

Effects of *Withania Somnifera* on Charcot-Marie-Tooth Disease Type 1A in the model organism *Eisenia Fetida*

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SUMMARY

Charcot-Marie-Tooth disease type 1A (CMT1A) affects 250,000 people in the United States alone. CMT1A causes abnormalities in the myelin sheath due to the duplication of PMP22 on chromosome 17. This duplication causes the breakdown of the myelin sheath that wraps the sensory and spinal cord motor neurons. CMT1A is commonly associated with symptoms that include bone deformities, pes cavus (a deformity in the plantar arch), and muscle weakness. This study focused on finding a correlation between *Withania somnifera* ingestion and the speed of the nerve signal. *W. somnifera* is a plant in the Solanaceae family, which grows in the Middle East, India, and parts of Africa, and it has been reported to be protective against demyelination. Our model organism, *Eisenia Fetida*, is a species of earthworm. Our hypothesis was that if *W. somnifera* is added to *E. fetida*'s diet, there would be a decrease in demyelination leading to a faster nerve conduction rate. From the experiment, we found that *W. somnifera* significantly increased the speed of the nerve conduction in *E. fetida*, making *W. somnifera* a potential option to investigate as a treatment for CMT1A.

INTRODUCTION

Charcot-Marie-Tooth disease type 1A (CMT1A) is an inherited neurological disorder that causes damage to the myelin sheath that wraps the neurons (1). CMT1A can be caused by somatic or germline mutations (2). Some of the common symptoms include bone deformities, pes cavus, loss in sensation of the fingers and toes, muscle weakness, and clawed fingers (2). This disorder affects an estimated 3 million individuals worldwide, with disease onset beginning at 10-20 years of age and slowly progressing throughout the lifetime (4, 5). CMT1A is caused by a duplication of the gene *PMP22* in the Schwann cells which causes damage to the myelin sheath (1). Myelin sheaths protect the nerves and allow electrical impulses to travel quickly through the axons (1). Damage to the myelin can make the transmission of nerve impulses slow and weak (1). Researchers have yet to find a cure and patients are advised to do physiotherapy, use various foot braces, and orthopedic devices (1, 2). Moreover, other treatment options like chemotherapy do not improve the conditions of CMT1A patients (10).

Withania somnifera (*W. somnifera*) is a plant in the Solanaceae family (6). Several studies show that *W. somnifera* contains chemical compounds like flavonoids, which help protect the nerves from neurotoxins that interfere with the

development and growth of the nerves (6, 7). *W. somnifera* also has many benefits due to its antimicrobial, anti-inflammatory, and neuroprotective properties (7). People diagnosed with CMT 1A tend to have high amounts of inflammation and *W. somnifera* can help to decrease inflammation. People also have oxidative stress and *W. somnifera* contains flavonoids that help to protect nerves from oxidative stress. The intake of *W. somnifera* aids in the development of the nerves.

Animal models are an excellent way to observe the effects of compounds. For studying CMT1A, zebrafish models have been utilized in the past by researchers. Zebrafish with a mutation in *Mfn2* display motor dysfunction by not being able to propel themselves in the water and are used to further understand the effects of CMT1A (8). The *Mfn2* gene is responsible for determining the structure of the mitochondria in our cells (8). Zebrafish are a great model organism because they develop rapidly and have a nervous system. As an alternative model, we used *Eisenia fetida* (*E. fetida*), which are a species of earthworm. *E. fetida* have three axons, one medial giant axon, and two lateral giant axons (9). *E. fetida*'s axons are covered by various glial cells which resemble vertebrate myelin (9).

Through the experiment, we investigated if *W. somnifera* helps to strengthen the nerves and transmit signals faster. If *W. somnifera* helps to increase the nerve signal then we can conclude that it helps to strengthen the myelin sheath that wraps around the neurons. The myelin sheath helps to conduct nerves faster by insulating the axon and allowing the signal to leap from one node to another (1). From our experiment, we saw the speed of the worm's nerve increase gradually over the course of 30 days, making *W. somnifera* a potential option to investigate as treatment for CMT1A. The nerve speed of the worms in the control group remained the same with fluctuations.

RESULTS

We investigated the effects of *W. somnifera* on *E. fetida* by having 4 groups. The 3 groups (1, 2 and 3), excluding the control group, were given different doses of *W. somnifera* every other day. They were given 1 gram, 1.5 grams, and 2 grams, respectively. We used a neuron SpikerBox to determine the nerve conduction of all the *E. fetida* (Figure 1). This was performed 3 times, on day 0, 15 and 30, over the course of 30 days (Figure 2, 3).

The results of the nerve conduction rate from the first to the final test in each group were statistically significant ($p < 0.05$) (Figure 4). On the last day of testing, all three groups that were given *W. somnifera* showed a significant increase in nerve conduction compared to controls that received no *W. somnifera*. In the ANOVA test, the p -values were less than 0.05 when the final nerve conduction speed of each

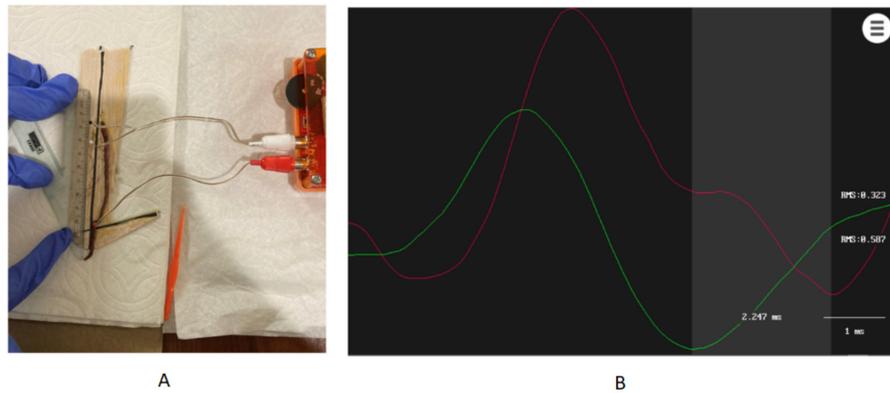


Figure 1: Example set up and data of measuring nerve conduction with SpikerBox. A. Experimental set up using *E. fetida* for the nerve conduction test. B. Action Potentials measured using the SpikerBox. The red line indicates the data collected from electrode 1 and the green lines indicate the data collected from electrode 2.

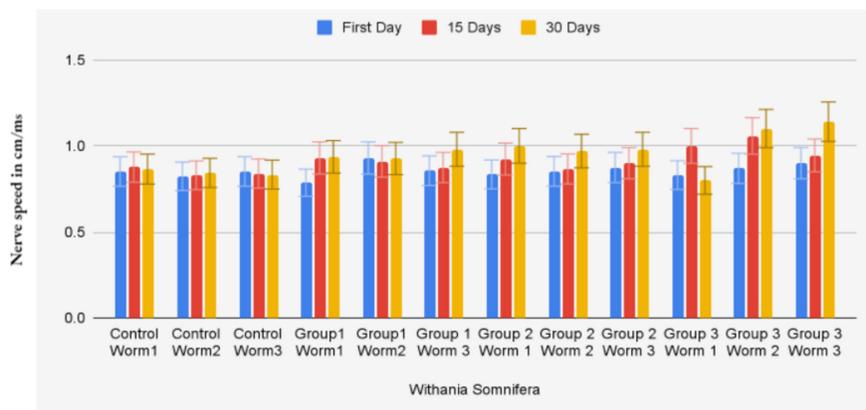


Figure 2: The average nerve conduction velocity (cm/ms) for the three groups and the control group. There was a gradual increase in the nerve speed after each test. The error bars represent the standard deviation.

treatment group was compared to the control group. The standard deviation for *E. fetida* in groups 1, 2, and 3 were 0.03394, 0.04515, and 0.07776.

DISCUSSION

Currently, there is limited knowledge about the various symptoms and causes of CMT1A, and ways to protect oneself from this progressive disease. In this study, we found that the *E. fetida* that were given more *W. somnifera* showed an increase in nerve conduction rate compared to *E. fetida* that were given 1 g to no *W. somnifera*. The groups that were given 1.5 g and 2 g of *W. somnifera* demonstrated faster nerve conduction rates, as the time it took the signal to travel from the posterior end to the anterior end decreased. Because the nerve conduction rate increased when *W. somnifera* was added to *E. fetida*'s diet, we can conclude that *W. somnifera* could be a potential option to investigate as treatment for CMT1A.

From our experiment, we found that the effect of *W. somnifera* on *E. fetida* on groups 1, 2, and 3 were statistically significant when compared to the starting results (the nerve conduction test taken on the first day) and the control group. During this study, we faced several issues that we had to overcome in order to obtain our results. In our experiment we struggled to maintain the *E. fetida* in the right temperature

and moisture level, and had a hard time in understanding and acquiring the data to find the nerve conduction rate. But after continuous trials and repetitive testing, we established a method for maintaining *E. fetida* and measuring its nerve conduction rate.

Some other factors that could have influenced the results were that the *E. fetida* had reproduced towards the end of the study. The reproduction of *E. fetida* could have caused some worms that were probably never exposed to *W. somnifera* to be accidentally used in a nerve conduction test. To minimize the possibility of using the incorrect worm, we had to spend the time to find the differences in the worms by looking at the length, width, and size of their clitellum. In addition, we identified the precise amount of time that the worms needed to be anesthetized. Using trial and error, we found that 4-6 minutes was an ideal range for the worms. The worms were anesthetized the right amount as we could still capture the electrical impulses and they did not interfere during the test.

In the future, we hope to experiment with different quantities of *W. somnifera* and test the effects on different invertebrates. This further experimentation will help us to determine if there is a correlation between the amount of *W. somnifera* ingested and the increase in nerve speed. Furthermore, we hope to research other beneficial properties of *W. somnifera* and whether it could help combat other neurological diseases,

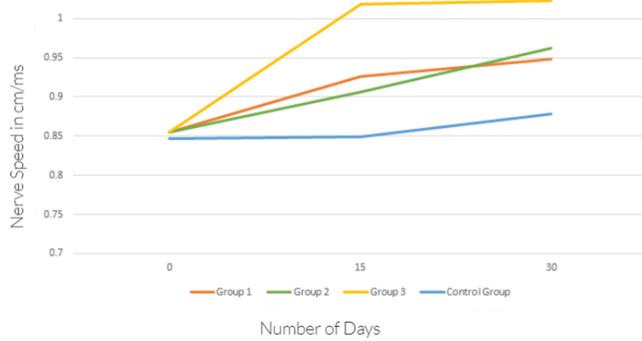


Figure 3: The average nerve conduction rate (cm/ms) for *E. fetida*. *E. fetida* in group 1 which received 1 g of *W. somnifera*, group 2 which received 1.5 g of *W. somnifera* and group 3 which received 2 g of *W. somnifera* were recorded after each test (0, 15, and 30th day).

including the various other subtypes in CMT.

Our results indicated that *W. somnifera* may help to increase the nerve speed in *E. fetida*. The findings of the research can be expanded to many neurological diseases as the *E. fetida* did not have CMT1a. The increase in nerve speed suggests that *W. somnifera* could be a potential option to investigate as a treatment for many demyelinating diseases such as multiple sclerosis, where it may help strengthen the myelin and increase the speed of the electrical impulses.

Other scientific research has been conducted to examine the neuroprotective properties of *W. somnifera*. The research used 30 albino mice to study the effects of *W. somnifera* on the behavior and memory of the mice. The mice were examined by using a y-maze and Morris water maze. The research found that *W. somnifera* has antioxidant properties which can help treat cognitive dysfunction (11). The research helps strengthen the correlation that *W. somnifera* can help with nerve degeneration.

MATERIALS AND METHODS

For this study, *E. fetida* was used to model the effects of *W. somnifera* on CMT1A. *E. fetida* were raised in four different groups and were placed in several different containers of rich mineral soil. Each group had three worms, and each worm had its own container, and the fourth group was the control group. Group 1, 2, and 3 excluding the control group were given 1 g, 1.5 g, or 2 g *W. somnifera*, respectively. All groups were given sanitized vegetable scraps as their main food source.

Soil Container

Holes were created on the bottom, top, and sides of the container, to make sure there was constant air circulation. The bottom layer of the container consisted of cardboard and paper, as this would help to absorb the moisture and wetness in the soil. Then fresh soil was placed on top of the paper and cardboard. Lastly, compost and 10 ml of water mixed with *W. somnifera* were added to the soil.

Withania Somnifera

For each group, the appropriate quantity of *W. somnifera* was mixed with 10 mL of water and was sprayed onto the soil every other day. This method dilutes the concentration of *W.*

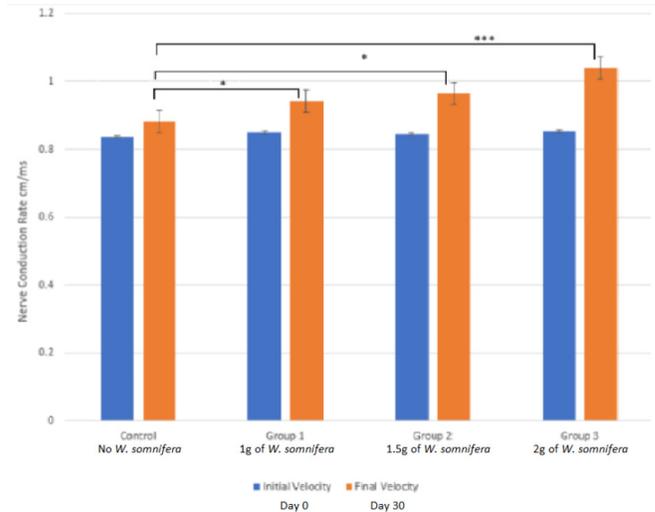


Figure 4: The average nerve conduction rate of the 3 worms in group 1, group 2, group 3 and control group for the initial test (day 0) and the final test (day 30). The error bars represent standard deviation. *** = $p < 0.005$, * = $p < 0.05$.

somnifera immensely as the water was sprayed onto 60 g of soil.

Data Collection

On the first day of the test, before any *W. somnifera* was given, a SpikerBox was used to find the nerve conduction speed of the worms.

To perform the test, we placed *E. fetida* in a container filled with sparkling water. The CO_2 in the water served as an anesthetic agent and stopped the movement of the worm. The worm was in the solution for 4-6 minutes, and then briefly rinsed off in tap water. Next, the worm was placed on balsa wood which was our recording base for the test.

The SpikerBox has two electrodes and one ground which were precisely placed on the worm. Then we gently tapped the *E. fetida* 3-4 times and the gap between each tap was 3-4 seconds (Figure 1). Immediately, the action potential from both the spikes was displayed on the app. Using the time difference between the troughs of each curve and using the distance, the speed was calculated (Figure 1).

The nerve conduction was measured for all groups of worms and their data was recorded. The data was recorded through the spike recorder app.

From then on, each *E. fetida* in groups 1, 2, and 3 were given *W. somnifera* in assigned quantities, every other day. Also, we fed the worms compost at the end of every week. After 15 and 30 days, the worms were removed from the rich mineral soil and the nerve conduction test was performed. We recorded the data and compared it to the previous data to determine if there were improvements in the nerve conduction. On the last test, we compared the results with the data collected on the first day to find the change in the speed of the nerve conduction (Figure 4).

Data Analysis

We used an ANOVA test to determine if the final nerve conduction speed was significant when compared with the initial nerve conduction test in each group. We also performed

an ANOVA where we compared the final nerve conduction rates in each group with the remaining groups. We also created charts and graphs to see the trend between the nerve conduction rates and *W. somnifera*. The standard deviation of the nerve conduction rate for all the *E. fetida* in each group was calculated to determine if there were any outliers in the nerve conduction rates.

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