

Methodology:

Role of Student vs. Mentor

I independently conducted the chemosensory and negative geotaxis assays, prepared the Ashwagandha concentrations, and induced the *Drosophila*. My mentor provided guidance by teaching me how to complete the assays and providing me with the appropriate materials. This project has been ongoing for four months during which I have conducted experiments and analyzed data.

Equipment and Materials

The study will use basic *Drosophila* research tools such as fly vials, culture media, and incubators with regulated temperatures to maintain the experimental groups. Moreover, the study will use behavior assays that include a chemosensory response test and a negative geotaxis assay to assess sensory responsiveness and motor function. Then, ashwagandha powder will be used for the experimental group of *Drosophila*.

Techniques

In this study, three important techniques were used to assess the outcome of GABAergic regulation in *Drosophila*: the negative geotaxis assay, which measures locomotor function by recording the fly's ability to climb after being tapped down; it helps in the evaluation of motor impairments and the possible restorative effects of Ashwagandha. The chemosensory assay-two-choice test explores the sensory response of *Drosophila* against sucrose and finds whether GABA deficiency affects their ability to respond to stimuli. Finally, inducing the *Drosophila* consists of keeping the flies under optimal conditions and adding Ashwagandha to their food to assess its impact on behavior. The methodologies mentioned have helped investigate the dysfunction in GABAergic function and potential interventions.

Negative Geotaxis Assay

The negative geotaxis test was conducted to assess the locomotor function in *Drosophila melanogaster* following treatment with different concentrations of Ashwagandha (0.3%, 0.6%, and 1.2%). Negative geotaxis is an innate escape behavior where flies climb upwards after being knocked to the bottom of a vial. This assay provided a quantitative measure of mobility and coordination by measuring the proportion of flies that could climb past a marked cutoff point within 10 seconds. Since impaired locomotion is a prominent characteristic of catatonia-like symptoms, this assay was essential for determining whether Ashwagandha treatment mitigated motor deficits associated with GABAergic dysfunction.

Chemosensory Assay

Two-choice chemosensory assay was carried out to evaluate the gustatory reaction of *Drosophila melanogaster* after treatment with different concentrations of Ashwagandha (0.3%, 0.6%, and 1.2%). The assay measured the ability of the flies to detect and respond to sucrose, a crucial behavior based on sensory detection and neural processing. Flies were kept in a chamber where they would be tested between sucrose solution or water, with positions taken at 1, 5, 7, and 10-minute intervals. Since impaired sensory processing is often associated with neurological dysfunction, this assay was critical for determining whether Ashwagandha enhanced chemosensory function in flies with reduced GABAergic activity.