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Abstract: Alcoholic beverage is any fermented liquor such as wine, beer, or distilled spirit that contains ethyl alcohol or ethanol as intoxicating agent. Daphnia (*Daphnia magna*) or water flea is a planktonic crustacean commonly found in canals and other waterways. It has been used as a standard organism for toxicity testing and toxicological reaction to environmental pollutants. The study is concerned on whether the brand and concentration of alcoholic beverages have an effect on the heart rate of Daphnia and verify if these factors can cause a significant difference. Samples of the organism were directly exposed to an aqueous environment with different concentrations of alcohol. The experiment used three different alcoholic beverages at three different concentrations in water. In the control group, no alcohol was added to the water. Results show that exposure of Daphnia to alcohol affects its heart rate. As the concentration of alcohol increases, the heart rate decreases. This observation is true for all the alcoholic beverages used in the experiment. **Keywords:**Toxicity, daphnia, alcoholic beverages, heart rate

1. Introduction

Alcoholism is a problem in our society and the age group drinking alcohol is becoming younger and younger. Alcohol can influence an individual's heart rate. Consumption of alcohol can become a cause of fatal and non-fatal heart attacks. A less serious condition referred to as "holiday heart syndrome" – an irregular heartbeat, is caused by too much alcohol in the body.

This study focuses on alcoholic beverage that mayaffect the heart-rate. The researchers chose this study because alcohol consumption is rampant all over the world and information on what brand and type of

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alcohol can change heart-rate the most proves beneficial to the consumers. The study will likewise benefit those who are interested in pursuing a similar study, giving them an idea of how alcohol can influence an organism's heart rate.

At different levels of intoxication, alcohol has different effects. Many drink alcohol to enjoy the relaxed euphoric sensation of being intoxicated. However, at higher levels of intoxication the stimulating effects of alcohol begin to fade as it becomes an anesthetic that acts as a depressant on the central nervous system. Alcohol can decrease heart rate and lower blood pressure and respiration rate to dangerous levels. When these effects begin, the drinker begins to feel tired. If enough alcohol is consumed, the drinker will fall asleep, or in extreme cases, may lapse into a coma. (Jackie Chew, <u>2001</u>).

Beer was selected as the test sample alcoholic beverage because it is proven to be the number one consumed alcoholic beverage in the world dominating 36% of all alcohol consumption yearly world wide.

The genome of Daphnia shows the strongest homology with the human genome. This characteristic of Daphnia makes it an ideal organism for biomedical research (N.S.Gaikwad, A.V. Panat and P. Augustine, 2012). This study aims to determine the effect of selected popular alcoholic beverages on the heart rate of Daphnia. Specifically, it seeks to answer the following questions: (a) what is the average heart rate of Daphnia exposed to a solution containing different concentrations (25%,50% & 75%) of alcoholic beverages (Brand A,B,C); (b) is there a significant difference in the heart rate of Daphnia exposed to the different concentrations of the alcoholic beverage in the solution? (c) Is there a significant difference in the average heart rate of the treated and the control samples of Daphnia?



Anatomical Parts of Daphnia



2. Methodology

2.1 Collection of Samples and Laboratory Preparations

The Daphnia samples were collected from drainage canals of residential houses in Tambacan, Iligan City. The samples collected were placed in plastic containers containing water. Because of their sensitivity to change in environment, the samples were collected 5 hours before the experimentation.

In the preparation of the aqueous solutions containing alcohol, three alcoholic beverages were used. Beverage A and B contains 5% alcohol and beverage C contains 6.8% alcohol. For each alcoholic beverages, three solutions with 25%, 50% and75% concentration of alcoholic beverage were prepared. The percentage concentration of alcoholic beverage is computed as 100 times the volume of the alcoholic beverage divided by the volume of the solution. The different solutions were placed in beakers and tests on the samples were done under normal laboratory conditions.

2.2. Determination of Heart Rate

From the plastic containing containing them, the untreated Daphnia samples were transferred to beakers containing solutions with different concentrations of alcoholic beverages.

The Daphnia samples were exposed to the solutions for one minute before being removed from the exposure using medicine dropperand examined for their heart rate under a microscope. With the aid of a video camera, the heart rate of the Daphnia were then obtained.

2.3 Statistical tools used for data analysis

The average heart rate of Daphnia samples exposed to a certain concentration of alcoholic beverage is obtained by dividing the sum of the data with the number of samples. For each concentration of alcoholic beverages, three trials were conducted. For each trial, 10 samples of Daphnia were observed, for a total of 30 Daphnia samples exposed to a certain concentration of alcoholic beverage.

Statistical software "Megastat" was used for the computation of the average heart rate and analysis of variance (ANOVA) to determine significant difference in the average heart rates of Daphnia samples exposed to different concentrations of alcoholic beverages.

3. Results and Discussion

Table 1 shows the average heart rate of Daphnia samples exposed to different solutions containing alcoholic beverages. It is observed that as the concentration of the alcoholic beverage in the solution is increased, the average heart rate of Daphnia samples decreases. The observation is true for the three alcoholic beverages used, although the decrease in heart rate caused by the concentration of each alcoholic beverages differs.

Alcoholic Beverage	Concentration of Alcoholic Beverages						
	25%	50%	75%				
А	146.7 bpm	137.3 bpm	138.7 bpm				
В	144 bpm	142.7 bpm	138.7 bpm				
С	152 bpm	134.7 bpm	130.7 bpm				
Note : Average heart rate of control Daphnia samples = 178							

Table 1. Average Heart Rate of Daphnia Samples Exposed to Aqueous Solutions Containing Alcoholic Beverages

Table 2 shows that there was no interaction between the brand and the concentration but there was significant difference between the treated and untreated samples regardless of the brands. This means that the brand does not play a significant role in affecting heart rate although concentration does. This can indicate that the more you drink the greater the effects. This means that an alcoholic beverage does have the ability to affect heart rate.

219

ANOVA Table								
Source	SS	df	MS	F	p-value			
Factor 1 (Brand)	24.89	2	12.444	0.29	0.7535			
Factor 2								
(concentration)	10,122.11	3	3,374.037	77.66	1.70E-12			
Interaction	302.22	6	50.370	1.16	0.3600			
Error	1,042.67	24	43.444					
Total	11,491.89	35						

 Table 2. Significance between the Treated and Controlled Samples

Table 3 shows that each of the concentrations 25%, 50%, 75% had a significant difference when compared to the controlled samples and also between 25% and 75% and between 25% and 50% has a significant difference between each other since their p-value are less than alpha while between 50% and 75% it has no significant difference since its p-value is greater than alpha. Meaning, the higher the concentration regardless of the brand, the greater the decrease of the heart rate. The heart rates of the Daphnia are affected by the concentrations of each of the brand.

Table 3. Post hoc AnalysisBetween the Concentrations

Post hoc analysis							
p-values for pairwise t-tests for Factor 2							
		75%	50%	25%	Controlled		
		136.0	138.2	147.6	178		
75%	136.0						
50%	138.2	0.4814					
25%	147.6	0.0011	0.0061				
Controlled	178.0	1.02E-12	3.24E-12	7.31E-10			

 Comparing the means of the heart rate at 25% and 50% concentrations, the p-value is 0.0061, which is less than the alpha of 0.05. Hence, the null hypothesis is rejected and therefore, there is a significant difference in the heart rate of Daphnia exposed to the two concentrations.

- 2. Comparing the means of the heart rate at 50% and 75% concentrations, the p-value is 0.4814, which is greater than alpha of 0.05. Hence, the null hypothesis is accepted and, therefore, there is no significant difference in the heart rate of Daphnia exposed to the two concentrations.
- 3. Comparing the means of the heart rate at 25% and 75% concentrations, the p-value is 0.0011 which is less than the alpha of 0.05. Hence, the null hypothesis is rejected and, therefore, there is a significant difference in the heart rate of Daphnia exposed to the two concentrations.

4. Conclusions

Based on the foregoing results and discussion, the following conclusions are made;

- 1. Alcoholic beverages have decreasing effect on the heart rates of Daphnia. As the concentration of alcoholic beverages increases, the heart rate of Daphnia decreases.
- 2. The results of the 2 way ANOVA, showed no interaction between the brand (factor 1) and the concentration (factor 2). It showed further that there was no significant difference among the three brands of alcoholic beverages, however, significant difference existed among the different concentrations.
- 3. Post hoc analysis showed significant difference in the following concentrations: 25% and 75% and between 25% and 50%.
- 4. Finally, there was a significant difference also between the treated and control samples.

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