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Applications of Slime Mold: The Use of Physarum polycephalum as an Alternative Clinical Trial Subject for Over-the-counter Drugs

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ABSTRACT

Globally, approximately 110 million lab animals die every year in clinical testing or from extermination post-experimentation (Diaz et al.). This issue has been debated and discussed in many areas of the medical and veterinary world. Along with this, prices to maintain live testing animals present financial burdens in research. An idea for a solution was the use of a unicellular organism known as slime mold between the in vitro and in vivo testing stages. While the name is misleading, slime mold is not a fungus. It is a form of protist. This protist has been documented in prior research navigating mazes, redesigning roads, along with learning and retaining information (Walecki). Based on these mind-boggling findings, an experiment was conducted to use the slime mold *Physarum polycephalum* as an addition to the clinical trial process. Four different solutions of water with dissolved over-the-counter drugs (Acetaminophen, Diphenhydramine, Dextromethorphan, and Caffeine) were tested on different samples of slime mold via their sustenance (oats), along with a positive control of water. The results collected over one week with a time-lapse camera indicated that the slime mold had different reactions to each of the four drugs used. It was proven that slime mold reacted as follows: Acetaminophen- attracted to the treatment and lingered on it; Diphenhydramine- attracted to the treatment and lingered; Dextromethorphan- attracted to the treatment and lingered; Caffeine the negative control- responded negatively as intended, eventually repelling the treatment; and water the positive control- responded positively as intended. In addition, the slime mold samples expressed different growth patterns seemingly out of randomness. These preliminary findings confirm that slime mold does react to over-the-counter drugs, as proven by observing the direct reaction to a substance over time. Therefore, with further research, slime mold could be a viable option for clinical trials, reducing subsequent animal deaths and research costs.

INTRODUCTION

In vitro – Latin for in-glass– clinical trials are the first step when formulating a new drug. In this step, the drug is administered to a culture of human cells or another microorganism. The reaction is studied to provide researchers with knowledge of the direct effect of the drug on living organisms. Next is the in vivo trial, where more complex organisms, like animals, are given drugs. There are many negative

outcomes in these trials. Many animals die and suffer while the drugs are being critiqued, and eventually, all tested subjects are euthanized. In these trials, plenty of resources and money are spent on the upkeep of all the animals as well. While this is the harsh reality for every drug seen on the market, there is a possible solution to decrease the suffering and animal deaths from these tests. *Physarum polycephalum*, or slime mold, is a unicellular organism that grows expansively with one membrane and thousands of nuclei. This extraordinary organism navigates environments with no central brain or basic sensory organs, yet it always moves in the most efficient paths and even holds memories. Slime mold once rebuilt the complex Tokyo subway map by simply searching for food. Much research has been conducted at Tufts University, determining that slime mold can sense objects from a distance and makes the choice to follow/ignore them (Walecki).

With efficient research, this intelligent microorganism can reshape the way drug clinical trials function. The slime mold *Dictyostelium* was used in a drug study led by Dr. Robin Williams of the UCL Department of Biology on depression drugs. Dr. Williams created a system to test these drugs on slime mold, as it had properties similar to those of depressed human brain cells. By documenting chemical changes in the slime mold, Dr. Williams determined that the slime mold was a reliable guide for depression drug testing (UCL). Using *Physarum*, a new system to test over-the-counter drugs more effectively between in vitro and in vivo trials can be pioneered. Administering commercially available drugs, with known effects, on slime mold and recording the physical reaction is the first step to improving clinical trials by reducing the suffering of test animals and the cost of clinical trials.

It was hypothesized alternatively that there would be a substantial and notable reaction to at least one of the treatments that indicated repulsion, similar to the negative control, caffeine. The null hypothesis stated that there would be no reaction at all to any of the treatments. Slime mold has been shown to react to psychoactive drugs and has documentation demonstrating behavior associated with organisms of higher intelligence. Therefore, a system using slime mold as a buffer between the in vitro and in vivo stages is perfectly feasible. The results of the experiment will advance our knowledge of this unique slime mold and provide a foundation for the advancement of clinical trials, preventing substantial lab animal suffering and reducing clinical trial costs.

METHODS AND MATERIALS

This research experiment involved using the following: commercially available slime mold cultures of *Physarum,* four over-the-counter drugs (caffeine, acetaminophen, diphenhydramine, and dextromethorphan), sterile agar petri dishes, oats, a front-opening terrarium, a heating pad, a hygrometer, a black trash bag, a thermometer, a micropipette, distilled water, beakers, forceps, mortars & pestles, and petri dish grid stickers. All slime mold cultures were grown in petri dishes with sterile agar and oats for 24 hours to reach their plasmodium states before the addition of treatments. To effectively document the movement and growth of the slime mold, a time-lapse camera was fixed to capture numerous photos over a week (Brownell). A cell phone camera was also used to capture the slime mold twice a day. A

microscope was used to view the microscopic changes of the slime mold cells. Throughout the study, standard safety equipment was used to prevent contamination or safety risks.

This experiment was divided into two parts. First, five slime mold cultures were grown from the dormant sclerotial stage to the active plasmodium stage. To begin the growth process, five sterile agar petri dishes were prepared (one for each treatment) for the introduction of slime mold by applying grid stickers. Next, the terrarium was sanitized and prepped for the slime mold with alcohol. Once clean, the heating pad and pan of distilled water were added to maintain consistent humidity and temperature through all trials, along with setting the camera up. The slime mold was added to the far top square of each petri dish and then placed inside the terrarium. One dish for each testing drug and two for the controls. These cultures were fed oats that were placed to create a desired path vertically along the dish. The cultures were left in the "incubator" for a day to establish and grow out.

In the second part, the treatments were curated and measured as follows: the single dosage based on box label recommendations was measured precisely, then each of the four drugs was dissolved into 240ml of warm(32°Celsius) distilled water, to ensure that drug dosages were controlled for each trial. Caffeine, diphenhydramine, and acetaminophen were all in tablet form and required a mortar and pestle to be pulverized before the addition to water to allow for a more even distribution. This process was not used for Dextromethorphan (DXM) because the drug was in liquid capsule form. Once all 4 solutions were mixed and cooled with a brief ice bath, the micropipette was used to extract 500 microliters of each solution and apply it to 3 oats, respectively, for all four treatments. The oats were soaked for 1 minute before being added to the respective slime mold subjects. Each of the five slime mold cultures was labeled with a respective drug, including the positive control (water) and negative control (caffeine). Positive control, meaning a control used as a comparison for attraction reactions, and negative control, meaning a control used as a comparison for repulsion reactions. Next, the properly treated oats were added to each dish, using sterile forceps. The cultures were left to interact with the oat/drug mixture by undergoing phagocytosis, when a protist feeds by engulfing its food with pseudopodia. The phagocytosis occurred over the remainder of the week (3 days). Over the course of digestion, the entire process was recorded for analysis. Every movement and action of the slime molds was captured by the time-lapse camera. After analysis, the reaction from each treatment was recorded based on attraction (+) or repulsion (-), which was determined by daily visual observations and movement towards or away from the treated oats (Adamatzky). The results were recorded in a table. On the final day of experimentation, all samples were removed from the incubator and autoclaved under standard conditions. The experiment was repeated twice following the same methods under similar conditions to increase the validity of the experiment.

RESULTS

Slime Mold Reactions to Each Drug Treatment (Attraction [+] or Repulsion [-])

Treatment Type	Trial 1	Trial 2	Trial 3
Water (control)	+	+	+
Caffeine (control)	-	-	-
Acetaminophen	+	+	+
Diphenhydramine	+	+	+
Dextromethorphan	+	+	+

Figure 1: Table recording the reactions to each treatment over multiple trials

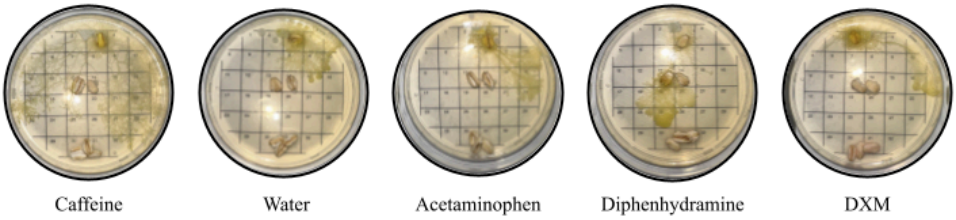

Figure 2A: Slime mold subjects one day into the treatments (Berman)

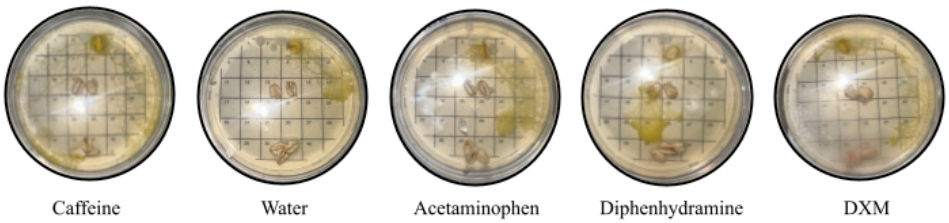

Figure 2B: Slime mold subjects two days into the treatments (Berman)

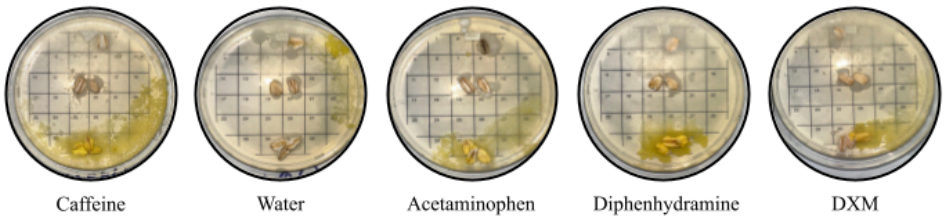

Figure 2C: Slime mold subjects three days into the treatments (Berman)

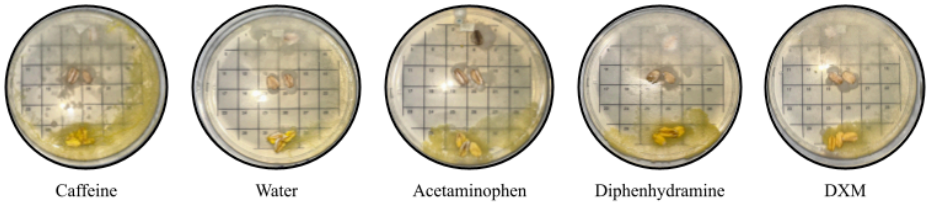

Figure 2D: Slime mold subjects four days into the treatments (Berman)

As seen above, the slime mold moves noticeably over the days in each picture, allowing proper recordings from the cameras. When slime mold was exposed to each drug, only caffeine caused a repulsion reaction from the slime mold, which was expected from the negative control. Meanwhile, every other treatment attracted the slime mold.

DISCUSSION AND CONCLUSION

The results of the experiment refuted the alternative hypothesis because none of the non-control treatments caused a repulsion reaction from the slime mold. As seen in the images above (Fig.2A-D), the three experimental drugs on the right caused similar reactions and patterns to the slime mold. These reactions aligned closely with each other, while also differing from the positive and negative controls, a result that was not expected. Further testing is needed to determine if these slightly differing reactions were due to the drugs themselves or the fillers within the drugs, like simple sugars. The initial reasoning for using caffeine as the negative control was due to former research performed by others that proved slime mold avoided caffeine and distanced itself from it, reflecting a negative reaction (Jaiswal et al.). In general, these results prove that slime mold does react differently to different drugs, as demonstrated by recordings of slime mold moving and actively responding to stimuli over a short period of time with minimal materials.

Over the course of this project, many things have been improved and altered with each trial, along with some unexpected weather changes. During the first trial, substantial bacteria/mold growth was noticed in the petri dishes after about 3 days into the experiment, as seen in Figure 2. This was likely due to the lack of proper sterility available in the high school lab. Therefore, in the following trials, everything was cleaned to the highest standard possible. Everything was cleaned with alcohol, for example, the forceps and beakers, and the oats used were microwaved to eliminate any bacteria carried into the petri dishes. It was also noted to keep the lid of the dishes open for as minimal time as possible to reduce any airborne contamination. When it comes to weather, the York region of South Carolina experienced a variety of conditions over the span of this experiment. The first trial was the most constant, until the last two days, when temperatures went below freezing outside, likely causing temperature dips indoors where the setup was. The second trial was more constant than the first, as the school interior was already acclimated to withstand the cold temperatures outside. Finally, the third trial occurred after a winter storm with the

coldest temperatures outside, which in turn caused warmer indoor temperatures. The third trial also experienced the most variation because live cultures were used for each of the treatment dishes due to a shortage of dry specimens from the supplier, along with the dramatic weather changes.

A possible restriction with this experiment could have been the quality of lab supplies, as all trials took place in a high school science lab with basic materials available. For example, an incubator was made with a heating pad, pan of water, and terrarium. This may have caused inconsistent temperatures, unlike an official incubator. If better quality materials and facilities were available, the outcomes may have been different. Another possible source of error may have been with the drugs themselves. Using over-the-counter drugs consisting of a majority of fillers (sugars, capsules, etc.) may have effects on the slime mold that counteract or enhance their reactions. The presence of fillers should be studied in continuation to determine if it may alter the data. In addition, pigments added to the drugs may have an effect on the slime mold to some extent.

From this research, slime mold is proven to show reactions to over-the-counter drugs. These preliminary findings show that more research is required to prove slime mold viability in clinical trial testing. Scientists can use this preliminary research to investigate other slime mold reactions, such as toxicity and pharmacological relevance, and how they relate to live animals and other in vitro tests. The findings of this experiment can provide a foundation for the improvement of clinical testing models in the future. The results gathered from this study show that a system of clinical trials using slime mold as a buffer between in vivo and in vitro studies can be effective. Further investigation and trials are recommended to validate our findings, likely resulting in the development of a new system that spares the lives of testing animals and saves money. In today's world, the ethics of live animal testing are debated and seen as immoral by many. This research can help remedy this problem and can challenge the traditional ways of current scientific processes, finding a more effective solution to prevent unnatural animal deaths and reduce the costs of drug research.

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