure of antibiotics, metabolize in the body and breakdown easier in the environment. The natural remedies can also be provided as dietary supplements to reduce oxidation and ailment over time.

Hypothesis

(4) State your hypothesis. Describe how your hypothesis could help solve your problem.

It is hypothesized that synthetic antibiotics will cause mitochondrial toxicity, therefore increasing the cell death, and the death of the mitochondria, or accelerated glycolysis of the mitochondria. Natural antibiotics will have no significant impact on the cell or the mitochondria.
It is also hypothesized that chlorofluoroquinones (ciprofloaxin) will have the fastest and most detrimental impact on the mitochondria, while the 'cillins' will have less impact. Antibiotics work by affecting things that bacterial cells have but human cells don't. They kill bacteria by neutralizing bacteria, tearing bacteria apart, or by disabling the bacteria. It is being determined that many antibiotics can cause mitochondrial toxicity, an affliction where the mitochondria of a body's cells become damaged or decline significantly in number, and therefore the cell ceases to function properly. This leads to a number of mystery ailments and even death. Chlorofluoroquinones (Cipro) and the stronger antibiotics meant for multi-level dosing or radical antibiotic dosing have the potential of wiping out the mitochondria, and causing a situation where the mitochondria never return. Natural antibiotics, while less aggressive on bacteria, are also more gentle to the system, and in particular, the cells, breaking down and metabolizing so that the bacterium is neutralized but the other cells are not confused as bacterium.

(5) Identify the independent variables and the dependent variables in your hypothesis.

The independent variables are the natural and synthetically produced antibiotics vs. the mitochondria and cellular reaction of the worm cells. Dependent variables are the cellular changes and the reaction of Janus Rhodamine in the examination of the mitochondrial behavior and toxicity reaction.

(6) How did you measure the validity of your hypothesis? (In other words, how did you determine that your hypothesis measures what it is SUPPOSED to measure?)

The hypotheses were measured by plating worm cells onto nutrient agar plates, and then determining their viability by using Rhodamine B. The mitochondria were then illuminated by injecting the cells with Janus B. The processing of ATP via glycolysis and therefore mitochondrial function is illuminated by the color change reaction instituted within the Janus B, and by the deterioration of the mitochondria. The cellular reaction to the solutions will also be monitored to determine cell death or continued cell function and viability. Antibiotics tend to kill off mitochondria, and other substances contribute to the continued existence of the mitochondria.

Experimental Design

(7) List the materials you used in your experiment. Include technologies you used (e.g., scientific equipment, internet resources, computer programs, multimedia, etc.).

See attached.

(8) Identify the control group and the constants in your experiment.

The control group were the cells as they were plated without any additions, the constants were the sterility of the cell harvesting and plating, as well as the sustainability of the cells' viability through dextrose feeding and incubation. The amount of solution added and the assays used were also constant. The overall testing environment was also constant.

(9) What was your experimental process? Include each of the steps in your experiment.

See attached.

Data Collection and Analysis

(10) Describe the data you collected and observed in your testing (use of data tables, charts, and/or graph is encouraged).

The data collected and observed in the testing was via observation through introduction of JanusB and Rhodamine viability, and through observation of mitochondrial survival and function in the presence of these dyes and in the presence of the solutions.
See attached.

(11) Analyze the data you collected and observed in your testing. Does your data support or refute your hypothesis? Do not answer with a yes or no. Explain your answer using one of the following prompts: 'Our data supports/refutes the hypothesis because...'

Our data supports the hypothesis because The first hypothesis stated that synthetic antibiotics will cause mitochondrial toxicity, therefore increasing the cell death, and the death of the mitochondria, or accelerated glycolysis of the mitochondria. Natural antibiotics will have no significant impact on the cell or the mitochondria, and is supported, as the cell death and mitochondrial death was increased by the synthetic antibiotics while the natural antibiotics had no change or a positive change in mitochondrial function. The second hypothesis indicated that chlorofluoroquinones (ciprofloaxin) will have the fastest and most detrimental impact on the mitochondria, while the 'cillins' will have less impact was also supported. The 'cillins' had a slower impact on the mitochondrial function and mortality than the chlorofluoroquinone; Cipro.

(12) Explain any sources of error and how these could have affected your results.

1) The initial experiment had to determine which cells of the worm had the most mitochondria; epidermal or neural, and the early epidermal cultures died in the presence of Janus B until more dextrose was added to the plates. This had little impact on the data, as the dextrose was moderated and the procedures were repeated.
2) Early natural solutions began to mold if not used right away and had to be replaced.This had no impact on the data because the solutions were replaced before they were used on the plates.
3) Overfeeding of the cell cultures with dextrose would cause the dye to flow over the entire plate rather than in the place needed. This did not impact the data as the trials were repeated.

Drawing Conclusions

(13) Interpret and evaluate your results and write a conclusion statement that includes the following: Describe what you would do if you wanted to retest or further test your hypothesis. Evaluate the usefulness of the data your team collected. What changes would you make to your hypothesis and/or experimental design in the future, if any?

Upon conducting the experiment and analyzing the data, it can be concluded that synthetic antibiotics have a greater potential of causing mitochondrial toxicity and mitochondrial loss, particularly Cipro, which eliminated most of the mitochondria in less than 5 minutes. The natural antibiotics had little to no impact on the mitochondria, their function or their mortality and in fact Rosemary and Cota increased mitochondrial function as indicated by the Janus B color change and cellular size and function. The first hypothesis stated that synthetic antibiotics will cause mitochondrial toxicity, therefore increasing the cell death, and the death of the mitochondria, or accelerated glycolysis of the mitochondria. Natural antibiotics will have no significant impact on the cell or the mitochondria, and is supported, as the cell death and mitochondrial death was increased by the synthetic antibiotics while the natural antibiotics had no change or a positive change in mitochondrial function. The second hypothesis indicated that chlorofluoroquinones (ciprofloaxin) will have the fastest and most detrimental impact on the mitochondria, while the 'cillins' will have less impact was also supported. The 'cillins' had a slower impact on the mitochondrial function and mortality than the chlorofluoroquinone; Cipro. This is a fairly accurate measure of mitochondrial toxicity. In the future, experimentation on vertebrate mitochondria may assist in determining actual viability of the development of natural antibiotics that will not impact mitochondria.

Uploaded Files:

- [**View**] **Bibliography** (By: mountainlion, 02/29/2016, .docx)

  Research sources for the experiment/project.
- [**View**] **Materials and Procedure** (By: mountainlion, 02/29/2016, .docx)

Materials and Procedure followed to conduct experimentation.

- [View] **Results** (By: mountainlion, 02/29/2016, .docx)

  The results of the experimentation

Community Benefit

(1) How could your experiments and data help solve your problem and benefit your community? Describe next steps for further research/experimentation and how you have or how you could implement your solution in the future.

Antibiotics work by affecting things that bacterial cells have but human cells don't. They kill bacteria by neutralizing bacteria, tearing bacteria apart, or by disabling the bacteria. It is being determined that many antibiotics can cause mitochondrial toxicity, an affliction where the mitochondria of a body's cells become damaged or decline significantly in number, and therefore the cell ceases to function properly. This leads to a number of mystery ailments and even death. This experimentation will help solve the problem that is being tackled, and could benefit our community through finding alternative medications that will be healthy at the cellular level, but also help cure mitochondrial toxicity. Many of the alternative medications are grown in the community, so therefore an industry could be made by growing and harvesting the natural medications to produce them on a mass basis. Further experimentation would be to determine higher and lower levels of the natural medications in dose format to determine their impact and threshold against the mitochondria. This would enable an actual dosage system and supplement suggestion to prevent cellular diseases through natural function.

Mission Verification

(1) Does your Mission Folder project involve vertebrate testing, defined as animals with backbones and spinal columns (which include humans)? If yes, team must complete and attach an IRB approval form.

No

(2) Did your team use a survey for any part of your project? If yes, team must complete and attach a survey approval form.

No

(3) You will need to include an abstract of 250 words or less. As part of the abstract you will need to describe your project and explain how you used STEM (Science, Technology, Engineering and Mathematics) to improve your community

Antibiotics work by affecting things that bacterial cells have but human cells don't. They kill bacteria by neutralizing bacteria, tearing bacteria apart, or by disabling the bacteria. It is being determined that many antibiotics can cause mitochondrial toxicity, an affliction where the mitochondria of a body's cells become damaged or decline significantly in number, and therefore the cell ceases to function properly. The purpose of this project is to determine the impact of antibiotics on mitochondrial function and then compare natural antibiotics and their impact on mitochondrial function.
Worm cells were grown on nutrient agar plates, and then exposed to the dye Janus B. The cells were monitored as were the mitochondrial mortality with antibiotic exposure and exposure to natural solutions that have antibiotic qualities.
It was determined that the antibiotics all had indications of mitochondrial toxicity, especially Cipro, which eliminated all the mitochondrial toxicity in a fast amount of time, while none of the natural antibiotics had any impact on the oxygen and sugar processing abilities of the mitochondria, indicating that natural antibiotics would be better to reduce mitochondrial toxicity.

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Administered by NSTA National Science Teachers Association

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Materials

Equipment	Chemicals	Biological
Dissection Table	Distilled water	Ginger
Scalpel	Rhodamine B	Rosemary
Mortar and pestle	Janus Green B	Garlic Flakes
Electronic scale	Dextrose	Osha
Weighing paper	Sodium Chloride	Cota
Micro-test tube	Isopropyl Alcohol	Worms
50 mL Beakers	Ethyl Alcohol	Nutrient Agar
150 mL Beakers	Tap Water	Trypsin
Parafilm	Antibiotics	Nutrient Broth
Hot Plate/Stirrer	Matches	
Thermometer	Silicon Based Hydrogel	
Aluminum Pan		
250 mL Beaker		
Centrifuge		
Centrifuge Capsules		
Pipettes		
Plastic Cups		
Autoclave		
250 mL Erlenmeyer Flasks		
1000 mL Beakers		
Ceramic Wire Gauze Pad		
Nutrient Agar Plates		
Bunsen Burner		
Inoculation Loop		

Sterile Glass Hockey Stick		
Incubator		
Refrigerator		
Screw-Cap Test Tubes		
Hypodermic Needle		
Digital Microscope		

Procedures

Luteolin Extract

[1.] Measure out 1g of cota flower and cota stem/leaf and mash with mortar and pestle.

[2.] Place in micro test tube with 5mL laboratory grade ethanol alcohol.

[3.] Cover with Parafilm, and place in water bath at 40^{o}C for 4 hours, gently agitating every hour

[4.] Remove from hot plate, pipette extract out from solid mass, discard solid mass.

[5.] Place liquid into centrifuge capsules and spin at 25000 rpm for 15 min.

[6.] Pipette liquid from remaining mass, separate mass to one test tube.

[7.] Cover and store in refrigerator at 4^{o}C until use.

Ginger, Rosemary, Garlic and Osha Solutions:

[1.] Mix one gram of each solid in 100 mL water, boil at 80^{o}C for 2 minutes. Allow to cool.

Antibiotics Solutions:

[1.] Mix one tab with 100 mL water, allow to set for one hour.

Nutrient Agar Procedure

[1.] Sterilize work area with isopropyl alcohol.

[2.] Weigh out 11.5 g of Nutrient Agar powder using electronic scale.
[3.] Measure 500 mL of distilled water.
[4.] Pour distilled water into 1000 mL beaker.
[5.] Place magnetic stirrer in beaker.
[6.] Place beaker with distilled water and magnetic stirrer on hot plate.
[7.] Turn heat up to setting of 10, turn stirrer up to setting of 5.
[8.] Add Nutrient Agar powder to distilled water.
[9.] Heat until liquid gently boils and turns slightly clear. (approx. 10 minutes)
[10.] Remove from heat using heat resistant glove.
[11.] Equally distribute Nutrient Agar solution into three screw cap Erlenmeyer flasks and place in autoclave.
[12.] Place 250 mL of tap water in autoclave.
[13.] Place 3 screw cap Erlenmeyer flasks in autoclave and seal.
[14.] Place pressure cooker onto hotplate, heat for 15 minutes after autoclave has attained 15 psi pressure and 121o C. Remove autoclave from heat and allow to cool.
[15.] With heat resistant glove, pull Nutrient Agar solution bottles from autoclave, and pour nutrient agar into Petri dishes. Allow Nutrient Agar to gel, invert and refrigerate until time of use.
[16.] Re-sterilize work area with isopropyl alcohol.

Nutrient Broth / Dextrose Solution

[1.] Weigh out 11.5 grams of Nutrient Broth powder using electronic scale.
[2.] Measure 500 mL of distilled water.
[3.] Repeat steps 3-15 of NAP procedure with Nutrient Broth solution.
[4.] Add 5mL .015 M Dextrose to solution.
[5.] Pour Nutrient Broth Solution into sterile Erlenmeyer flask and refrigerate until time of use. Allow to come up to room temperature before use.

Worm Culturing Procedure:

Part I

(The following procedures should take place within a fume hood and anything that is placed within shall be sprayed with alcohol to maintain sterility. Gloves shall be worn.)

[1.] Make a 1% dextrose/ nutrient broth solution, a 1% trypsin solution, and a 1% saline solution.

[2.] Sterilize six screw cap test tubes, and 20 centrifuge capsules.

[3.] Sterilize work area with 70% isopropyl alcohol.

[4.] Place 5mL of the 1% dextrose solution into 3 sterilized screw cap test tubes. Then place .5mL of trypsin solution into each test tube.

[5.] Get an earth worm (night crawler) and clean soil off of the worm with distilled water.

[6.] Place worm into a 50mL beaker with 10 mL of 70% isopropyl alcohol for 15 seconds.

[7.] Dry worm with a paper towel and place on dissection tray.

[8.] Collect three tissue samples from the epidermis and place into isopropyl alcohol for 5 seconds, then place into a screw cap test tube with dextrose solution.

[9.] Place screw cap test tubes in incubator for 24 hrs. at 37°C.

Part II

[1.] Sterilize work area with 70% isopropyl alcohol.

[2.] Take the screw cap test tubes out of the incubator.

[3.] With a sterilized glass pipette place 1mL of a tissue sample solution into centrifuge capsule.

[4.] Place capsule into centrifuge. Repeat step 3 until all the tissue sample solution is within centrifuge capsules.

[5.] Centrifuge at 25000 rpm for 15 min.

[6.] After the 15min there should be a pellet at the bottom of the centrifuge capsule. Remove nutrient broth from the centrifuge capsule; make sure to leave not to disturb the pellet. Place nutrient broth into a waste beaker.

[7.] Place 1mL of saline solution into centrifuge capsule and place into centrifuge.

[8.] After doing steps 6-7 for all the capsules run the centrifuge for 15min at 25000 rpm.

[9.] After the 15min take a capsule and remove the saline solution making sure not to disturb the pellet. Place saline solution into a waste beaker.

[10.] Place 1mL of dextrose nutrient cell broth into centrifuge capsule and gently agitate the pellet. Then place the dextrose nutrient cell broth into a sterilized screw cap test tube.

[11.] Repeat steps 3-10 for the rest of the tissue sample solutions, then place screw cap test tubes in incubator at 37°C. Every two weeks repeat this process to ensure that the cells remain viable.

[12.] When ready for experimentation, add cells to nutrient agar plates, using inoculation loop to spread.

[13.] Place hydrogel cover over the top of the plate.

[14.] Allow to incubate for 24 hours at 37^{o}C.

[15.] Place 50% Rhodamine B solution on top of cells and allow to set for 5 minutes to determine absorption and viability.

[16.] Place one microdrop of each solution/strip segment on top of cell, through silicon hydrogel layer, with hypodermic needle, observe cellular change.

Experimental Procedure

Part One

[1.] Sterilize work area with 70% isopropyl alcohol.

[2.] Place two drops of worm epidermal cell solution onto nutrient agar plates.

[3.] Spread using inoculation loop.

[4.] Add 10 drops dextrose/ nutrient broth solution and spread.

[5.] Place in Incubator at 37°C. for 24 hours.

[6.] Remove from incubator and place 50% Rhodamine B solution on top of cells and allow setting for 5 minutes.

[7.] Place stained agar plate onto the stage of a microscope and observe under at least 100x TM.

[8.] In logbook, draw diagram of cell health before being exposed to any solutions.

[9.] While recording, place one micro-drop of capsaicin solution on top of a viable cell, and observe immediate changes in cell health.

[10.] Record observations in log book.

[11.] After 5 minutes, end recording and draw new diagram of the cell. If the cell is still responding, extend time to ten minutes and continue to observe morphology.

[12.] Repeat steps 8-11 on five cells.

[13.] Measure size change of cells and record in logbook.

[14.] When done, store plates in refrigerator and rehydrate periodically with dextrose/nutrient broth solution until next use.

Experimentation

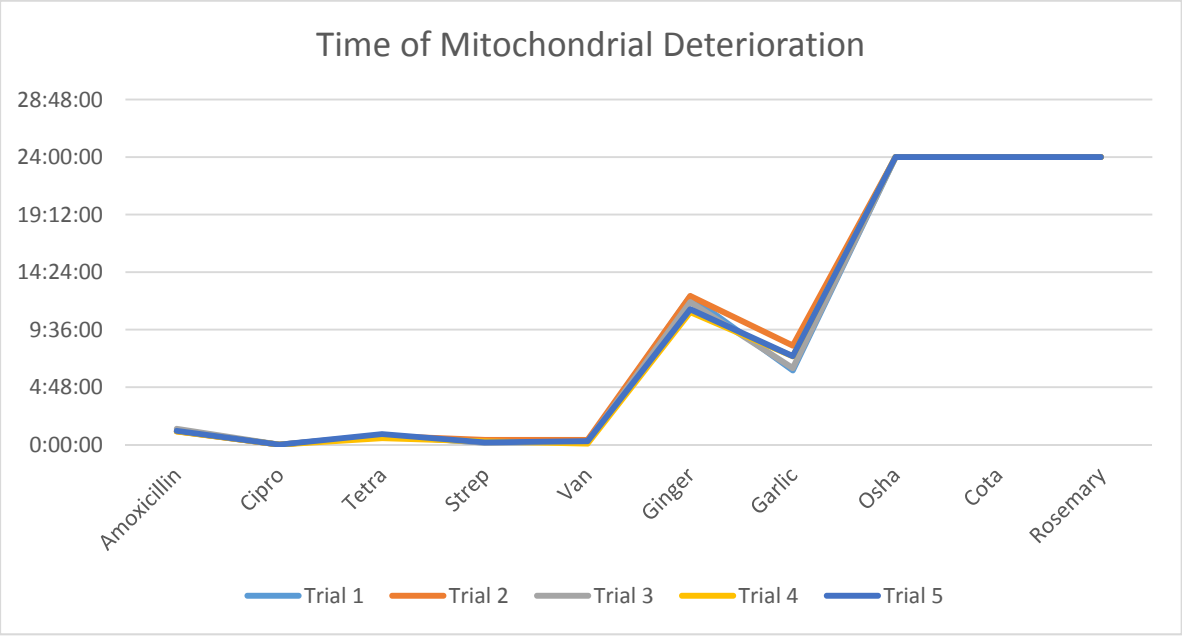
* conduct experimentation through hypodermic injection through the parafilm/hydrogel membrane, observe through inverted microscope. Experimenter will be wearing gloves that can prevent accidental needle penetration.

1. Load approximately 1mL of 50% diluted Rhodamine B or Janus Green B into hypodermic. Expel air bubbles.
2. Saturate cell area with dye and allow absorbing for 10 minutes to determine viability.
3. Load approximately 1 mL of each solution through hypodermic needle. Expel air bubbles.
4. Gently penetrate membrane above cells.
5. Gently press plunger on hypodermic to produce 1 micro drop (md). Place drop on top of cell.
6. Observe changes to cell, time how long it takes for the cell to regress back to normal, or determine how fast the death of the mitochondria occur.
7. Remove hypodermic; cap needle.
8. Repeat steps above for other trials and repeat with other solutions.
9. Discard plates as per proper autoclaving protocol, in excess of 121^{o}C for 30 minutes to 1 hour.

Risk Assessment

The risk for the procedures within this project is minimal. All safety precautions for chemical and biological safety will be observed ranging from personal protective gear including goggles, aprons/jackets, nitrile gloves, and face shields /masks to laboratory protective gear ranging from chemical (alcohol) and Ultraviolet sterilization, sealing of the solutions and plates to avoid cross contamination, fume hood, splash guards and constant adult supervision. All disposal protocols according to BSL-1 standards and chemical disposal will be observed.

Observations were taken by observing the cellular and mitochondrial reaction to the different solutions while under the presence of Janus Green B Dye which tested the viability and continued ability of the mitochondria to process glucose and to continue the process of glycolysis and oxygen processing through a colormetric reaction. The Rhodamine B worked to determine the viability of the cell to continue cellular processes.



Time of mitochondrial deterioration was the amount of time it took for the mitochondria to deteriorate in the presence of each solution. The antibiotics were all within a few minutes or hours, while the natural solutions had a much longer survival rate. Solutions listed at 24 hours plus, had no mitochondrial deterioration and the cells remained viable after 24 hours. The amount of mitochondria was also counted but variable.