



The effect of *Gelsemium sempervirens* extract on social interaction of a traumatic injury induced *Drosophila melanogaster* offspring model (mimicking preterm birth defects)

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ABSTRACT

Every year, approximately 15 million infants are born prematurely, leading to various birth defects such as reduced social interaction or increased anxiety. *Drosophila melanogaster*, or fruit flies, are used as a model organism as they display behaviors such as anxiety and agoraphobia which mimic premature birth defects like reduced social interaction. Traumatic injury induced on maternal flies using a High Impact Trauma (HIT) device shows reduced social interaction in offspring flies. Social interaction can be measured using a social space assay where each fly's nearest neighbor is considered a social space. Smaller distances between flies represent higher social interaction with increased in aggregation of flies. *Gelsemium sempervirens* (GS) extract counteracts increased anxiety levels and increases interaction levels in mice. I hypothesize that if the extract is administered to the maternal fruit flies induced with traumatic injury, then the offspring will have improved social interaction levels. There was a significant difference found in social interaction when the extract is applied in a trauma scenario. The hypothesis is supported as offspring social interaction was increased when extract was administered to trauma induced maternal flies. The research can shine light on the possibility of further research on neonates, specifically, improving their social interactions.

Keywords: Behavioral neuroscience, social interaction, preterm birth, *Drosophila melanogaster*, maternal trauma

INTRODUCTION

1.1 Premature Birth

Approximately 15 million babies are born premature every year (WHO, 2022). Premature neonates are known to experience multiple birth defects that can impact overall health and quality of life including reduced social interaction and increased anxiety (Crnic *et al.*, 1983). The behavior of decreased social interaction among neonates is often seen in lower parent-infant interaction (McCollum, 1984). In a study on the cognitive and behavioral outcomes of very preterm (VPT) children,

it was found that preterm children exhibit decreased cognitive ability in adolescence relative to children born at full term (Johnson, 2007). This decrease in cognitive ability was concomitant with a reduction in socioemotional development for VPT children. Reduced socioemotional development in VPT children can result in decreased social competency and interactive nature based on a study on early-life children (Groh *et al.*, 2017).

1.2 Maternal Trauma

Maternal traumatic injury (TI) is a significant risk factor during pregnancy, leading to developmental disabilities in children. Approximately 50% of injuries occur due to traumatic injury incidents such as motor vehicle accidents (Esposito *et al.*, 1989). Research on pregnant women has demonstrated that women who experience physical traumatic injury during pregnancy have a higher likelihood to early preterm birth due to fetomaternal hemorrhage (Drost *et al.*, 1990). Currently, there is no treatment for developmental defects in children that follow in the neonate post maternal trauma. TI in pregnant women have also been known to result in a delay in socioemotional development for infants (Goodwin & Breen, 1990).

1.3 Model Organism

The common fruit fly, *Drosophila melanogaster*, is an accessible model organism for studying developmental behavior as due to their behaviors mimic its many social behaviors in humans. *D. melanogaster* has been used to model human behaviors that are associated with preterm birth, such as depression, anxiety, and agoraphobia (Meichtry *et al.*, 2020). By studying these behaviors in fruit flies, we can gain insight into the effects of birth complications on social interactions (Chauhan & Chauhan, 2019).

1.4 Current Research



Figure 1. High Impact Trauma device for maternal trauma.

Researchers have focused on studying the effects of TI in fruit flies and its effects on social behavior of their progeny. TI can be induced on flies using a High Impact Trauma (HIT) device, using a closed head traumatic brain injury method (shown in *Figure 1*) by (Katzenberger *et al.*, 2013). In a study focusing on maternal TI on fruit flies, it was found that there was a decrease in social interaction among resulting offspring (Chauhan & Chauhan, 2019). A social space assay was utilized to measure individual social interaction levels through social space distances. A fly's distance to its nearest neighbor is known as a social space distance. Fruit flies that remained socially isolated displayed the behaviors of increased anxiety and timidness. A smaller distance between flies and closer aggregation of flies was a representation of high social interaction levels. The triangular shaped test chamber was utilized to measure social space distances, as this was the most consistent model for measuring social interaction based on the relative aggregation of flies (Simon *et al.*, 2012). Ongoing research is being conducted on how the traumatic brain injury model can be used for studying therapeutic intervention treatments as the model mimics similar neurodegenerative diseases and related conditions (Katzenberger *et al.*, 2013). Currently, there are no treatments alleviating the decrease in social interaction due to TI for offspring flies.

1.5 Gelsemium sempervirens Extract

Gelsemium sempervirens (GS) extract is a known herbal remedy in adult humans to alleviate anxiety. GS has anxiolytic-like properties which are known to alleviate anxiety symptoms (Bellavite *et al.*, 2018). In the model organism of mice, GS has decreased anxiety levels and improved social behavior (Bellavite *et al.*, 2018). Since GS extract has been known to decrease anxiety levels and increase interaction levels in mice, it can be hypothesized that GS extract administration to maternal flies with TI, their offspring will have increased social interaction.

1.6 Novelty & Purpose

This study investigates GS extract as a potential treatment to improve social interaction of a TI induced fly offspring model. This study is novel in that GS has not been utilized in flies as a therapeutic nor has it been explored at alleviating preterm birth defects such as reduced social interaction. Ultimately, this research can provide neonates who experience such premature

birth defects like reduced social interaction with a means for therapy.

MATERIAL AND METHODS

2.1 Stock Maintenance

Wild Type Oregon-R flies were maintained at 22°C on a standard cornmeal/ agar diet and kept on a 12-hour dark and 12-hour light cycle. Fruit flies were tapped into new plastic vials with foam flugs every four days and fresh food vials every three weeks. Flies were frozen at -20°C for around an hour to dispose of the flies.

2.2 Fly Food Preparation

The masses of the following dry ingredients were first measured using an electronic balance, scoopula, and weigh boat: 6.75 grams yeast, 3.90 grams soy flour, 28.50 grams yellow cornmeal, and 2.25 grams agar. The following wet ingredients were then measured using a graduated cylinder: 30 mL light corn syrup and 390 mL distilled water. The wet and dry ingredients were mixed in a 500 ml beaker using a stirring rod. The 500 ml beaker was then placed in the microwave and warmed through 30 second increments until a rolling boil was achieved. Between each increment, a glass stirring rod was used to thoroughly stir the mixture. After the mixture was boiled, the food was cooled down and checked with a thermometer. After cooling down, a micropipette was used to administer 1.88 ml of 10% propionic acid. Finally, the fly food was poured into around 30-40 vials and covered with a cheesecloth to prevent contamination as the food cooled to room temperature. Vials were monitored daily for mold or contamination and disposed of, if

needed. For the treatment food, the GS extract was added at a 20-microliter dosage when the propionic acid was added to the food.

2.3 Cold Sorting

To cold sort, the vials of flies were first submerged in an ice bucket for around 2 minutes and then using a feather they were sorted for gender on the *Teca* cold sorting machine at 2°C. Female flies were isolated and cold anesthetized for ease of transfer to social space assay apparatus.

2.4 High Impact Trauma Device

The High Impact Trauma (HIT) device was constructed to induce traumatic injury (TI) in female fruit flies. The HIT device is used as a traumatic brain injury model. Refer to the above experimental setup (*Figure 2*) when building a HIT device. The HIT procedure is an adapted version of the method used by (Katzenberger et al., 2013; Chauhan & Chauhan, 2019) which consists of a 32 cm screen door spring, platform jack, lab ring stand and clamps. For each trial, the vial attached to the spring was released five consecutive times against the platform jack to induce trauma in five strikes to maintain a constant amount of force in each trial.

2.5 *Gelsemium sempervirens* Extract

Gelsemium sempervirens extract was administered in the fly food for female fruit flies and obtained from *Boiron USA* at a 30-centesimal dilution. The extract was further diluted for flies based on the mass-to-dosage ratio for humans. After increasing a hundred-fold from the initial calculation of 0.02 microliters for one batch of food, 20 microliters were administered to a batch of 100 food vials using a micropipette.

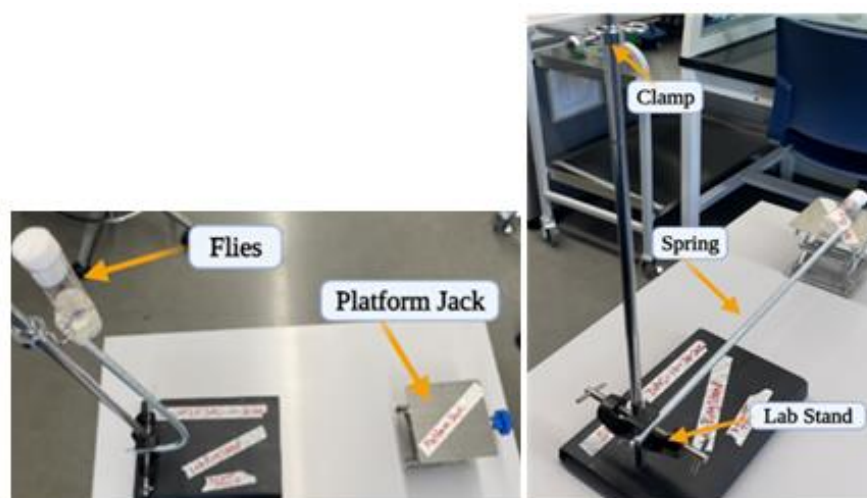


Figure 2. Maternal trauma device constructed

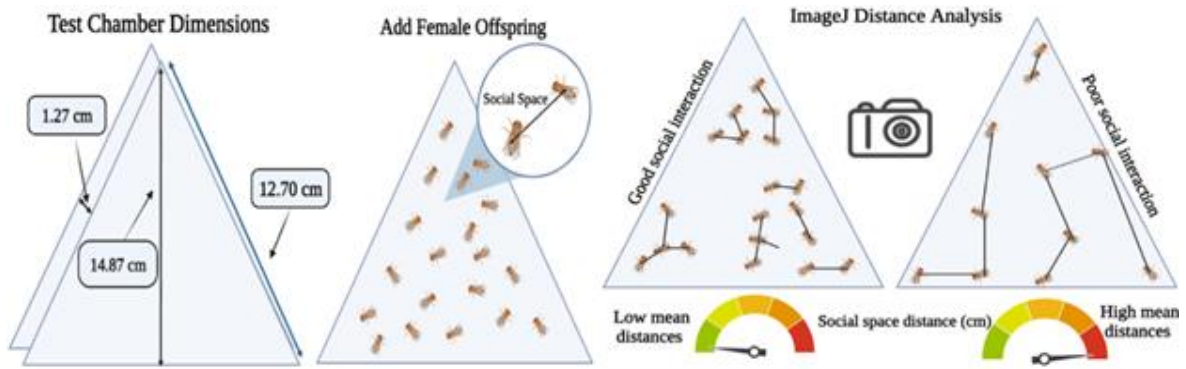


Figure 3. Social space assay methodology

2.6 Social Space Assay

5-6 days old female offspring were sorted and transferred to the triangular test chamber (14.87 cm x 12.7 cm x 12.7 cm) after cold sorting. Female flies were used as they kept a more consistent level of interaction and movement during the socialization period inside the triangular test chamber when compared to male flies. The flies were in the triangular test chamber for 20 minutes to allow for adequate social interaction and a consistent social space distance. After the socialization period was completed, an image was captured of the aggregation of the flies (refer to Figure 3). The sample size for each social space assay is around 15-20 flies. The image was uploaded to *ImageJ* software (with plug-ins) and a distance analysis was conducted at a set scale of 14.87 centimeters (height of

the test chamber). The mean distance of all the social space measurements of the female offspring was recorded as one trial. When the offspring were closer together and aggregated, there was a higher social interaction level represented by lower mean social space distances. If the flies are more isolated, there are higher mean social space distances, indicative of poorer social interaction levels.

3. RESULTS

3.1 Data

Ten trials of social space assays were conducted for each group shown in Figure 4. The mean social space distances from *ImageJ* were recorded and are shown in Table 1.

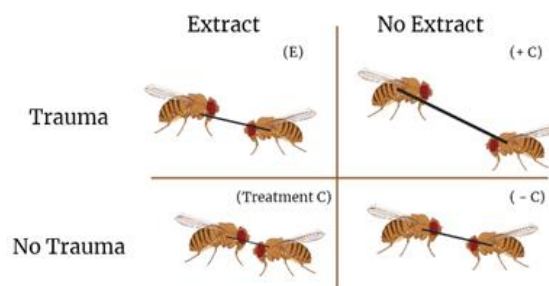


Figure 4. Groups and expected results

Table 1. Mean social space distances (cm)

Trial	No Trauma & No Extract	No Trauma w/ Extract	Trauma & No Extract	Trauma & Extract
1	2.9863	2.1106	5.2145	0.6656
2	3.309	1.538	3.5605	0.9402
3	2.3557	1.071	1.953	1.3334
4	1.5925	0.7563	1.8702	1.5165
5	0.9155	1.0034	1.7705	1.1942
6	1.4245	1.0467	1.9121	1.4208
7	1.2396	0.9819	1.5211	1.823
8	1.2445	1.8731	2.2017	1.1996
9	1.4212	1.5532	1.6074	1.0351
10	1.4174	0.9156	1.8251	0.921
Group Averages (cm):	1.7985 cm	1.2849 cm	2.34361	1.2049 cm

3.2 Graph of Average Social Space Distance Comparison

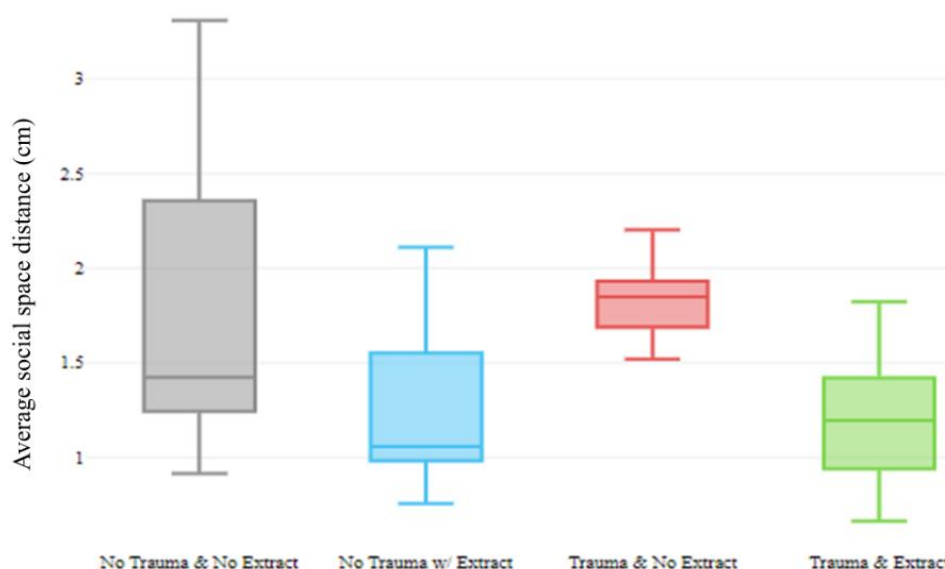


Figure 5. GS significantly improves social interaction

Figure 5 corresponds to the data shown in Table 1, showing the distribution of the average social space distances across ten trials in each data collection group. Table 2 shows the p-values from the Mann-Whitney U Test conducted for statistical analysis. When calculating the p-values in relation to the Trauma & No Extract group, the outliers of 5.23145 and 3.5605 cm were removed as they were around two standard deviations away from the mean in that specific group.

3.3 Statistical Analysis

Table 2. Mann-Whitney U Test P-values

Comparison	P-value
No Trauma & No Extract VS. No Trauma & Extract	0.1655
Trauma & No Extract VS. Trauma & Extract	0.0433*
Trauma & No Extract VS. No Trauma & No Extract	0.01828*
No Trauma & No Extract VS. Trauma & Extract	0.06432

*All p-values in boldface indicate a significant result where $p < 0.05$ for a two-tailed test.

DISCUSSION

4.1 Data Analysis

The p-value of 0.1655 shows that there is no significant difference in social interaction when the extract is used during non-trauma case, which shows treatment is not toxic. P-value of 0.0433 suggests that there is a significant difference in social interaction when the extract is applied in a trauma scenario. A p-value of 0.01828 indicates there is significant difference in social interaction which validates the trauma model with a decrease in social interaction. The p-value of 0.06432 shows that there is no significant difference in social interaction, which shows that the extract in a trauma model brings interaction levels close to when there was no extract and trauma.

4.2 Connection to Current Research

As GS was found as a potential treatment for improving social interaction in offspring flies of the maternal trauma model, the research can shine light on the possibility of further research on neonates, specifically, improving their social interactions. Since GS was found to improve social interaction, this research can apply in the future to current studies among VPT (very preterm) children and improving their parent-infant interaction. In addition, the intervention of fruit flies through the administration of the GS extract shows how the social interaction

outcomes of the progeny can be changed. This elucidates the potential for further research on pregnant women using dietary supplements containing the GS extract to alleviate reduced social interaction in neonates.

4.3 Limitations & Errors

A possible limitation could be the dosage of the GS extract as it was not sourced from its pure form and there are no previous studies for GS on flies. This was a challenge as it was hard to find a benchmark for the right level of toxicity for the flies. Standard delivery of the level of trauma induced by the HIT device was limited as it was difficult to control for the same amount of force being induced on the flies. However, this was mitigated by using a platform jack and clamps to have an approximately constant spring force.

4.4 Future Work

Possible future work involves studying alternatives to GS extract such as *Lycopodium* extract, which has been known to improve stage fright in humans. This suggests the possibility of countering increased anxiety and improving social interaction. Other areas of future research could be investigating other mimicked birth defects in flies such as decreased climbing ability in relation to reduced motor skills in neonates because of premature birth.

5. CONCLUSION

Based on the results, when maternal fruit flies experienced TI and were subsequently treated with GS extract, offspring social interaction increased relative to untreated, injured maternal flies. Therefore, hypothesis is supported, and the scope of the project has been met as the GS extract has been found to be a valid treatment for improving social interaction for offspring flies under the maternal traumatic injury model. The research provides path for the future possibility of research on neonates and improving their social interactions.

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Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

- Bellavite P, Bonafini, C & Marzotto M (2018) Experimental neuropharmacology of Gelsemium sempervirens: Recent advances and debated issues. *Journal of Ayurveda and Integrative Medicine*, 9(1), 69–74. <https://doi.org/10.1016/j.jaim.2017.01.010>
- Chauhan V & Chauhan A (2019). Traumatic injury in female *Drosophila melanogaster* affects the development and induces behavioral abnormalities in the offspring. *Behavioral and Brain Functions*, 15(1). <https://doi.org/10.1186/s12993-019-0163-1>
- Crnic KA, Ragozin AS, Greenberg MT, Robinson NM & Basham RB (1983) Social Interaction and Developmental Competence of Preterm and Full-Term Infants during the First Year of Life. *Child Development*, 54(5), 1199. <https://doi.org/10.2307/1129675>
- Drost TF, Rosemurgy AS, Sherman HF, Scott LM & Williams, JK (1990) Major Trauma in Pregnant Women: Maternal/fetal Outcome. *Journal of Trauma and Acute Care Surgery*, 30(5), 574. https://journals.lww.com/jtrauma/Abstract/1990/05000/Major_Trauma_in_Pregnant_Women_Maternal_fetal.8.aspx
- Esposito TJ, Gens DR, Smith LG & Scorpio R (1989) Evaluation of Blunt Abdominal Trauma Occurring during Pregnancy. *Journal of Trauma and Acute Care Surgery*, 29(12), 1628. https://journals.lww.com/jtrauma/Abstract/1989/12000/Evaluation_of_Blunt_Abdominal_Trauma_Occurring.6.aspx
- Goodwin TE & Breen MS (1990) Pregnancy outcome and fetomaternal hemorrhage after noncatastrophic trauma. *American Journal of Obstetrics and Gynecology*, 162(3), 665–671. [https://doi.org/10.1016/0002-9378\(90\)90979-h](https://doi.org/10.1016/0002-9378(90)90979-h)
- Groh AM, Fearon RMP, van IJzendoorn MH, Bakermans-Kranenburg MJ & Roisman GI (2017) Attachment in the Early Life Course: Meta-Analytic Evidence for Its Role in Socioemotional Development. *Child Development Perspectives*, 11(1), 70–76. <https://doi.org/10.1111/cdep.12213>
- Johnson, S (2007) Cognitive and behavioural outcomes following very preterm birth. *Seminars in Fetal and Neonatal Medicine*, 12(5), 363–373. <https://doi.org/10.1016/j.siny.2007.05.004>
- Katzenberger RJ, Loewen CA, Wassarman DR, Petersen AJ, Ganetzky B & Wassarman DA (2013) A *Drosophila* model of closed head traumatic brain injury. *Proceedings of the National Academy of Sciences*,

110(44), E4152–E4159.
<https://doi.org/10.1073/pnas.1316895110>

McCollum JA (1984) Social interaction between parents and babies: validation of an intervention procedure. *Child: Care, Health and Development*, 10(5), 301–315.
<https://doi.org/10.1111/j.1365-2214.1984.tb00188.x>

Meichtry LB, Poetini MR, Dahleh MMM, Araujo SM, Musachio EAS, Bortolotto VC, de Freitas Couto S, Somacal S, Emanuelli T, Gayer MC, Roehrs R, Guerra GP & Prigol M (2020) Addition of saturated and trans-fatty acids to the diet induces depressive and anxiety-like behaviors in *Drosophila melanogaster*. *Neuroscience*, 443(5), 164–175.
<https://doi.org/10.1016/j.neuroscience.2020.07.042>

Simon AF, Chou MT, Salazar ED, Nicholson T, Saini N, Metchev S & Krantz DE (2012) A simple assay to study social behavior in *Drosophila*: measurement of social space within a group. *Genes, Brain, and Behavior*, 11(2), 243–252.
<https://doi.org/10.1111/j.1601-183X.2011.00740.x>

WHO. (2022, November 14). *Preterm birth*. www.who.int; World Health Organization.
<https://www.who.int/news-room/fact-sheets/detail/preterm-birth#:~:text=An%20estimated%2015%20million%20babies%20are%20born%20too%20early%20ever>
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