



Do atropine and diphenhydramine, two anti-cholinergic drugs, interact to affect heart rate?

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Abstract

Pharmaceuticals are often prescribed together to treat varying symptoms, so understanding how drugs interact mechanistically is important for guiding a safe and effective treatment plan. Two common drugs, atropine and diphenhydramine are both classified as anti-cholinergic, which means they block the action of acetylcholine resulting in an increase in heart rate. However, these drugs are prescribed for different ailments, so they may be administered concurrently, which poses the risk for unintended side effects. The goal of this study was to investigate atropine and diphenhydramine both independently and in combination by measuring their effects on heart rate using the model organism *Daphnia magna*. Atropine increased heart rate by 17% compared to control, while diphenhydramine increased heart rate by 27% compared to control. In combination, heart rate increased by 30%, which was similar in magnitude to the effect seen by diphenhydramine alone. In a second trial, diphenhydramine increased heart rate even in the presence of a drug that reduces its ability to bind to one of its receptors suggesting its mode of action is more complicated. However, because heart rate increased similarly when drugs were administered independently or in combination, evidence does not suggest the two drugs interact to adversely affect heart rate.

Introduction

A pharmaceutical is a substance that is used to treat an illness or alleviate symptoms by interacting with specific target molecules. Depending on the active ingredient, pharmaceuticals work by targeting various enzymes, receptors, transporters, and ion channels found within the body to induce a physiological effect. By utilizing these mechanisms, pharmaceuticals can work to target a specific region or organ within the body, but because the certain receptors or channels with which they interact are often found throughout the body, pharmaceuticals may work on other regions of the body, which may result in unintended side effects. Furthermore, multiple pharmaceuticals are often prescribed together. In these cases, their effects may be independent of each other, or one of three primary types of interactions can occur. An additive interaction is when the effect of two chemicals is equal to the sum of the effect of the two chemicals taken separately, whereas a synergistic interaction means that the effect of two chemicals taken together is greater than the sum of their separate effect at the same doses. An antagonistic interaction means that the effect of the two chemicals is less than the sum of the effect of the two drugs taken independently of each other (Yeh et al., 2007). Understanding how two or more

drugs interact is important to guide effective and safe treatments.

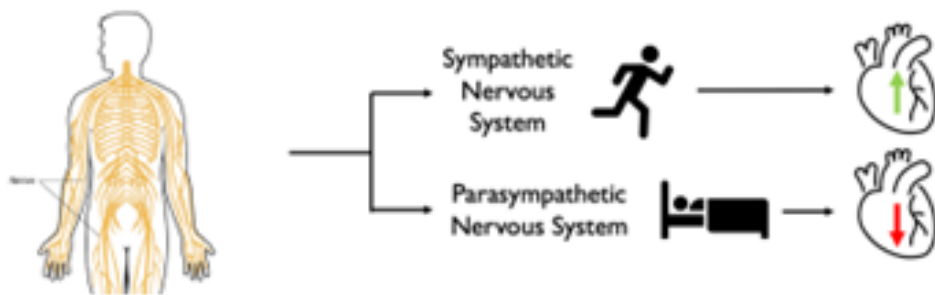


Figure 1. Visual representation of the two branches of the autonomic nervous system and how they affect heart rate



Figure 2. Anatomical structure of the *Daphnia magna*

One of the most common classifications of drugs are parasympathetic nervous system agents. The parasympathetic nervous system is a branch of the peripheral nervous system within the autonomic nervous system (Fig. 1). The parasympathetic nervous system is responsible for stimulating digestion and initiating relaxation throughout the body. Organs receive parasympathetic innervation by receiving messages from various nerves. A common nerve that is associated with parasympathetic innervation is the vagus nerve. Specifically, the heart is an organ that is innervated by parasympathetic cholinergic nerves that are derivatives of the vagus nerve. Here, acetylcholine is released by these nerve fibers and binds to the M2 muscarinic receptors (Bardal et al., 2011). In the cardiac muscle tissue, the primary regions that are under innervation from the vagus nerve are the sinoatrial (the pacemaker) and atrioventricular nodes. When stimulated, the vagus nerve produces a negative chronotropic effect, resulting in decrease in heart rate.

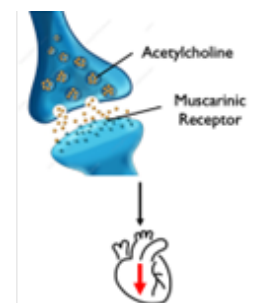


Figure 3. Visual representation of acetylcholine's mechanism to initiate a decrease in heart rate.

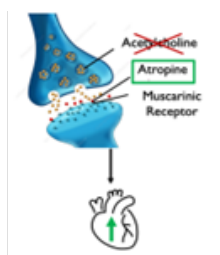


Figure 4. Visual representation of Atropine (ATR) competing for the muscarinic receptor by blocking the binding of acetylcholine, resulting in an increase in heart rate.



Figure 5. Visual representation of diphenhydramine (DPH) and atropine competing for muscarinic receptors blocking the binding of acetylcholine.

Daphnia magna are a type of water flea that serve as beneficial testing organisms because they are cost efficient, easy to handle and manipulate, and have similar cellular receptors as humans (Fig. 2) (Tkaczyk et al., 2021). *Daphnia* are the oldest and most utilized testing organisms for various aspects of biological research (Tkaczyk et al., 2021). Throughout the past ten years, the use of *Daphnia* as a model testing organism has become more popular in pharmacological research because of their sensitivity to chemicals. It has been found that the *Daphnia* heart is partially innervated by the vagus nerve, which is the same nerve that innervates the human heart

(Bekker et al., 1951). Therefore, *Daphnia* may be ideal organisms to test questions regarding drugs that work through the vagus nerve or by similar mechanisms to initiate their effects.

Acetylcholine is the chemical messenger that allows the neural impulse from the vagus nerve to communicate with the muscular tissue to initiate its effects, therefore it acts as a neurotransmitter. Once acetylcholine is released, it binds to muscarinic receptors within the heart, which then initiates a signal within the heart muscle tissue leading to contraction of smooth muscles, dilation of blood vessels, and bradycardia (decreased heart rate) (Fig. 3) (Bekker et al., 1951).

Atropine is an anticholinergic chemical that has many clinical uses. Primarily, atropine is used to reduce bronchiole secretions, but it is also used to treat bradycardia (low heart rate). As it pertains to this project, atropine works as a muscarinic receptor antagonist, meaning it blocks the action of acetylcholine therefore working to inhibit vagus nerve stimulation (Fig. 4) (Carvalho et al., 2003). Since the vagus nerve works through the parasympathetic nervous system to slow heart rate, atropine works to counter the effects of the parasympathetic nervous system to increase heart rate (Lomba et al., 2020). There is little research published on the effects of atropine on the *Daphnia* heart rate, and within these experiments, there appears to be contradictory results of the effect of atropine on the *Daphnia* heart rate (Baylor et al., 1942) (Bekker et al., 1951) (Villegas-Navarro 2003).

Diphenhydramine is classified as both a first generation H1-antihistamine and an anticholinergic drug that also works by targeting muscarinic-3 receptors (Fig. 5) (Sicari & Zabbo, 2020). Diphenhydramine, the primary ingredient in Benadryl[®], is most utilized for its antihistamine properties (Kristofco et al., 2014) as it also blocks histamine receptors, therefore it is able to be utilized for many other conditions such as motion sickness, difficulties sleeping, and can even help manage symptoms of Parkinson's disease. Since there are widespread bodily effects from diphenhydramine, there is an increased possibility for an interaction with other anticholinergic drugs to occur. In a case report conducted by Abdi et al. (2014) it was found that diphenhydramine toxicity presents with symptoms such as tachycardia, encephalopathy, and dry mucous membranes. These symptoms are due to the anticholinergic nature of diphenhydramine.

The objective of my study was to investigate atropine and diphenhydramine both independently and in combination by measuring their effects on heart rate using the model organism *Daphnia magna*. Given that diphenhydramine and atropine are both anticholinergic, I hypothesized they would both increase heart rate independently and when combining these drugs, a synergistic interaction will occur, meaning the increase in heart rate caused by these two drugs concurrently will be greater than when taking the two drugs separately.

Materials/Methods

First, an atropine dose-response was conducted. Using preliminary trials, it was found that concentrations below 10^{-8} M atropine did not initiate a response, so 10^{-8} M was chosen to be the most dilute concentration. Dilutions were made starting with 10^{-2} M atropine and were diluted by one order of magnitude until 10^{-8} M atropine (10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M). Next, a diphenhydramine dose-response was similarly conducted with diphenhydramine at the following concentrations: 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-4} M.

Following the independent dose-response trials, *Daphnia* were treated to combinations of atropine and diphenhydramine to evaluate potential interactions. First, 10^{-8} M diphenhydramine was administered to the *Daphnia* as a background with atropine increasing in concentrations

by one order of magnitude (10^{-8} M- 10^{-5} M atropine) at each step resulting in the following dose-response trial concentrations: 10^{-8} M diphenhydramine/ 10^{-8} M atropine, 10^{-8} M diphenhydramine/ 10^{-7} M atropine, 10^{-8} M diphenhydramine/ 10^{-6} M atropine, and 10^{-8} M diphenhydramine/ 10^{-5} M atropine.

Since diphenhydramine has two drug classifications, a third experiment was conducted to expose the role of the histamine receptors in diphenhydramine's mechanism of action. To do this, carbachol, an acetylcholine mimic, was added to compete with diphenhydramine. This limits its ability to bind to muscarinic receptors, thus exposing the magnitude of response from diphenhydramine binding to histamine receptors alone. To begin, a carbachol dose response was conducted at 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M and 10^{-4} M. The dose response trial revealed that 10^{-7} M carbachol initiated the greatest effect, so in the interaction trial, 10^{-7} M carbachol was held constant as the background with increasing concentrations of diphenhydramine, yielding the following experimental treatment solutions: 10^{-7} M carbachol/ 10^{-10} M diphenhydramine, 10^{-7} M carbachol/ 10^{-8} M diphenhydramine, 10^{-7} M carbachol/ 10^{-6} M diphenhydramine, and 10^{-7} M carbachol/ 10^{-6} M diphenhydramine.

Using preliminary time trials, it was determined the adequate exposure times for each drug (Table 1). Each *Daphnia* were exposed to 100 μ L of the treatment solutions in increasing concentrations with pond water as the control. Since the *Daphnia* heart beats too rapidly at room temperature to accurately visualize the heart under a microscope, the *Daphnia* were chilled to a constant 10 degrees Celsius while obtaining measurements. Upon exposing the *Daphnia* to the solution, heart rate was obtained by counting the number of times the heart physically beat by visualizing the heart through the microscope in the designated amount of time determined by preliminary trials. Data were analyzed by Repeated Measures ANOVA with Tukey HSD post-hoc tests (JMP software, SAS Institute Inc.).

Table 1. Adequate exposure time for drugs determined by preliminary time trials

Drug	Exposure Time (seconds)
Atropine	60
Diphenhydramine	120
Atropine + Diphenhydramine	120
Carbachol	120
Carbachol + Diphenhydramine	120

Results

Atropine

When exposed to increasing concentrations of atropine, *Daphnia* heart rate increased significantly (Fig. 6). On average, heart rate increased by 17% compared to control ($F = 3.32$, $P = 0.0041$). The 10^{-7} M concentration of atropine was found to be the threshold dose response. In an attempt to discover whether the effects of this drug are reversible, *Daphnia* were re-exposed to the control solution, pond water. This effect appeared to be reversible as heart rate declined during the washout recovery.

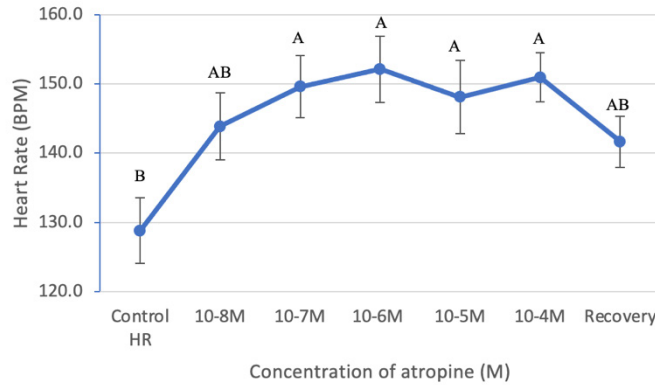


Figure 6. Mean heart rate (beats per minute) of *Daphnia magna* treated with increasing atropine concentrations (pond water, 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M). *Daphnia* were maintained at 10°C and exposed to each concentration of atropine for 60 seconds before counting heart beats. Each point represents the mean heart rate of 25 *Daphnia* ($F=3.32$, $P=0.0041$). Error bars represent the standard error. Means with different letters are significantly different at $p<0.05$ (Tukey HSD).

Diphenhydramine

When exposed to increasing concentrations of diphenhydramine, *Daphnia* heart rate increased significantly (Fig. 7, $F= 11.097$, $P= <0.001$). On average, heart rate increased by 27% compared to control. Using preliminary dose response trials (data not shown), it was determined that 10^{-8} M was the threshold concentration for diphenhydramine. The increase in heart rate in response to diphenhydramine was reversible.

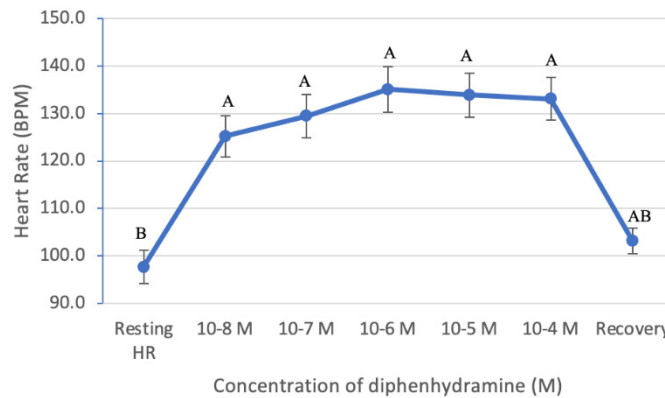


Figure 7. Mean heart rate (beats per minute) of *Daphnia* treated with increasing diphenhydramine concentrations (pond water, 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M). *Daphnia* were exposed to each concentration of diphenhydramine for 120 seconds before counting heart beats. Each point represents the mean heart rate of 15 *Daphnia magna* ($F=11.097$, $P<0.001$). Error bars represent the standard error. Means with different letters are significantly different at $p<0.05$ (Tukey HSD).

Atropine and Diphenhydramine in Combination

When exposed to increasing concentrations of atropine and diphenhydramine, heart rate significantly increased. On average, heart rate increased by 30% compared to control (Fig. 8, $F= 26.67$, $P<0.001$). Atropine was exposed to the *Daphnia* increasing in concentration by two orders of magnitude, while diphenhydramine was in the background. It was found that atropine and diphenhydramine increased heart rate, but the magnitude of response was similar in magnitude to diphenhydramine alone.

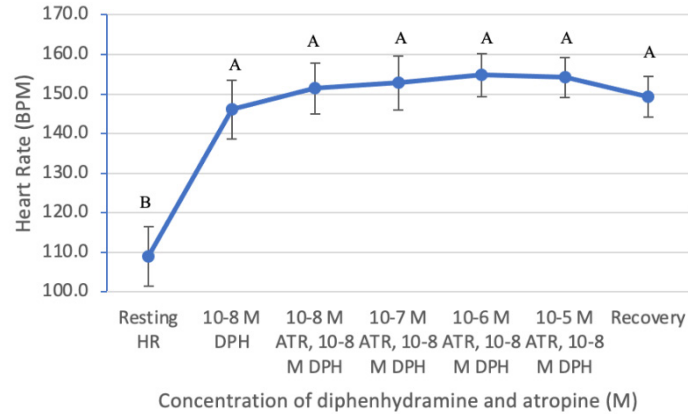


Figure 8. Mean heart rate (beats per minute) of *Daphnia* treated with increasing concentrations of atropine (ATR, 10^{-8} - 10^{-5} M) against a constant 10^{-8} M diphenhydramine (DPH) background. *Daphnia* were exposed to each concentration of diphenhydramine for 120 seconds before counting heart beats. Each point represents the mean heart rate of 15 *Daphnia magna* ($F=26.67$, $P<0.001$). Error bars represent the standard error. Means with different letters are significantly different at $p<0.05$ (Tukey HSD).

Carbachol

When exposed to increasing concentrations of carbachol, an acetylcholine mimic, heart rate significantly decreased (Fig. 9, $F= 19.28$, $P<0.001$). On average, heart rate decreased by 28% compared to control (Fig. 8, $F= 19.28$, $P<0.001$). Carbachol at 10^{-7} M was found to be the threshold dose response to initiate a decrease in heart rate, while 10^{-5} M exhibited the greatest decrease in heart rate. This confirms carbachol decreases heart rate as would be expected by acetylcholine in *Daphnia*.

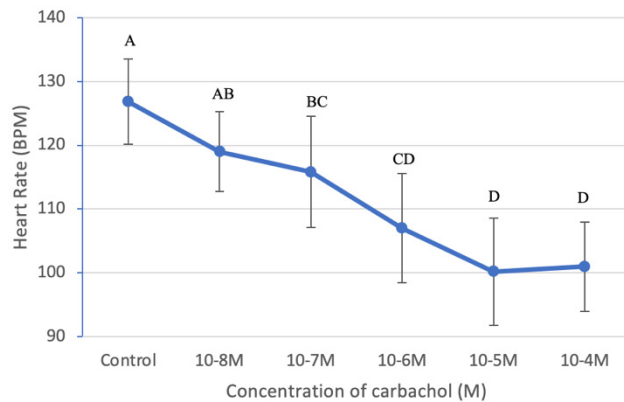


Figure 9. Mean heart rate (beats per minute) of *Daphnia* treated with increasing carbachol concentrations (pond water, 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M). *Daphnia* were exposed to each concentration of carbachol for 120 seconds before counting heart beats. Each point represents the mean heart rate of 15 *Daphnia magna* ($F=19.28$, $P<0.001$). Error bars represent the standard error. Means with different letters are significantly different at $p<0.05$.

Carbachol and Diphenhydramine in combination

When exposed to increasing concentrations of diphenhydramine with carbachol at a constant background concentration of 10^{-7} M, *Daphnia* heart rate significantly increased. On average, heart rate increased by 14% compared to control (Fig. 10, $F=12.75$, $P<0.01$). The presence of carbachol reduced the effect of diphenhydramine by approximately 50% compared to the effect

of diphenhydramine alone (Fig. 7).

Carbachol and Diphenhydramine in combination

When exposed to increasing concentrations of diphenhydramine with carbachol at a constant background concentration of 10^{-7} M, *Daphnia* heart rate significantly increased. On average, heart rate increased by 14% compared to control (Fig. 10, $F=12.75$, $P<0.01$). The presence of carbachol reduced the effect of diphenhydramine by approximately 50% compared to the effect of diphenhydramine alone (Fig. 7).

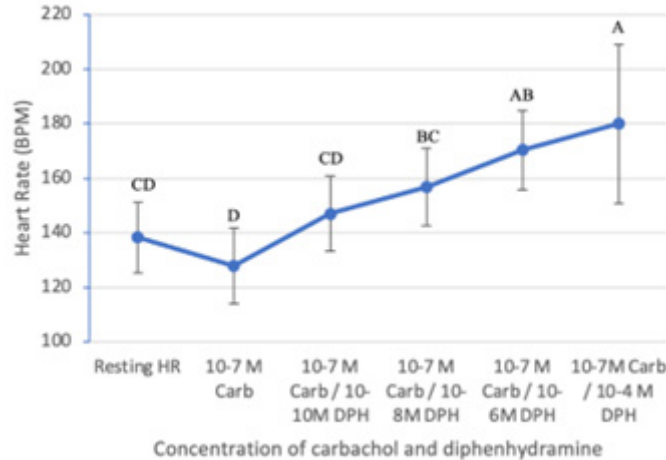


Figure 10. Mean heart rate (beats per minute) of *Daphnia* treated with increasing concentrations of diphenhydramine combined with a constant carbachol concentration (pond water, 10^{-7} M carbachol, 10^{-7} M carbachol/ 10^{-10} M diphenhydramine, 10^{-7} M carbachol/ 10^{-8} M diphenhydramine, 10^{-7} M carbachol/ 10^{-6} M diphenhydramine, and 10^{-7} M carbachol/ 10^{-4} M diphenhydramine). *Daphnia* were exposed to each concentration of carbachol and diphenhydramine for 120 seconds before counting heart beats. Each point represents the mean heart rate of 15 *Daphnia magna* ($F=12.75$, $P<0.01$). Error bars represent the standard error. Means with different letters are significantly different at $p<0.05$ (Tukey HSD).

Discussion/Conclusion

Atropine and diphenhydramine, two-anticholinergic drugs, were exposed to the *Daphnia magna* both independently and in combination to shed light upon the potential interaction between drugs with a similar mechanism of action. Both atropine and diphenhydramine increased *Daphnia* heart rate independently, and when applied in combination, their effects were similar in magnitude to when exposed to diphenhydramine alone. In addition, this study evaluated whether *Daphnia magna* are prime test subjects for anti-cholinergic drug testing. Because the *Daphnia* are easy to maintain, cost efficient, and responded in a similar manner to humans, it was determined *Daphnia* are prime test subjects for anti-cholinergic drug testing.

Since the pharmaceutical industry is rapidly developing and is in need of cost-efficient testing organisms (Tkaczyk et al., 2021), it was pertinent to discover if *Daphnia* are under innervation of a nerve similar to the vagus nerve, since that is the mechanism of action in the parasympathetic nervous system of humans. Evidence from this study supports the hypothesis that *Daphnia* have a nerve similar to the vagus nerve because the *Daphnia magna* responded to atropine, diphenhydramine, and carbachol similar to the response observed in humans.

This current study showed that atropine significantly increased heart rate when exposed to increasing concentrations of atropine similar to findings from other studies (Villegas-Navarro 2003). However, these results contradict the findings in Bekker et al., (1951). Therefore, this supports the hypothesis that atropine blocks the deaccelerating effects of the parasympathetic nervous system, resulting in an increase in heart rate. Similarly, when *Daphnia* were exposed to increasing concentrations of diphenhydramine, heart rate increased significantly. These data support the hypothesis that atropine and diphenhydramine, via their anti-cholinergic properties, both independently increase heart rate.

Although atropine and diphenhydramine are both classified as anti-cholinergic drugs and pose an interaction risk if consumed together (Yeh et al., 2007), evidence supports that these drugs in combination, do not interact. When *Daphnia* were exposed to the drugs in combination, the impact was similar in magnitude to when exposed to diphenhydramine alone. These data answered the question of whether an interaction occurs, but it is unclear as to why diphenhydramine independently increased heart rate 10% more than atropine.

There are likely several possible explanations as to why diphenhydramine caused a greater increase in the heart rate of *Daphnia* than atropine. First, it is possible that diphenhydramine has a greater affinity for the muscarinic receptors compared to atropine, meaning the molecules more readily bind to the muscarinic receptors, thus initiating a greater response. To test this hypothesis, the chemical kinetics would need to be evaluated. This would include obtaining the chemical dissociation constant for atropine binding to the muscarinic receptor. Understanding the kinetics would best inform how readily these molecules interact with their receptors.

The second explanation as to why diphenhydramine initiated a greater response may be related to its antihistamine properties. While diphenhydramine interacts with muscarinic receptors, it also binds histamine receptors that may also lead to an increase in heart rate via a mechanism independent of its action as an acetylcholine antagonist. This study found that when carbachol, an acetylcholine mimic, was added in combination to diphenhydramine, *Daphnia* heart rate still increased by 12%, which is a lesser extent than when exposed to diphenhydramine alone.

Even in the presence of a drug that reduces the ability of diphenhydramine to bind to muscarinic receptors as part of the parasympathetic pathway, diphenhydramine was still able to increase heart rate. Therefore, histamine receptors likely have a role in the response. The extent of the involvement of the histamine receptors in the response of diphenhydramine, in addition to the affinity of diphenhydramine for the histamine and muscarinic receptors remain unknown. Berninger et al., (2011) found that diphenhydramine initiated a greater response in *Daphnia magna* compared to other aquatic organisms, but the reason why this occurred is unknown. There is little research investigating the effects of antihistamines on the *Daphnia magna* heart rate, thus future studies could investigate the kinetics of these reactions. Also, additional information about the role of diphenhydramine binding to the histamine receptors in the acceleration response in *Daphnia* is needed and would inform our understanding of how atropine and diphenhydramine

should best be used to guide safe and effective treatments in humans.

Acknowledgements

Thank you to the UWEC diversity mentoring program, UWEC Ronald E. McNair program, Dr. Kelly Wonder, Dr. Ka Vang, and Dr. Brad Carter for their support and guidance through this project. Thank you to my mentor, Dr. Tali Lee, for her mentorship, diligence, and dedication.

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