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Article

How planarians are affected by mouthwash and cough syrup

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SUMMARY

Cough syrup and mouthwash are commonly used items and often end up flushed down the drain or toilet. These substances can become dangerously addictive to humans over time and eventually get into freshwater waterways which can be harmful to many marine organisms, such as planarians (aquatic flatworms). To investigate the affects of these substances on planarians, we tested six different concentrations of Listerine mouthwash and its active ingredients and ten different concentrations of Robitussin cough syrup and its active ingredients. We used a behavioral assay to test the effects of mouthwash and cough syrup or their individual active ingredients on planarian behavior. Active ingredients of cough syrup but not mouthwash detrimentally affected planarian behavior. In light of our results, we provide recommendations for the disposal of cough syrup to lessen the environmental effect it can have on aquatic life.

INTRODUCTION

When most people think of personal care products (PCPs), cosmetics and personal hygiene come to mind. Some of the leading PCPs found in most households include mouthwash and cough syrup. Mouthwash is a product that people use in everyday hygiene to sip, gargle, and spit down the drain. Cough syrup can be extremely beneficial when experiencing respiratory agitation. Unfortunately, in addition to being helpful for humans, cough syrup and mouthwash can become dangerously addictive and both PCPs are also known as non-beverage alcohol substances often abused by humans.

Not only do mouthwash and cough syrup affect people who abuse it, but they can contaminate water sources, such as streams and rivers. Both cough syrup and mouthwash can negatively impact water quality and affect the behavior of aquatic organisms after they make their way from the drain to streams or rivers. For example, anything that contains over 24% ethanol is hazardous and should not be disposed of down the drain (1). As discussed by Stevenson and Beane, concentrations above 3% ethanol can inhibit gross and fine motor skills and be lethal to planarians (2). Mouthwash, for instance, includes 26.9% denatured alcohol (1). Unfortunately, many U.S. city sewer systems are unable to afford to filter out PCP contaminants, such as mouthwash (3). As a result, these PCPs are in aquatic environments at concentrations capable of causing detrimental effects to aquatic organisms (4). It is imperative that the effects of these substances on marine organisms are studied further. For our experiments, we studied two products, mouthwash and cough syrup, and their active ingredients to determine how they affected the behavior of planarians.

Planarians are flatworms found in freshwater and typically live under rocks and debris (5). These photophobic organisms prefer dark environments and will move to cover when exposed to light (6). Furthermore, almost any freshwater fish will eat planarians, making these organisms an important food source (7). The study of planarians has led to a better understanding of the evolution of the human brain (8). Research has also shown that these organisms display responses that are similar to the human nervous system when exposed to chemical substances, such as nicotine and sucrose (9-10). We selected planaria for this study because they are established as an animal model for the study of toxicology (11). For example, ethanol, an active ingredient in mouthwash, has been shown to slow regeneration and cause death in planarians (11-12). Specifically, we explored how the active ingredients in cough syrup and mouthwash may change planarian behavior and the implications these chemicals have on their aquatic environment.

The goal of our investigation was to raise awareness about the harmful effects of some PCPs on planarian life. Particularly, we provide a rationale for humans to change PCP disposal methods. These substances are disposed of in sinks and toilets. Our recommendation is a logical course of action that lessens the effect of these PCPs on planarians, which could also impact other forms of aquatic life. We investigated the effects on photophobicity by hypothesizing that exposure to cough syrup, mouthwash, or their active ingredients causes behavioral changes in planarians. We used a conditioned place preference assay to test the effects of cough syrup, mouthwash, and their active ingredients on planarian behavior. Our major findings indicated there was a statistically significant difference using the conditioned place preference protocol under conditions of 0.5 mM guaifenesin, 0.675 mM dextromethorphan (DXM), and 0.3375 mM DXM compared to the control of spring water. The results indicate that exposure to specific concentrations of the active ingredients of cough syrup affects the behavior of planarians.

We recommend any unused or expired cough syrup should be disposed of in medication drop boxes at local pharmacies.

RESULTS

We tested mouthwash, cough syrup and their active ingredients on the behavior of *Dugesia dorotocephala* planarians. To determine if these substances have any negative effects to these organisms. We hypothesized that exposure to cough syrup, mouthwash or their active ingredients would cause behavioral changes in planarians. The active ingredients of cough syrup are guaifenesin, DXM and phenylephrine. The active ingredients of mouthwash are eucalyptol, thymol, and ethanol. We conducted the experiment to collect data using conditioned place preference protocol on planarians to determine if Robitussin cough syrup, Listerine mouthwash or their active ingredients would cause harmful behaviors compared to the spring water control.

Planarians typically prefer darkness and will normally spend a greater amount of time on the dark side of a half-covered dish (split petri dish) when given the choice. Conditioned place preference starts by setting a baseline for planarian behavior with a pretest. A planarian is placed in the middle of a split petri dish and timed while given free access to roam. After being exposed to a new substance such as Listerine mouthwash or Robitussin cough syrup and conditioned through several rounds of light or dark exposure, the planarian will be timed in a split petri dish again. If their exposure causes a change in their behavior, then they will spend more time in the light than in the dark during this posttest.

We used conditioned place preference protocol with spring water only as a control for these investigations. We hypothesized that there would be a statistically significant difference in the amount of time the planarians spend in the light between the organisms that are and are not exposed to Robitussin cough syrup, Listerine mouthwash, or their active ingredients.

Planarians spent slightly more time in the light compared to the spring water control and conditioned place preference protocol for the cough syrup and its active ingredients which included 5% cough syrup, 2.5% cough syrup, 2% cough syrup, 1% cough syrup, 0.25 mM guaifenesin and 0.1688 mM DXM. The planarians spent slightly less time in the light compared to spring water control and conditioned place preference protocol for the active ingredient in cough syrup of 0.06 mM phenylephrine. Therefore, we found no significant differences in planarian behavior between the spring water control and the conditioned place preference protocol for cough syrup under the conditions of 5% cough syrup, 2.5% cough syrup, 2% cough syrup, 1% cough syrup, 0.25 mM guaifenesin, 0.1688 mM DXM, and 0.06 mM phenylephrine.

Additionally, planarians spent slightly more time in the light compared to the spring water control and conditioned place preference protocol for the mouthwash and its active ingredients which included 0.001% eucalyptol, 0.1% ethanol, 2.5% ethanol and 1.5% Listerine. The planarians spent slightly less time in the light compared to spring water control and conditioned place preference protocol for the active ingredient in mouthwash 1% ethanol and 0.625% thymol. Thus, there was no significant difference for 0.001% eucalyptol, 0.1% ethanol, 2.5% ethanol and 1.5% Listerine, 1% ethanol and 0.625% thymol. However, there were statistically significant differences using the conditioned place preference protocol under conditions of 0.5 mM guaifenesin (p=0.0041), 0.675 mM DXM (p=0.0066), and 0.3375 mM DXM (p=0.0301) compared to the control of spring water.

We collated the data for both Robitussin cough syrup in **Table 1** and Listerine mouthwash in **Table 2**.

Substances	N	Mean time in light (seconds in light) (Pre)	SD(Pre) (seconds in light)	Mean(Post) (seconds in light)	SD(Post) (seconds in light)	*t* value	df	*p* value (significance)
spring water	10	58.40	42.66	77	37.33	1.3875	18	0.1987
5% cough syrup	10	47.10	54.16	93.20	128.36	1.1492	9	0.2801
2.5% cough Syrup	10	31.80	42.69	40.10	34.77	.6615	9	0.5249
2% cough Syrup	12	23	22.45	67.67	110.78	1.5758	11	0.1434
1% cough syrup	10	31.80	29.70	40.10	34.77	.7455	9	0.475
0.5 mM guaifenesin	10	29.90	28.36	86	55.62	3.8257	9	0.0041
0.25 mM guaifenesin	10	23.90	14.87	40.1	39.38	1.3423	9	0.2124
0.675 mM DXM	10	85.40	95.78	222.50	125.05	3.551	9	0.0066
0.3375 mM DXM	10	34.4	25.79	108.1	97.42	2.5722	9	0.0301
0.1688 mM DXM	10	48	25.06	91.50	84.24	1.4038	9	0.1939
0.06 mM phenylephrine	10	75.9	79.04	44.9	70.61	2.1417	9	0.0609

Table 1: Conditioned place preferencece of Robitussin and Robitussin's active ingredients. $p<0.05$= statistically significant. N = number of worms. The pretest (pre) is when the planarian is given free access to roam both sides of the chamber of spring water (a split petri dish=one side covered and the other side uncovered). The posttest (post) is essentially the pre-test repeated after conditioning, the planarian will now tend to spend a greater amount of time in the original least-preferred side of the split petri dish.

Substances	N	Mean(Pre) (seconds in light	SD(Pre) (seconds in light)	Mean(Post) (seconds in light)	SD(Post) (seconds in light)	*t* value	df	*p* value
spring water	10	36.4	52.85	67.5	34.81	2.0049	9	0.076
eucalyptol 0.001%	8	43.5	43.81	86	90.72	1.1125	7	0.3027
thymol 0.0625%	9	54.11	48.6	40.11	54.42	0.6678	8	0.523
ethanol 0.1%	9	46.33	43.6	62.78	53.54	1.0671	8	0.3171
ethanol 1%	9	61.44	59.07	26.11	30.94	2.1359	8	0.0652
ethanol 2.5%	9	16.44	17.95	66.22	66.19	2.2565	8	0.054
Listerine 1.5%	8	32.75	45.19	126.38	114.22	2.2871	7	0.056

Table 2: Conditioned place preference of Listerine and Listerine's active ingredients. $p<0.05$= statistically significant. N = number of worms. The pretest (pre) is when the planarian is given free access to roam both sides of the chamber of spring water (a split petri dish=one side covered and the other side uncovered). The posttest (post) is essentially the pre-test repeated after conditioning, the planarian will now tend to spend a greater amount of time in the original least-preferred side of the split petri dish.

DISCUSSION

Our results indicate, via the conditioned place preference assay, that exposure to specific concentrations of the active ingredients of cough syrup affected the behavior of planarians. Specific active ingredients in PCPs like cough syrup, also known as non-beverage alcohols, can change a planarian from an organism that prefers an environment of darkness to an organism that prefers a light environment which may make this organism more vulnerable to predators. Our major findings indicate there was a statistically significant difference using the conditioned place preference protocol under conditions of 0.5 mM guaifenesin (p=0.0041), 0.675 mM DXM (p=0.0066), and 0.3375 mM DXM (p=0.0301) compared to the control of spring water. The results indicate, via the conditioned place preference assay, that exposure to specific concentrations of the active ingredients of cough syrup affects the behavior of planarians. Cough syrup is disposed of in toilets and sinks which makes its way into water drain systems. This can pose a threat to planarians since they reside in a variety of water systems, including sewer systems, which receive water and sewage from water drains (14).

The Environmental Protection Agency regulates wastewater management systems for pathogens, such as Escherichia coli but not all compounds that may be hazardous to aquatic life. Therefore, many chemicals found in waterways are unregulated. Additionally, when river or stream levels get low and the weather is warm, it is a challenge to remove contaminants that negatively affect biological life. However, some areas, such as the Chesapeake Bay, plan to have controls in place by 2025 so that water from municipal sewer systems no longer gets piped directly into rivers after a major storm, which may affect marine organisms, including planarians. Some argue that humans should make smarter choices about PCP disposal to lessen the negative impact on aquatic organisms (15).

We believe our findings will be crucial in raising awareness about the results of this investigation so that people can avoid contributing to the problem. There was a study that examined the function of the CYP2D6 enzyme to assess the body's ability to eliminate drugs through metabolism based on several physiological variables including sex hormone levels. Researchers found the average ratio of DXM to dextrorphan, a metabolite of DXM, in urine samples of 56 women and 86 men to be 0.008 and 0.02, respectively. These findings suggest these drugs are polluting the waterways because they cannot be completely metabolized by the body (16). Thus, this can have adverse effects on marine life. As previously discussed, studies have also examined the negative effects several chemicals in waterways have on fish, but our unique study focuses specifically on the effects of mouthwash and cough syrup on planarians (3-15).

To further decrease negative change in behavior, cough syrup should be placed in biohazard containers, which can lessen contamination of the environment (17). Environmental agencies focused on water quality would determine our findings important for regulations to prevent aquatic organisms from being at risk. Based on our findings, we recommend any unused or expired cough syrup should be disposed of in medication drop boxes that many pharmacies provide. It is suggested that products made of all-natural ingredients be substituted for substances deemed dangerous to aquatic life, and people should not use nonessential substances daily (18). We also recommend not to rinse PCPs down the drain to prevent the toxic or sometimes deadly biological effects of these substances (3-18).

This study was conducted using *D. dorotocephala* planaria. In future studies we could test the effects of mouthwash and cough syrup on other species of planaria and other marine life. Furthermore, we could also conduct the conditioned place preference protocol and make observations using other brands of these substances on other species of marine organisms.

MATERIALS AND METHODS

The materials needed to make this experiment successful include planarians (*Dugesia Dorotocephala*), Petri dishes, pipettes, and scoopulas, all of which were purchased from Carolina Biological. Grid paper, black construction paper, timers, and spring water were purchased from Walmart. The Robitussin cough syrup and Listerine mouthwash were purchased from CVS drug store and made into varying concentrations. The chosen concentrations were those in which the planarians could survive for 30 minutes. The

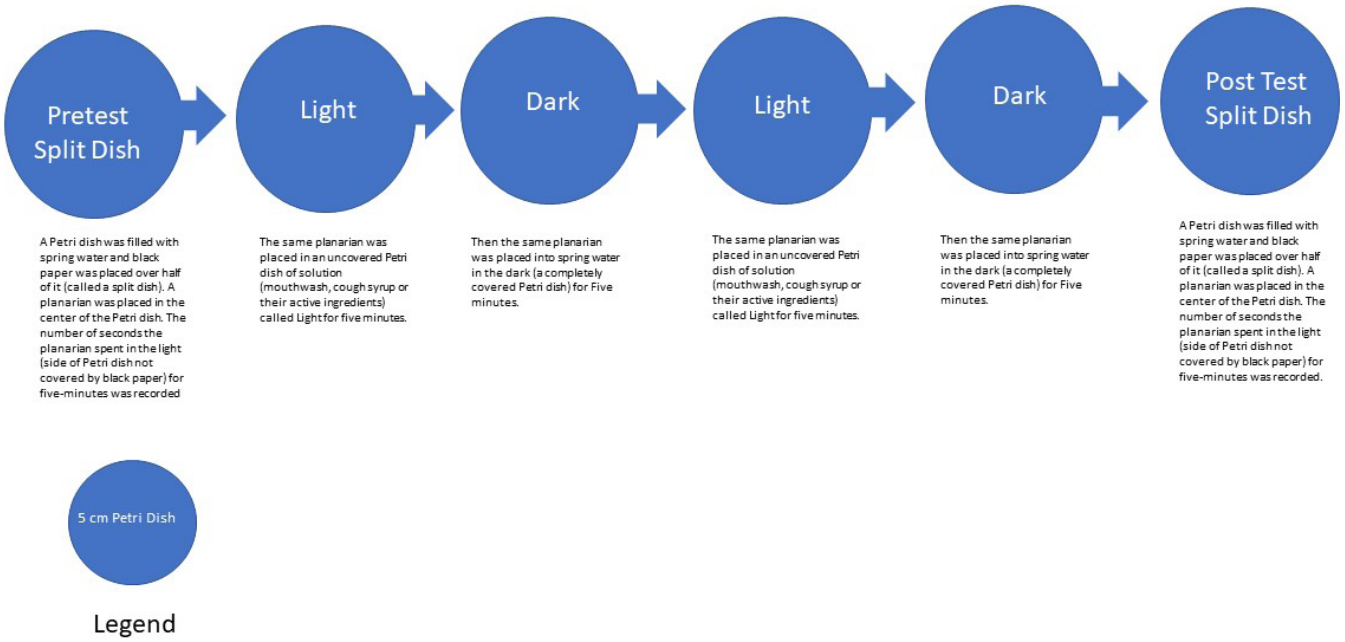

Figure 1: Conditioned place preference protocol.

rationale for how concentrations were determined is very logical. We diluted cough syrup, mouthwash and the active ingredients in each to find concentrations that were not lethal to the planarians, used that concentration as a ceiling concentration, and then diluted to lower concentrations for testing. We chose to test the survival of a planarian for 30 minutes for 50% dilution of the Listerine and Robitussin. If a planarian did not survive at a concentration for the designated time interval, then we diluted the concentration by 50 % again. This process of dilution continued until the planarians survived for the designated time in the following concentrations: 5% cough syrup, 2.5 % cough syrup, 2% cough syrup, 1% cough syrup, and 1.5 % Listerine (higher and lower concentrations of Listerine were tested, but not found effective). Remaining solutions of guaifenesin, DXM, phenylephrine, thymol, and ethanol were purchased from Sigma Aldrich and made into varying concentrations: 0.5 mM guaifenesin, 0.25 mM guaifenesin, 0.675 mM guaifenesin, 0.675 mM DXM, 0.3375 mM DXM, 0.1688 mM DXM, 0.06 mM phenylephrine, 0.001% eucalyptol, 0.0625% thymol, 0.1% ethanol, and 2.5 % ethanol.

Before conducting the investigation with mouthwash and cough syrup, we completed the assay for conditioned place preference from SEADAP Lesson Outline (19). As noted on the labels of the mouthwash and cough syrup, only active ingredients were included in the protocol and not inactive ingredients, such as sucrose. All active ingredients were dissolved in spring water except for eucalyptol, which was soluble in ethanol.

To begin, 10 mL solutions of mouthwash, cough syrup, or their active ingredients were poured into 5-cm Petri dishes. A second Petri dish was filled with spring water and black paper was placed on half of it (called a split dish). A planarian was then placed into the center of the split dish. The number of seconds the planarian spent in the light (side of Petri dish not covered by black paper) for five-minutes was recorded. The same planarian was put into the solution in light (uncovered Petri dish) for five minutes and next this planarian was placed into spring water and the Petri dish was completely covered for five minutes. These two previous steps were repeated. Finally, the planarian was placed in the split dish for five-minutes and the seconds the planarian spent in the light were recorded. The procedure is illustrated in **Figure 1**.

We used a paired *t*-test. The *p* value, the degrees of freedom (*df*), and standard deviation (*SD*) were calculated using Microsoft Excel. The *t*-test analysis was used to interpret the results. The test was conducted for each condition comparing post-test versus pre-test. We initially used Microsoft Excel to calculate the mean of the pre-test data and the mean of post-test data for conditioned place preference trials. The calculated results were also confirmed by entering data in GraphPad using Quickcalcs. Our null hypothesis was that there is no statistically significant difference between the amount of time the planarian spent in the light before the conditioned place preference pre-conditioning (pre-test) compared to post-conditioning (post-test). An independent sample *t*-test was used for comparing group difference in the change (post test score – pretest score). The change between pre and post score was the dependent variable.

The null hypothesis for two conditions where the planarians were placed in the non-spring water solutions (i.e. 5% cough syrup, 2.5 % cough syrup, 2% cough syrup, 1% cough syrup, 0.5 mM guaifenesin, 0.25 guaifenesin, 0.675 mM guaifenesin, 0.675 mM DXM, 0.3375 mM DXM, 0.1688 mM DXM, 0.06 mM phenylephrine, 0.001% eucalyptol, 0.0625% thymol, 0.1%

ethanol, 2.5 % ethanol, 1.5 % Listerine) or what is called a treatment should have the same mean as the planarians in the spring water. We collated the data for both Robitussin cough syrup in **Table 1** and Listerine mouthwash in **Table 2**.

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