

WHEATGRASS: A NEUROPROTECTIVE AND NEUROMODULATOR IN CENTRAL NERVOUS SYSTEM DISORDERS

Manesh B. Kokani¹, Meena K. Yadav^{*2}

¹Research Scholar, Department of Pharmacology, Nims Institute of Pharmacy, Nims University Rajasthan, Jaipur, Rajasthan, India.

²Professor, Department of Pharmaceutical Chemistry, Nims Institute of Pharmacy, Nims University Rajasthan, Jaipur, Rajasthan, India.

*Corresponding Author: Meena K.Yadav. Email ID: meenayadav82@gmail.com

ABSTRACT

Background: Triticum aestivum, or wheatgrass, is well known for its high antioxidant profile and possible medical uses. Using a variety of validated behavioural models, this study examines its antidepressant, anxiolytic, antioxidant, and anti-compulsive properties in mice.

Objectives: To assess wheatgrass extract's neuropsychopharmacology effects in mouse models, such as anxiety, depression, compulsive behaviour, and motor coordination, as well as its antioxidant capability using the DPPH assay.

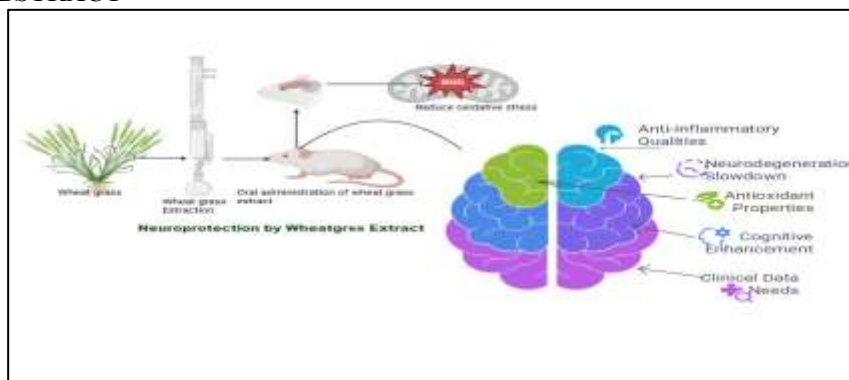
Methods: DPPH radical scavenging assay was used to measure antioxidant activity. The Elevated Plus Maze was used to assess anxiolytic activity (EPM). The Rotarod assay was used to measure motor coordination. The Marble Burying Test (MBT) and Nestlet Shredding Test (NST) were used to examine compulsive behavior. The Forced Swim Test (FST) was used to test antidepressant-like action.

Results: Wheatgrass extract was administered to mice at 50 and 100 mg/kg in contrast to a negative control and common medications like fluoxetine. Similar to ascorbic acid ($IC_{50}=2.5\mu g$), wheatgrass extract demonstrated dose-dependent antioxidant activity ($IC_{50}=3.8\mu g$).

Conclusion: In mice, wheatgrass extract has strong anxiolytic, anti-depressive, anti-compulsive, and motor-enhancing effects in addition to its strong antioxidant qualities. Its promise as a natural treatment agent for neurobehavioral disorders is supported by these findings. Although encouraging, there is little clinical data, and further study is required to validate its therapeutic potential. The current study is emphasized on wheatgrass and its antioxidant activity which benefited in neurodisorders.

KEYWORDS: Neurodisorders, Wheatgrass, Antioxidant, Neurobehavioral study

GRAPHICAL ABSTRACT



INTRODUCTION:

Neurological disorders are linked to some of the highest rates of illness, disability, and mortality in the world and represent a serious threat to cognitive function. The most prevalent neurological disorders include spinal cord, intracranial tumour, autoimmune, cerebral vascular, neurodegenerative, and craniocerebral trauma. Their patterns of infection have changed significantly over time, with the main concerns being the rise in neurological diseases caused by aging, urbanization, and population growth, as well as the rise in life expectancy cases and the ensuing worldwide illness burden (Huang et al., 2023). The study of disease epidemiology generates statistics on incidence, prevalence, risk factors, and distribution within a population; however, these data are not very helpful for population health unless they are connected to a program of treatment (or prevention) and the use of resources. Research that concentrates on broad categories that are skewed toward social and economic issues fails to adequately consider the effects of sickness on patients and caregivers. According to a Global Campaign study, the treatment gap for epilepsy might be closed by about 20% by implementing basic techniques (Shorvon et al., 2016). Oxidative stress or free radicals may be a basic

mechanism behind a number of neurological disorders in humans. Antioxidants, which are free radical scavengers, can be used in therapy to prevent, delay, or improve a variety of neurological conditions. However, because oxidative pathobiology has a complex biochemistry, the best antioxidant treatment approaches may differ and must be customized for each condition. According to their mode of action, antioxidants can be used to treat a number of neurological conditions, including Parkinson's, Alzheimer's, and Huntington's diseases (Sindhu et al., 2022).

The antioxidants in wheatgrass are evaluated analytically for their capacity to scavenge free radicals, including superoxide anion radical (O_2^-), hydroxyl radical, peroxy radical ($ROO^\bullet$), and DPPH. The results of measuring the antioxidant activity of extract components can vary depending on the particular free radical reactant that is used. A variety of reactants, including malondialdehyde (MDA), thiobarbituric acid-reactive compounds (TBARS), 2-azobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-azobis (2-amidinopropane) (AAPH), are used to measure activity levels (Kulkarni et al., 2006; Paritet et al., 2018; Şavşatlı, 2020; Devi et al., 2019; Durairaj et al., 2014; Wang et al., 2024). Wheatgrass Length Effects on Antioxidant Activity and Total Phenolic Content in Wheatgrass (*Triticum spp.*). Neuroprotection of age-associated amnesia induced by scopolamine in mice by wheatgrass. Scopolamine, which is an anticholinergic drug that causes Alzheimer's disease-like molecular and behavioral features. Since it might be associated with the ERK/Akt-CREB-BDNF pathway and also attenuates tau pathology, it might have therapeutic potential in the treatment of age-associated amnesia. Wheat grass can be an excellent source of nutrients like calcium, cobalt, iron, phosphorus, potassium, sulfur, and zinc, along with antioxidants like vitamin A, B, C, and E, along with antioxidant enzymes like superoxide dismutase (SOD), and cytochrome oxidase. From the outcomes, it can be concluded that wheat grass may be used for the treatment of Alzheimer's disease (Tauqueeret al., 2022; Katiyar et al., 2022; Suriyavathanaet al., 2016; Stevenson et al., 2012; Khan et al., 2013; Kokani et al., 2024; Minocha et al., 2022). The preclinical assessment of wheatgrass extract and its neuroprotective and antioxidant properties was the main focus of the current investigation (Latha et al., 2024; Annapurna et al., 2018; Kamal et al., 2024)

MATERIAL AND METHODS

Chemicals and Drugs:

All the chemicals and drugs used for the experiment were purchase from the local market and with lab grade analysis.

Animals:

The animals used in the current experiment were albino mice of both genders, with an approximate age of 8 weeks. The specific strain that was employed in this experiment was the Swiss albino. The weight of the mice used was 25-30 grams. From the observation of the general look and behavior of the mice, as well as lack of visible signs of diseases, the mice were found to be in good health condition. Equal numbers of males and females were in each group. Acclimatization of the mice before commencing the treatment. For acclimatization and throughout the experiments, the mice were held in a controlled environment in an animal laboratory where the temperature was $25 \pm 1^\circ\text{C}$ and humidity 55.5%. The light and dark phases were 12 hours each. Laboratory food and water were available freely.

The current experiment was approved by the Institutional Animal Ethics Committee (IAEC). The protocol number is TRS/PT/025/067-C. All protocols followed in the current experiment have been in agreement with the relevant institutional guidelines for the care and use of laboratory animals.

Extraction of Wheatgrass:

A market survey was carried out in order to collect commercially available wheatgrass powder made up of *Triticum aestivum* L. (wheatgrass). The product selection was made based on declaration made on the label, composition, packaging and no addition of any substances. The product which had been declared to be made of 100% wheatgrass powder with no use of preservatives, artificial colors, flavoring agents, sugars, filling agents and other substances was selected for the experiment.

The selected wheatgrass powder was bought from the local market of Nandurbar and was transferred to the laboratory in the same packaging. Details of the product like batch number, date of manufacture, expiry date, manufacturer details and ingredient composition of the product were noted down. It was confirmed that the powder contained only the *Triticum aestivum* wheatgrass and no other ingredients as declared in the product label. The sample was kept in a closed container until analyzed.

Antioxidant DPPH Assay:

Fill a test tube with 1.5 ml of samples at different concentrations. Next, add 1.5 ml of DPPH solution. Lastly, add 1.5 ml of 95% ethanol to both the test and blank. Set aside at room temperature for 20 minutes. At 517 nm, the solutions' absorbance (A) was measured in relation to the corresponding blanks (Kato et al, 1988; Molyneux. 2004).

Experimentation Design:

The treatment schedule for the experiment is as follows:

- Group 1: Treated with AlCl_3 at the dose by 100 mg/kg po
- Group 2: Treated with 50 mg/kg wheatgrass extract + AlCl_3 at the dose by 100 mg/kg po
- Group 3: Treated with 100 mg/kg wheatgrass extract + AlCl_3 at the dose by 100 mg/kg po (10-12)
- Group 4: Treated with 10 mg/kg Fluoxetine + AlCl_3 at the dose by 100 mg/kg po (Khalil et al., 2022)

A negative control is supposed to have only the vehicle applied to it while the other group should be exposed to $AlCl_3$. Vehicle controls are essential since they help in identifying the baseline effects and separate them from the effects induced by the mode of application. The previous animal studies employed the use of $AlCl_3$ for inducing either neurotoxicity or changes associated with Alzheimer's disease, although the methods have differed greatly.

Elevated Plus Maze Test:

The test compound's anxiolytic effect in adult albino mice was assessed using the Elevated Plus Maze (EPM) test. The device was 50 cm above the ground and had two open arms (50×10 cm) and two closed arms ($50 \times 10 \times 40$ cm) arranged in a plus (+) form. Each group received treatment as depicted above. After 30 minutes of intraperitoneal drug administration, each mouse was positioned separately in the middle of the maze, facing one of the open arms. Five minutes were spent observing each animal's behaviour. Time spent in the open and closed arms, as well as the quantity of entries, were noted. When an animal inserted all four paws into an arm, it was considered to have entered. Anxiolytic activity was thought to be indicated by longer duration and more entries in the open arms. In order to remove olfactory cues, the maze was cleaned with 70% ethanol in between trials, and all studies were carried out in the light phase of the cycle under consistent ambient circumstances.

Rotarod Test:

To assess motor coordination, a rotarod treadmill was utilized. Three days before the trial, the animals were taught on the device. The rotarod was set up. In order to remove olfactory cues, the maze was cleaned with 70% ethanol in between trials, and all studies were carried out in the light phase of the cycle under consistent ambient circumstances to progressively increase the acceleration over a 10-second period from 10 to 30 rotations per minute, with a 300-second test duration. The animals were placed on the horizontal rod for the duration of the trial, and each animal's falling-off time was noted. Every trial was conducted once a week and three times. For analysis, the average duration of the three trials was employed (Abu-Elfotuh et al., 2021).

Marble Burial Test:

Test should be performed in standard polycarbonate rat cages with dimensions of $26 \text{ cm} \times 48 \text{ cm} \times 20 \text{ cm}$ and with filter top lids. In order to ensure flat surface of bedding, a second cage of equal dimension should be placed on top of bedding and new odorless mouse bedding material should be placed in each cage to a depth of 5 cm. Moreover, it will create parallel lines on the bedding surface which can be used for placing marbles. Standard glass toy marbles (different shapes and colors with diameter of 15 mm and weighing 5.2 g) should be laid on bedding surface in five rows of four marbles. Nestlet Shredding Test: The standard polycarbonate rat cages ($19 \text{ cm} \times 29 \text{ cm} \times 13 \text{ cm}$) with fitted filter top cover should be used for nestlet shredding test. New, odorless mouse bedding should be filled in each test cage up to 0.5 cm depth. Then, bedding should be packed by placing another cage of equal dimensions over the bedding. Commercially available cotton fibernestlets ($5 \text{ cm} \times 5 \text{ cm} \times 5 \text{ mm}$ thick and weighing approximately 2.5 g) should be weighed using analytical balance and then placed one per test cage. Then, lay one nestlet on top of the bedding in each test cage. Set stop watches or timers for timing session lengths (30 min for each test). Keep mice in a room with a 12-hour light/dark cycle, either alone or in groups. Give every mouse unlimited food and water. Three to five days before the test, handle the mice with care. For both tests, use mice of either sex. Conduct behavioral testing from 9:00 am to 4:00 pm, and move all mouse home cages to the testing area 60 minutes before to the test's start time (Alavi et al., 2024).

Nestlet Shredded Test:

Put a single mouse in a cage with a single nestlet that has been previously weighed, and 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 over, take the mouse out and put it back in its cage. Using forceps, remove the cage's remaining intact nestlet material, 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 initial weight. If necessary, utilize a specific 5-point nest rating scale¹⁷ to score the shape of the nest the subject mouse created, keeping in mind that this scale is typically meant to evaluate nest construction rather than compulsive shredding. Discard remaining shredded nestlet material and bedding (Pyatha et al., 2023).

Forced Swim Test:

A transparent cylindrical glass container measuring 25 cm in height and 10 cm in diameter was used for the test, and it was filled with water up to 15 cm deep. To avoid hypothermia, the water's temperature was kept at $23 \pm 1^\circ\text{C}$. For a total of six minutes, each mouse was put into the container separately and watched. The first two minutes were for acclimatization, while the last four minutes were for immobility. An animal was said to be immobile if it was merely floating in the water and moving only the bare minimum 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.

Statistical Analysis

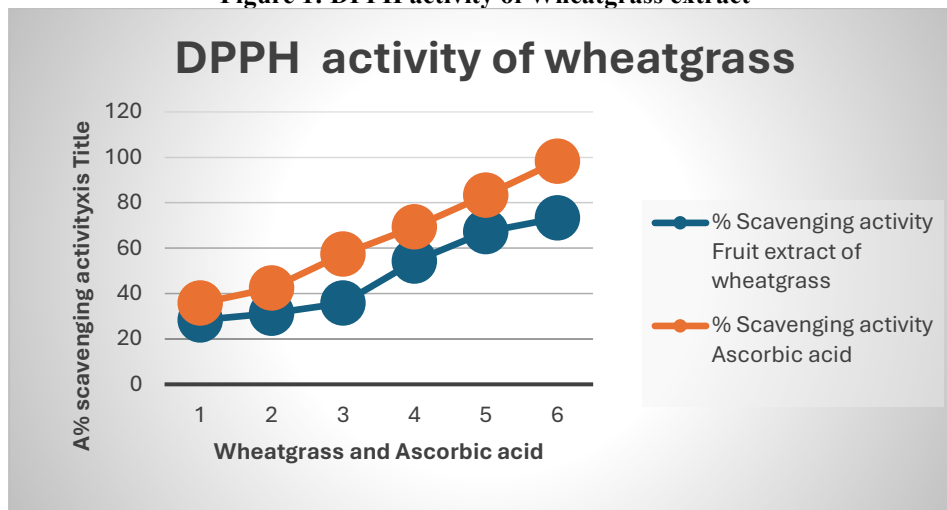
Each treatment group was compared with negative control. The data were presented as mean $\pm$ SD for each experimental group. The statistical significance of the mean between experimental groups was determined using the one-way ANOVA test. Here, the experimental group served as the independent variable, while the measure of behavior

was the dependent variable. If the one-way ANOVA test showed statistically significant differences among the groups, then Tukey's Honestly Significant Difference (HSD) test was used for multiple comparisons. The statistical significance level was $p < 0.05$. The exact p-values, as well as values of ANOVA and degrees of freedom, were provided. The data were presented in Mean $\pm$ SD * $p < 0.05$ and ** $p < 0.01$ in one-way Analysis of Variance (ANOVA) with Tukey's multiple comparison test.

RESULT

DPPH Assay

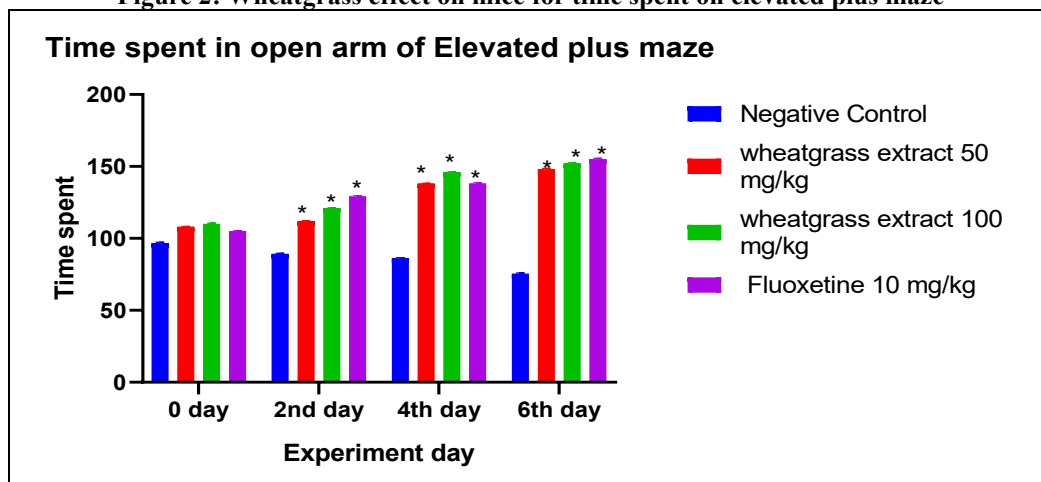
Figure 1: DPPH activity of Wheatgrass extract



As depicted in Figure 1 graph demonstrates a concentration-dependent increase in DPPH radical scavenging activity both wheatgrass extract and ascorbic acid, a common antioxidant, exhibit concentration-dependent scavenging activity, according to the DPPH assay graph. For both compounds, the percentage of scavenging action rises with increasing concentration. The scavenging activity of wheatgrass extract increased from about 28% at low concentration to roughly 74% at the highest measured concentration. Wheatgrass extract reaches 50% scavenging around concentration 3.5–4, suggesting its $IC_{50} \approx 3.8 \mu g$ and ascorbic acid reaches 50% scavenging at around 2.5 μg , giving an $IC_{50} \approx 2.5$ units.

Elevated Plus Maze:

Figure 2: Wheatgrass effect on mice for time spent on elevated plus maze



The Figure no 2 demonstrate the Wheatgrass extract demonstrated dose-dependent anxiolytic-like activity as shown by increased time spent in the open arm of the Elevated Plus Maze. The percentage of open arm admissions and the amount of time spent in open arms were both markedly increased by wheatgrass at 50 and 100 mg/kg, suggesting anxiolytic-like activity. The effects of the high dose (100 mg/kg) were similar to those of the common anxiolytic medication, fluoxetine. There was no significant change in total arm entries, indicating that the impact is exclusive to anxiety and not brought on by an increase in locomotor activity.

Rotarod Test:

Figure 3: Effect of Wheatgrass extract on motor coordination of mice

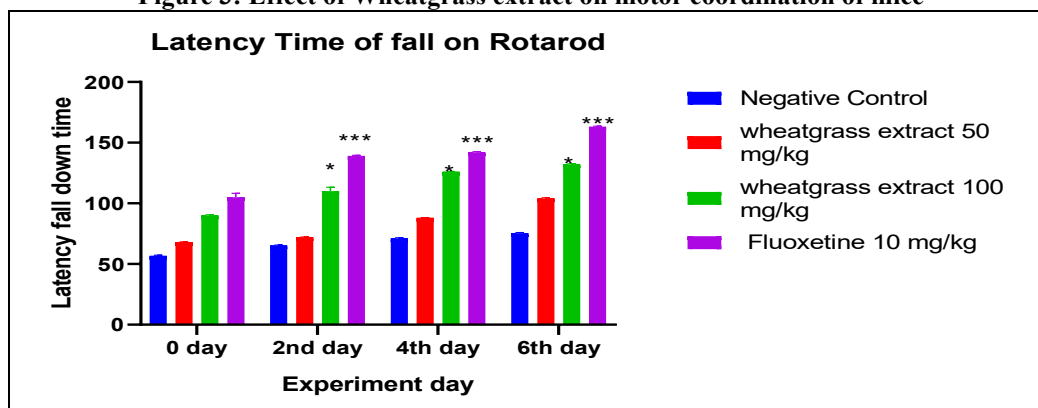


Figure 3 indicates that Wheatgrass extract enhances motor coordination and muscle endurance in a dose-dependent manner. When compared to the control group, the wheatgrass-treated group at dose of 50 mg/kg and 100 mg/kg showed a statistically significant increase in latency to fall ($p < 0.05$), suggesting enhanced motor function. This impact increased with continued dosing and was dose-dependent. Throughout the course of treatment, no negative effects were noticed.

Marble Burying Test:

Figure 4: Effect of Wheatgrass extract on marble buried test

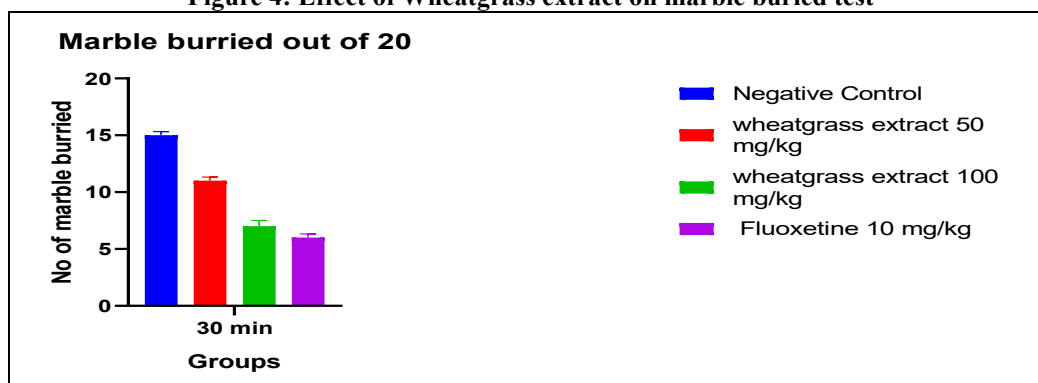
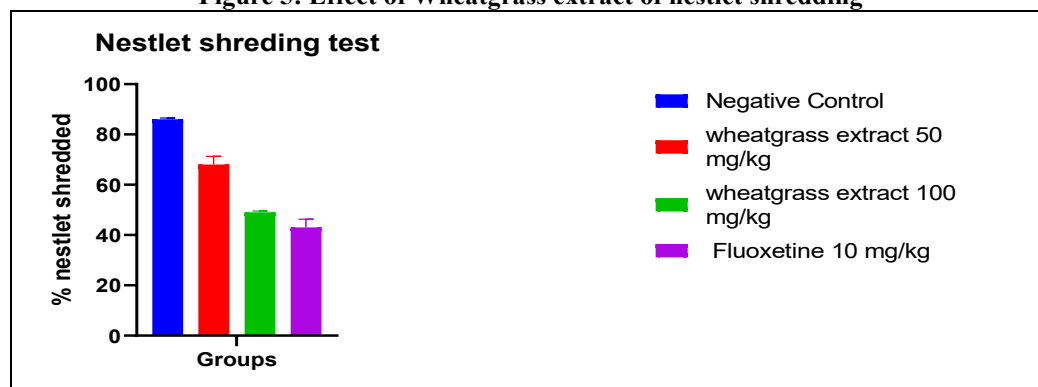


Figure 4 explains that wheatgrass extract dramatically lowers marble-burying activity in rodents, exhibiting dose-dependent anxiolytic-like behaviour. Mice were given a 30-minute marble burying test to evaluate obsessive-compulsive and anxiety-related behaviour. For every group, the number of marbles buried (out of 20) was noted. With an average of 15 marbles buried, the negative control group exhibited the highest marble burying behaviour. Marble burial was considerably decreased to around 11 marbles after treatment with 50 mg/kg of wheatgrass extract, and the number was further decreased to about 7 marbles after treatment with 100 mg/kg of wheatgrass extract. Fluoxetine (10 mg/kg)-treated positive control group buried only roughly 6 marbles, demonstrating a similar decrease.

Nestlet Shredding:

Figure 5: Effect of Wheatgrass extract of nestlet shredding



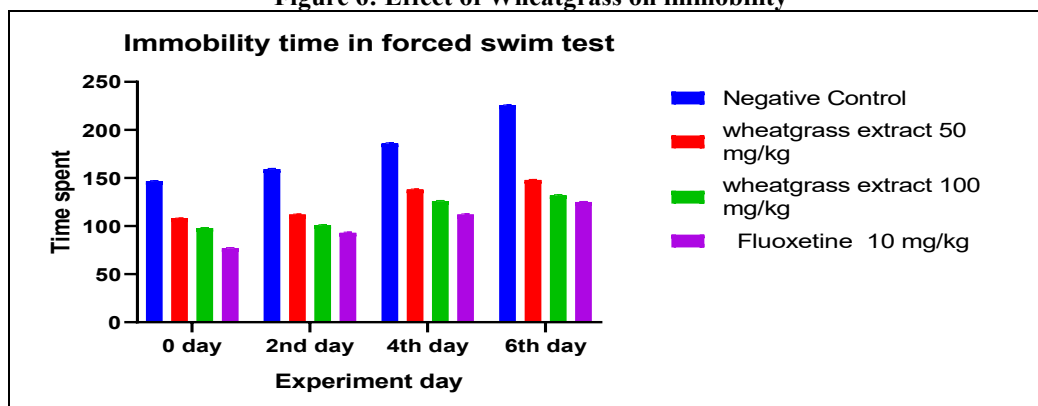
The Figure 5 indicates Nestlet Shredding Test in mice in various treatment groups were evaluated for compulsive-like behaviour using the proportion of nestlets that were shredded as a behavioural index. With over 85% of the nestlet

shredded, the Negative Control group had the highest nestlet shredding behaviour, suggesting increased repetitive or compulsive activity.

About 70% of the nestlets were shredded in mice given 50 mg/kg of wheatgrass extract, indicating a moderate decrease in shredding propensity. Nestlet shredding decreased to about 50% in the group that received 100 mg/kg of wheatgrass extract, a more noticeable reduction. With about 45% of the nestlets shredded, the positive control group that received fluoxetine (10 mg/kg) had the least amount of shredding activity, demonstrating the medication's effectiveness in lowering obsessive behaviour (Angoa-Pérez et al., 2013).

Forced Swim Test:

Figure 6: Effect of Wheatgrass on immobility



As depicted in Figure 6 shows that dose dependant effect of wheatgrass extract on immobility test. Immobility time was measured on days 0, 2, 4, and 6 of the Forced Swim Test (FST) to evaluate antidepressant-like activity in mice in different treatment groups. All groups showed similar immobility periods on Day 0, which suggests baseline behaviour. The Negative Control group's immobility time increased gradually throughout the experiment, reaching about 220 seconds by Day 6, indicating behavioural despair and a lack of coping strategies. The groups who received wheatgrass extract, on the other hand, showed a dose-dependent reduction in immobility time. By the sixth day the 50 mg/kg wheatgrass group showed a moderate reduction (~150 seconds), 100 mg/kg wheatgrass group exhibited a more significant decrease (~130 seconds), Fluoxetine (10 mg/kg) group, used as the positive control, had the lowest immobility time (~120 seconds), consistently lower across all time points compared to the control.

DISCUSSION

Wheatgrass extract lengthens the time spent in the open arms, according to the results of the Elevated Plus Maze test, indicating an anxiolytic (anxiety-reducing) effect. The 100 mg/kg group exhibited more open-arm activity than the 50 mg/kg group, indicating that this effect was dose-dependent. Similar to the common anxiolytic medication fluoxetine, both wheatgrass-treated groups showed noticeably more open-arm exploration by the sixth day. Because of its strong antioxidant and neuroprotective properties, wheatgrass may be a natural anxiolytic, according to these findings.

Anxiety and repetitive behaviour in rodents can be evaluated using the validated marble burying test; a greater number of buried marbles generally denotes stronger anxiety or obsessive-compulsive tendencies. A dose-dependent decrease in marble burying behaviour was seen in this study upon administration of wheatgrass extract, indicating possible anxiolytic and anti-compulsive effects. Marble burying in the group given 100 mg/kg of wheatgrass extract was similar to that of the common medication fluoxetine, indicating a significant improvement in behaviour. The anti-inflammatory, neuroprotective, and antioxidant components of wheatgrass, including as flavonoids, polyphenols, and chlorophyll, may be responsible for the effects that have been seen.

A typical test for assessing compulsive-like behaviour in rodents that is frequently associated with disorders related to stress and anxiety is the nestlet shredding test. A possible anti-compulsive impact was suggested by the study's findings that wheatgrass extract dramatically decreased nestlet shredding in a dose-dependent way. Results were similar to those of the conventional medication fluoxetine at the higher dose (100 mg/kg), indicating that wheatgrass may have neurobehavioral effects through neuromodulatory, anti-inflammatory, and antioxidant pathways. The therapeutic potential of wheatgrass in treating anxiety-related and compulsive disorders is supported by these studies.

Increased immobility time in the Forced Swim Test, a popular model for assessing antidepressant activity, is indicative of behavioural despair. Wheatgrass extract dramatically shortened immobility time in this trial in a dose-dependent fashion, suggesting possible antidepressant-like effects. Results from the 100 mg/kg dose were similar to those of the common medication fluoxetine, indicating that wheatgrass may have an impact on the neurochemical pathways linked to mood regulation. This impact is probably caused by its high antioxidant content and neuroprotective qualities, which suggest its potential use in treating depressed symptoms. Our results are in agreement with previous results (Kothari et al., 2023; Mishra et al., 2025; Jorjic et al., 2015)

CONCLUSION

Mice given wheatgrass extract showed a progressive improvement in latency to fall, fall down time from rotaroad, immobility in forced swim test as well as marble burring and nestlet shredding. According to the results of the Rotarod test, suggesting improved neuromuscular strength and motor coordination. The 100 mg/kg dose performed better than the 50 mg/kg group, indicating that the impact was dose-dependent. The 100 mg/kg wheatgrass group began to function similarly to the usual medication fluoxetine, which consistently had the longest delay time, by the sixth day. These results support wheatgrass extract's potential use in the treatment of neurodegenerative or neuromuscular diseases by indicating that it may have neuroprotective and motor-enhancing qualities. These findings suggest that wheatgrass, through lowering oxidative stress and increasing GABAergic activity, may alter neurotransmitter systems implicated in anxiety and compulsive behaviour. To clarify the precise mechanism, more research using biochemical markers and receptor-binding assays is advised.

DECLARATIONS

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Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request. The accompanying author can provide the data that support the study's conclusions upon reasonable request.

Ethics Approval: Every animal experiment was carried out in compliance with the approval No: TRS/PT/025/067-C. The study conforms with the CPCSEA guidelines for the use and care of laboratory animals.

Declaration of Competing Interest: The authors declare that there are no conflicts of interest.

Consent to Publish: The authors attest that ready give consent to publish the data and conclusions in this study. Bothco-authorshave given their approval for the paper to be submitted and consents to its publication.

List of Abbreviations:

DPPH- 2	2-Diphenyl-1-picrylhydrazyl
EPM	Elevated Plus Maze
MBT	Marble Burying Test
NST	Nestlet Shredding Test
FST	Forced Swim Test
Po	Per Oral
O ₂	superoxide anion radical
ROO	Peroxyl radical
MDA	Malondialdehyde
TBARS	Thiobarbituric acid-reactive compounds
ABTS	2-azobis (3-ethylbenzothiazoline-6-sulfonic acid)
AAPH- 2	2-azobis (2-amidinopropane)

REFERENCES

1. Huang Y, Li Y, Pan H, Han L (2023) Global, regional, and national burden of neurological disorders in 204 countries and territories worldwide. *J Glob Health*13:13001-09.
2. Shorvon S. (2016) Neurology worldwide: The epidemiology and burden of neurological disease. *Neurol Queen SqTextb* 2016:1–10.
3. Sindhu RK, Kaur P, Kaur P, Singh H, Batiha GES, Verma I (2022) Exploring multifunctional antioxidants as potential agents for management of neurological disorders. *Environ SciPollut Res Int.*29(17):24458–77.
4. Kulkarni SD, Tilak JC, Acharya R, Rajurkar NS, Devasagayam TPA, Reddy AVR. Evaluation of the antioxidant activity of wheatgrass (*Triticum aestivum* L.) as a function of growth under different conditions. *Phytother Res.* 2006;20(3):218–27.
5. Parit SB, Dawkar VV, Tanpure RS, Pai SR, Chougale AD. Nutritional quality and antioxidant activity of wheatgrass (*Triticum aestivum*) unwrap by proteome profiling and DPPH and FRAP assays. *J Food Sci.* 2018;83(8):2127–39.
6. Şavşatlı Y. The effects of wheatgrass length on antioxidant activity and total phenolic content in wheatgrass (*Triticum* spp.). *Turk J Agric For.* 2020;44(3):271–7.
7. Devi CB, Bains K, Kaur H. Effect of drying procedures on nutritional composition, bioactive compounds and antioxidant activity of wheatgrass (*Triticum aestivum* L.). *J Food Sci Technol.* 2019;56:491–6.

8. Wang V, Hoda M, Shakya G, Babu SP, Rajagopalan R. Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass. *Asian Pac J Trop Med*. 2014;7(Suppl 1):S398–404.
9. Wang CY, Wang ML, Li QQ, Yan Y, Hao S, Gou JY. Enhancing the antioxidant potential of wheatgrass to improve nutrient value. *Seed Biol*. 2024;3(1):100019.
10. Tauqueer M, Pathan MA, Shaikh MRN. Antidiabetic activity of wheat grass juice. *World J Pharm Res*. 2023;12(3). doi:10.20959/wjpr20233-27146.
11. Katiyar P, Rathore AS, Banerjee S, Nathani S, Zahra W, Singh SP, et al. Wheatgrass extract imparts neuroprotective actions against scopolamine-induced amnesia in mice. *Food Funct*. 2022;13(16):8474–88.
12. Suriyavathana M, Roopavathi I, Vijayan VINU. Phytochemical characterization of *Triticum aestivum* (wheat grass). *J PharmacognPhytochem*. 2016;5(1):283–6.
13. Stevenson LE, Phillips F, O'Sullivan K, Walton J. Wheat bran: its composition and benefits to health, a European perspective. *Int J Food Sci Nutr*. 2012;63(8):1001–13.
14. Khan GM, Ansari SH, Ahmad F. Pharmacognostic standardization, antioxidant and free radical scavenging activity of the seeds of *Triticum aestivum* L—A dietary staple. *J Young Pharm*. 2013;5(2):54–9.
15. Kokani MB, Mittal A. Systematic review on *Triticum aestivum* as an adjuvant in neurological disorder treatment and its pharmacological activities. *Biochem Cell Arch*. 2024;24(2):000–000.
16. Bitra VR, Rapaka D, Mathala N, Akula A. Effect of wheat grass powder on aluminum induced Alzheimer's disease in Wistar rats. *Asian Pac J Trop Med*. 2014;7(Suppl 1):S278–81.
17. Minocha N, Saini S, Pandey P. Nutritional prospects of wheatgrass (*Triticum aestivum*) and its effects in treatment and chemoprevention. *Explor Med*. 2022;3(5):432–42.
18. Latha JD, Obilineni I, Nadh AVS, Ramachandran V, Reddy CS, Bhuvaneshwari K, et al. Evaluation of the effect of wheatgrass powder on stress-induced depression and memory loss in mice. *Res J Pharm Technol*. 2024;17(5):2315–9.
19. Annapurna A, Vishala TC, Bitra VR, Rapaka D, Shaik A. Cerebroprotective actions of *Triticum aestivum* Linn powder and *Bauhinia purpurea* flower powder in surgically induced cerebral infarction in rats. *Pharmacogn Mag*. 2018;13(Suppl 4):S737–43.
20. Visternicu M, Rarinca V, Miron I, Kamal FZ, Guenne S, Ciobica A. Nutritional benefits of chlorophyll and mineral elements in wheatgrass. *Ann Acad Rom Sci Ser Biol Sci*. 2024;13(2):19–37.
21. Kato K, Terao S, Shimamoto N, Hirata M. Studies on scavengers of active oxygen species. 1. Synthesis and biological activity of 2-O-alkylascorbic acids. *J Med Chem*. 1988;31(4):793–8.
22. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol*. 2004;26(2):211–9.
23. Khalil MG, Ali AA, Hassanin SO, Al-Najjar AH, Ghosh S, Mahmoud MO. Comparative study on the effect of EGCG and wheat grass together with mental and physical activities against induction of Alzheimer's disease in both isolated and socialized rats. *Phytomed Plus*. 2022;2(1):100146.
24. Abu-Elfotuh K, Ragab GM, Salahuddin A, Jamil L, Abd Al Haleem EN. Attenuative effects of fluoxetine and *Triticum aestivum* against aluminum-induced Alzheimer's disease in rats: The possible consequences on hepatotoxicity and nephrotoxicity. *Molecules*. 2021;26(21):6752.
25. Alavi MS, Al-Asady AM, Fanoudi S, Sadeghnia HR. Differential effects of antiepileptic medications on neurogenesis: Evidence from cells to animals. *Heliyon*. 2024;10(4):e23394.
26. Pyatha S, Kim H, Lee D, Kim K. Co-exposure to lead, mercury, and cadmium induces neurobehavioral impairments in mice by interfering with dopaminergic and serotonergic neurotransmission in the striatum. *Front Public Health*. 2023;11:1265864.
27. Angoa-Pérez M, Kane MJ, Briggs DI, Francescutti DM, Kuhn DM. Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviors in mice. *J Vis Exp*. 2013;(82):e50978.
28. Kothari S, Gupta A. Experimental evaluation of anxiolytic and antidepressant activities of methanolic extract of *Triticum aestivum* (wheatgrass) in albino mice. *J Appl Pharm Res*. 2023;11(3):41–7.
29. Mishra N, Tripathi R, Pandey D, Shah K, Chauhan NS. Wheatgrass (*Triticum aestivum*): a miraculous microgreen: an overview. *J Future Foods*. 2025;5(3):239–47.
30. Jorige A, Akula A. Neuroprotective role of wheatgrass powder in experimental diabetic neuropathy via modulating oxidative stress markers in rat sciatic nerves. *Am J Phytomed Clin Ther*. 2015;3(7):529–40.