Combined Effect of Ethanol and Acetaminophen on the Central Nervous System of *Daphnia magna*

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The combined consumption of acetaminophen (APAP) and ethanol (EtOH) has been an issue with clinical implications. Previous findings regarding the simultaneous consumption of APAP and EtOH have reported harmful effects on the liver and stomach; however, little is known about the effects on the central nervous system (CNS). We hypothesized that EtOH and APAP will have a synergistic effect on the CNS of *Daphnia magna* (*D. magna*), causing a pronounced decrease in heart rate at a toxic dose of EtOH. To better understand the effects of the combined consumption of EtOH and APAP on the CNS, the heart rates of *D. magna* were measured under a dissection microscope after exposure to EtOH, APAP, or a combined EtOH-APAP solution. Interestingly, the average heart rates of *D. magna* exposed to the EtOH-APAP solution and *D. magna* exposed only to APAP were approximately the same. Although our results did not support our original hypothesis, the data demonstrated that APAP exerted a dominant effect over EtOH. APAP and EtOH are known to have inhibitory effects on the CNS. Therefore, these findings suggest that APAP and EtOH may compete against each other on similar pathways to be the substance that exerts an inhibitory effect in the CNS.

Keywords: depressant, heart rate, liver, serotonergic descending inhibitory pathways, stomach, synergistic effect

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**Introduction**

Acetaminophen (APAP) is a common ingredient in over-the-counter painkillers such as Tylenol®. It is used to relieve headaches, fevers, and general aches. When used as directed, the effects are generally considered harmless. However, the FDA advises against taking APAP in conjunction with other substances, particularly alcohol (U.S. Food and Drug Administration, 2003). Combining APAP and ethanol (EtOH) is a common mistake among consumers, who are often unaware of its consequences (Prescott, 1999). Side effects are seen in the liver and stomach (Maze et al., 1998), but are unknown in the central nervous system.

The nervous system can be affected by chemicals that act as depressants or stimulants, either to slow or accelerate processes in the brain and body. The medulla oblongata, part of the brain stem, is responsible for autonomic body functions including the control of heart rate (Korpelainin et al., 1999).

The organism *D. magna* is an ideal specimen to use to study the effects of chemical substances on the nervous system. *D. magna* are filter feeders normally found in temperate fresh waters (Soares et al., 2008). The heart of a *D. magna* is easily viewed under a dissection microscope due to its transparent body, making it well-suited for this experiment (Chapman, 1976). As its heart rate is modified by the nervous system (McMahon, 2001), the heart is a useful organ to study in order to further understand the effects of APAP and EtOH on the nervous system. In addition, the heart of *D. magna* behaves like a vertebrate heart (Bekker and Krijsgman, 1951) and is thus comparable to the human heart.

In addition to its analgesic effects, APAP also acts as a depressant on the CNS. More specifically, APAP is believed to demonstrate a positive effect on the serotonergic descending inhibitory pathways (Smith et al., 2009), thereby producing its analgesic effects. Thus, it is important to note that serotonin and
its related pathways are highly responsive to APAP (Anderson, 2008). Analgesics work by inducing the release of opioid peptides, which activate the descending inhibitory system in the spinal cord (Mukaida et al., 2007). Between the inhibitory effects and the release of the opioid peptides, physiological reactions to APAP have an overall depressant effect. There is thus an inhibitory reaction to APAP in all bodily functions controlled by the brain stem and spinal cord, including the heart rate. It has been reported that APAP causes antiarrhythmic effects on the heart of the dog (Merrill et al., 2007) and causes an increase in cardiac output and mean arterial pressure in sheep (Leshnower et al., 2006).

Another commonly used effector of the CNS is EtOH. Typically EtOH triggers an increase in rate heart. Previously, it has been shown that alcohol causes marked tachycardia in erect and supine postures (Scott et al., 1987). While EtOH triggers an increased heart rate, consumption of a toxic dose of EtOH may cause bradycardia. It has been demonstrated that upon administration of EtOH to female rats, there is a corresponding decrease in mean arterial blood pressure (El-Mas et al., 2005). Further research indicates that EtOH acts as an agonist on GABAergic receptors; inducing inhibitory postsynaptic potentials in postsynaptic neurons (Theile et al., 2008).

The goal of this study is to determine how APAP and EtOH affect the nervous system when taken simultaneously. The commercial alcoholic beverage, Absolut™ Vodka 80 proof, was used in place of laboratory grade EtOH because it is more similar to what individuals would consume on a college campus. There are known side effects in the stomach and liver, but there is little research on the effects of this combination in the nervous system. Our hypothesis is that EtOH and APAP will act synergistically on the CNS of D. magna causing a pronounced decrease in heart rate, mimicking consumption levels of a toxic dose that would result in heart rate depression.

Materials and Methods

Optimal Concentration Determination

D. magna were placed on Vaseline™-coated depression slides with 0.171 M, 0.342 M, or 0.684 M EtOH solutions to determine the optimal EtOH concentration. Optimal concentration is defined as the highest concentration of the medium that did not result in lethality. After five minutes of acclimation in the solution, the heart beats of the D. magna were observed under a dissection microscope. Ten trials were used to determine optimal EtOH concentration. The same procedure was repeated for 32 mg/mL, 3.2 mg/mL, and 0.32 mg/mL APAP solutions to determine the optimal APAP concentration. All D. magna used in this trial were used once and only in this trial. Trials were conducted at 20-22 °C.

Heart Rate

D. magna were transferred from a culture of wild D. magna (Ward’s Natural Science, Rochester, NY) to a Vaseline™-coated Fisher-brand depression slide (Fisher Scientific, Pittsburgh, PA). After five minutes of acclimation on the slide, its heart beats were counted for two minutes under a Nikon SMZ645 dissection microscope (Nikon, Melville NY) using a manual palm counter (Fisher Scientific, Pittsburgh, PA) and a digital timer (Fisher Scientific, Pittsburgh, PA). The heart rates of ten D. magna were counted, using five trials for each D. magna. In this procedure and the procedures listed below, each D. magna was kept in a liquid medium at all times. All D. magna used in this trial were used only in this trial. Trials were conducted at 20-22 °C.

Ethanol Treatment

D. magna were transferred to a Vaseline™-coated depression slide. Excess water was removed while 0.342 M EtOH (Absolut™ Vodka, 80 Proof, 40% EtOH by volume, produced by Pernod Ricard, Paris, France) was added immediately afterwards. The D. magna were allowed to acclimate to the new medium on the depression slide for five minutes. Their heart rate was then measured following the control procedure. All D. magna used in this
trial were used only in this trial. Trials were conducted at 20-22 °C.

**Acetaminophen Treatment**

*D. magna* were placed in a container of 3.2 mg/mL liquid APAP (Children’s acetaminophen oral suspension liquid, 32 mg/mL, Target Corporation, Minneapolis, MN) for two minutes. *D. magna* were then transferred to an untreated slide in which excess APAP was removed while deionized water was immediately added. This exchange was performed to improve visibility of the heart due to the cloudy nature of APAP. The *D. magna* were then transferred to a Vaseline™ coated depression slide. The protocol for measuring heart rate was the same used in the control procedure. All *D. magna* used in this trial were used only in this trial. Trials were conducted at 20-22 °C.

**Ethanol and Acetaminophen Combined Treatment**

*D. magna* were placed in a mixed solution of 0.342 M EtOH and 3.2 mg/mL APAP (EtOH-APAP) for five minutes. After the five minute incubation period, the *D. magna* were placed on a Vaseline™-coated depression slide with deionized water in order to improve visibility of the heart. Following a two minute acclimation period, the heart rate was counted using the control procedures. All *D. magna* used in this trial were used only in this trial. Trials were conducted at 20-22 °C.

**Statistical Analysis**

Microsoft Office Excel 2007 with the Data Analysis add-in tool pack was used to determine F, T, and P values for each dataset.

**Results**

**Optimal Concentration Determination**

The average heart rate of untreated *D. magna*, measured in beats per minute (bpm), was 221.06 ± 5.75 bpm, n = 50 (average ± SEM, n = number of subjects) (Fig. 1), was used as the control throughout the rest of this study. EtOH and APAP both demonstrated inhibitory effects on the *D. magna* heart rate; as the APAP and EtOH concentration increased, the heart rate dropped drastically (Fig. 1). *D. magna* administered with 0.171 M EtOH experienced the least change in heart rate (176.20 ± 15.56 bpm, n = 10) when compared to the control group. The 0.342 M EtOH concentration had the most influence on heart rate (97.30 ± 12.62 bpm, n = 10). The highest concentration, 0.648 M EtOH, resulted in 90% lethality, with the surviving individual expressing a heart rate of 28 bpm, n = 10 (Fig. 1A). Similarly, *D. magna* administered with 0.32 mg/mL APAP concentration showed the least amount of change in heart rate (179.2 ± 18.0 bpm, n = 10) in comparison to the control group. *D. magna* treated with 3.2 mg/mL APAP exhibited the largest change in heart rate without lethality (151.5 ± 30.9 bpm, n = 10) in comparison to the control. The 32 mg/mL concentration of APAP was lethal for all *D. magna* (Fig. 1B).

![Figure 1](https://impulse-journal.com/images/Impulse_2010/16/16A.png)

**Figure 1.** *D. magna* heart rate response to varying concentrations of EtOH and APAP, n = 10 at each condition. A. Compared to control average heart rate, an EtOH concentration of 0.171 M demonstrated a slightly decreased heart rate (F = 4.61, t stat = 5.28, P(F≤f) = 0.009, P(T≤t) = 1.15 x 10⁻⁵), 0.342 M EtOH resulted in the largest decrease in heart rate (F = 4.63, t stat = 14.80, P(F≤f) = 0.009, P(T≤t) = 2.37 x 10⁻¹⁰) with no lethality, 0.648 M EtOH resulted in 90% lethality. B. Compared to control average heart rate, an APAP concentration of 0.32 mg/mL demonstrated slightly decreased heart rate (F = 3.93, t stat = 4.71, P(F≤f) = 0.016, P(T≤t) = 3.57 x 10⁻³), 3.2 mg/mL APAP had the greatest decrease in heart rate (F = 1.21, t stat = 4.94, P(F≤f) = 0.401, P(T≤t) = 3.53 x 10⁻⁴) without resulting in lethality, 32 mg/mL resulted in lethality for all *D. magna*. 
**Ethanol and/or Acetaminophen Treatment**

*D. magna* administered with 3.2 mg/mL APAP experienced an average heart rate of 153.03 ± 5.11 bpm, n = 50. Individuals exposed to APAP demonstrated a slightly non-rhythmic heart rate. *D. magna* exposed to 0.342 M EtOH experienced a decreased heart rate of 107.04 ± 3.10 bpm. Individuals treated with EtOH also exhibited a slightly non-rhythmic heart rate. The average heart rate of *D. magna* treated with both 3.2 mg/mL APAP and 0.342 M EtOH was 160.75 ± 7.35 bpm (Fig. 2). The average heart rate when tested with the EtOH-APAP solution was lower than the average heart rate of the control group, but was higher than the average heart rates of *D. magna* treated with APAP or EtOH individually (Fig. 2). There was a more evident non-rhythmic heart rate seen in *D. magna* exposed to the EtOH-APAP combined solution.

![Figure 2](image-url)

**Conclusions**

The *D. magna* treated with the combined solution of 0.342 M EtOH and 3.2 mg/mL APAP had a similar heart rate to the *D. magna* treated with 3.2 mg/mL APAP alone. On the other hand, *D. magna* treated with EtOH alone had a pronounced decrease in heart rate as compared to APAP- or EtOH-treated *D. magna*, as well as compared to controls. Untreated *D. magna*, used as the control group, expressed an average heart rate of 221.06 ± 5.75 bpm. This agrees with previous experimental data of 222.74 bpm (Sreekala et al., 1991), measured for *D. magna* in a 29.5 °C H2O medium. *D. magna* in an 0.342 M EtOH medium, 3.2 mg/mL APAP medium, and EtOH and APAP (0.342 M + 3.2 mg/mL) combined medium experienced a 52%, 31%, and 27% decrease in heart rate compared to control, respectively.

The use of the 3.2 mg/mL APAP solution ensured the vitality of *D. magna*. Previous experiments with *D. magna* have found 48 hour EC50 values of 9.2 mg/L (Kühn et al., 1989), 50 mg/L (Henschel et al., 1997), and 30.1 mg/L (Kim et al., 2007). These EC50 values represent the APAP concentration where 50% of its maximal effect is observed in 48 hours. A more potent concentration of 3.2 mg/mL APAP was chosen to visualize a considerable change in heart rate over a shorter time span of five minutes. Previous experiments administered a continuous supply of lower concentrations, a method that is not normally practiced in humans. The 3.2 mg/mL APAP concentration was chosen on the basis that humans take a concentrated amount of APAP in one dose in order to alleviate general pains.

The original hypothesis was that the *D. magna* exposed to toxic doses of the EtOH-APAP combined solution would have a synergistic depressant effect, resulting in a heart rate lower than those tested with APAP or EtOH alone. However, our findings demonstrate that the administration of the EtOH-APAP combined solution resulted in a heart rate similar to *D. magna* treated with only APAP. Since both APAP and EtOH can have inhibitory effects (Smith et al., 2009; Theile et al., 2008) on the nervous system, the two substances may operate on similar pathways.

Therefore, since we now suggest that APAP and EtOH utilize similar pathways, the presence of one substance could mask the effects of the other. In this case, the effect of EtOH was found to be masked by the presence of APAP, potentially explaining the similar average heart rate of *D. magna* treated with the EtOH-APAP solution and *D. magna* treated with APAP alone. More research must be conducted to better...
understand the interaction of the two inhibitory pathways involved in EtOH and APAP nervous system responses.

The decreased heart rate of *D. magna* administered with an EtOH-APAP solution was approximately the same as that of the *D. magna* treated with APAP alone. Interestingly, this finding suggests that in regards to the CNS, simultaneously consuming EtOH and products containing APAP will generate the same effect as ingesting APAP alone. This conclusion does not take into account the possible effects on other systems of the body caused by the intake of the two substances concurrently.

Previous research shows compelling evidence that the stomach and liver are damaged when EtOH and APAP are consumed in combination (U.S. Food and Drug Administration, 2003). However, there is limited research demonstrating the effects of the two substances on the nervous system, thus further studies are necessary to fully understand the effects of this combination on the CNS.

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