# Investigating the effects of herbs on reducing symptoms associated with schizophrenia in the model organism *Caenorhabditis elegans*

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## Abstract

Schizophrenia is a mental disorder characterised by the breakdown in the relation between a person's thoughts, emotions and behaviour. Symptoms include hallucinations, social withdrawal and memory defects. Schizophrenia is a prevalent issue, with over 70 million people worldwide suffering from this illness. Currently, the cost of treating schizophrenia is exorbitantly high. In this study, we will be using two herbs, namely *Rhodiola rosea* and *Curcuma longa*, and testing their effectiveness in treating symptoms associated with schizophrenia. Our proposed treatment is promising as these herbs have been previously shown to help with depression and anxiety in the past and have also been found to decrease glutamate-induced excitotoxicity, and hence has potential to be a low cost and effective alternative for treatment of schizophrenia. The model organism *Caenorhabditis elegans* was used to model schizophrenia. *C. elegans* CB1372 *daf-7(e1372)* display increased social feeding due to a higher level of physical discomfort towards atmospheric oxygen and a lower average moving velocity as a result of increased glutamatergic signalling. in reducing symptoms associated with schizophrenia in *C. elegans* CB1372 *daf-7(e1372)*. This research concludes that both herbs, *Curcuma longa* and *Rhodiola rosea*, are able to effectively reduce social feeding and increase the average moving velocity of *C. elegans* CB1372 *daf-7(e1372).* Furthermore, there appears to be no significant side effect on the behaviour of wild type *C. elegans* N2 when the herb extracts of *Rhodiola rosea* and *Curcuma longa* were added to the set-ups. These results suggest that the herbs can potentially be used in treating schizophrenia, replacing the use of antipsychotic drugs.

## 1. Introduction

Schizophrenia is a chronic mental disorder characterized by an inability to connect with reality. While the genetic cause of schizophrenia is unknown, a major hypothesis

investigates the dysregulation of the neurotransmitter glutamate in patients with schizophrenia The glutamate hypothesis states that negative and cognitive symptoms of schizophrenia, such as social withdrawal and memory defects, are associated with increased glutamate levels in the brain, which causes neurodegeneration through a process called excitotoxicity (Howes, McCutcheon, & Stone, 2017; Mei, Wu, & Zhou, 2018).

Neuroscience is increasingly utilising smaller, non-rodent models to understand mechanisms related to psychiatric disorders. One such model organism is the nematode *Caenorhabditis elegans* which comprises 959 somatic cells, 302 of which are neurones. The chemical properties of *C. elegans* neurones are similar to those of mammalian neurones with synapses utilising chemical neurotransmitters such as acetylcholine, glutamate, dopamine, serotonin and gamma-Aminobutyric acid. Despite the simplicity of its nervous system, *C. elegans* shows well-defined behaviours, such as the ability to find food sources, detect and escape noxious substances, find mating partners, display social or solitary feeding, and locate optimal oxygen concentration and temperature (Burne et al., 2011).

*C. elegans* isolates exhibit either social or solitary feeding on bacteria. Solitary feeders such as the wild type *C. elegans* N2 strain disperse across a bacterial lawn, while social feeders tend to aggregate together (de Bono & Bargmann, 1998). Social feeders also accumulate on the border of a bacterial lawn. Social feeding is induced by nociceptive neurones that detect adverse or stressful conditions, hence social feeding is probably due to the sensation of noxious chemicals by certain sensory neurones (de Bono, Tobin, Davis, Avery, & Bargmann, 2002).

According to Chang, Chronis, Karow, Marletta and Bargmann (2006) social feeding is a suitable protophenotype for social withdrawal, a component of schizophrenia. *C. elegans* CB1372 *daf-7(e1372)* has lower daf-7 activity. Since daf-7 negatively regulates daf-3 activity, *C. elegans* CB1372 *daf-7(e1372)* has higher daf-3 activity, leading to an overexpression of tph-1 in ADF neurons (sensory neurone). This further leads to higher serotonin levels which act as a stress signal for *C. elegans*. Hence, *C. elegans* CB1372 *daf-7(e1372)* shows increased agitation, physical discomfort and is more hyperoxia (excess oxygen) avoidant and aggregate more to reduce exposure to ambient oxygen. Excess oxygen

or hyperoxia is toxic for *C. elegans* as it promotes the production of toxic oxygen species (López-Puebla, A., Mayoral-Peña, Z., Gómez-Cepeda, K., & Arellano-Carbajal, F. ,2019)

Thus, aggregation in *C. elegans* is not mediated via bonding or positive motivation, but by avoidance and safety-in-numbers considerations. Moreover, likewise in *C. elegans*, stress-induced serotonergic overdrive leads to symptoms of schizophrenia like psychosis and cognitive impairment in humans (Eggers, 2013). Antipsychotic drugs like clozapine used to treat schizophrenia in humans also inhibit serotonin receptors due to their high affinity to serotonin receptors. Moreover, these same drugs inhibit social feeding in *C. elegans* with the *daf-7(e1372)* mutation (Dwyer, 2017).

Furthermore, according to McGehee, Moss, and Juo (2015) and Zheng et al. (1999), the hypofunction of NMDA receptors in humans causes excessive glutamate release and hyper-glutamatergic functions, leading to disease progression from prodromal symptoms to psychosis in humans. *C. elegans* CB1372 *daf-7(e1372)* with the *daf-7* mutation have increased GLR-1 transcription, leading to increased glutamatergic signaling as well. *C. elegan*s CB1372 *daf-7(e1372)* have a disrupted mechanosensation and a disrupted escape response to mechanical stimuli to the nose and, as a result, rapidly alternate between forward and backward movement. Hence, symptoms of schizophrenia manifest as more random locomotion and a lower average moving velocity in *C. elegans* CB1372 *daf-7(e1372)*.

Essentially, through the social feeding and locomotion protophenotypes, *C. elegans* CB1372 *daf-7(e1372)* serves as an excellent model organism to replicate schizophrenia in humans.

*Rhodiola rosea* and *Curcuma longa* are two natural compounds which have been shown to help with depression and anxiety in the past and have also been found to decrease glutamate-induced excitotoxicity (Yeonju et al., 2013; Chen, An, Tie, Pan, & Li, 2015). Hence, this study aims to investigate if the extracts of these two compounds are able to reduce social feeding and increase the average moving velocity of *C. elegans* CB1372 *daf-7(e1372)*.

## Objectives and Hypothesis

Our objective is to investigate the extent in which the extracts of *Curcuma longa* and *Rhodiola rosea* are able to reduce social feeding and normalise the locomotion patterns in *C. elegans* CB1372 *daf-7(e1372)* by determining the number of *C. elegans* aggregates on the plates and the increase in its average velocity of locomotion.

We hypothesise that both *Curcuma longa* and *Rhodiola rosea* are able to reduce social feeding and normalise the locomotion patterns of *C. elegans* CB1372 *daf-7(e1372)* by reducing the number of aggregates observed and increasing its average velocity.

## 2. Materials and Methods

### 2.1 Materials

*Rhodiola rosea* herb extract was procured from Swedish Herbal Institute. Aquacumin (water-soluble *Curcuma longa* herb extract) was procured from Ava Plant. Luria-Bertani (LB) agar broth powder was procured from Becton, Dickinson and Company. Bacto-peptone powder was procured from Oxoid Company.

### 2.2 Preparation of herb extracts

Aquacumin powder was dissolved in water at 5mg/ml. *Rhodiola rosea* powder was dissolved in water at 5mg/ml. Filtration was conducted to remove undissolved residue.

### 2.3 Preparation of Nematode Growth Medium (NGM)

0.9 g NaCl, 7.5 g agar, 0.75 g bacto-peptone were added with 291.6 ml water. After autoclaving, 0.3 ml of cholesterol (5 mg/ml), 0.3 ml of $MgSO_4$ solution (1M), 0.3 ml $CaCl_2$ solution (1M), 7.5 ml of $KH_2PO_4$ solution pH 6.0 (1M) were added.

### 2.4 Growth of bacterial culture

*Escherichia coli* OP50 was inoculated and grown in 10 ml of LB broth overnight at 30℃ in a shaking incubator. The absorbance of the bacterial culture at 600 nm was standardised at 0.8 units.

### 2.5 Growth of *E. coli* OP50 on NGM plates

100µl of extract solution (test) or sterile water (control) was spread on the NGM plates and left to dry. 50µl of *E. coli* OP50 was added in the centre of the NGM plates. The plates were incubated for 1 day at 32℃.

### 2.6 Growth of *C.elegans* on prepared NGM plates

A block of agar containing *C.elegans* N2 or *C. elegans* CB1372 *daf-7(e1372)* was cut and placed in the centre of the NGM plates. The plates were incubated at 20°C for 2 days. The number of aggregates of *C. elegans* was then determined. 6 set-ups were prepared, as shown in Table 1.

| | *Rhodiola rosea* treatment | *Curcuma longa* treatment | No treatment (Sterile water) |
|---|---|---|---|
| Healthy organisms- *C. elegans N2* | Control *(group 1)* | Control *(group 2)* | Control *(group 3)* |
| Mutated organisms- *C. elegans CB1372 daf-7 (e1372)* | Experimental *(group 4)* | Experimental *(group 5)* | Control *(group 6)* |

*Table 1*: Set-ups prepared

The purpose of the N2 controls (set-up 1 and 2) is to compare with the wild type N2 *C. elegans* to test for side effects of using the herb extracts. Experimental set-ups 4 and 5 will be used to test our hypothesis. Control set-up 6 will be used to compare with control set-up 3 to show the presence of our dependent variables that we will be testing.

### 2.7 Data analysis

For the social feeding assay, the number of aggregates of *C. elegans* were counted using a microscope. The standard error was determined and plotted as error bars. The software, Celleste Image analysis, was used to study the locomotion patterns of *C. elegans* using the manual tracking function. The Kruskal-Wallis statistical test was conducted to determine if there were significant differences between the mean values of the set-ups.

## 3. Results and Discussion

### 3.1 Social feeding assay

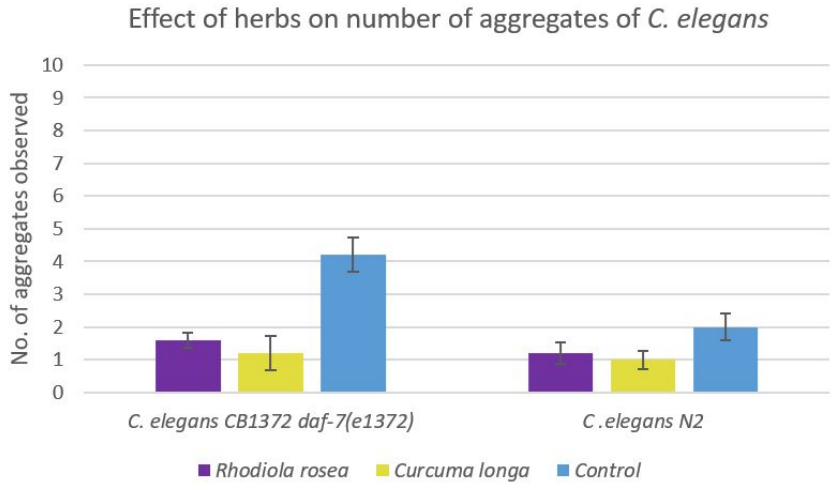


*Fig. 1:* Graph of number of *C. elegans* aggregates observed in respective NGM plates

Figure 1 shows the mean number of *C. elegans* aggregates observed in the NGM plates across all 6 set-ups. It can be observed that after treatment with *Curcuma longa* and *Rhodiola rosea*, the social feeding behaviour of *C. elegans* CB1372 *daf-7(e1372)* was significantly altered and the worms were more dispersed. A p-value of 0.011<0.05 suggests that there is a significant difference between the mean values of the test set-ups and control set-ups for *C. elegans* CB1372 *daf-7(e1372)*. Whereas, a p-value of 0.242>0.05 suggests that there is no significant difference between the mean values of the control and test set-ups for wild type *C. elegans* N2.

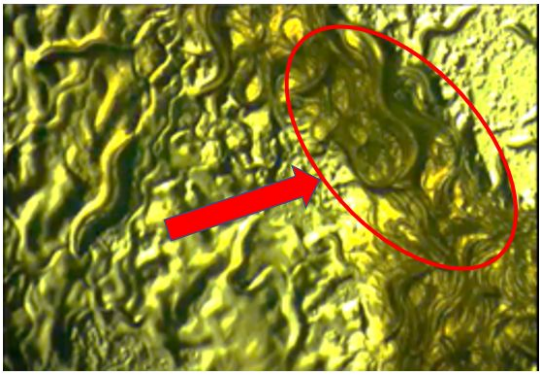

*Fig. 2(a):* Photograph illustrating social feeding behaviour in the control set-up of *C. elegans* CB1372 *daf-7(e1372)*

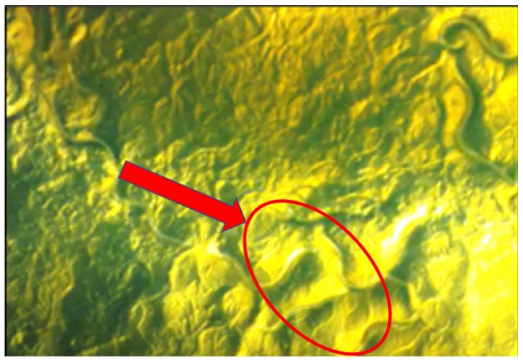

*Fig. 3(b)*: Photograph illustrating solitary feeding behaviour in the experimental set-up of *C. elegans* CB1372 *daf-7(e1372)* with *Curcuma longa* treatment

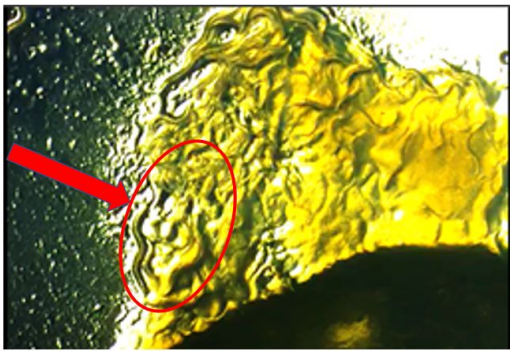

*Fig. 3(c)*: Photograph illustrating diminished social feeding behaviour in the experimental set-up of *C. elegans* CB1372 *daf-7(e1372)* with *Rhodiola rosea* treatment

Curcumin, an active ingredient of *Curcuma longa*, has shown to have anti-inflammatory and antioxidant effects (Liao. *et al*, 2011). Research done by Monroy, Lithgow and Alavez (2013) also suggests that curcumin reduces inflammatory responses and activates neuroprotective genes in *C. elegans*.

By its chemical structure, curcumin may act as a natural free radical (ROS) scavenger. Acting through the Neurotrophic factor κB (NF- κB), curcumin can decrease the release of different interleukins (inflammatory compounds). Curcumin could act as a stress response mimetic that induces protein homeostasis network. This induction requires the transcription factor SKN-1 in *C. elegans*. Additionally, curcumin could act as a DR mimetic to activate this transcription factor through the AMPK pathway. Hence, *Curcuma longa* treatment through reducing inflammation (which has been previously linked to high glutamate levels and depression in humans by Emory Health Sciences (2016)) and offering neuroprotection

may be able to decrease the ill-effects of increased glutamate levels as a result of the aforementioned *daf-7* mutation. This reduces the social feeding behaviour observed in *C. elegans* CB1372 *daf-7(e1372)*.

Research by Weigrant. et al (2008) suggests that *Rhodiola rosea* induces translocation of the DAF-16 transcription factor from the cytoplasm into the nucleus in *C. elegans*, leading to a reprogramming of transcriptional activities favoring the synthesis of proteins involved in stress resistance and longevity. The increased stress resistance of *C. elegans* CB1372 *daf-7(e-1372)* after undergoing *Rhodiola rosea* treatment means that it is now less aversive to the threat of hyperoxia and, hence, has reduced social feeding.

### 3.2 Locomotion assay

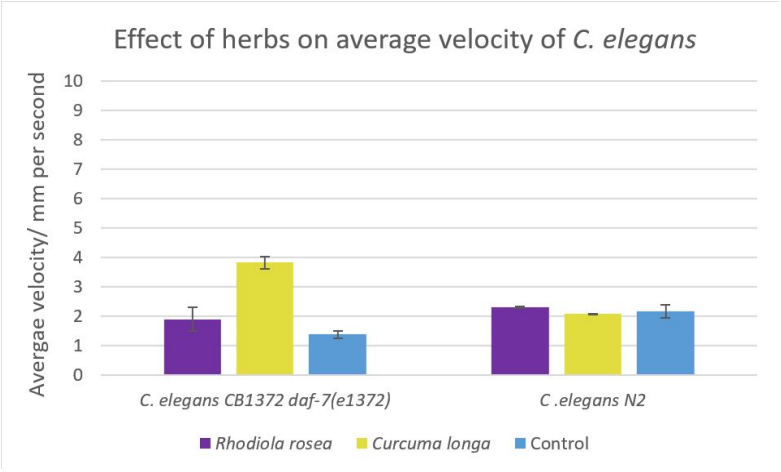


*Fig. 4:* Graph illustrating the average velocity of *C. elegans* N2 and *C. elegans* CB1372 *daf-7(1372)* across all 6 experimental groups

As observed from Figure 4, both *Curcuma longa* and *Rhodiola rosea* are able to increase the average velocity of *C. elegans* CB1372 *daf-7(e1372)*. The Kruskal-Wallis statistical test indicated a p-value of 0.016 suggests that there is indeed a significant difference between the mean velocity of set-ups for mutated *C. elegans*. Additionally, the p-value of 0.365 for the mean velocity of set-ups with wild type *C. elegans* N2 suggests that the herbs do not have a significant side effect on locomotion.

Research done by Satapathy and Chinnu (2016) suggests that curcumin treatment is able to restore dopamine levels in *C. elegans* previously exposed to neurotoxins, hence, curcumin offers neuroprotective efficacy to *C. elegans*. Increased neuroprotection accrued by curcumin may be able reduce the ill-effects of increased glutamate levels, leading to more normalised locomotion patterns in *C. elegans* CB1372 *daf-7(e1372)*. This manifests as a higher average velocity for *C. elegans* CB1372 *daf-7(e1372)* after *Curcuma longa* treatment as compared to the control set-up.

Research by Weigrant. et al (2008) suggests that *Rhodiola rosea* increases stress resistance and longevity of *C. elegans*. Increased stress resistance may mean that *C. elegans* CB1372 *daf-7(e1372)* is less susceptible to the ill-effects of increased glutamate levels and have normalised locomotion patterns. *C. elegans* CB1372 *daf-7(e1372)* treated with *Rhodiola rosea* had a higher average velocity as compared to the control set-up. However, it remains unknown as to why *C. elegans* CB1372 *daf-7(e1372)* treated with *Curcuma longa* treatment had a significantly higher average velocity than the control set-up with *C. elegans* N2.

## 4. Conclusion

Both *Curcuma longa* and *Rhodiola rosea* are effective in reducing social feeding in *C. elegans* CB(1372) *daf-7(e-1372)* to levels seen in the control set-up of *C. elegans* N2. Both herbs are also effective in normalising locomotion patterns in *C.elegan*s CB(1372) *daf-7(e-1372)*. Hence, the usage of *Curcuma longa* and *Rhodiola rosea* are successful in reducing symptoms associated with schizophrenia in *C.elegans* CB(1372) *daf-7(e-1372)*; and, they, too, are potentially novel and cheap methods to replace expensive psychotic drugs in treating human patients suffering from schizophrenia.

## 5. Limitations

The first limitation was that the growth rate of *C. elegans* CB1372 *daf-7(e-1372)* was observed to be lower than that of *C. elegans* N2. There was difficulty in standardising growth stages of mutated and N2 *C. elegans*, which could have affected the results obtained. Furthermore, each batch of *C. elegans* used may have subtle biological differences (e.g. social feeding behaviour and locomotion patterns).

## 6. Recommendations for future work

In the future, the optimum dosage and concentration of *Curcuma longa* and *Rhodiola rosea* to reduce symptoms associated with schizophrenia in *C.elegans* CB(1372) *daf-7(e-1372)* can be determined. This can be done by altering dosage and concentration of herbs, and subsequently determining the exact dosage and concentration which reduces social feeding and increases average velocity most effectively. On top of this, the effectiveness of *Curcuma longa* and *Rhodiola rosea* in alleviating symptoms of human patients with schizophrenia can be determined through clinical trials. As schizophrenia is caused by changes in serotonin and glutamate levels, we can test the effect of these herbs on the neurotransmitters, and determine its effectiveness in alleviating symptoms of schizophrenic human patients.

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