

# The Neurophysiological Effects of Guarana and Ethanol Intake on *Daphnia magna*

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In recent years, the consumption of energy drinks and alcoholic beverages has become a prevalent habit, especially among younger generations. However, there is little scientific research surrounding the interaction of ethanol and the natural stimulant guarana, which is being utilized more frequently as the main caffeine source in energy drinks. This study utilized *Daphnia magna* (*D. magna*) as a model organism to observe alterations in the functioning of the central nervous system when exposed to both ethanol and guarana in a series of time trials. As expected, ethanol significantly decreased the overall heart rate of the *D. magna*, while guarana increased it. In combination, the depressant effects of ethanol decreased the stimulating effects of guarana, as our results displayed a statistically significant reduction of heart rate. Therefore we propose that our findings indicated that the alcohol effects may be stronger than the effects of guarana.

Keywords: calcium-dependent neurotransmitter release; energy drinks; GABAergic transmission; vagal center; vasomotor center

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## Introduction

The ingestion of alcoholic beverages and “energy drinks” has been increasing in popularity (Ferreira et al., 2004). Marketed energy drinks most often include taurine, carbohydrates, gluconolactone, inositol, niacin, pantothenol, B-complex vitamins and some variety of caffeine (Ferreira et al., 2006). Users of this combination have frequently reported a reduction in sleepiness and an increase in the sensation of pleasure when consuming both alcoholic beverages and energy drinks containing caffeine and taurine (Ferreira et al., 2006). These findings suggest that energy drinks, and more specifically caffeine, may reduce the depressant effects and/or increase the excitatory effects of alcohol. However, even

though previous reports have considered the effects of combined ingestion of alcoholic beverages and energy drinks containing taurine and caffeine, there is a lack of scientific evidence on the interaction between alcoholic substances and energy drinks containing the natural stimulant guarana.

Ethanol, a common chemical component within alcoholic beverages, has been found to have many effects on both neurological and physiological processes within the body. A few of the most studied and prominent properties of alcohol consumption are progressive weakness, difficulty in walking, and lowered heart rate (Ferreira et al., 2006). Ethanol inhibits calcium-dependent neurotransmitter release, therefore

inhibiting excitatory and inhibitory postsynaptic potentials in cultured spinal cord neurons. This inhibitory effect of ethanol thus suppresses such physiological functions, such as in the heart (Deitrich and Erwin, 1996). GABA, the main inhibitory neurotransmitter in the adult mammalian central nervous system (CNS), is also affected by alcohol ingestion. Ethanol has been shown to enhance GABAergic function significantly in several CNS areas, and can also increase GABAergic transmission on postsynaptic receptors (Nie et al., 2009). Enhancement of GABAergic activity is a common property of many sedative and hypnotic drugs, including ethanol (Siggins et al., 2005). Thus, alcohol has been widely studied and has consistently demonstrated the ability to affect a number of channels, potentials and processes within the CNS when introduced into the body.

Guarana is a naturally occurring plant in the Sapindaceae family, where each one of its seeds within the fruit contains approximately five times more caffeine than coffee beans, and is known to cause increased physical capacity when an individual is subjected to a stressor (Espinola et al., 1997). Guarana has been utilized as a popular medicine as well, mainly for its stimulating effects (both mental and physical), and for its suggested rejuvenation and aphrodisiac effects (Bydlowski et al., 1988). It is predicted that guarana affects the same processes as caffeine, only at an increased level since guarana contains a significant source of caffeine as the stimulating agent. High doses of caffeine have been found to stimulate respiratory, vasomotor and the vagal center within the human body (Bolton and Null, 1981). The vagal center, which is part of the autonomic nervous system and located in the peripheral nervous system, controls involuntary functions, including digestion and heart rate. Ultimately, extracts of guarana have been used as popular stimulants in many nonalcoholic “healthy”

drinks, and more recently have been reported to be used by those with demanding exercise routines or those who wish to stay awake all night at parties (Bempong, 1992).

For this study, *D. magnawere* utilized as a model organism to observe changes in the functioning of the heart when in the presence of both alcohol and guarana. *D. magna* have been used by many biologists in various types of experimental investigations due to their short life span, quick maturation period, manageable size and transparency that allows for the use of limited technology to observe physiological processes within the organism (Anderson and Jenkins, 1942). Even though *D. magna* possess a neurogenic mechanism of heart beat control which is entirely different from the myogenic ‘pacemaker’ of the vertebrate heart, *D. magna*’s neurogenic pacemaker has some key properties in common with the autonomic nerve system of vertebrate animals, and responds similarly in the presence of specific toxins and drugs (Bekker and Krijgsman, 1951). More specifically, *D. magna*’s nervous system responds quite similarly to a wide range of agonists and antagonists that affect the heart rate, rhythm and the vagal center of humans, and are therefore comparable when observing cardiovascular movements of the heart (Villegas-Navarro et al., 2003).

As guarana is being utilized more frequently in energy drinks, it is important to uncover its cardiovascular and CNS effects. Overall, we have found a lack of scientific evidence surrounding the combined ingestion of alcohol and the highly concentrated caffeine in guarana. Previous studies concentrating on the interaction between alcohol and energy drinks containing both caffeine and taurine demonstrated the overpowering effects of alcohol over the stimulating effects of the energy drink within physiological processes (Ferreira et al., 2006). Therefore, in this study we attempted to see if guarana would demonstrate similar

properties by reducing the depressant effects or increase the excitatory effects of alcohol within *D. magna*. These results could then be used to further understand the neurophysiological processes affected by these two chemicals. It was hypothesized that even though guarana contains a significant concentration of caffeine, alcohol would still overpower the stimulant and produce overall depressant effects within the *D. magna* when compared to controls. However, we also believed that guarana would slightly reduce the depressant effects of alcohol when in combination, and demonstrate a slight increase in heart rate when compared to alcohol intake alone. To test this hypothesis of alcohol and guarana ingestion, this study compared *D. magna* heart rates after being separately subjected to the depressant and stimulant. Then *D. magna* were introduced to a combination of both alcohol and guarana to observe the subsequent effects on the cardiovascular system to infer effects occurring within the CNS.

## Materials and Methods

### *Animal Model*

*D. magna* were ordered from a biological supply company (Ward's (Rochester, NY)). The control trial consisted of *D. magna* being placed on a depression slide in deionized water, and the number of heart beats in one minute were counted. To get a baseline heart rate of *D. magna*, 100 control trials were conducted. The dissection microscope (Nikon (Melville, NY) SMZ645) with a Nikon (Melville, NY) NI-150 high intensity illuminator was utilized to perform the trials.

### *Alcohol*

The alcohol used in the trial was Absolut® vodka (Stockholm, Sweden). Absolut® Vodka (80 proof, 40% alcohol) is a

commercial alcohol and may contain unknown impurities and contaminants as compared to laboratory grade ethanol. The reason the commercial alcohol was chosen was to represent a model in *D. magna* that mimics the effects seen on humans when alcohol is ingested.

A trial consisted of measuring the heart rate by counting the beats per minute (bpm) of *D. magna*. Before the bpm could be determined a period of time had to pass so that the ethanol could assert its effect on *D. magna*. A series of trials were conducted with varying periods of time before the bpm was obtained. For each time trial 10 µL of 2% alcohol was utilized. Ten trials were conducted for each of these varying time periods; 1 minute, 2 minutes, 3 minutes, and 4 minutes. The optimal reaction period was chosen to be the period in which the bpm was the lowest because a lower bpm indicates a greater effect of ethanol at that time. The optimal period was then employed in conducting concentration trials. To perform these concentration trials, a serial dilution was carried out to create 1.25%, 2.5%, 5% and 10% from the original 40% alcohol. Each concentration was tested in 10 µL of the alcohol at the varying concentrations for 10 trials. The optimal concentration was the concentration in which the lowest bpm was seen without killing the subjects. The optimal reaction period, 2 minutes, and 10 µL of the optimal concentration, 2.5%, was then tested for 50 trials, each trial being conducted on a different *D. magna* subject.

### *Guarana*

Nature's Answer (Hauppauge, NY) Guarana [liquid guarana herbal extract, USP purified water, coconut glycerin, and 12-15% certified organic alcohol] was utilized as the guarana in this study. The same methods used in the alcohol trials were utilized to determine the optimal reaction period and concentration of guarana. The optimal reaction period and

concentration for guarana was chosen based on the greatest number of heart beats measured in one min. The concentrations tested were 10 mg/mL, 100 mg/mL, and 500 mg/mL. The optimal reaction period, 1 minute, and 10  $\mu$ L of the optimal concentration, 500 mg/mL, were then tested for 50 trials.

### Combination Guarana and Alcohol

The combination solution was added in a staggered, two-step fashion so that the optimal effects of both the ethanol and guarana would be seen while counting the number of heart beats in the *D. magna* subject. First, 5  $\mu$ L of 5% ethanol was added. A 5  $\mu$ L dose of guarana at 1000 mg/mL was added one minute later. This produced a final concentration of 2.5% alcohol and 500mg/mL guarana when exposed to the *D. magna* subject. After an additional minute had elapsed, total two minutes from initial exposure to the ethanol, the number of heart beats in the *D. magna* subject was counted. This process was repeated for 50 trials.

### Statistical Analysis

Statistical analysis was done using Microsoft  $\text{\textcircled{C}}$  Excel 2007. Analyses were calculated using student t-test protocols assuming unequal variances at  $\alpha = 0.05$ . Data were interpreted for one-tailed significance.

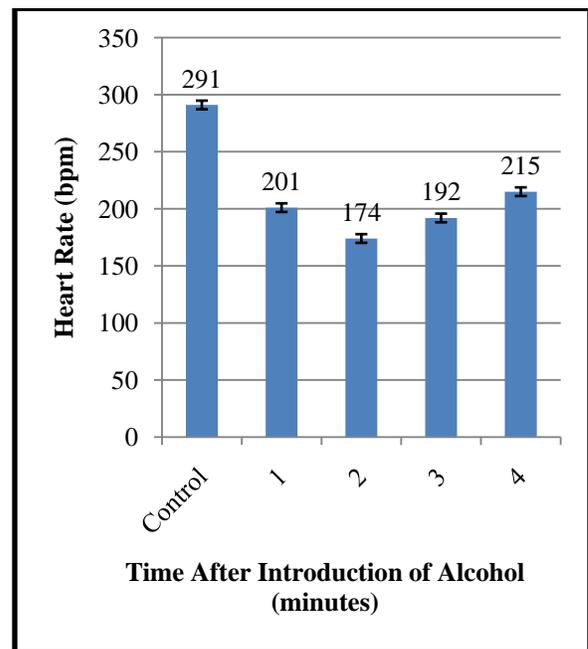
## Results

### Control

Control heart rates were calculated using 100 *D. magna*. Control heart rate was found to be 291 bpm (SEM  $\pm$  1.40). The control heart rate was then used for comparison in the following experimental protocols.

### Alcohol

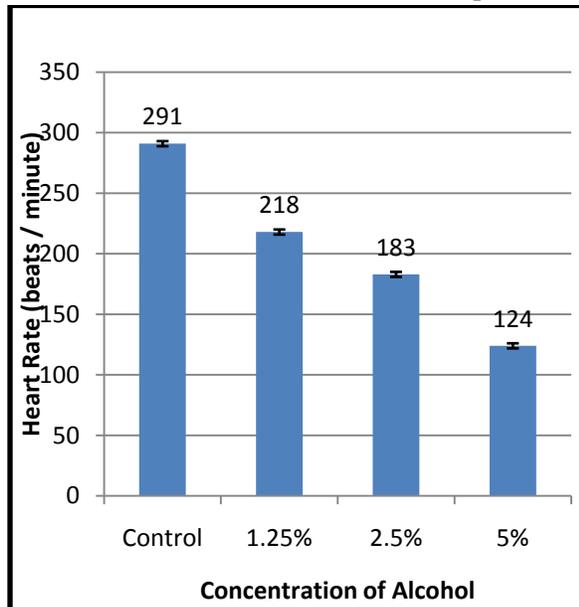
Time series tests of 2.5% alcohol showed that the optimal time to wait between the introduction of the alcohol to *D. magna* and measuring heart rate is two minutes (Fig. 1). The 2.5% concentration of alcohol was chosen arbitrarily, because at this point a dilution series had not been performed. Two minutes produced the greatest decrease in heart rate, since at this time the heart rate was 174 bpm, and after this point the heart rate increased.



**Figure 1.** Time series effect of 2.5% alcohol on *D. magna*. The greatest depressant effect after the introduction of alcohol was seen at two minutes. Heart rate decreased until 2 minutes, and then started to increase again after two minutes. Error bars reflect SE and n=11 for each condition and n=100 for control.

Dilution series tests showed that the optimal concentration of alcohol was 2.5% (Fig. 2). The series was done using 1.25%, 2.5%, 5% and 10% alcohol. All of the *D. magna* used for the 10% alcohol died (data not shown). Exposure to 5% alcohol produced an average heart of 124 bpm (SEM  $\pm$  3.03), however the average was based on eight samples versus ten because two *D. magna* died. All *D. magna* tested

with 2.5% alcohol survived and had an average heart rate of 183 bpm (SEM  $\pm$  3.20), and also had a lower heart rate in comparison to those tested with 1.25% alcohol (218  $\pm$  2.27 bpm).



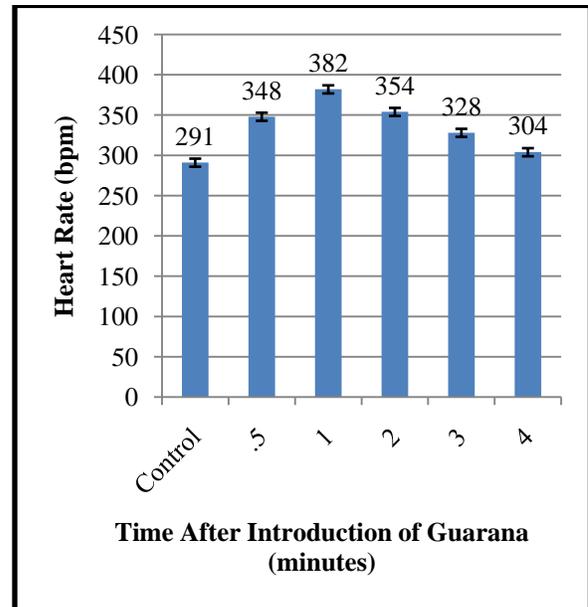
**Figure 2.** Dilution series effect of alcohol on *D. magna*. Heart rates were the lowest using 5% alcohol, however some *D. magna* died with this concentration. The 2.5% had a higher heart rate than the 5% affected *D. magna*, but it was utilized since all *D. magna* survived. Error bars reflect SE and n=10 for each concentration.

Using 50 *D. magna* in a solution of 2.5% alcohol with heart rate measured at two minutes after introduction showed that the average heart rate of *D. magna* was 177bpm (SEM  $\pm$  1.15). Using student t-test statistical analysis, it was found that this heart rate is significantly less than the average heart rate of control *D. magna*, which was 291bpm ( $p < .001$ ).

### Guarana

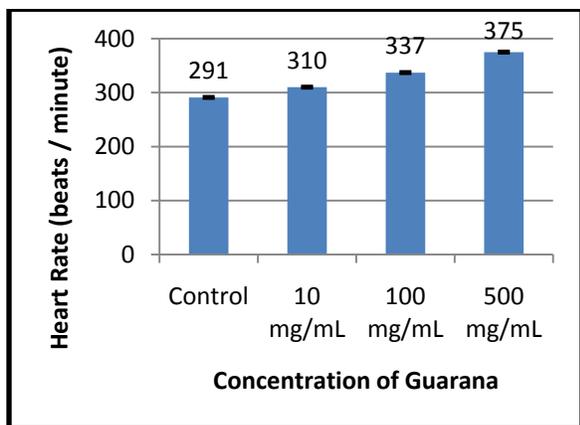
Time series of 500 mg/mL of guarana showed that one minute after the introduction of the guarana was the optimal time to measure heart rate (Fig. 3). Optimal time was determined as the time at which heart rate had increased to the highest level. Just as in the alcohol protocol, 500 mg/mL was chosen arbitrarily as the concentration used in the time series. After one

minute, the heart rate decreased back towards baseline control values.



**Figure 3.** Time series effect of 500 mg/mL of guarana. The heart rate increased the most at one minute after introduction and then decreased back to baseline. One minute was the optimal time to wait after introduction of guarana to *D. magna* since after this time heart rate started to approach control baseline heart rate. Error bars reflect SE and n=10 for each condition and n=100 for control. After three minutes, heart rate was significantly higher than control heart rate ( $p < 0.001$ ) using student t-test analysis.

Dilution series tests determined that 500 mg/mL was the most favorable concentration to use due to the greatest increase in heart rate (Fig. 3). Again, the most favorable concentration was determined as the concentration that caused the greatest increase in heart rate. Dilution series was performed with 10 mg/mL, 100 mg/mL, and 500 mg/mL of guarana. The 500 mg/mL concentration of guarana had the greatest stimulant effect, because it increased the heart rate the most in comparison to the other two concentrations tested.



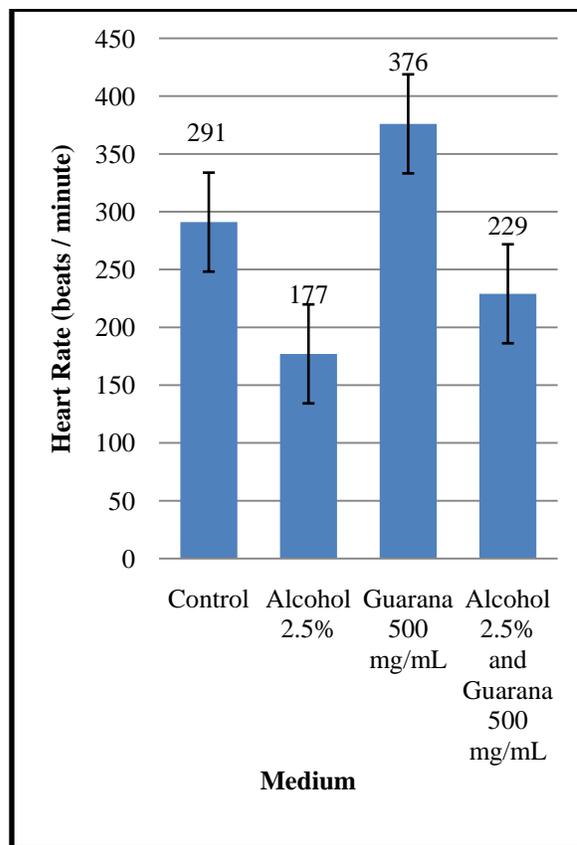
**Figure 4.** Dilution series effect of guarana from 500 mg/mL to 10 mg/mL. Guarana at a concentration of 500 mg/mL increased the heart rate the most in comparison to the heart rates affected by 100 mg/mL and 10 mg/mL. Error bars reflect SE and n=10 for each concentration.

The average heart rate for *D. magna* measured at one minute after introduction of 500 mg/mL of guarana was 376 bpm for the 50 trials (SEM  $\pm$  1.33) using 50 *D. magna*. This was significantly greater than the control baseline heart rate ( $p < .0001$ ) as calculated by student t-test statistical analysis.

#### Combination Guarana and Alcohol

*D. magna* treated with a combination of guarana and alcohol after 50 trials had an average heart rate of 229 bpm (SEM  $\pm$  0.89) (Fig. 4). Guarana and alcohol together did affect the heart rate of *D. magna*. The heart rate of *D. magna* treated with the combination lowered the heart rate when compared to the control trials. However, the combination did not lower the heart rate to the same level as induced by alcohol alone (177  $\pm$  1.15 bpm).

The combination heart rate (229  $\pm$  0.89 bpm), using student t-test statistical analysis, was found to be significantly less than control heart rate (291  $\pm$  1.40 bpm) ( $p < .0001$ ).



**Figure 5.** Average heart rates for control, 2.5% alcohol, 500 mg/mL of guarana, and a final combination of 2.5% alcohol and 500 mg/mL of guarana. The combination heart rate was significantly lower than the control heart rate using student t-test ( $p < .0001$  In control, n=100, and n=50 for alcohol, guarana, and combination. Error bars reflect SE.

## Discussion

The findings of this study are among the first on the combined reaction to guarana and alcohol within the central nervous system by analyzing *D. magna* heart rate. The ethanol demonstrated depressant effects, having decreased the average heart rate from 291 bpm (SEM  $\pm$  1.40) in the control trials to 177 bpm (SEM  $\pm$  1.15) in the ethanol trials (Fig. 5). The original 291bpm (SEM  $\pm$  1.40) from the control trials increased to 376 bpm (SEM  $\pm$  1.33) with the addition of guarana (Fig. 5). This demonstrates the stimulatory effects of the

guarana on the *D. magna*. The average heart rate in the combination trials, 229 bpm (SEM  $\pm$  0.89), was still lower than the control average of 291 bpm (SEM  $\pm$  1.40), but not as low as the ethanol average of 177 bpm (SEM  $\pm$  1.15) (Fig. 5).

The fact that the heart rate in the combination trials ( $229 \pm 0.89$  bpm) was in-between the control trials ( $291 \pm 1.40$  bpm) and the ethanol trials ( $177 \pm 1.15$  bpm), demonstrated that guarana antagonized the depressant effect of ethanol on heart rate in *D. magna*. This result is similar to a previous study done on rats on the co-ingestion of ethanol and energy drinks containing both caffeine and taurine (Ferreira et al., 2006). In this previous study the authors found that a 10.71 mL/kg dose of energy drink antagonized the depressant effects of ethanol in the locomotor activity of the rats (Ferreira et al., 2006).

A possible reasoning for the overall slight decrease in heart rate in the combination trials is that both the alcohol and guarana affect the nervous system of the *D. magna* but the methods in which they exert their influence on the nervous system are different. The alcohol tends to have an overall depressive effect on the entire nervous system (Nie et al., 2009) while guarana stimulates more specific areas of the brain such as the vagal center (Bolton and Null, 1981). The combined ingestion of guarana and ethanol must affect the nervous system differently than when ethanol is ingested alone. This could provide a possible explanation for why the alcohol seems to have a stronger affect on the *D. magna* when ingested alone versus the co-ingestion of guarana and ethanol. More testing is needed to determine the specific effects of guarana on the nervous system both with and without the presence of alcohol in order to prove this theory.

The Ferreira et al., (2006) study reported that when consuming energy drinks with alcohol, as compared to alcohol alone, the

personal perception of intoxication became impaired. When alcohol was accompanied by energy drinks the subjects felt less intoxicated than when they ingested the same amount of alcohol alone. Although the subjects felt less intoxicated, their nervous system was still impaired, as evidenced by the reduction in the subjects' objective motor coordination and visual reaction time. The results also demonstrated that the subject's blood alcohol content had not changed when the energy drink was co-ingested with alcohol as compared to alcohol alone. The results here from the combined solution trials may give a possible reason for the reduced perception. We propose that physiological effects, such as heart rate, may be part of the perception of intoxication. Reduced perception when under the effects of alcohol co-ingested with energy drinks may be caused by an increase in physiological functions. As shown in our *D. magna* study, the heart rate, a physiological function, does not decrease as much while under the effects of a mixed solution of guarana and alcohol as opposed to alcohol alone. The fact that *D. magna* respond similarly to toxins such as ethanol as do humans leads us to infer that the lack of reduced perception of intoxication accompanied by the co-ingestion of alcohol and energy drinks is derived from the slight increase in physiological functions such as heart rate. However, more research is needed on the specific effects of the co-ingestion of ethanol and guarana on the CNS and physiological functions to uncover causal links between the two and their effects on the perception of intoxication. Additionally, further research is necessary to enable individuals to make informed decisions regarding the co-ingestion of alcohol and energy drinks containing guarana based upon its neurophysiological effects.

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