RESEARCH PAPER

Neuroprotective Activity of Botanical Study of *Barleria Sahydrica* from the Ambai Region of Maharashtra

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Abstract:
The purpose of this study was to assess the effects of Barleria sahyadrica extract on neuroprotection, anxiety, depression, and motor coordination in experimental animal models. Levetiracetam (10 mg/kg, standard) or Barleria sahyadrica extract (100 and 300 mg/kg, p.o.) were administered to groups of albino rats. The Elevated Plus Maze (EPM), Rotarod Test, Marble Burying Test, Nestlet Shredding Test, and Forced Swim Test (FST) were used to evaluate behaviour. The data were compared to a negative control and presented as mean ± SEM. The extract from Barleria sahyadrica showed notable neurobehavioral effects that were dose-dependent. Groups treated with extract exhibited more open-arm exploration in the EPM, which is indicative of anxiolytic action. The Rotarod Test showed an improvement in motor performance. In the Marble Burying and Nestlet Shredding Tests, obsessive-compulsive behavior was decreased, and the 300 mg/kg dosage had effects similar to those of levetiracetam. The extract demonstrated antidepressant-like effects in the FST by dramatically reducing immobility time. The 300 mg/kg dose continuously showed greater efficacy than the 100 mg/kg dose across all models. The results demonstrate that Barleria sahyadrica has strong neuroprotective potential with anxiolytic, depressive, and anti-compulsive effects. These effects are probably caused by the phytoconstituents in the plant that have neuromodulatory and antioxidant qualities. These findings support its traditional medical application and point to its potential as a natural therapeutic option for the treatment of neuropsychiatric and neurodegenerative illnesses.

KEYWORDS: Barleria Sahyadrica, Neuroprotective Activity, Antioxidant, Antianxiety
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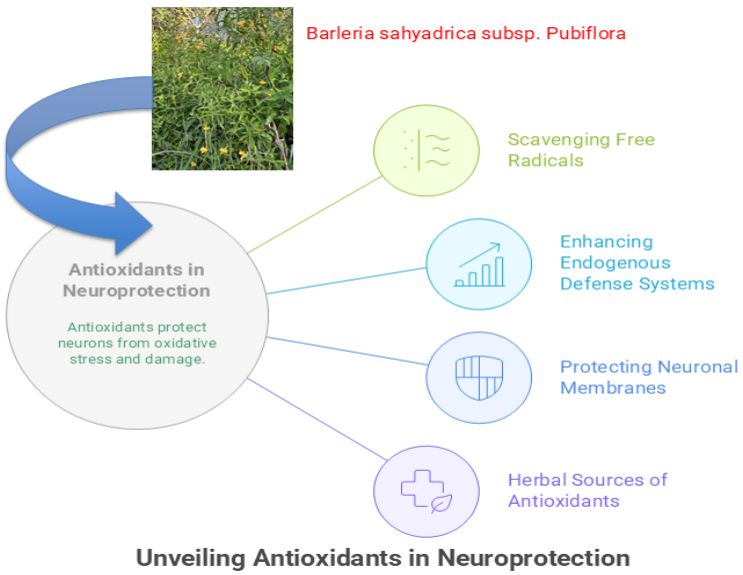
Graphical Abstract:



1. Introduction:

The most recent world Burden of Disease (GBD) 2021 studies have shown that neurodegenerative diseases in particular, Alzheimer's disease, various dementias, and Parkinson's disease—are becoming increasingly important problems and burdens on world health. These illnesses have become more common and severe, and their growing impact is intimately related to the demographic trend toward an elderly population and the possible long-term effects of the COVID-19 pandemic. such is the growing prevalence of neurodegenerative illnesses worldwide, unmet medical and social demands

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in the current healthcare systems, the particular and heightened difficulties brought on by the COVID-19 pandemic, and possible approaches to improving healthcare policy and practice. In order to successfully address the growing burden of neurodegenerative illnesses and enhance the quality of life for patients and their caregivers, we emphasize the critical need for coordinated, interdisciplinary approaches across the medical, scientific, and policy domains (Wang et al., 2024).

A class of illnesses known as neurodegenerative diseases exhibits a number of neuropathological signs. Serious or even fatal degenerative nerve disorders are possible. These age-dependent illnesses are growing increasingly prevalent as a result of the recent increase in the number of elderly people. According to the WHO, neurodegenerative illnesses will surpass cancer as the second most common cause of death in a few years, with cardiovascular disorders coming in first. The fact that all neurodegenerative disorders are linked to some kind of gene mutation that results in subsequent protein malfunction unites them. Alzheimer's disease, Parkinson's disease, Huntington's disease, spinal muscular atrophy, spinocerebellar ataxia, and prions disease are the six neurodegenerative disorders covered in this article (Zaib et al., 2023).

Numerous illnesses have been connected to oxidative stress, which has been implicated in the onset and/or progression of a number of neurodegenerative conditions. The purpose of this study is to provide an overview of some of the research that links the pathogenesis of Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis to an excess of reactive oxygen species. It has also been observed that oxidative stress alters the inflammatory response. Despite being two completely distinct disease processes, oxidative stress and neuroinflammation are connected and have an impact on each other. To create effective treatments, however, a number of factors pertaining to the development and progression of neurodegenerative illnesses still require understanding. The most popular antioxidants, both enzymatic and non-enzymatic, have been highlighted in this study as potential treatments for the disorders under discussion since they can change oxidative stress and reduce the symptoms of certain neurodegenerative diseases (Teleanu et al., 2022; Domanskyi et al., 2022; Sienes et al., 2022).

On the other hand, the imbalance between the production of ROS and their elimination by the body's own antioxidant defence mechanism and antioxidant treatment given to them. The immunological mechanisms and functioning of the cardiovascular systems are harmed by the association between anxiety disorders, depression, and sleeplessness. Numerous phytochemical bioactive and multimodal cellular modes of action, including antioxidant, anti-inflammatory, vasorelaxant, detoxifier, antidepressant, anxiolytic, and cell-rejuvenator capabilities, were found in herbal medicine, according to the evidence gathered from the databases. Other herbal remedies act as agonists of the GABA-A receptor. For this reason, we suggest that theranostics has promise and can be used in future studies to enhance human well-being (Heyat et al., 2022; Sultana et al., 2022).

With over 300 species, mostly found in tropical Africa, South Africa, and Asia, Barleria Linnaeus is one of the most species-rich genera in the Acanthaceae. In India, the genus has 26 species, one subspecies, and one variant (Karthikeyan et al., 2009; Shendage et al., 2010). Barleria prionitis L. is a perennial, bushy, prickly herb that has long been prized for its therapeutic properties. Although it is mostly found in India, it is also widely dispersed throughout Bangladesh, Pakistan, Malaysia, Sri Lanka, the Philippines, and tropical Africa (Banerjee et al., 2021). Current studies indicate that Sahyadrica Barleria prionitis is a significant source of a wide variety of phytochemicals. It has been demonstrated that many secondary metabolites present in plants, such as tannins, alkaloids, flavonoids, and terpenoids, have antibacterial properties. Knowing how plants are used in traditional medicine is beneficial to healers as well as the pharmaceutical industry (Talukdar et al., 2015; Chanda, 2014; Gangaram et al., 2021).

Many of the therapeutic chemicals that are the foundation of contemporary medications have long been thought to originate mostly from plants. The phytochemical richness of Barleria prionitis, sometimes referred to as Sahyadrica locally, has been the subject of extensive research. According to recent research, this plant is an important source of a variety of bioactive substances, such as terpenoids, flavonoids, alkaloids, and tannins. The wide range of pharmacological characteristics of these secondary metabolites, especially their antibacterial and antioxidant capabilities, are well known. The development of neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, and other age-related cognitive deficits is largely influenced by oxidative stress. In order to prevent oxidative damage to neurons, antioxidants are essential for scavenging free radicals. Barleria prionitis is a promising option for research into its neuroprotective effects because of its well-established antioxidant activity.

2. Material And Methods:

2.1 Chemicals and medications:

Every substance and medication used in the experiment was purchased from the neighborhood market and examined by a lab.

2.2 Animals:

The animals were grouped in distinct treatment groups Each group consists of six animals. Eight-week-old boys and girls were given time to acclimate to their surroundings. The animals had unrestricted access to food and water, a strict 12:12 h light-dark cycle, and a temperature-controlled environment (25±1ºC, 55.5% humidity). The experimental protocol, with protocol number TRS/PT/025/067-E, was approved by the IAEC committee.

2.3 Extraction:
Fresh Barleria prionitis (Sahyadrica) plant material was gathered from Ambai region of maharashtra. The Botanical Survey of India Koregaon in Pune verified the authenticity of the specimens that were gathered. The plant material (leaves or entire plant) was rinsed with distilled water after being properly cleaned with running tap water to get rid of dust and debris. For ten to fifteen days, the cleaned material was shade-dried at room temperature (25 to 30 °C) until its weight remained constant. A mechanical grinder was then used to coarsely powder the dried material, which was then kept in an airtight container at 4 °C until it was needed again. 500 g of powdered plant material was first macerated for 24 hours using Pet. Ether (1:10 w/v) to defatten it before the solvent was disposed of. Then, using a Soxhlet apparatus, the defatted marc was extracted with ethanol (1:15 w/v) for 8–10 hours, or until the siphon turned colorless. To produce a semi-solid mass, the mixed extracts were filtered and concentrated in a rotary evaporator at 40 °C under decreased pressure. After being weighed to determine the yield percentage, the dried extract was kept in amber-colored glass vials between 2 and 8 °C until it was needed again.

2.4 Antioxidant DPPH Assay:
Add 1.5 ml of samples in varying concentrations to a test tube. Add 1.5 ml of the DPPH solution after that. Finally, mix the test and blank with 1.5 cc of 95% ethanol. Leave for 20 minutes at room temperature. The absorbance (A) of the solutions was measured at 517 nm in comparison to the equivalent blanks (Kato et al., 1988; Molyneux, 2004).

2.5 Experimental design:
The treatment schedule for the experiment is as follows:
Negative control: Treated with $AlCl_3$ at the dose by 100 mg/kg *po*
Test low dose: Treated with 100 mg/kg Barleria sahyadrica extract +$AlCl_3$ at the dose by 100 mg/kg *po*
Test high dose: Treated with 300 mg/kg Barleria sahyadrica +$AlCl_3$ at the dose by 100 mg/kg *po* (10-12)
Standard: Treated with 10 mg/kg Levetiracetam +$AlCl_3$ at the dose by 100 mg/kg *po* (Khalil et al., 2022).

2.6 Elevated Plus Maze Test:
Using the Elevated Plus Maze (EPM) test, the test compound's anxiolytic impact in adult albino mice was evaluated. The apparatus featured two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) arranged in a plus (+) shape, and it was 50 cm above the ground. As shown above, each group received treatment. A 30-minute intraperitoneal drug administration period was followed by the individual placement of each mouse facing one of the open arms in the centre of the maze. We watched the behaviour of each animal for five minutes. We recorded the number of entrances and the amount of time spent in the open and closed arms. An animal was said to have entered an arm when it inserted all four paws.

2.7 Rotarod test:
They used a rotarod treadmill to test motor coordination. The devices were used to teach the animals three days prior to the testing. They had the rotarod ready. The maze was cleaned with 70% ethanol between trials to eliminate olfactory cues. All experiments were conducted in the light phase of the cycle with constant ambient conditions to gradually increase the acceleration over a 10-second period from 10 to 30 rotations per minute, with a test duration of 300 seconds. The animals spent the entire trial on the horizontal rod, and the time it took for each to fall off was recorded. Three trials each day, once a week, were carried out. The average length of the three trials was used for analysis (de Figueiredo et al., 2023; Abu-Elfotuh et al., 2021).

2.8 Marble Burying Test:
For the marble burial test, polycarbonate rat cages with filter-top lids are typically utilized. They measure 26 cm by 48 cm by 20 cm. To level the bedding surface, place a second cage of the same size on top of the first one. Next, add brand-new, odourless mouse bedding to each cage until it is 5 cm deep. This has the benefit of leaving a parallel line template on the bedding surface that may be utilized for marble installation. On the bedding surface, gently place the typical glass toy marbles in five rows of four. They weigh 5.2 g, have a diameter of 15 mm, and come in a variety of colors and shapes (Witkin et al., 2022).

2.9 Nestlet Shredding test:
For the nestlet shredding test, use standard polycarbonate rat cages (19 cm x 29 cm x 13 cm) with appropriate filter-top coverings. Fill each cage with brand-new, odourless mouse bedding until it is 0.5 cm deep. Next, place a second cage of the same size over the bedding to pack it. Use an analytical balance to weigh cotton fiber nestlets that are sold commercially. After placing one mouse in a cage with a nestlet that has been previously weighed, cover the cage with the filter top. During the test, avoid eating or drinking anything. For half an hour, leave the mouse alone in the cage with the nestlet. After the test is finished, take the mouse out and put it back in its cage. Use forceps to remove any leftover intact nestlet material from the cage, then let it air dry. To determine the proportion of nestlet that has been shredded, weigh the remaining unshredded nestlet and divide the weight by the beginning weight. In a room with a 12-hour light/dark cycle, mice can be housed alone or in groups. Provide a limitless supply of food and water for every mouse. Three to five days before the test, handle the mice with care. For both tests, use mice of either sex. Conduct behavioural testing from 9:00 am to 4:00 pm, moving all mouse home cages to the testing area 60 minutes before the test starts (Kwon et al., 2022).

2.10 Forced swim test:
A transparent glass container of 25 cm in height and 10 cm in diameter was filled with water up to 15 cm deep for the test. To avoid hypothermia, the water's temperature was kept at 23 ± 1°C. For a total of six

minutes, each mouse was kept in the container separately. The first two minutes were spent acclimating, while the latter four minutes were spent immobilizing. An animal was deemed motionless if it was simply floating in the water and only moved slightly to maintain its head above the surface. It was thought that a reduction in immobility time relative to the control group was a sign of antidepressant-like activity.

2.11 Statistics Analysis:
Each treatment group was compared using a negative control. One-way Analysis of Variance (ANOVA) and Tukey's multiple comparison test were used to report all values as Mean ± SD *p < 0.05 and **p < 0.01.

3. RESULTS:

3.1 Extraction:
The practical extraction yield of Barleria sahyadrica in ethanol was found to 29 %.

3.2 Antioxidant DPPH Assay:
The DPPH activity of Barleria sahyadrica was shown that IC50 valu of Barleria sahyadrica was found to be 45 µg/ml as compared to Ascorbic acid which was 28 µg/ml as depicted in Fig. 1.

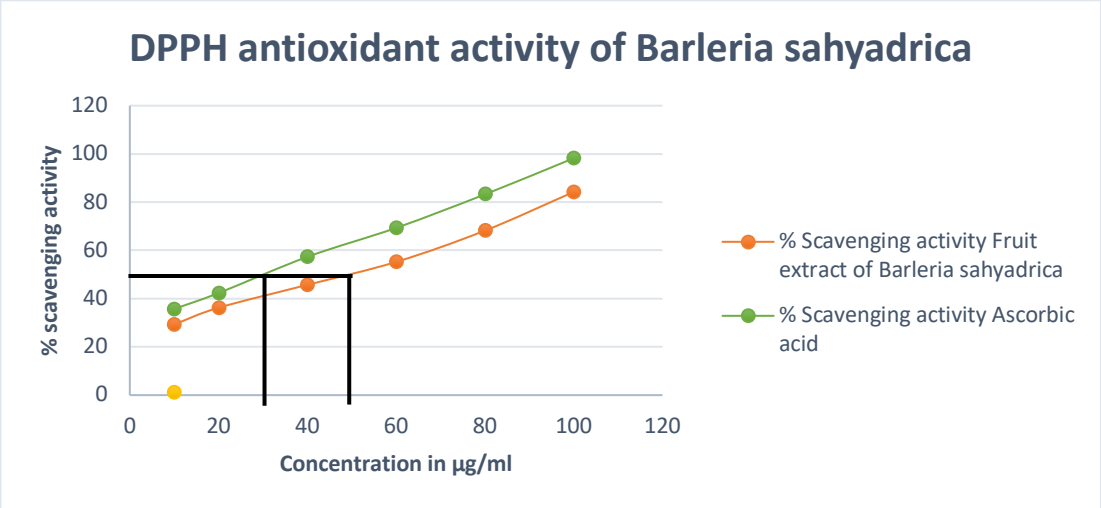

Figure 1: Antioxidant DPPH activity of Barleria sahyadrica

3.2 Elevated Plus Maze Test:
Over the course of six days, the impact of Barleria sahyadrica extract on the amount of time spent in the Elevated Plus Maze's (EPM) open arms was assessed. Between 80 and 95 seconds was the average amount of time spent in the open arms by the negative control group. Open-arm exploration (110–125 seconds) increased somewhat but significantly in mice given 100 mg/kg of Barleria sahyadrica extract. Animals spent roughly 140–150 seconds in the open arms at the higher dose (300 mg/kg), which produced a more noticeable impact. Over 150 seconds were spent in the open arms on all test days, demonstrating the greatest efficacy of the conventional medication, levetiracetam (10 mg/kg). The effect of *Barleria sahyadrica* extract was dose-dependent, with the 300 mg/kg group showing results closer to the standard drug as depicted in Figure 1.

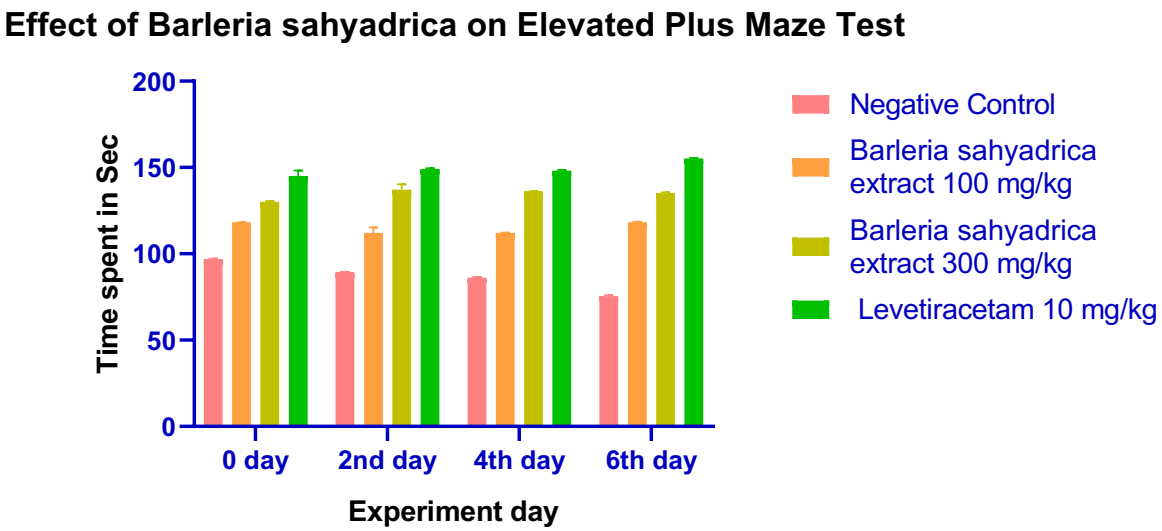

Figure 2: Effect of Barleria sahyadrica extract on Elevated Plus Maze Test

3.3 Rotarod Test:

The negative control group continuously showed the shortest retention time on the rotating rod (60–70 seconds) in the Rotarod test, indicating weaker muscles and poorer motor coordination. The animals that received 100 mg/kg of Barleria sahyadrica extract shown a slight improvement, holding their stance for about 80 to 90 seconds. With retention periods ranging from 120 to 130 seconds over the course of the experiment, a greater dose of 300 mg/kg had a more noticeable effect. Animals consistently stayed on the rod for more than 150 seconds, demonstrating the highest efficacy of the conventional medication, levetiracetam (10 mg/kg). Barleria sahyadrica's effects were dosage-dependent, with the 300 mg/kg dose nearly matching the effectiveness of the reference medication.

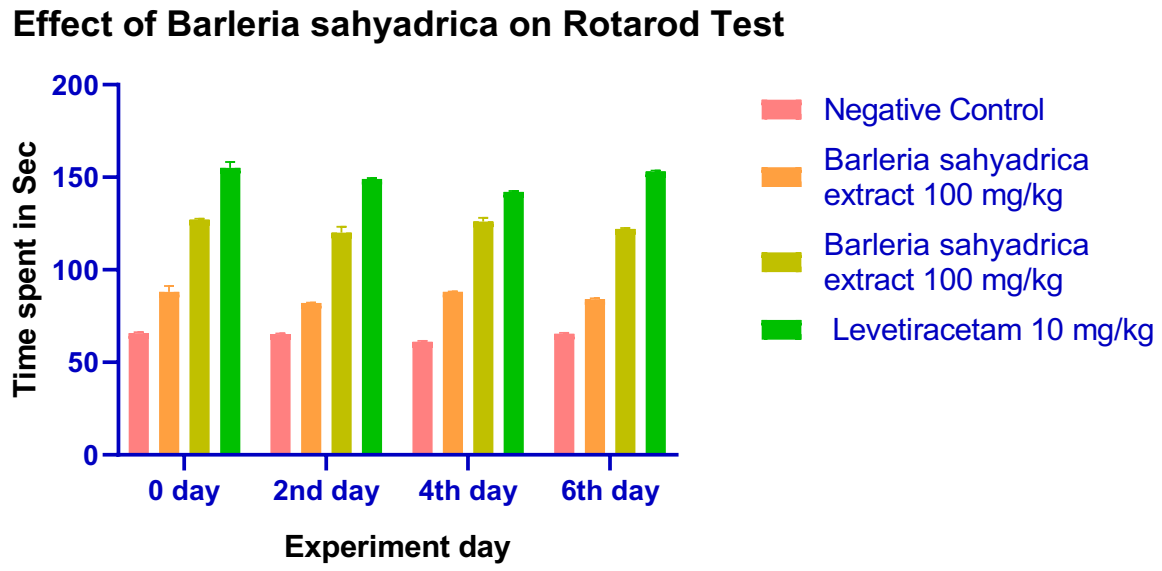

Figure 3: Effect of Barleria sahyadrica extract on Rotarod Test

3.4 Marble burying Test:

The negative control group buried the most marbles (about 16–17) in the marble burying test, suggesting a higher degree of obsessive and anxiety-like behaviour. The number of marbles buried was significantly decreased (≈10) by administering 100 mg/kg of Barleria sahyadrica extract, although the reduction was greater (≈8) at the higher dose of 300 mg/kg. Animals only buried roughly six marbles when using the conventional medication, levetiracetam (10 mg/kg), which had the largest effect. These results imply that extract from Barleria sahyadrica decreases anxiety-related and compulsive behavior in a dose-dependent way as shown in Fig. 4.

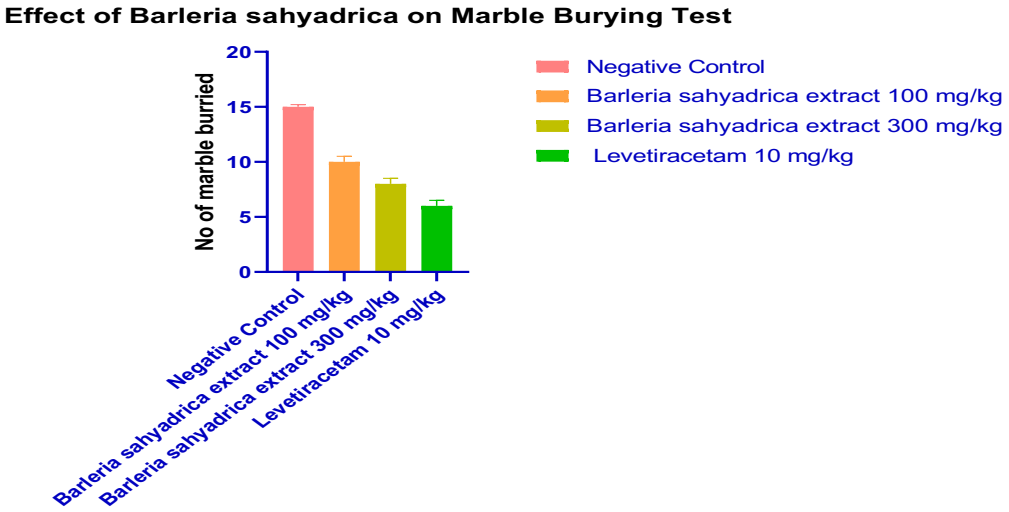

Figure 4: Effect of Barleria sahyadrica extract on Marble Burying Test

3.5 Nestlet Shredding Test:

The results of the marble burying test verify that the extract from Barleria sahyadrica has strong anti-compulsive and anxiolytic properties. The higher dose (300 mg/kg) demonstrated a noticeable improvement that was equivalent to that of the common medication Levetiracetam. The impact was dose-dependent. These results support more mechanistic research and the possible use of Barleria sahyadrica in the treatment of anxiety and obsessive-compulsive disorders by bolstering the evidence for its neuroprotective and behavioural effects.

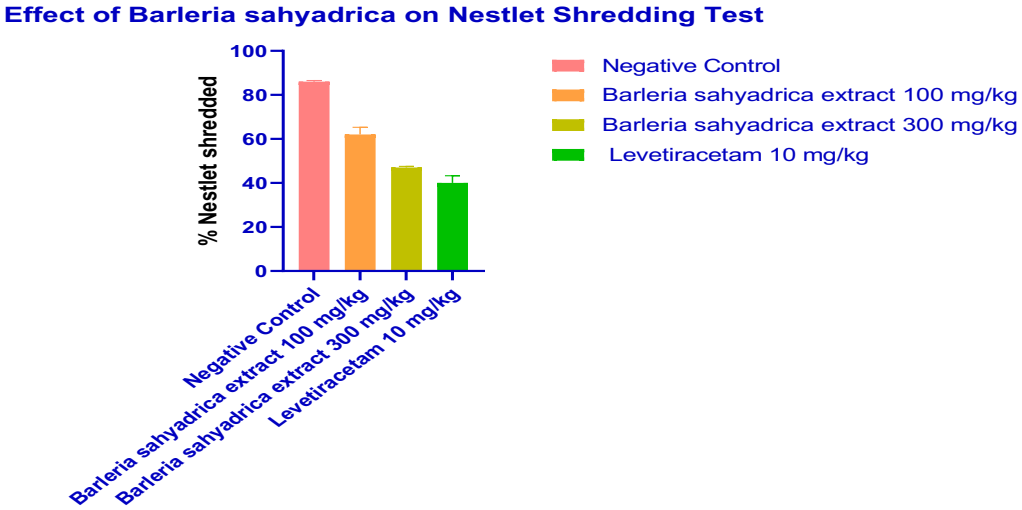

Figure 5: Effect of Barleria sahyadrica extract on Nestlet Shredding Test

3.6 Forced Swim Test:

The negative control group showed the longest immobility time (≈180–200 sec) in the Forced Swim Test, indicating more depressive-like behaviour. Immobility time was moderately reduced (≈120–130 sec) by administering 100 mg/kg of Barleria sahyadrica extract, and further reduced (≈100–110 sec) by administering 300 mg/kg. The greatest reduction was obtained with the usual medication, levetiracetam (10 mg/kg), and the immobility period was continuously kept between 80 and 90 seconds on all experimental days. The 300 mg/kg extract of Barleria sahyadrica showed results that were comparable to the conventional therapy, indicating that the effect was dose-dependent as shown in fig. 6.

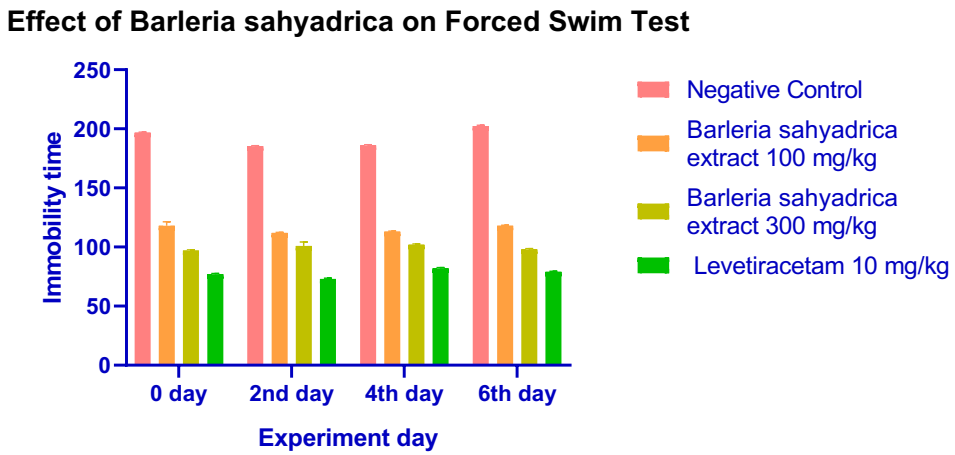

Figure 6: Effect of Barleria sahyadrica extract on Forced Swim Test

4. Discussion:

A popular behavioural model for assessing neuroprotective and anxiolytic activity is the Elevated Plus Maze test, in which more time spent in the open arms indicates less anxiety and better cognitive performance. Barleria sahyadrica showed a notable improvement in open-arm exploration in the current investigation, especially at the dose of 300 mg/kg. This implies that the extract may have neuroprotective and anxiolytic properties.

The action could be explained by the presence of phytoconstituents such terpenoids, tannins, and flavonoids, which have been shown to have neuroprotective and antioxidant properties by scavenging free radicals and altering neurotransmitter pathways. The findings suggest that extract from Barleria sahyadrica can reduce neuronal dysfunction and oxidative stress, two factors that significantly contribute to anxiety and neurodegeneration.

It's interesting to note that the results of the higher dose (300 mg/kg) were almost identical to those of the normal Levetiracetam, demonstrating the drug's potential as a plant-derived treatment. The dose-dependent pattern reinforces the extract's pharmacological significance.

The Rotarod test is frequently used to assess neuromuscular integrity, balance, and motor coordination. While longer memory times suggest protective or improving effects on motor function, shorter retention times signal poorer coordination. Barleria sahyadrica showed a notable improvement in retention time in this trial, especially at the higher dose of 300 mg/kg.

The extract's flavonoids, tannins, and terpenoids—which are known to boost neuronal survival, lower oxidative stress, and strengthen neuromuscular function—are responsible for this improvement. The found neuroprotective effect is consistent with the plant's antioxidant capacity, indicating that the extract maintains motor function when neurons are under stress. The plant's pharmacological significance is shown by the dose-dependent trend, with the 300 mg/kg extract exhibiting effects that are comparable to those of levetiracetam.

A common method for evaluating anxiety and obsessive-compulsive behaviour in rodents is the marble burying test. Anxiolytic or anti-compulsive activity is indicated by a decrease in the number of marbles buried, whereas a higher number represents increased anxiety or compulsive tendencies. Marble burying was significantly reduced in the current study by Barleria sahyadrica extract, with the 300 mg/kg dose having a greater effect than the 100 mg/kg dose.

The phytoconstituents of Barleria sahyadrica, such as flavonoids, tannins, and terpenoids, which have neuroprotective and antioxidant qualities, are responsible for this anxiolytic action. These substances have been shown to lower oxidative stress and alter neurotransmitter systems (including serotonergic and GABAergic pathways), all of which are linked to anxiety and obsessive disorders. The extract's promise as a natural medicinal drug is supported by its dose-dependent effect, which is comparable to that of standard levetiracetam.

A behavioural assay that has been thoroughly validated for evaluating anxiety, obsessive behaviour, and stress-induced activity in rodents is the Nestlet Shredding Test. Higher percentages of shredding are indicative of more obsessive and anxious behaviours. Compared to the negative control, rats treated with Barleria sahyadrica in this study exhibited a substantial decrease in shredding behaviour; the 300 mg/kg extract exhibited higher anxiolytic action than the 100 mg/kg dose. Because of their antioxidant and neuroprotective properties, phytochemicals such flavonoids, tannins, and terpenoids are responsible for this effect. In order to alleviate anxiety and obsessive behaviors, these substances may alter neurotransmitter systems, lower oxidative stress, and enhance neural resilience. Further demonstrating the extract's medicinal potential are its dose-dependent efficacy and resemblance to levetiracetam at higher doses.

A proven model for assessing antidepressant and neuroprotective effects is the Forced Swim Test, in which a shorter immobility period is associated with less behavioural despair and better coping skills. Compared to the negative control, Barleria sahyadrica dramatically

decreased immobility time in the current investigation, indicating possible neuroprotective and antidepressant-like effects. Plant phytochemicals with antioxidant, anti-inflammatory, and neuromodulatory qualities, including flavonoids, alkaloids, tannins, and terpenoids, are probably responsible for the effect that has been observed. Neuronal resilience and mood-related behaviour may be enhanced by the extract through lowering oxidative stress and modifying neurotransmitter systems, including serotonin and noradrenaline. A dose-dependent pharmacological response was demonstrated by the increased effects of the higher dose (300 mg/kg), which performed similarly to levetiracetam. Collectively, the results show that Barleria sahyadrica has multifaceted neuroprotective effects that include antidepressant, anti-compulsive, anxiolytic, and motor-coordination-enhancing qualities. Barleria sahyadrica rich phytochemical composition, which includes flavonoids, alkaloids, tannins, terpenoids, and phenolic compounds all of which have anti-inflammatory, neuromodulatory, and antioxidant properties may be the cause of the reported activity. The antidepressant-like effect in the Forced Swim Test may be explained by the modulation of monoaminergic neurotransmitters (serotonin, dopamine, and noradrenaline), whilst antioxidant activity may lessen oxidative stress in neural tissues.

5. Conclusion:

According to the study, Barleria sahyadrica extract has important psychopharmacological and neuroprotective qualities. Anxiolytic action (EPM, Marble Burying, Nestlet Shredding) is one of its effects. action similar to an antidepressant (Forced Swim Test). Motor coordination has improved (Rotarod). These findings support Barleria sahyadrica's traditional medical applications and point to the plant's potential as a source of new neuroprotective compounds. To investigate its clinical value in treating neurological and mental illnesses, more research is necessary, including the isolation of active phytoconstituents, assessment of the molecular mechanism, and long-term neurotoxicity studies.

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Conflict of Interest:

Authors are hereby declaring that there is no conflict of interest.

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