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The Effect of G. Lucidum on the Lifespan of Caenorhabditis Elegans modeling Duchenne Muscular Dystrophy

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ABSTRACT

Duchenne muscular dystrophy (DMD) is an X chromosome-linked disease characterized by progressive physical disability, immobility, and premature death in affected boys. Underlying the devastating symptoms of DMD is the loss of dystrophin, a structural protein that connects the extracellular matrix to the cell cytoskeleton and provides protection against contraction-induced damage in muscle cells, leading to chronic peripheral inflammation. However, dystrophin is expressed in neurons within specific brain regions, including the hippocampus, a structure associated with learning and memory formation. Linked to this, a subset of boys with DMD exhibit progressing cognitive dysfunction, with deficits in verbal, short-term, and working memory. Furthermore, in the genetically comparable dystrophin-deficient mouse model of DMD, some, but not all, types of learning and memory are deficient and specific deficits in synaptogenesis and channel clustering at synapses has been noted. Little consideration has been given to the cognitive deficits associated with DMD compared with the research conducted into the peripheral effects of dystrophin deficiency. Therefore, this review focuses on what is known about the role of full-length dystrophin (Dp427) in the hippocampal neurons. In this experiment, I hypothesized that 100 ug/ml of G. Lucidum would extend the lifespan and too much concentration of this herbal medicine would lose its efficacy in treating this disease. A study was conducted through the reactions and lifespan of Caenorhabditis Elegans exhibiting the lack of dystrophin to the different concentrations of G. Lucidum. As a result, the effect of G. Lucidum on the Caenorhabditis Elegans modeling Duchenne Muscular Dystrophy was astonishing as 100 ug/ml of G. Lucidum helped prolong the lifespan of these nematodes by 20%. This data can be reflected onto the lifespan of humans with DMD as the 20% increase in lifespan of these nematodes could mean the prolonged life of 6-8 years for humans. However, too much concentration of G. Lucidum was shown not to affect the life of the worms. The hypothesized argument was proven correct as the results show the 20% increase of lifespan for the 100ug/ml of G. Lucidum concentration and the effect of too much concentration of this herbal method. Moreover, the use of herbal medicine like G. Lucidum could be a new inexpensive and attainable method of treatment for those diagnosed with DMD. The importance of dystrophin in learning and memory is assessed, and the potential importance that inflammatory mediators, which are chronically elevated in dystrophinopathies, may have on hippocampal function is also evaluated.

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Introduction

Duchenne Muscular Dystrophy falls in the varied group of 8 other similar diseases under the name of Muscular Dystrophy. All muscular dystrophies vary by their different gene imperfections. Though Muscular Dystrophy is rare, there has been progress in researching the impacts of these different variants of the disease. Many variants of Muscular Dystrophy include Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), Myotonic (MMD), Limb-Girdle (LGMD), Facioscapulohumeral (FSH), Congenital (CMD) and myopathies, Distal (DD), Oculopharyngeal (OPMD), and Emery-Dreifuss (EDMD). Both Duchenne Muscular Dystrophy and Becker Muscular Dystrophy are similar variants of Muscular dystrophy where the gene for the protein dystrophin is absent, so the lack of dystrophin in one's system causes their m21uscles cells to fall apart and degenerate over a while. However, there are variants like the Myotonic Dystrophy where the gene DPMK which codes for the protein kinase is affected and subsequently, results in a mutated gene. Myotonic Dystrophy is the most common out of all the variants of Muscular Dystrophy. Additionally, the similarities between all these variants of muscular dystrophy are the degeneration of muscle and skeletal tissue. Moreover, this paper investigates the possible solutions and validations for Duchenne Muscular Dystrophy.

Background

The history of Duchenne Muscular Dystrophy goes all the way back to the 1860s when the French neurologist Guillaume Benjamin Amand Duchene first described this disease. However, there was not much information on this disease until the late 1980 were when MDA-supported researchers identified a particular gene on the X chromosome that was mutated. They concluded that Duchenne Muscular dystrophy was caused by the DMD mutation on the X chromosome. DMD is usually carried by females who have a normal dystrophin gene on one X chromosome and another abnormal dystrophin gene on their other X chromosome. Most carriers do not experience any symptoms or signs of the disease but, rarely carries can experience symptoms of this deadly disease. Scientific Studies have shown that symptoms can range from mild skeletal muscle and cardiac involvement to severe cardiac effects in children or even adults. Before any advances in cardiac and respiratory care, those who were diagnosed with this disease would not make it past their teen years. However, with many advances in medicine, those diagnosed with this disease would be able to make it past their 30's. Since then, researchers have been trying many different ways to treat this disease by going through gene

therapy to gene repair. Duchenne Muscular Dystrophy is an inherited and progressive disease affecting children. This debilitating disease limits a person's muscular motion as their muscles start to degenerate as a result of this disease. Based on studies conducted by the Centers for Disease Control and Prevention showed that in 2007, 349 out of 23.7 million males were diagnosed with DMD and through statistics, it was shown that 15 out of every 100,000 males 5-27 were living wivr`th DMD in the US. If 15 out of 100,000 males were diagnosed with DMD that year and if the rate of diagnosis were the same about 48,000 males would be diagnosed with DMD as the US has a living population estimated close to 320 million people. Additionally, another study by the Centers for Disease Control and Prevention found that 233 out of 1.49 million males were diagnosed with Duchenne Muscular Dystrophy in 2009 in the United Kingdom. It was statistically shown that 16 out of 100,000 males were found living with this debilitating disease. If this rate were the same every year, 10,560 males would be affected by DMD in the United Kingdom as the UK has a population of 66,300,000 people. People with Duchenne muscular dystrophy face symptoms of weakness even before the age of 5 and only live up to their early 30 's. Duchenne Muscular Dystrophy is usually an early onset disease where individuals began experiencing symptoms of enlarged calves as a result of abnormal muscle and scar tissue also known as pseudohypertrophy. Additionally, as individuals grow up, easy movements such as walking and running prove to be difficult. As these individuals progress, they gradually switch to wheelchairs so that those individuals will be able to conserve their energy. However, by the teen years, many everyday activities such as raising an arm or running will be difficult, and additional assistance will be required. From studies by the muscular dystrophy association, it has been observed that most individuals with Duchenne Muscular Dystrophy do not experience pain, but those who do can be easily treated by over the counter pain medications. Additionally, the nervous system is not directly affected by DMD, so there is normal nerve function and has proper sensation. However, studies have shown that DMD is shown to affect the functions of the heart and the respiratory system. Since there is an absence of dystrophin, the muscle layers of the heart start to degenerate and as a result, lead to cardiomyopathy. Cardiomyopathy cannot be reversed or cured, but it can be controlled with a healthy lifestyle and prevention of water retention. The lack of dystrophin also causes respiratory functions to fail gradually. Respiratory functions usually require the help of the diaphragm and other muscles which gradually degenerate because of DMD. The degeneration of lung muscles causes poor respiratory function which affects individuals with symptoms like headaches, mental dullness, difficulty concentrating, and even nightmares. With the weakened respiratory system, the risk for serious respiratory illnesses increase as a simple action like coughing is very difficult. For example, a person with DMD with cold is in a greater risk of developing pneumonia than a normal person without DMD with a cold. Though this disease is incurable, there are treatments to control the symptoms and improve the quality of life for that individual with DMD. Physical therapy, occupational therapy, and medicines like steroids are available to treatments for individuals with DMD.

Ganoderma Lucidum

Commonly known as Reishi mushrooms or Lingzhi mushrooms, G. Lucidum is a species of fungus that is known for its miraculous health benefits. It has been used as a medicinal supplement for years in Asia and has been proven to help with many diseases, including cancer. There have even been unproven reports of the mushroom significantly improving the state of DMD patients. Reishi Mushrooms has become increasingly popular among herbal users as these herbal mushrooms have shown to improve the vital functions of the immune system significantly. There has not been much research on the effect of Reishi mushrooms on Duchene Muscular Dystrophy. However, with the minimal research given, it is shown that reishi mushrooms have a promising position in the treatment of symptoms of Duchene Muscular Dystrophy.

Caenorhabditis Elegans

C.elegans or Caenorhabditis Elegans are non-hazardous,non-infectious, non-pathogenic, and a non-parasitic organism. Caenorhabditis Elegans is conceived as a single cell which then undergoes the complex process of development beginning with embryonic cleavage to then to morphogenesis and then to an adult worm. However, after these worms reproduce they gradually age and die. The astonishing thing about these organisms is that their brains are wired to rudimentary learning as it can produce sperm, eggs, and even mates. They are made up of 959 somatic cells or cells that are not needed to function reproductively. Out of their 959 cells, 300 of them are neurons that mediate response to taste, smell, and touch. Additionally, 81 of the 959 cells are muscle cells as Caenorhabditis Elegans move by the four bands of muscle paired by their sub-dorsally and sub-ventrally. Many scientific studies have shown that their average lifespan is about 2-3 weeks. This was the best option as Caenorhabditis Elegans is very similar to an organism that exhibits similar essential biological characteristics that are central problems of human biology. This history of Caenorhabditis Elegans goes all the way back up to 1994 when thousands of scientists all around the world began to investigate these creatures. Additionally, between October 1994 and January 1995, 73 scientific articles about Caenorhabditis Elegans were published by scientists in international science journals.

Objective: To evaluate the efficacy of Ganoderma Lucidum on the lifespan of Caenorhabditis Elegans modeling Duchenne Muscular Dystrophy.

Experimental Methods

To investigate the efficacy on DMD, I have developed an experimental hypothesis stating the G. Lucidum powder will help gradually increase the lifespan of the Caenorhabditis Elegans as the powder concentration increases until it reaches a threshold at which it will start to reduce the lifespan. I began my experimentation where I used three sets of 5 plates. There would be one plate which was Caenorhabditis Elegans without any G. lucidum powder serving as the control. The independent variable would consist of four plates containing Caenorhabditis Elegans with various concentrations of G. lucidum added to the growth medium (50 ugs/mL, 100 ug/mL,150 ug/mL, and 200 ugs/mL). The lifespans of the worms on each plate would serve as the dependent variable. Standard culturing methods will be used. To detect whether the Caenorhabditis Elegans are living or dead I checked for any responses of external stimuli by gently prodding worms and seeing if they respond by moving. If they do not show signs of movement, they are dead and will be counted as such. Additionally, the constant variable of this experiment would be the type of Caenorhabditis Elegans and the type of plates used in this experiment. To prepare for the experiment, I had to prepare agar and Luria broth plates for the E.coli, which was served as food for the Caenorhabditis Elegans. To prepare for this plate, I had to mix 20 mL of Luria broth with 3 grams of agar in a 500 mL Erlenmeyer flask and heat until boiling on a hot plate. After, I used a thermometer to measure to 50 C and let it cool at this temperature. I then, poured enough of the mixture into the plate to cover the bottom and let it cool. Using a sterile spreading rod, I was able to streak plate with bacteria from the starter culture. I

then placed the plates in the incubator to Incubate for 24 hours at 37 Celsius. After 24 hours, I was able to Isolate single colonies on the streak plate and with a single colony, I used an inoculation loop to inoculate a liter of liquid Luria Broth. I left the plates to incubate at 37 Celsius overnight. After preparing the agar and Luria broth plates for the E.coli to grow, I started with the Nematode Growth plates or NGM plates which were essential for the growth of the Caenorhabditis Elegans. To prepare these plates, I labeled the bottom of 5 Petri dishes "control," 50 ugs/ml of G. lucidum powder, 100 ug/ml of G. lucidum powder, 150 ug/ml of G. lucidum powder, and 200 ug/ml of G. lucidum powder. I then heated 1 liter of NGM solution until it was boiling on a hot plate. After, I let the NGM solution Cool to 50 C. I poured the NGM solution into the labeled plates until about ⅔ full. I then stored the plates at room temperature for 2-3 days in case of contaminants. In order to see the effects of the G. Lucidum powder on the Caenorhabditis Elegans, I had to seed the NGM plates with the G. Lucidum powder. To seed the NGM plates I had to add .05 mL of the liquid culture to each of the five screw cap vials, and I used a digital balance to measure 2.5,5, 7.5, and ten micrograms of G. Lucidum. I then added water to the G. Lucidum powder and use a micropipette to add 2.5, 5, 7.5, and ten micrograms of G. lucidum powder to each of four of the screw- cap vials. I included the contents of each screw cap vial to its corresponding plate. The vial without any powder corresponded to the "Control" plate. I used a sterile spreading rod to spread bacteria. After seeding the NGM plates, I left the plates overnight at room temperature. After finishing both the NGM plates and the Luria Broth/ Agar plates, I transferred the Caenorhabditis Elegans into the NGM Petri dishes. When transferring the Caenorhabditis Elegans, I used a worm pick to transfer 10 Caenorhabditis Elegans to each petri dish. I then stored the Caenorhabditis Elegans in the incubator at 15 C for the rest of the day to help the Caenorhabditis Elegans grow. Each day, I observed the behavior of these worms and their reactions to this experiment. To check whether the worms were alive and dead, I had to prod each worm individually and check for a response. If they did not move at all, they were considered dead.

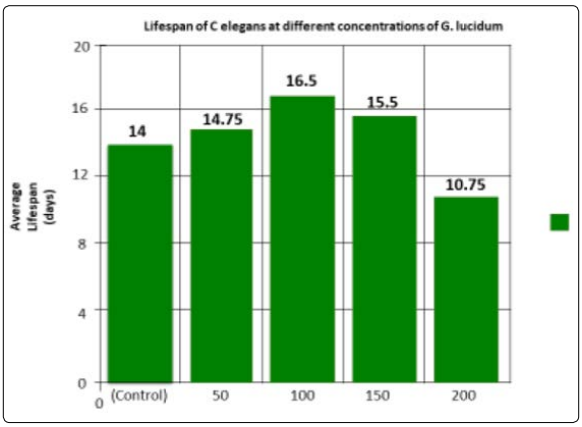

Figure 1: The results of the experiment processed in a graph

Figure 1: The results of the experiment processed in a graph

	Control	50ug/ml	100 ug/ml	150 ug/ml	200 ug/ml
Plate 1	13	14	16	15	10
Plate 2	15	15	18	16	12
Plate 3	14	16	17	16	11
Plate 4	14	14	15	15	10

Figure 2: The table above shows the approximate average lifespan of worms in days for the four plates.

After testing three trials to procure accurate results, I took the average of each plate that is shown in the above graph. The results procured from this experiment were astounding. Rather than, the highest amount of concentration of G. Lucidum powder providing an increase of lifespan it was the 100 ugs/ml of G. Lucidum which showed a dramatic increase compared to the rest of the plates. Additionally, as the concentration of G. Lucidum increased the lifespan of Caenorhabditis Elegans decreased. The control plate lived an average of about 14 days. I expected this as the average lifespan of the strain of Caenorhabditis Elegans was about 18 days. Because of environmental factors like the fluctuating outside temperature, the worms stayed on the lesser side of this average.

The smallest concentration, 50 ug/ mL showed a slight increase in lifespan. This falls in line with my hypothesis that the G. lucidum would increase the lifespan of the Caenorhabditis Elegans to a certain point. However, an increase of just .75 days is not conclusive evidence that the mushrooms are indeed increasing lifespan. Fortunately, this trend continues and supports our hypothesis. The next largest concentration, 100 ug/ mL, showed the highest increase in lifespan at 16.5 days — this almost a 20% increase from the average lifespan. In humans with DMD, 20% could add 6-8 years to a patient's life. The 150 ugs/ mL concentration was also promising with an average of 15.5 days. This is more than a 10% increase in lifespan. The last and largest concentration, 200 ug / mL caused a decline in the lifespan of the Caenorhabditis Elegans with an average of 10.75 days. This data supports the second part of my hypothesis that states that the G. lucidum will lose effectiveness as the concentration increases until eventually hurting the lifespan of the worms.

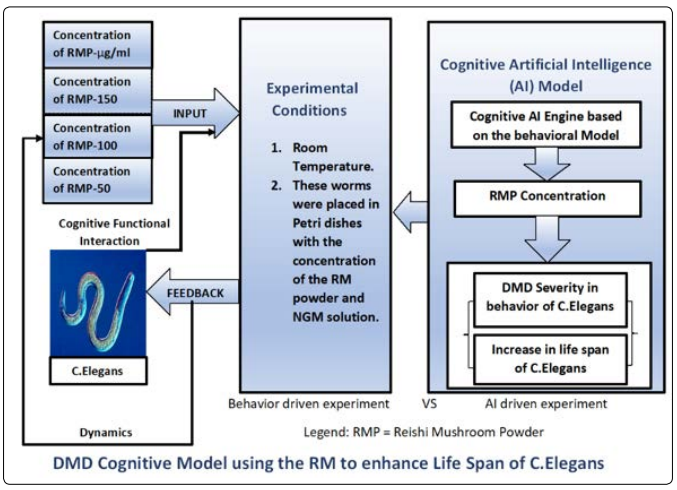

Figure 3: DMD Cognitive Artificial Intelligence Model using Reishi Mushrooms to enhance and elongate the Lifespan of Caenorhabditis Elegans

DMD is cognitive. Reishi Mushroom Powder with different ratios was used to study the behavior of the DMD parameter variations that were used in the experiment. This particular disease occurs when there is an absence of dystrophin, the structural protein that connects the extracellular matrix to the cell cytoskeleton and as a result, there is no protection against contraction-induced damage in muscle cells. When we used the correct concentration ratio of Reishi Mushroom powder, the lifespan of the Caenorhabditis Elegans increased. The results match with the cognitive AI model presented in this paper with the Caenorhabditis Elegans experiment performed.

Conclusion

The results completely supported my hypothesis. The lifespan of the Caenorhabditis Elegans increased as the concentration increased, eventually reaching a concentration at which time the lifespan began to reduce. I found a consistent trend which shows this is true. While the differences in lifespan did not seem immense enough to constitute a real change, the short lifespan our control Caenorhabditis Elegans had was taken as a consideration. Even these small differences are large percentages of the worms' natural lifespan. This, combined with the constant trend, makes me believe that G. lucidum does have the potential to be used as a cheap and attainable treatment for people with Duchenne Muscular Dystrophy. I hope to extend this experiment and conduct further research with more trials and more concentrations to find the ideal concentration for treating DMD in our model organisms [1-7].

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