

Impact of the extracts and fractions of *Pennisetum purpureum* on the locomotive ability of *Drosophila melanogaster* under oxidative stress condition

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ABSTRACT

Oxidative damage can adversely affect the climbing performance and acetylcholine levels of *Drosophila melanogaster*. This study evaluated the protective effects of *Pennisetum purpureum* on climbing performance and acetylcholine levels in fruit flies exposed to hydrogen peroxide (H_2O_2). In this study, fruit flies were exposed to various concentrations of extracts and fractions of *P. purpureum*. Climbing ability was assessed using the method described by Abolaji et al. (2020) and Sharma et al. (2022), while the acetylcholine assay was conducted following the protocols of Ream et al. (2003) and Makos et al. (2009). The results revealed a significant increase in climbing performance and acetylcholine levels in flies treated with various concentrations of extracts and fractions compared to the control ($P > 0.05$). The H_2O_2 treated control group exhibited the lowest values, which were significantly different from those of the treated groups. These findings suggest that the extracts and fractions of *P. purpureum* enhanced climbing ability and increased acetylcholine levels in *D. melanogaster*, indicating a protective effect against oxidative damage-induced locomotive impairment.

Keywords: Climbing performance, hydrogen peroxide, acetylcholine, *P. purpureum*, *D. melanogaster*, oxidative damage.

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INTRODUCTION

Drosophila has a well-developed nervous system and offers several advantages for studying the nerve physiology of behavioral traits, as well as the effects of genetic and environmental factors (Mishra and Barik, 2018). Locomotion is a robust motor pattern that reflects the health of an organism's neuronal system.

The climbing assay is a behavioral and neurological parameter used to measure the climbing rate (locomotor performance) of flies. This is determined by either the number of flies that climb beyond a specific distance within a set time or the time it takes to climb a defined distance (Alexander et al., 2019). The climbing assay is widely used in research on various central nervous system (CNS) diseases and neurodevelopmental disorders. Neurobehavioral changes following brain injury can also be effectively identified using this assay (Rubela

et al., 2019).

Oxidative stress can cause cellular damage, affecting various physiological processes, including muscle contraction and coordination. Studies have shown that increased oxidative stress correlates with reduced climbing performance in *Drosophila*, making the climbing assay a valuable tool for evaluating the impact of oxidative stress on motor function (Zhu et al., 2025). Climbing ability has been shown to be impaired by oxidative damage resulting from exposure to toxic chemicals. Under oxidative stress conditions, such as exposure to reactive oxygen species (ROS), the locomotive ability of flies may decline due to damage to muscle and nervous system tissues (Abolaji et al., 2020; Jordan et al., 2012). The climbing assay can also be used to assess the efficacy of potential antioxidant

treatments or dietary interventions aimed at mitigating oxidative stress. By comparing the climbing performance of treated and untreated flies, researchers can determine the protective effects of specific compounds against oxidative damage (Zhu et al., 2025).

Acetylcholine is a neurotransmitter involved in regulating motor functions and neuronal activity in the brain. Acetylcholinesterase, on the other hand, is an enzyme that breaks acetylcholine down into acetic acid and choline (Anadozie et al., 2019; Chen et al., 2022; Scholastical et al., 2024). Assessing acetylcholine levels can provide insights into how oxidative stress affects the nervous system and, consequently, the locomotive ability of *D. melanogaster*. Hydrogen peroxide (H₂O₂) is known to induce oxidative stress, which can impair neurotransmitter release and function. By measuring acetylcholine levels, researchers can determine the extent of neurotoxicity and its correlation with locomotion deficits.

The acetylcholine assay in this study can help elucidate the mechanisms through which oxidative stress affects neuronal function and how the plant extracts or fractions under investigation may counteract these effects. This understanding is crucial for developing potential therapeutic strategies for neurodegenerative conditions.

The efficacy of plant extracts or fractions can be solvent-dependent, as different solvents may influence biological activity (Gonçalves et al., 2024). In this study, various extracts and fractions of the plant were administered to fruit flies to identify the specific treatment(s) responsible for the observed locomotive effects, determine whether certain fractions enhance or diminish activity, and obtain a more comprehensive profile of the bioactive compounds present in the plant. This approach increases the likelihood of identifying effective compounds (Lee et al., 2022; Park et al., 2011; Venkatesan et al., 2019) and understanding their mechanisms of action (Zhao et al., 2019).

Different compounds have varying solubilities depending on the solvent used. By employing multiple solvents, a broader range of active compounds can be extracted from the plant or material being studied (Gonçalves et al., 2024). Solvents differ in polarity, which affects the types of compounds they extract. For example, polar solvents may extract hydrophilic compounds, while non-polar solvents are more effective for hydrophobic compounds. This approach allows for a more comprehensive analysis of the extract's effects (Park et al., 2011).

MATERIALS AND METHODS

Preparation of *Pennisetum purpureum* Extracts

The extraction was performed using the cold maceration

method as described by Garcia et al. (2025). The leaves were removed from the whole plant, sliced, chopped, and air-dried at room temperature for 21 days. The dried parts were then coarsely powdered.

A total of 400 mL of the extracting solvent was added to 20 g of *Pennisetum purpureum* (1:20, w/v) in a 1,000 mL beaker. The mixture was then macerated in a water bath (LABEC, Marrickville, Australia) at 70 °C for 40 minutes. Subsequently, the mixture was homogenized at a temperature of 55–80 °C by constant shaking for 4 hours using a homogenizer (IKA, Germany). The filtrate was separated from the residue by filtration using filter paper. This process was repeated three times to obtain the extract exhaustively. The extract solution was concentrated using a rotary evaporator (Pollab, India) at 40 °C and dried at room temperature. The dried extract samples were stored in airtight containers at 4 °C. The same procedure was repeated to obtain the aqueous extract (Garcia et al., 2025).

Fractionation by liquid-liquid extraction

The methanol extract of *Pennisetum purpureum* (PP) was dissolved in distilled water in a separating funnel, equilibrated, and successively extracted with n-hexane, ethyl acetate, and methanol (85:15, v/v) (Sigma-Aldrich, Singapore) to obtain fractions of various polarities (Huseynov, 2022). Fractionation was performed in triplicate. The organic fractions (n-hexane, ethyl acetate, and methanol) were then concentrated under reduced pressure using a rotary evaporator. The fractions were dried at a temperature of 25 °C and stored in the refrigerator until required for use (Huseynov, 2022).

Lethal concentration (LC50) determination

The LC50 is defined as the concentration of substances in a normal fly food medium that causes 50% of fly deaths in 7 days. The concentration used in this study was obtained by exposing flies to different concentrations of the plant extracts and fractions for 7 days, as described by Afolabi et al. (2019). Thereafter, the LC50 of the plant extracts and fractions was determined as follows: 300.61 mg per 10 g diet for the methanol extract, 320.94 mg per 10 g diet for the aqueous extract, 150.08 mg per 10 g diet for n-hexane, 480.24 mg per 10 g diet for the ethyl acetate fraction, and 820.25 mg per 10 g diet for the methanol fraction. These values indicate that the extracts and fractions are relatively safe.

Climbing assay (Behavioral assay)

The climbing assay was performed based on the method

described by Abolaji et al. (2020) and Chaudhari et al. (2002). Briefly, fifty (50) flies, in triplicate, were fed different concentrations of methanol extract, aqueous extract, n-hexane, ethyl acetate, and methanol fractions of the plant for 7 days. To induce oxidative stress, flies in all treatment groups were exposed to hydrogen peroxide (Ullah et al., 2021; Sharma et al., 2022). The treated and untreated flies were immobilized under mild ice anesthesia and placed separately in labeled vertical glass columns (length: 15 cm, diameter: 1.5 cm).

After a recovery period of about 20 minutes, the flies were gently tapped to the bottom of the vials, and the number of flies able to climb 5 cm in 6 seconds was recorded at each interval. The assay was repeated three times at 1-minute intervals. The scores represent the mean number of flies at the top (n_{top}) and the bottom (n_{bot}), expressed as a percentage of the total number of flies (n_{tot}). Results are presented as the mean \pm SEM of the scores obtained in three independent experiments. For each experiment, a performance index (PI) was calculated as follows:

$$PI = \frac{1}{2} [(n_{tot} + n_{top} - n_{bot}) / n_{tot}] \quad (\text{Chaudhari et al., 2002}).$$

Acetylcholine (ACh) activity

After 7 days of extract/fraction treatment, flies were anesthetized on ice, homogenized in 1:10 volumes of 100 mM phosphate-buffered saline (pH 7.4), and centrifuged (Eppendorf AG, 5227 R, Germany) at 4 °C for 10 minutes at 4,000 rpm. The supernatant was cooled and used for the determination of ACh activity, following the method described by Ream et al. (2003) and Makos et al. (2009), with slight modifications.

To the reaction mixture containing 285 μ L of distilled water, 180 μ L of 100 nM potassium phosphate buffer (pH 7.4), 60 μ L of 10 mM DTNB, and 15 μ L of sample, 60 μ L of 8 mM acetylcholine was added. The change in absorbance was examined at 412 nm for 2 minutes at 10-second intervals using a UV-VIS spectrophotometer (Jenway 7315). The total protein concentration of the fly homogenate was determined using total protein kits (Randox) according to the manufacturer's instructions. Data were calculated using blank and sample blanks, and results were normalized to protein content. Acetylcholine activity was expressed as micromoles/min/mg of protein (Ream et al., 2003; Makos et al., 2009).

Statistical analysis

The results were expressed as mean \pm SEM, where applicable. The data were subjected to ANOVA (Analysis of Variance) using SPSS software (version 20). When ANOVA was significant, a post hoc test (Fisher's least

significant difference) was carried out. A difference was considered statistically significant at $P < 0.05$.

RESULTS

The locomotor function, evaluated by the climbing performance of different concentrations of the n-Hexane fraction of *Pennisetum purpureum*, showed a significant difference at ($p < 0.05$) when compared to the control. There was also a significant difference between the different concentrations of the n-Hexane fraction. Furthermore, the decline in the climbing ability of flies increased with concentration, with LC6.25 showing the best climbing ability, followed by LC12.5, then LC25, and the least at LC50. This suggests that flies fed with the n-Hexane fraction had a lower climbing ability than the control, and the observed effect was concentration-dependent. The performance index for the different concentrations of n-Hexane ranged between 28% and 74% (Figure 1).

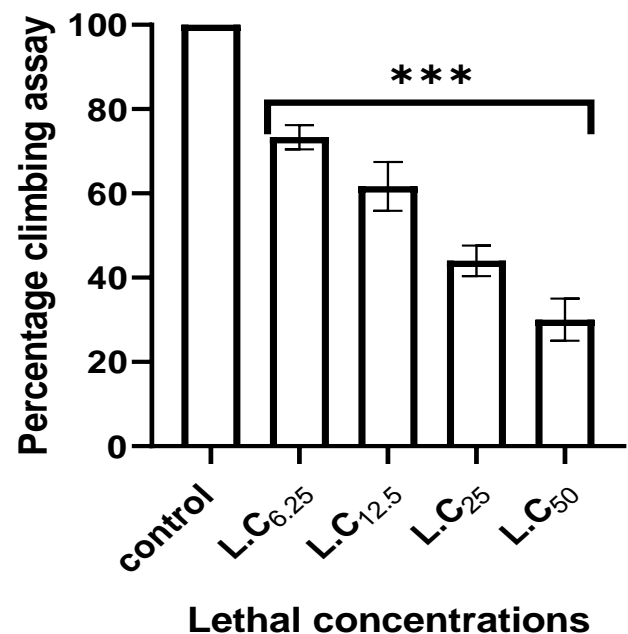


Figure 1. The climbing performance of the lethal concentration of n-Hexane fraction of *Pennisetum purpureum* in *Drosophila melanogaster*.

For the ethyl acetate fraction, there was no significant difference between the control and the lowest concentration of the fraction (LC6.25). However, there was a significant difference between the control and the remaining concentrations of the ethyl acetate fraction (LC12.5, LC25, and LC50) (Figure 2).

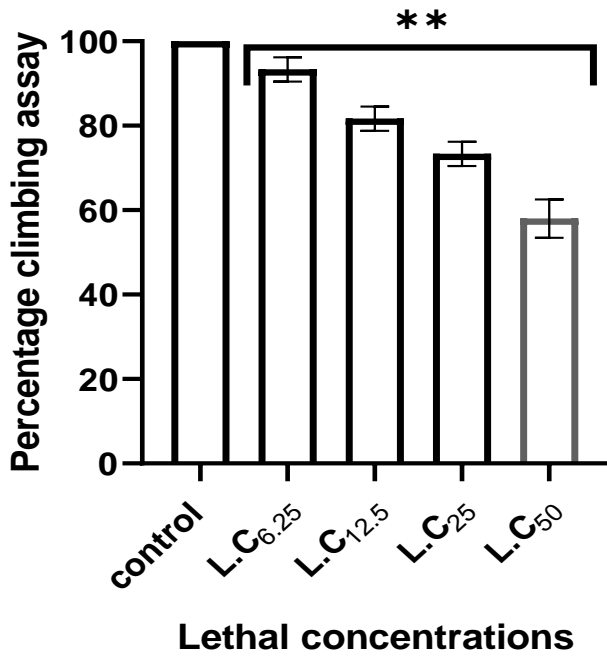


Figure 2. The climbing performance of the lethal concentration of ethyl acetate fraction of *Pennisetum purpureum* in *Drosophila melanogaster*.

This suggests that the climbing ability of the flies decreased with increasing concentration. The percentage decrease in this case was not as pronounced as that observed for the n-Hexane fraction, indicating that flies fed with the ethyl acetate fraction had better climbing ability than those fed with the n-Hexane fraction. The performance index for the various concentrations of the ethyl acetate fraction ranged between 55% and 92% (Figure 2) when compared to the normal control (flies fed with the vehicle alone).

In the case of the methanol fraction, there was no significant difference between flies fed with three different concentrations of the methanol fraction (LC6.25, LC12.5, and LC25) and the normal control (Figure 3). A significant difference was observed only between the normal control and the highest concentration of the fraction (LC50) and between the two highest concentrations (LC25 and LC50). The performance index for the various concentrations of the methanol fraction ranged between 92% and 100% when compared to the normal control (Figure 3).

For the aqueous crude extract, there was no significant difference between flies fed with various concentrations of this extract and the control (Figure 4). The performance index ranged between 97% and 100% (Figure 4), with no significant difference compared to the control.

For the methanol extract, there was no significant difference between *Drosophila melanogaster*

supplemented with three different concentrations of the crude extract (LC6.25, LC12.5, and LC25) and the control (Figure 5).

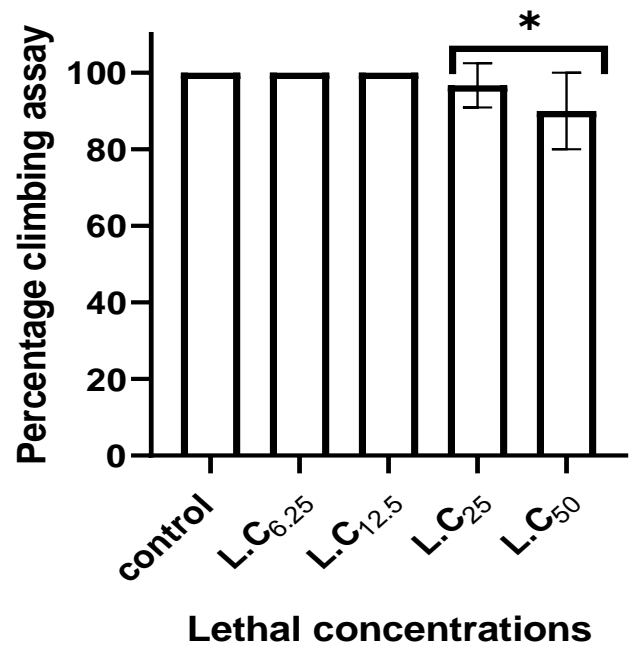


Figure 3. The climbing performance of the lethal concentration of methanol fraction of *Pennisetum purpureum* in *Drosophila melanogaster*.

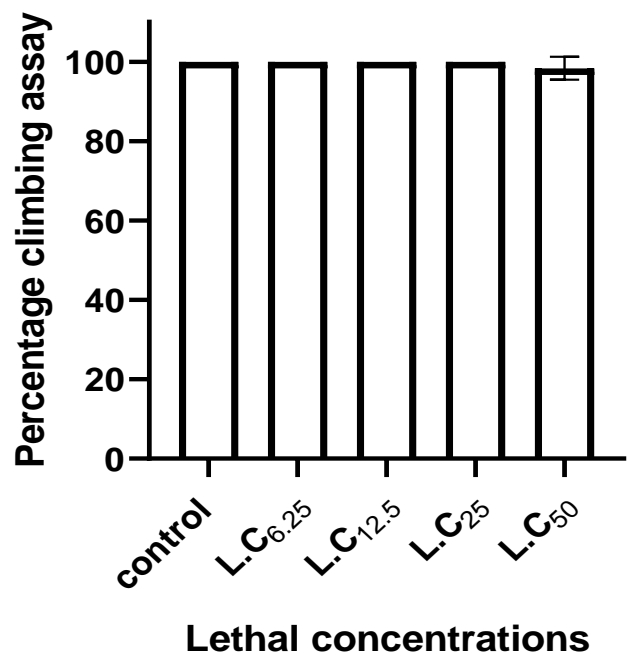


Figure 4. The climbing performance of the lethal concentration of aqueous of *Pennisetum purpureum* in *Drosophila melanogaster*.

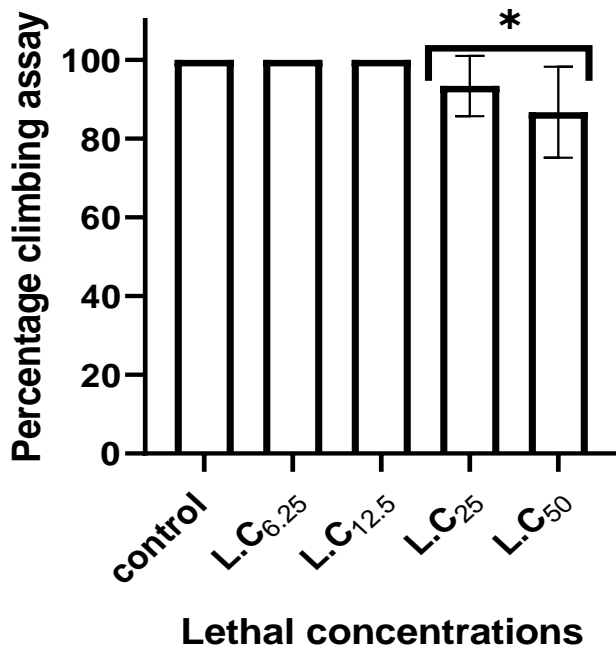


Figure 5. The climbing performance of the lethal concentration of methanol extract of *Pennisetum purpureum* in *Drosophila melanogaster*.

A significant difference was observed only between two concentrations of the crude extract (LC25 and LC50). Higher climbing performance was observed at lower concentrations of the extract (LC6.25 and LC12.5), and as the concentration increased (LC25 and LC50), the climbing ability decreased, though not remarkably. The performance index for the various concentrations of the methanol extract ranged between 85% and 100% when compared to the normal control (Figure 5).

For the standard drug (Ascorbic acid), there was no significant difference between *Drosophila melanogaster* supplemented with three different concentrations of the standard drug (LC6.25, LC12.5, and LC25) and the control (Figure 6). A significant difference was observed only between two concentrations of the standard drug (LC25 and LC50). Higher climbing performance was observed at lower concentrations of the standard drug (LC6.25 and LC12.5), and as the concentration increased (LC25 and LC50), the climbing ability decreased, though the effect was not remarkable. The performance index of the various concentrations of the standard drug ranged between 90% and 100% (Figure 6), when compared to the normal control.

There was a significant difference between the different concentrations of the toxicant group (H_2O_2) and the normal control. Additionally, there was a decline in the climbing ability of flies with increasing concentration, with LC6.25 showing relatively fair climbing ability, followed by LC12.5, then LC25, and the least at LC50 (Figure 7).

These results suggest that H_2O_2 significantly decreased the climbing ability of the flies in a concentration-dependent manner, implying that as the concentration of the toxicant increased, the climbing ability of the flies decreased. The performance index for the different concentrations of the toxicant group ranged between 7% and 62% (Figure 7), when compared to the normal control.

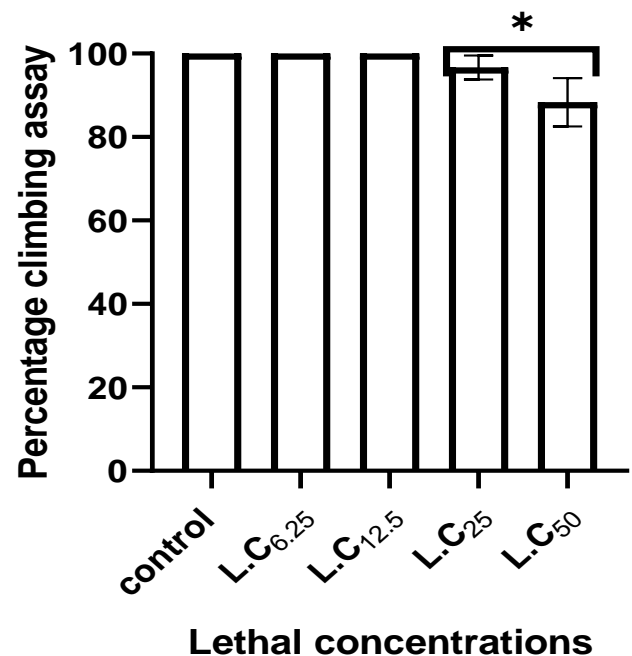


Figure 6. The climbing performance of the lethal concentration of Ascorbic acid in *Drosophila melanogaster*.

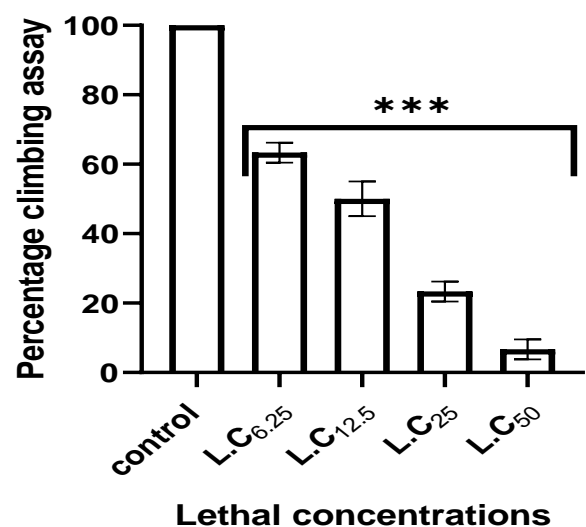


Figure 7. The climbing performance of the lethal concentration of hydrogen peroxide in *Drosophila melanogaster*.

Acetylcholine activity

Table 1 shows the acetylcholine activity (mmol/min/mg protein) at the lethal concentrations of *Pennisetum purpureum* fractions in *D. melanogaster*. The results revealed that H₂O₂ (the toxicant group) caused a significant decrease in acetylcholine activity across all concentrations (LC6.25, LC12.5, LC25, and LC50). However, there was a significant decrease in acetylcholine activity across all treatment groups supplemented with *Pennisetum purpureum* extracts and fractions.

At LC6.25, the methanol extract had a significantly

higher effect than all other extracts and fractions of *Pennisetum purpureum*, and its effect was greater than that of the standard drug (Ascorbic acid). At LC12.5, the methanol extract also had a significantly higher effect compared to other extracts and fractions. At LC25, the aqueous extract had a significantly higher effect than all other *Pennisetum purpureum* extracts and fractions, with the observed effect greater than that of the standard drug (Ascorbic acid). Both the aqueous extract at LC25 and the methanol extract at LC6.25 had significantly higher effects than the standard drug. At LC50, the methanol extract had a significantly higher effect than other extracts and fractions of *Pennisetum purpureum*.

Table 1. Acetylcholine activity (mmol/min/mg/protein) of lethal concentration of *Pennisetum purpureum* fractions in *Drosophila melanogaster*.

Fractions	LC _{6.25}	LC _{12.5}	LC ₂₅	LC ₅₀
Ethyl acetate	1.33±0.01 ^h	0.95±0.02 ^h	0.75±0.02 ^h	0.72±0.01 ^h
n-Hexane	1.92±0.02 ^g	1.87±0.01 ^g	1.93±0.01 ^g	1.82±0.03 ^g
Methanol	2.36±0.03 ^f	2.07±0.01 ^f	1.95±0.01 ^f	1.87±0.01 ^f
Aqueous	1.87±0.01 ^e	1.94±0.01 ^e	2.75±0.01 ^{e#}	1.85±0.01 ^e
Methanol crude	2.64±0.01 ^{d#}	2.54±0.02 ^d	2.73±0.02 ^{d#}	2.23±0.02 ^d
Standard	2.44±0.02	2.74±0.01	2.52±0.03	2.44±0.01
H ₂ O ₂	0.66±0.01	0.66±0.01	0.66±0.01	0.66±0.02
Control	1.45±0.01	1.45±0.01	1.45±0.01	1.45±0.01
F	1571.938	3666.065	5563.766	3356.449

Means under the same column tagged with different letter alphabets compares significantly different with ^aControl, ^bH₂O₂, ^cStandard.

[#]Means under the same column with significant higher intended effect than standard fraction.

DISCUSSION

Drosophila has a well-developed nervous system and provides several benefits for studying the nerve physiology of behavioral traits and the endpoints of genetic and environmental factors (Mishra and Barik, 2018). Locomotion is a robust motor pattern that indicates the health of an organism's neuronal system. The performance index in the climbing assay is used to assess the effects of drug treatment on fly locomotor function. Climbing assays performed in various studies offer insights into how the performance index can be classified based on experimental data.

The classification of the performance index into categories such as excellent, good, average, and poor was first proposed by Gargano et al. (2005). This classification is based on comparing the climbing abilities of different groups of flies, typically determined by setting cutoff values for the climbing time or heights reached by flies and then categorizing their performance accordingly (Simon et al., 2009; Gargano et al., 2005; Coulom and Birman, 2004).

In the current study, the climbing ability of the organism after exposure to various concentrations of *Pennisetum purpureum* (aqueous extract, methanol extract, and

fractions) for seven days was assessed. The results showed an insignificant change in the climbing behavior of the organism in most treatment groups compared to the control. For the n-hexane and ethyl acetate fractions (Figure 1-2), there were significant differences between various concentrations of the fractions and the control, with the n-hexane fraction showing a greater significant difference than the ethyl acetate fraction. However, considering the performance index, the n-hexane fraction had a range of 0.28–0.74, which indicates moderate performance. Flies in this range exhibit a moderate level of motor function and climbing ability, which could be observed in younger or mildly affected flies. This could also represent an intermediate stage of age-related decline or the result of partial rescue or improvement in motor function (Tinkerhess et al., 2012).

The ethyl acetate fraction had a performance index of 0.55–0.92 (Figure 2), indicating good performance. Flies in this range exhibit robust motor function and climbing ability, considered within the “normal” or “wild-type” range. This is typically observed in young, healthy flies or in those with interventions that enhance locomotor performance (Stats et al., 2018; Rogina et al., 2000).

The aqueous extract, methanol extract, and fraction had performance indices of 0.97–1.0, 0.85–1.0, and

0.92–1.0 (Figure 4, 5 and 3), respectively, which are interpreted as excellent performance. Flies in this range display exceptional motor coordination and climbing ability, often seen in genetically or pharmacologically enhanced individuals. This high-performance range may be the target for interventions aiming to maintain or improve motor performance in *Drosophila melanogaster*.

A study by Moskalev et al. (2019) showed that the overexpression of the Ryanodine receptor protein in *Drosophila melanogaster* muscles led to a climbing performance index of around 95–100% (Figure 7), compared to control flies. For the toxicant group (H_2O_2), the performance index ranged between 0–62%. Compared to the control group, this range includes very poor performance (0–30%) to moderate performance (50–69%). Flies in the poor performance range exhibit severe difficulties in coordinating movement and climbing against gravity, indicating severe locomotor dysfunction and impairment. This condition is often observed in highly aged or severely compromised flies, including transgenic models with specific manipulations that impair motor functions (Feany, 2000). A study by Rogina et al. (2000) found that the climbing performance of *Drosophila melanogaster* with a mutation in the *Indy* gene decreased by around 10% at 50 days of age compared to wild-type flies, which maintained a performance index of approximately 80–90%.

The results in all treatment groups are comparable to studies conducted by Asejeje et al. (2024), Pratap et al. (2021), and Singh et al. (2022) on the climbing performance of flies treated with different extracts and fractions. These studies collectively indicate that treatment with specific plant extracts and fractions can lead to significant improvements in the locomotive abilities of *Drosophila melanogaster*, particularly when compared to untreated groups exposed to toxicants. This suggests that the various extracts and fractions helped mitigate the locomotor impairments caused by the toxicant.

Acetylcholine is a neurotransmitter involved in the regulation of motor functions and neuronal activity in the brain. Acetylcholinesterase, on the other hand, is an enzyme that breaks acetylcholine into acetic acid and choline (Anadozie et al., 2019; Chen et al., 2022; Scholastical et al., 2024). In this study, *Pennisetum purpureum* extracts and fractions demonstrated a protective effect on the decreased acetylcholine activity caused by the exposure of flies to H_2O_2 (Table 1). This suggests that *Pennisetum purpureum* may have neuroprotective and locomotion-enhancing properties. The study also complemented the results of the Negative Geotaxis study (Climbing Assay), where *Pennisetum purpureum* restored the impaired locomotor activity caused by H_2O_2 toxicity. The decreased locomotor activity of flies exposed to H_2O_2 suggests that this toxicant can impair locomotor function in *Drosophila*

melanogaster. Flies exposed to H_2O_2 exhibited higher mortality, which may be attributed to oxidative stress. A similar finding was reported by Abolaji et al. (2020), where a toxicant impaired the upward movement of flies, thereby affecting their climbing ability.

Other studies conducted by Avallone et al. (2024); Lopez-Ortiz et al. (2023); and Luna et al. (2021) demonstrated significant increases in acetylcholine (ACh) levels in *Drosophila melanogaster* extract-treated groups when compared to untreated hydrogen peroxide toxicant groups. This increase was found to correlate with enhanced climbing ability, indicating that certain plant extracts not only counteracted toxic effects but also improved locomotion through increased ACh availability. Several mechanisms by which plant extracts and fractions improve locomotion in *Drosophila melanogaster* have been proposed:

- 1. Antioxidant Properties:** Many plant fractions possess antioxidant properties that help alleviate oxidative stress caused by toxicants like hydrogen peroxide. By neutralizing reactive oxygen species (ROS), these compounds can protect neuronal tissues and improve overall motor function (Saha et al., 2020).
- 2. Neuroprotection:** Certain phytochemicals may exert neuroprotective effects by reducing neuroinflammation and preventing neuronal cell death, which is crucial for maintaining nervous system integrity and directly affects locomotion (Kumar et al., 2021).
- Mitochondrial Function Enhancement:** Some plant extracts can improve mitochondrial function and energy production within cells. Improved energy metabolism translates to better muscle function and increased locomotion, as the fly's muscles receive adequate energy for movement (Ghosh et al., 2022).
- 3. Neurotransmitter Modulation:** Plant fractions may influence neurotransmitter levels or receptor activity in the central nervous system. By enhancing signaling pathways involved in motor control, these compounds can improve locomotor activity (Singh et al., 2023).
- 4. Hormonal Influence:** Certain plant-derived compounds can affect hormonal pathways that regulate growth and development, thereby inducing muscle development and coordination, which are crucial for locomotion (Patel et al., 2019).
- 5. Gene Expression Modulation:** Plant fractions can induce or inhibit the expression of genes associated with stress response, neuroprotection, and muscle function, leading to improved locomotor outcomes (Verma et al., 2022).

CONCLUSION

This study indicates that *Pennisetum purpureum* leads to a significant increase in the climbing ability of *Drosophila*

melanogaster across all treatment groups when compared to the negative control. However, the aqueous extract, methanol extract, and fraction exhibited the most significant climbing performance. The study also revealed an improvement in acetylcholine levels across all treatment groups when compared to the negative control, with the methanol extract and fraction showing the most remarkable elevation in acetylcholine levels. The difference in both instances was found to be statistically significant ($P < 0.05$). This suggests that the plant extracts and fractions exert a protective effect on the organism's locomotive performance.

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