The Effectiveness of Diphenhydramine (a sleeping pill)
on Mice (*Mus musculus*) Consuming Caffeine

Senior Thesis Proposal

Introduction to Scientific Research

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INTRODUCTION

Reduction of sleep quality and efficacy is characterized as a sleeping disorder, otherwise known as insomnia (Alexandru 2009). Sleeping disorders are very prevalent among Americans, with 58% of adults reporting that they have difficulty sleeping (Cochran 2003). Five percent of the American population is known to have insomnia, which indicates that a majority of the population self-diagnose and self-treat. A common self-treatment involves the consumption of sleeping pills. Diphenhydramine is an antihistamine, more commonly known as Benadryl, and is also an over the counter sleeping pill used to treat insomnia (Diphenhydramine 2011). Diphenhydramine also acts as a depressant. Those who suffer from insomnia develop the daytime sleepiness and tend to combat it with caffeine. Caffeine acts as a stimulant. Possible alternative treatments of insomnia could involve a reduction in the consumption of caffeine. However, because of the addictiveness of caffeine, this method is rarely used (Youngberg, et al 2011). The self-diagnosis of insomnia could also be incorrect because individuals may be overly stimulated with caffeine, and to treat this self-diagnosed insomnia a sleeping pill may be used.

It is important to study the combination of depressants and stimulants due to the high prevalence of self-treatment of insomnia utilizing both types of compounds because of the opposite effects they have on the central nervous system and possible synergistic activities. The reason this combination is studied is to help fill the gaps in the medical field.

The objective of my study is to determine the effects of diphenhydramine, a commercially available sleeping pill, on mice sleeping behavior after the consumption of caffeine. This experiment hopes to help future studies, which are related to the combination of depressants and stimulants.
REVIEW OF LITERATURE

The sedative and depressant effects of diphenhydramine that humans utilize are relevant to my research topic. Diphenhydramine was used as a treatment of children's nocturnal and upper respiratory infections that affected sleep difficulty in a study completed by Paul (2004). Although the parent's overall sleep quality was assessed, this article showed that the antihistamine was a significant sedative. Children’s and parent's sleep quality was better for the children who received diphenhydramine compared to the children who received the dextromethorphan or placebo. This study further proves that diphenhydramine is a valid treatment for insomnia. However, diphenhydramine should only be used as a short term treatment. In a study completed by Williams, their results showed that tolerance to diphenhydramine was built by the second day of administering the drug (1997). The depressant effects of diphenhydramine are best explained by how humans metabolize the drug. Humans metabolize diphenhydramine into its N-demethylated metabolite by the cytochrome P450 isoenzyme CYP2D6 (Akutsu 2007). Akutsu’s study concludes that the sedative effects of diphenhydramine could be caused by increased plasma concentrations of diphenhydramine associated with CYP2DC inhibitors/substrates (2007).

The depressant effects that diphenhydramine has on human sleep quality are quite different from the stimulant effects that caffeine has on human sleep quality. Caffeine is one of the most common drugs that is extensively studied due to its influence on human performance. Tea and coffee are the two most common caffeinated beverages that are normally consumed because of the dose and time of daily consumption (Hindmarch 2000). A study that compared the natural consumption of caffeinated beverages vs. non caffeinated beverages to sleep quality proved that caffeinated beverages reduced the ease of getting to sleep and the quality of sleep.
Although, these numbers varied as the amount of caffeine was consumed, they both showed a significant reduction in overall sleep quality (Hindmarch 2000). It has been recommended that groups of people, which include military personnel, consume 100-600mg of caffeine to help maintain cognitive performance during sleep deprivation (Ruxton 2008). Sleep deprivation is commonly combated with caffeinated beverages and is said to keep the individual awake, which disrupts normal sleep-wake cycles.

In my study, the mouse (*Mus musculus*) will be used as the study model to determine the effectiveness of diphenhydramine. There is limited literature that is associated with diphenhydramine and rodents. However, there in one study that focused on the sedative effects on the central nervous system caused by depressants and the interaction with a natural sedative. This study used depressants, which on their own produce sedative effects, and mixed them with a plant (*Ternstroemia pringlei*) known to also produce a sedative effect and treat insomnia (Balderas, et al 2008). Table 1 depicts the 95% confidence limits for *Ternstroemia pringlei* extracts and the effective dose, which produced 50% of sedation, and the other drugs. Balderas’s study showed that the combination between the drug depressants and the plant depressant interacted in a complex way (2008). My proposed study will help fill in the gaps that are missing due to the lack of research on this particular topic.

The research between rodents and diphenhydramine is lacking, however there is a vast amount of research on caffeine and rodents. The literature that is important to my study involves caffeine-related sleep studies using rodents as a model. One study used caffeine as the stimulant to keep rats awake and showed that the caffeine did not work to eliminate sleep drive, but it did result in fewer attempts to sleep after administering the caffeine (Wurts and Edgar 1999). Results from this study are important to the proposed study because they help to support the hypotheses
and although the caffeine stimulant will reduce the opposite effects of the diphenhydramine, the caffeine will not eliminate the sedative effects from the diphenhydramine. A sleep study on mice that consumed caffeine observed that after 120 minutes of administering the caffeine to mice, the amount of sleep was reduced significantly (Fisher, et al 2012). This study also showed that the sleep patterns of the caffeinated mice were not returned to normal (the sleep patterns of the control mice) until 2 hours after the caffeine was given (Fisher, et al 2012). A study done by Patterson, reported that sleep-promoting agents can reverse insomnia present in rats and humans that is onset by caffeine (2009). The proposed study will fill in the gaps that are missing from the mentioned studies by observing the synergistic effects of stimulant and depressant.

Understanding the sleep-wake cycle of the mouse is critical information for the proposed study. Fisher's study on the sleep-wake cycles in mice uses two different approaches to monitoring the sleeping patterns of mice (2012). One mechanism is EEG/EMG, which uses surgery to implant electrodes that record the sleep-wake behavior. This approach is very invasive, but has increased our understanding of sleep. The other mechanism used is a video analysis, which is non invasive and much cheaper than EEG/EMG recordings (Fisher, et al 2012). While the animal may be stationary during the study, it may not be sleeping; for example, if the animal is grooming or feeding, it is not sleeping. The animal activity tracker that will be used for the proposed study records this information that will help determine the actual time the animal spent sleeping.

Although there has been research on the effect that diphenhydramine has on sleep and the effect that caffeine has on sleep, there has never been a study that links the two together. Therefore, it is the goal of this study to test the effects of diphenhydramine on mice that are consuming caffeine. The study will be observing the amount of time the mouse is lethargic after
doses of the sleeping pill are given to determine how effective the depressant is against the stimulant. This research will address the hypotheses that diphenhydramine will be effective on the mice consuming caffeine and that there will be a difference in resting times negatively correlated with caffeine dosage. By addressing these hypotheses, it will help to fill the gaps of research that has been completed on sleep quality, diphenhydramine, and caffeine. This hypothesis will also allow for the popular combination of depressant and stimulant to be more fully understood.

DESIGN AND METHODS

Completion of this study will be done at the York College campus in York, Pennsylvania. This study will require the use of the York College animal laboratory room, and can be completed without assistance.

Pilot Study

A pilot study will be necessary for this research in order to assess how the animal activity tracker is used and how the data is displayed and can be interpreted. It will be important to compose a protocol on how the tracker is set up to start the monitoring process. A trial run of the tracker will use a control mouse (no caffeine, no diphenhydramine) to practice the use of the machine. Data from this trial run will not be used. The steps used to accomplish this tracking process will be determined in the pilot study and will act as the standard protocol for tracking the behavior of the 15 mice in the study. The pilot study will also help the researcher become familiar with the equipment, which will help with the efficiency of this study.

A second pilot study will need to be completed in order to determine a protocol to dissolve the diphenhydramine in a saline solution for injection and to dissolve the caffeine in
water. After the injection solution is prepared, the researcher will practice injecting a mouse. These tasks are very frequent in this study, and the purpose of this pilot study will help the researcher to become more familiar with these methods.

**Actual Study**

Fifteen mice will be sorted using a random sampling method to be placed into three test groups, while there are six experimental groups the mice will be re-used because the drugs used have a short half life and are not toxic. The design of this research can be easily displayed in figure 1. The first test group (n=5) will be a control group where the mice will receive neither caffeine nor diphenhydramine HCl. The second test group (n=5) the mice will receive the low dose caffeinated water (0.15 mg/mL). The third test group (n=5) the mice will receive the high dose caffeinated water (0.30 mg/mL). The fourth test group (n=5) the mice will receive the diphenhydramine HCl (25 mg/kg) only, not caffeine. The fifth test group (n=5) the mice will receive diphenhydramine HCl (25 mg/kg) and a low dose of caffeine (0.15 mg/mL). The sixth and final test group (n=5) the mice will receive diphenhydramine HCl (25 mg/kg) and a high dose of caffeine (0.30 mg/mL).

The effectiveness of the diphenhydramine will be recorded by using an animal activity tracker. The data that will be collected, which will be important to my study will be the resting time, distance traveled, and the time spent in locomotion. The test groups that will receive the caffeine will be administered caffeine via caffeinated water. The caffeinated water will be given 24 hours before the diphenhydramine injection and placement into animal activity tracker. The low dose group will contain 0.15 mg caffeine/mL water and the high dose group will contain 0.30 mg caffeine/mL water; these dosages are based off the study done by Arendash, which states that the average mouse consumes approximately 5 mL water/day (2009). The study
completed by Arendash also mentions that the high caffeine dose is equivalent to the human consumption of 500 mg caffeine (about 5 cups of coffee) and the low caffeine dose is equivalent to the human consumption of 250 mg caffeine (about 2.5 cups of coffee) (2009). Administering the diphenhydramine will be done by dissolving the diphenhydramine HCl (25 mg/kg) in a saline solution (0.9%) similar to a study completed in 2008 (Balderas, et al 2008). Balderas’ study showed that this value was greater than the amount of the drug that produced 50% of sedation. Diphenhydramine also has a long half life of 8.5 hours, which will allow the drug to be in the blood stream longer, prolonging the sedative effects (Williams 1997). The mice will receive a single IP injection of diphenhydramine HCl serum in a volume of 0.1 mL/10g body weight before the individual will be placed in the animal activity tracker. The mouse will be given at least five minutes to rest after the injection has been given, this time will allow the mouse to recuperate and time for the drug to disperse. The mouse will be kept in the animal activity tracker over a 24 hour period. Computer analysis data of the mouse in the animal activity tracker will be recorded. The data important to this study will include: total time spent resting (sec), total distance traveled (cm), and the total time spent in ambulatory movement (sec). Each mouse from the test groups will be tested multiple times to ensure a large sample size. Test cycles will be completed to make certain that each mouse was placed in the animal activity tracker only once.

It is unknown how long the mice will be kept for this study. However, while the mice are not being monitored in the animal activity tracker, they will be housed individually and marked according to their test group. All mice will be fed a mice chow and will have their water changed on a daily basis.
LITERATURE CITED


Table 1. ED$_{50}$ values and 95% confidence limits of the sedative effect induced by *Ternstroemia pringlei* extracts and different central nervous system depressant drugs in mice.

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED$_{50}$ (mg/kg, i.p.)</th>
<th>CL 95%</th>
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<tbody>
<tr>
<td>Hexane extract</td>
<td>446.68</td>
<td>307.70–648.3</td>
</tr>
<tr>
<td>Dichloromethane extract</td>
<td>345.93</td>
<td>150.20–795.6</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>232.80</td>
<td>178.40–304.1</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>77.58</td>
<td>50.21–124.1</td>
</tr>
<tr>
<td>Buspirone</td>
<td>1.04</td>
<td>0.73–1.46</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1.22</td>
<td>0.97–1.49</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>17.05</td>
<td>15.70–18.53</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1938.83</td>
<td>1617.65–2323.77</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.42</td>
<td>0.30–0.55</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>11.86</td>
<td>10.53–13.34</td>
</tr>
</tbody>
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(Balderas, et al 2008).
Figure 1. Research design flow chart for the proposed study. 15 Mice will be purchased and randomly sorted into four research groups. The first group is a control group (n = 5), mice will receive no diphenhydramine or caffeine. The second group is a low dose caffeine group (n = 5), mice will only receive a low dose caffeine (0.15 mg/mL). The third group is a high dose caffeine group (n = 5), mice will only receive high dose caffeine (0.30 mg/mL). The fourth group is a diphenhydramine only group (n = 5), mice will receive diphenhydramine (25 mg/kg) and no caffeine. The fifth group is a low dose caffeine group (n = 5), mice will receive diphenhydramine (25 mg/kg) and caffeine (0.15 mg/mL). The sixth group is a high dose caffeine group (n = 5), mice will receive diphenhydramine (25 mg/kg) and caffeine (0.30 mg/mL). One mouse will enter the animal activity tracker for monitoring purposes. Data collected from tracker that is important to the study is distance traveled (cm), resting time (sec), and ambulatory time (sec).