Shift of Acetaminophen Dosages on Liver, Kidney, and Nerve Cells

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Abstract

The purpose of this research was to observe the effect of off label dosages of Acetaminophen on Liver, Kidney, and Nerve cells. Two separate hypotheses were used. The main hypothesis was that Acetaminophen applied at irregular dosage amounts would have a noticeable effect on the cells. The null hypothesis, for statistical testing, stated that irregular doses of Acetaminophen will have no effect on the cells. Treatments of Acetaminophen were calculated in to four treatment groups: control, low (0.05mg/mL), medium (0.058 mg/mL), and high (0.58 mg/mL). On Day 1 the cells were placed in the well plates. On Day 2 treatments were sterilized and then added before overnight incubation. On Day 3 the trypsin and trypan blue were added to the cells to prepare them for counting. Using a hemacytometer, the viability of the cells were counted. For data analysis, ANOVA tests were ran. The data showed that increasing dosages of Acetaminophen killed the liver cells the most (F= 7.4 , df= 15 , P=0.005 ). This was somewhat expected because Acetaminophen is processed in the liver. Differences observed in the kidney and nerve cells were not statistically significant. The kidney cells were not affected whatsoever, and had viable cells in the thousands. It was concluded that acetaminophen does have an effect on nerve and liver cells. Further research is needed to determine if efforts need to be made to reduce irregular acetaminophen dosing.

Introduction

After a long day at the gym, arms are sore, and legs are even worse. Stumbling occurs as the normal struggle to get out of bed occurs. Crawling towards the kitchen, the medicine cabinet is already open. It takes a while to reach the sore arm up to the second shelf, but it happens, and what medicine is pulled out? That is right, the Acetaminophen. Four pills are taken, but is that healthy? The dosage amount is only two pills every 6-8 hours. Is that good for the cells and organs that process the medicine? If Acetaminophen is not taken at the right amount, it could cause many long-term effects, such as acute liver failure, kidney failure, and even cancer. Acetaminophen has proved to be the number one use to commit suicide. (Acetaminophen, 1979).

 The research problem was: Shift of Acetaminophen Dosages on Liver, Kidney, and Nerve Cells. Acetaminophen works to relieve pain caused by conditions such as headache, osteoarthritis, muscle pain, and to reduce fever caused by infection (Acetaminophen 2008). It is a commonly used medicine that is the first treatment people use for minor pains. Since Acetaminophen is a relatively weak medicine, people tend to take more of it as needed. This is called overdosing.

 The purpose of this research was to determine the effects of the different dosage amounts of Acetaminophen on Liver, Kidney, and Nerve cells. This research was beneficial to society by determining the amount of Acetaminophen different types of cells can handle. If the society took precaution of the dangers over dosages of Acetaminophen can happen, it could possibly decrease many health risks including cancer. It could prevent the over multiplying of the cells, which potentially creates cancer. The main hypothesis was that Acetaminophen applied at irregular dosage amounts would have a noticeable effect on the cells. The null hypothesis, for statistical testing, stated that irregular doses of Acetaminophen will have no effect on the cells.

The problem was tested by having the three different types of cells in three separate well plates. The certain dosage amount of Acetaminophen was applied to each of the cells. They were incubated overnight. Afterwards, a hemacytometer was used to count the viable cells. The process was repeated 4 times, increasing the dosage amount each time to observe how the cells react. Observations were made to determine how many cells are viable, or living.

 This research project consisted of beneficial research to all society. Visually displaying the harmful affect Acetaminophen had on cells will hopefully increase the awareness of people the next time they take it.

Literature Review

There are trillions of cells in the human body (Lodish 1986). These cells consist of 200 different types of cells, with 20 different structures and organelles. (AAAS 2013). Out of these trillions of cells, adults lose about 96 million per minute. (AAAS 2013). There is no need to lose more cells than needed; they are vital to human life.

 There was little known about Acetaminophen before this research was conducted. Many people do not know the dangers of Acetaminophen. When it made its first debut in the 1970s, doctors encouraged it as the safest medicine available. Not safe, not safer, but safest. In 2011, Acetaminophen was named the most dangerous over-the-counter medicine. (Palmer, B. 2011). Since Ibuprofen is a weaker pain killer, society tends to take more of it at once. When people take Acetaminophen, it can produce long-term effects such as liver failure, kidney failure, and even cancer.

Once research started, it was found that Acetaminophen had very harmful effects on cells. For the research, liver cells, kidney cells, and nerve cells were used. These cells come from mice. Liver cells were used because they are one of the first sets of cells to begin processing the Acetaminophen. The Acetaminophen causes horrible damage to kidneys and can cause acute kidney failure, fluid loss, etc. (National Kidney 2010). The reason the Acetaminophen causes so much damage is because these types of pain killers can only be excreted through the kidneys. Acetaminophen has a very important part with nerve cells. When the body is injured, special nerve endings send messages to your brain (Science Museum 2013). Pain killers either go to the injury itself, or through the spinal cord. These are nerves. Too much Acetaminophen at one time can cause damage to the nerves.

 One of the topics of literature review is called Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver. It did not just talk about the liver; it talked about all different types of cells and the effects they had after drugs and chemicals. According to the book, not taking proper amounts of dosage caused potential hepatotoxic (Hepatotoxicity). Hepatotoxicity meant toxic to the liver

The reason this research was being performed is to test the effects of Acetaminophen in 4 treatment groups on kidney, liver, and nerve cells. The average dosage was from 250 mg-450 mg every 6-8 hours. People were likely to take more than the recommended amount of Acetaminophen depending on the amount of pain experienced. The viability of cells were tested during the research. Four trials per treatment group were conducted on each of the cell lines. There were a total of 48 trials. The independent variable was the dosage size of the Acetaminophen on the different types of cells. The dependent variable was the reactions of the cells to the different dosage amounts. How many were viable? Did any cell line process the Acetaminophen better than the other? A hemacytometer was used after the cells incubate to count the cells that survived. This research was important to society because it might be able to prevent higher statistics of kidney failure, or liver disease, or other diseases related to pain killers. This research could encourage people to only take the directed amount, and not overdose. This research could be a great contribution to society.

Methodology

Procedure Summary:

 Treatments of Acetaminophen were added in 4 different treatment groups to Liver, Kidney, and Neuron cells. They were incubated overnight, then using a hemacytometer. This measured the viability of the cells under high Acetaminophen conditions. Each cell line was experimented on for 3-5 days. The entire experimentation period took about three weeks.

Safety and Waste Disposal:

 Cells, and any other item that touched the cells were placed in a container of 10% bleach and water. Any disposable materials were disposed of in a trash can. There were no large safety risks in this procedure. Do not drink Acetaminophen. Take caution when handling cells.

Materials:

* Acetaminophen
* Kidney cells
* Liver cells
* Nerve cells
* Well plates
* Micropipettes/tips
* Treatments
* Growth Medium
* Trypan Blue
* Laminar Flow Hood
* Gloves
* Goggles
* Lab coat
* Ethanol
* Distilled Water
* Hemacytometer
* Waste Beakers
* Auto Clave
* Timer
* Sample tubes
* Camera (to take pictures of materials)

Detailed Procedures

 Aseptic technique was used at all times. Gloves and lab coats were worn. All well plates going in and out of the Laminar Flow Hood were sterilized with 70% Ethanol. Sterile micropipette tips were used, and were changed if the tip is touched anywhere else.

 The first day consisted of making the treatments. The acetaminophen was diluted to the right amount for cells. The end result for the high concentration was 0.58 mg/mL. For the medium concentration, it was 0.058 mg/mL. For the low concentration, it was 0.005 mg/mL. The control group had no acetaminophen in it. Afterwards, the treatments were sterilized using filters. The acetaminophen treatments were kept in the cell lab fridge until used for the cells.

 The first cell line that was tested is the nerve cells. These cells came from mice. A sterile well plate was taken out of its wrapper, wiped with ethanol, and placed in the Laminar Flow Hood. Only sixteen of the twenty-four wells will be filled. Trypsin was added to the tube of nerve cells to shake them free from the bottom of the tube. They were in the CO2 incubator for 5 minutes. A small sample of cells were taken to test the ratio of the cells to the media. The cells were then added to the media in the well plates and kept in the CO2 incubator overnight.

 The next day, the treatments are added to the neuron cells. The well plate was wiped with 70% ethanol before being placed under the Laminar Flow Hood. The four treatments were taken out of the lab fridge and placed in 38.1C water. This is to warm the treatments before adding them to the cells. 55mL were added to each well according to the amount of acetaminophen required. The wells were gently shaken to ensure the treatments were mixed evenly with the cells. The well plate was gently wiped with 70% ethanol and left in the CO2 incubator overnight.

 The next day, the cells were counted for viability. Again, the edges of the well plates were wiped with 70% ethanol before being placed under the Laminar Flow Hood. A pipette was set to 600mL to ensure that all the old media was removed from the well plate. After drawing the old media out, the well plate looked completely empty. A thin film was seen at the bottom of each well; this indicated the nerve cells were stuck to the well plates. 250mL of Trypsin were added to each well. Then the well plate was wiped with ethanol and placed in the incubator for 5 minutes. When time was up, the cells were observed under a microscope to make sure they were not still stuck to the bottom of the wells. If they were, they were placed back in the incubator for another 5 minutes. The edges of the well plate were wiped with ethanol and placed under the Laminar Flow Hood. 250mL of cell media was added to neutralize the Trypsin. The well plate was shaken gently to ensure the cell media has indeed neutralized the Trypsin.

 Counting the cells was the last step. 16 small sample tubes were placed into a sample tube holder. Little movement needs to be made. This decreased the risk of accidentally knocking over the cell samples. A micropipette was used to pull 200mL of cell suspension out of each well and placed them in the sample tubes. The micropipette was changed after each treatment group. 200mL of Trypan Blue was then added to each sample. A Hemacytometer was cleaned with ethanol and distilled water before containing the cells. A 10 mL sample of cells was pulled and placed into the Hemacytometer. Two samples went in the hemacytometer because the hemacytometer had 2 sides. The Hemacytometer was placed under a microscope on the medium magnification setting (yellow). Quadrants A, B, C, and D on the hemacytometer were visible through the microscope. There were 16 squares in each quadrant. These were the cells that need to be counted. A cell counter was used to help remember the viable cell count number. All four quadrants were counted. The number of viable cells was written for each treatment group. When finished counting the cells, the sample tubes and well plate were placed in a bucket of bleach. The Hemacytometer was washed again with distilled water and 70% ethanol. Aseptic technique was then used to clean the workspace.

 The same procedures and techniques were used for Kidney and Liver cells. Aseptic technique continued to be used for the remainder of the experimentation period.

Data Analysis

The data analysis was performed using tests in ANOVA, including One-Way ANOVA, and Basic Descriptive Statistic tests. Graphs of this data were made with Microsoft Excel. This analysis will explain the results of experimentation, and the hypothesis that was most supported.

 In each cell line, all four trials for each control group were averaged together. The Kidney cells had a p-value of 0.996, which determined that they were not statistically significant. There was not an identified trend in the data. It was not expected that the kidney cells would be statistically significant. The number of viable cells in the raw data of each Trial was in the thousands. Different dosages of Acetaminophen had no effect on the Kidney cells. The kidney is the organ that processes the Acetaminophen, so it is thought that the Kidney has a high tolerance for Acetaminophen as well as other medicines.

These results were similar to the Nerve cells. They also were not statistically significant, and had a p-value of 0.884. However, there was a trend in the data until it reached Trial three. It was expected that Acetaminophen was going to have a greater effect on nerve cells because the nervous system is the part of the body that transfers the Acetaminophen to the affected area.

In contrast, the Liver cells had a trend in the data. This cell line proved to be statistically significant with a p-value of 0.005. Acetaminophen had a very noticeable effect on Liver cells. Between the Control trial and the Low trial, the number of viable cells decreased by about 15 cells (per mL).

 As a result, my main hypothesis was mainly supported. Acetaminophen had a noticeable effect on the liver and nerve cells, but not the kidney cells.

Conclusions

The purpose of this research was to determine the effects of what irregular dosages of Acetaminophen would do to Kidney, Liver, and Nerve cells. The major findings of the research were that different dosages of Acetaminophen had a noticeable effect on the liver and nerve cells, but not the kidney cells. Only the liver cells were statistically significant. The main hypothesis was mostly supported with the liver and nerve cells, but the null hypothesis supports the kidney cells.

 Sources of error could include not measuring correctly, miscalculations, or contamination from other bacteria.

 A possible explanation for the greatest effect on Liver cells is that Acetaminophen is processed by the liver. The liver could not handle the over dosages, which supports the theory of the harmful effects of over dosing.

 For the next step of this project, it would be interesting to gradually add greater amounts of Acetaminophen to the same cell lines to somehow determine long-term effects. This would examine further into the damage of over dosing, and expand further on the Liver cell results.

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I would like to thank Mrs. Mary Mansell for helping me with cell choices.

Appendix A

Experimental Design Diagram

|  |  |  |  |
| --- | --- | --- | --- |
| IV: Dosage Amount (mg) | .058 mg (High) | 0.058 mg (Medium) | 0.005 mg (Low) |
| Group: | Liver Cells (TIB-73) | Kidney Cells (CRL-6436) | Nerve Cells (HB=12317) |
| # of Trials: | 4 | 4 | 4 |

Constants: location, pipettes, medicine brand

DV: type of cells, cell reproduction, cell functions, cell division, colony count

Appendix B

**Equipment & Instruments Needed**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***Equipment Name*** | ***Size/******Details*** | ***Current Location or Order Information\**** | ***Length of Time Needed***  | ***Additional Notes*** ***(Note if you will need exclusive use of the equipment during testing and explain why)*** | ***Numbered step(s) in your detailed procedure where this will be used (must be listed specifically)***  |
| Incubator | 3 well Plates | Cell Lab | 2-3 Weeks |  | Incubation |
| Hemacytometer | Cell counter | Cell Lab | Whenever counting cells |  | Counting live cells. |
| Micropipettes | 1-2 | Cell Lab | Whenever in lab |  | Using to insert treatment. |

**Incubator Space Needed**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Type of Incubator*** | ***Incubator Temperature*** | ***Length of Incubation*** | ***Space Required*** ***(ie: Number of Test tubes or plates)*** | ***Additional Notes*** |
| CO2 | 99.3-101.3 degrees Fahrenheit | Different lengths based on time. | 3 well plates |  |
|  |  |  |  |  |

**Reusable Materials Needed**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Material Name*** | ***Quantity Needed*** | ***Current Location or Order Information \**** | ***Length of Time Needed*** | ***Numbered step(s) in your detailed procedure where this will be used (must be listed specifically)*** |
| Micropipettes | 1-2 | Cell Lab | Whenever in lab |  |
|  |  |  |  |  |
|  |  |  |  |  |

**Disposable Materials Needed**

|  |  |  |  |
| --- | --- | --- | --- |
| ***Material Name*** | ***Quantity Needed*** | ***Current Location or Order Information \**** | ***Numbered step(s) in your detailed procedure where this will be used (must be listed specifically)*** |
| Trypan Blue/ Trypsin | 5 mg | Cell Lab | Dye cells to see how many are dead/ to shake the cells loose from the bottom of the well plate. |
| Micropipette Tips | 10-20 | Cell Lab | Applying treatment to cells. |
| GlovesAcetaminophen | 5-10 pairs1 bottle | Acetaminophen bought from target, cost $3.34 | Working in lab. |

**Budget:**

Total Cost of Reusable Materials & New Equipment Ordered: $ 0.00

Total Cost of Disposable Materials Needed (cannot be reused): $ 3.34

Appendix C

Aseptic technique was be used at all times, especially before handling cells or anything in the lab. Everything was as sterile as possible. Latex gloves, lab coats, and goggles were worn whenever in the lab. Gloves and counters were wiped with 70% ethanol before working in the lab and under the laminar flow hood.

The materials that were used in this research consist of many items. There were over the counter medicines and disposal materials. The medicine used was Acetaminophen. This was a common pain reliever medicine used to reduce fever and relieve pain. The Science Fair fund did not pay for over the counter medicine, the Acetaminophen was purchased by the researcher. The Acetaminophen that was bought is a dye free, liquid Acetaminophen. The Acetaminophen was applied to 3 different types of cells. These cells were Liver, Kidney, and Nerve cells. The cells came from mice. Micropipettes were used to insert the cells into the well plates in each trial. They were also used to apply the certain dosage of Acetaminophen to the cells. Well plates were used to hold the cells and the Acetaminophen once applied to them. A laminar flow hood was used to hold the cells during experimentation. Trypan blue was used to dye the cells after they have incubated to determine how many cells survived the dosage. A Hemacytometer was used to count the cells. All hazardous materials were disposed of properly. There discarding areas with bleach were used for the cells and anything that touched them.

 The procedures of this research were a precise process. Everything was with caution or the research can risk being ruined. All measurements of both the cells and Acetaminophen had to be perfect to get the most accurate results possible. The measure of Acetaminophen for the high treatment was 0.58 mg per micro liter. The medium treatment was a tenth of that (0.05), was and the low treatment was a tenth of that (.005). The control treatment had no Acetaminophen in it. All treatments had a buffer added to them, and then sterilized using sterile filters. The treatments were labeled and kept in a fridge in the lab until cells are added. This pre-cell process was repeated before all types of cells are tested.

The neuron cells were tested first. Then the kidney cells, then the liver cells. They grew in a Co2 incubator until they were ready to be applied to the treatments. The entire experimentation process should have lasted no longer than 4 weeks.

Sanitation of all materials and work places was emphasized to prevent skewing the data. All well plates and instruments had to be as sterile as possible to prevent infecting the cells and killing them.

Experimental Design Diagram

|  |  |  |  |
| --- | --- | --- | --- |
| IV: Dosage Amount (mg) | 0.58 mg (High) | 0.058 mg (Medium) | 0.005 mg (Low) |
| Group: | Liver Cells (CRL-1571) | Kidney Cells (CRL-1548) | Nerve Cells (CRL-1721) |
| # of Trials: | 4 | 4 | 4 |

 Each of these were applied to Liver, Kidney, and Nerve cells using the micropipette. This took place in separate well plates. The well plates were then placed under the laminar flow hood and allowed to incubate for a certain amount of time (depending on lab times). This was completed in a school lab. There were 4 different labs, but this experiment took place in the Cell lab.

 After the cells incubated with the dosage of Acetaminophen depending on the trial, small amounts of Trypan Blue were used to dye the live cells. Then the live cells were able to be counted. A Hemacytometer was used to count the live cells.

 After all the data for Trial 1 has been collected, it can be entered into a logbook for later data analysis and conclusions. The data was analyzed using ANOVA, which included one-way ANOVA and descriptive statistics tests. These steps were repeated for trials 2-3. The only thing that changed is the dosage amount of Acetaminophen.

 The estimated time for the experimentation process was three weeks. Each type of cell was tested 4 times with each dosage level.

**Appendix D: Raw Data Tables**

Neuron Cell Raw Data-Number of Cells Counted Based on Level of Treatment

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |   |  |  |  |  |
| Tria­l # | Control | Low | Medium­­ | High |  |
| 1 | 79 | 70 | 73 | 39 |  |
| 2 | 31 | 82 | 79 | 122 |  |
| 3 | 196 | 71 | 107 | 159 |  |
| 4 | 85 | 106 | 61 | 123 |  |
|  | Liver Cell Raw Data-Number of Cells Counted Based on Level of Treatment |  |  |  |  |
|  |  |  |  |  |  |
|  |   |  |  |  |  |
| Tria­­­­l­­ #­ | Control | Low | Medium | High |  |
| 1 | 31 | 12 | 25 | 14 |  |
| 2 | 46 | 16 | 32 | 25 |  |
| 3 | 40 | 31 | 20 | 18 |  |
| 4 | 45 | 30 | 18 | 19 |  |
|  | Kidney Cell Raw Data-Number of Cells Counted Based on Level of Treatment |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
| Tria­l # | Control  | Low | Medium | High |  |
| 1 | 399 | 326 | 492 | 684 |  |
| 2 | 255 | 526 | 454 | 358 |  |
| 3 | 1015 | 1364 | 486 | 1284 |  |
| 4 | 1096 | 664 | 1174 | 502 |  |
|  |  |  |  |  |  |

**FLOW CHART FOR CELL CULTURING EXPERIMENTS**

Wipe with ethanol.

Disposable materials taken care of properly.

Place cells in 10% bleach.

Analyze using ANOVA

Prepare Hemacytometer

Add Trypan Blue

Pipet some into tube

Add Trypsin

Remove old media.

Wipe down

Add treatments and place back in incubator.

Observe wells for error.

Wipe with 70% ethanol

Wipe Plate with 70% ethanol and incubate overnight.

Add growth medium

Label Well Plate

Sterilize with 70% Ethanol

Autoclave Materials

Goggles, Lab Coat, Gloves

Aseptic Technique

Well Plate Preparation

Day 1: Cell Culturing

Treatment Preparation

Day 2: Add Treatments

Day 3: Collect Cells

Count Cells

Analyze Data

Clean Up Protocol

Aseptic aTechnique

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