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Research Article

Evaluation of the Potential Role of Beetroot as an Anxiolytic at Different Doses in a Rodent Model

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Abstract

There has been an increase in the population suffering from anxiety episodes worldwide. Hence, the present study was intended to assess the effects of different doses of lyophilized *Beta vulgaris* (beetroot) powder (500mg/kg and 1000mg/kg) on various anxiety models. Albino mice weighing 20-25g were selected for the study and divided into 4 groups. Group I was administered distilled water, and 500mg/kg and 1000mg/kg of *B. vulgaris* were given to groups II and III, respectively, orally. Group IV was administered lorazepam 0.03mg/kg orally, while 3 groups comprising Wistar rats were also taken for the testing of anxiety and comprised of 3 groups, i.e., control given distilled water, Treated I and II administered with 500mg/kg and 1000mg/kg *B. vulgaris* powder. Head dip and cage crossing tests were performed on mice, whereas open field and light and dark testing were done on the rat model. The results showed a highly significant decline in cage crossings, head dip, and peripheral square crossings, and an increase in central square crossings and time spent in a light area as compared to the control. In comparison to the standard, a significant anxiolytic effect was observed. Among treated groups, the 1000mg/kg dose showed better anxiolytic potential at chronic dosing. From our study, we came to conclude that *B. vulgaris* showed anxiolytic potential in an animal model, and future studies should be conducted to focus on the mechanistic and clinical data.

Keywords: Anxiety, beetroot, betaine, cage crossing, head dip, open field

1. Introduction

Today, people are living in a world that is growing anxious, and the feelings of nervousness, worry, and fear are common observations in everyday lives. Anxiety is a mental condition that encompasses excessive apprehension and a feeling of worry. There are many general reasons behind this condition, such as stress, financial issues, education, emotional events, family, and personal life. These conditions often lead to anxiety. Extreme situations often further worsen the condition of different anxiety disorders like panic attacks, phobic disorders, agoraphobia, and generalized anxiety disorders (Bandelow et al. 2017). Anxiety is also a side effect of certain medications, but in most scenarios, this is reversible. Anxiety may also lead to some physiological changes, such as increased blood pressure, effects on the heart, diarrheal conditions, and more. These conditions are co-related and may need co-treatment with anxiety(Celano et al. 2018).

The conventional anxiolytics, such as Selective Serotonin Reuptake Inhibitors, Benzodiazepines, and Selective Norepinephrine Reuptake Inhibitors, are commonly prescribed, but the patients face several issues, such as cognitive impairment, withdrawal symptoms, sedation, and tolerance ("Mechanisms of Action of Anxiolytics | Springer Nature Link," 2021). Many studies are being conducted on the symptoms, cures, and improving the condition with medicines, lifestyle changes, and dietary products (Arimitsu et al. 2019). The trend of using medicinal plants and dietary vegetables for the reduction of anxiety and stress-related disorders is not new. Literature studies have shown that flavonoids, phenolic acids, alkaloids, and terpenoids present in the plants possess anxiolytic potential, and the effects are modulated through the serotonergic, gamma-aminobutyric acid (GABA), and antioxidant pathways. Previous literature on extracts from *Lactuca sativa* (lettuce), *Matricaria chamomilla* (chamomile), and *Passiflora incarnata* (passionflower) on animal behavior models has demonstrated significant reductions in anxiety. Similarly, green leafy vegetables rich in antioxidants and polyphenols have been associated with improved mood and reduced oxidative stress. However, despite growing evidence supporting plant-based anxiolytics, the specific anxiolytic potential of *Beta vulgaris* (beetroot) remains largely unexplored.

B. Vulgaris is a root vegetable commonly known as Red Beet, Table Beet, Garden Beet, or just Beet. It belongs to the family Chenopodioideae, and the subfamily is Amaranthaceae. It is a high source of fiber, vitamin B_9, manganese, potassium, iron, and vitamin C (Kujala et al. 2000). *B. vulgaris* main constituents are betanin, betalain, and inorganic nitrates, which turn into nitric oxide that is important for many body functions (Sarfaraz et al. 2020). It is commonly used for detoxification of the body by eliminating all the toxins of the body through the colon. *B. vulgaris* is good for losing weight as it is high in fiber (Clifford et al. 2015). It may also relax and dilate the blood vessels because nitric oxide helps lower blood pressure and heart disease (Zamani et al. 2021). Betanin is a potent antioxidant; therefore, *B. vulgaris* is also a valuable dietary product for antioxidation purposes. Betaine is noted as an anticoagulant agent when administered at 6g once daily (PRYOR et al. 2017). It is a blood tonic because of the presence of iron (Christensen et al. 2023). Betalaine is also useful in reducing stress; it binds to GABA receptors to produce its action. *B. vulgaris* is also used as an anti-inflammatory agent and analgesic agent (Sarfaraz and Najam 2017,Sarfaraz and Ikram 2019).

It also possesses 5,5,6,6-tetrahydroxy-3,3-biindolyl, cyclo-DOPA (Dihydroxyphenylalanine), dihydroxyindole, betavulgarin, and phenolic amides. Besides these, cyclo-DOPA -5-o glycoside L-tryptophan, P-coumaric acid, ferulic acid, ferulyl glucose, carboxylic acid, and flavonoids have also been reported (Neelwarne and Halagur 2013). Despite its biochemical profile suggesting potential effects on mood and neuronal excitability, the anxiolytic activity of lyophilized *B. vulgaris* powder has not been thoroughly evaluated in standardized rodent behavioral models. Existing literature provides minimal and fragmented evidence, with no comprehensive assessment across multiple anxiety paradigms or using dose-comparative chronic treatment. The current study was designed to focus on evaluating the anxiolytic effect of *B. vulgaris* by using different models, and it is hypothesized that due to its antioxidant-containing neuroactive constituents, *B. vulgaris* will show significant anxiolytic effects.

2. Materials and Method

2.1 Selection of Animals and Housing Conditions

Rodents were taken to conduct this study, including mice (20-25 grams) and rats weighing about 200- 250g of both sexes. Both sexes were incorporated for generalization purposes. Animals were placed in proper plastic cages (n=6) of specific gender to avoid behavioral influence in the animal house of the Department of Pharmacology, University of Karachi. They were acclimatized for one week before the experimentation started. The temperature and humidity were controlled and kept to a standard that is 25±2° C and 50-65% respectively. The light and dark cycle of 12 hours was also monitored critically. Proper care of food (standard rodent diet) and water was also taken to reduce any environmental errors.

2.2 Animal Handling Guidelines and Ethical Approval

For the handling of animals, the proper guidelines of the National Institute of Health (NIH) guide for the Care and Use of Laboratory Animals (Research 2011) were followed. This study was legally authorized by the Institutional Board of Advanced Studies, and Research vide Resol. No. 10 (P).

2.3 Lyophilized Powder of *Beta vulgaris* (Beetroot)

The *B. vulgaris* lyophilized powder was bought from Sun Rise NutraChem Group, Qingdao, China. The product Lot number was Ctc 2015 0320. To maintain its purity, a proper Ziplock plastic bag was used to store and pack the material, and aluminum foil was also used to avoid degradation from sunlight.

2.4 Drugs and Chemicals

All analytical grade chemicals were used in this study. For the standard in the study, Lorazepam (2 mg Wyeth Pakistan LTD) was used. Distilled water was used as a solvent for all preparations; no suspending agents were used.

2.5 Designing the Model and Dosing Schedule

To design this study, albino mice were taken and assembled into IV groups. (n=6 per group)

Control (Group I)	Distilled water
Group II	*Beta vulgaris* (Beetroot) powder (500mg/kg) (Jain et al. 2011).
Group III	*B. vulgaris* powder (1000mg/kg)
Standard	Lorazepam (2mg/ 60kg = 0.03mg/kg)

The Control group was given distilled water because all the solutions of different doses were prepared in distilled water. A stock solution of 5000mg/25ml was prepared in distilled water before administration. The solution was given to mice according to their body weights (10ml/kg dosing volume). A stock solution of lorazepam was prepared (12mg/60ml) in distilled water and was also given orally.

Albino Wistar rats were selected and divided into three groups. The Control group was labeled as Group I and given distilled water. Treated groups were Groups II and III, which were given 500mg/kg and 1000mg/kg doses, respectively (Jain et al. 2011). All drugs were given one hour before carrying out the test for a period of 2 months by oral route.

Mice and rats were both used for the study as some of the testing parameters are species-validated models, where cage crossing and hole board test are more well established in mice, and open field and light and dark testing have more reliability and sensitivity when done in rats. The mice underwent a cage crossing test first, followed by a gap period of 2 hours, and then the hole board test was performed. Similarly, for rats, there was a 2-hour gap between the open field test and the light and dark test.

2.6 Cage Crossing Activity

According to the international standards to evaluate the anxiety models in rodents, the cage crossing method is used. The apparatus is placed in a calm place to avoid any external disturbance. The animals were comfortable with the environment and were kept in the cage. The readings were noted for a duration of 5 minutes (Sarfaraz et al. 2015).

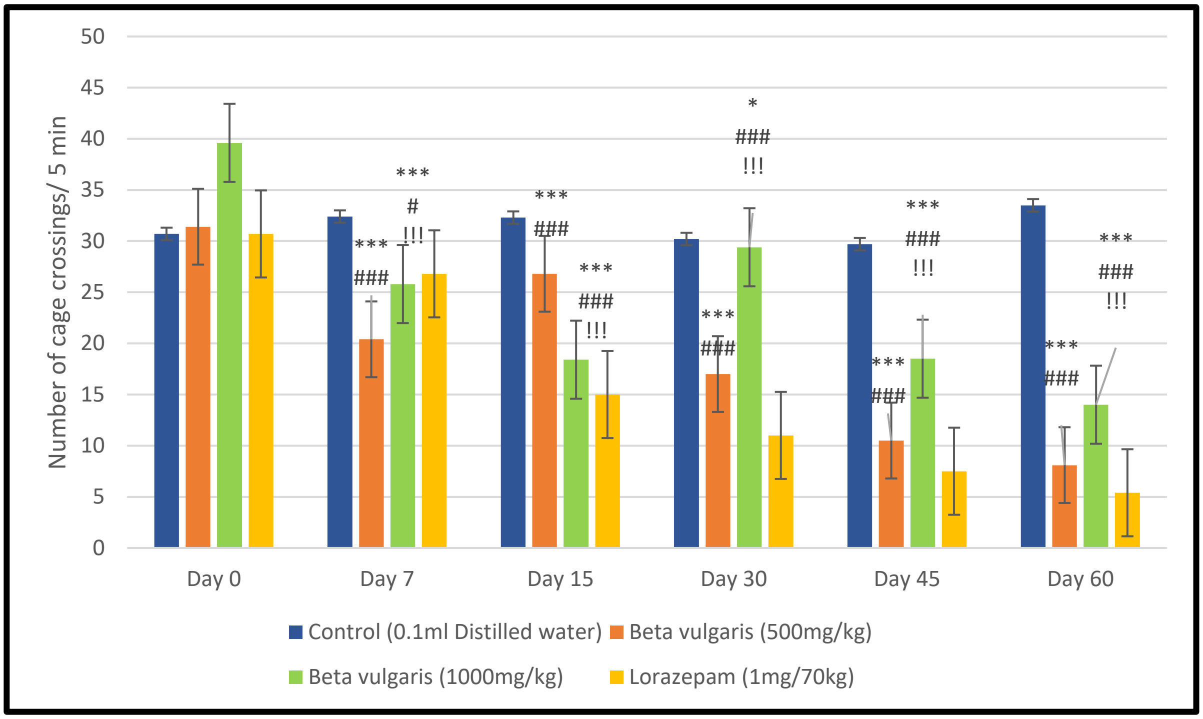
2.7 Hole Board Test

The apparatus consists of a board that is rectangular in shape, wooden, and enclosed with dimensions of (35cm×45cm×45cm). It contains 3 holes on all sides, which are about 2.5cm in diameter. The rodent was placed in the center of the apparatus, and it was allowed to explore for the duration of 5 minutes. The snout poking was observed.

2.8 Open Field Activity

An open field test was also used to evaluate the locomotor and behavioral activity in the anxiety model. The floor comprises 25 squares. Every

Figure I: Effect on the number of cage crossings on mice by *Beta vulgaris* at different doses.



where ***, * represent p≤ 0.001 and p≤ 0.05 as compared to control, ###, # represent p≤ 0.001 and p≤ 0.05 as compared to standard, and !!! represent p ≤ 0.001 when compared among treated groups. (n=6)

square diameter is 15cm. The Middle area also contains the central square of 15×15 cm. The rats are placed individually in the center of the open field for the duration of 10 minutes, and the total number of times the rats crossed the central and peripheral squares is noted (Strickland 2014).

2.9 Evaluation of Anxiety by the Light and Dark Model

Light and dark is designed to evaluate the anxiety behavior in rodents. The light compartment has transparent sides, which are uncovered, and a lamp is placed to provide brightness. The dark compartment is painted black and is enclosed by a black lid. To observe the anxiolytic effect, the rats were placed in the light area for ten minutes, and the time spent in the light area was recorded. The willingness of rats to explore the light-unprotected area shows the anxiolytic effect, and it is the feature that is to be monitored by the researcher.

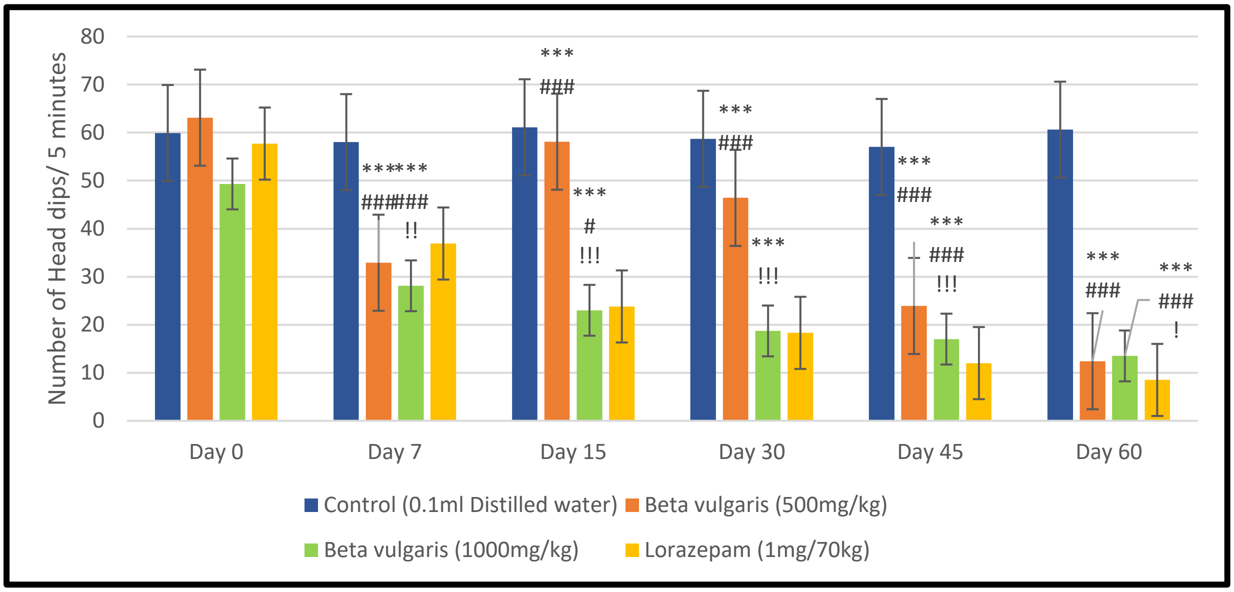
2.10 Statistical analysis

Two-way analysis of variance (ANOVA) followed by post-Tukey's test for multiple comparisons was used for assessing the mean ± SD between different groups by using statistical software SPSS 26. P ≤ 0.05 was considered statistically significant.

3. Results

Figure I displays the effect of orally administered ***B. vulgaris*** powder in mice at different doses on cage crossing activity. As compared to control analysis displayed a more notable (p≤0.001) decrease in the number of cage crossings by both doses throughout the treatment period. When equated with lorazepam, a more noteworthy increase in the number of cage crossings was observed in comparison to both doses throughout the treatment period. When the 500mg/kg dose was compared with the 1000mg/kg dose, after 15th days, 500mg/kg showed a substantial reduction compared to 1000mg/kg.

Figure II: Effect of Hole Board Activity on Mice by *B. vulgaris* at different doses.



where ***, * represent p≤ 0.001 and p≤ 0.05 as compared to control, $^{###}$, $^{#}$ represent p≤ 0.001 and p≤ 0.05 as compared to standard, and $^{!!!}$ represent p ≤ 0.001 when compared among treated groups. (n=6)

Figure II displays the effect of *B. vulgaris* powder on hole board activity in mice. When compared with the control, more noteworthy reductions in the number of head dips were observed by both doses of *B. vulgaris* throughout the treatment period. After day 15th, a more noteworthy rise in head dip was observed by both doses as compared to lorazepam. Comparison among the doses showed that a more substantial drop in head dip was observed by the 1000mg/kg dose till day 45, as compared to the 500mg/kg dose. However, on the 60th day of dosing, a 500mg/kg dose produced a more noteworthy effect as compared to the higher 1000mg/kg dose

Tables I and II show the effect of *B. vulgaris* on central square crossing and peripheral square crossings in Open field activity in rats. In comparison to control, a more substantial rise in the central square crossing was observed in both doses, whereas a more substantial decline in peripheral square crossings was noted throughout the treatment period.

Comparison among the treated groups showed insignificant differences on day 30th and day 45 of dosing. Whereas a more noteworthy incline in central crossings was observed on the 7th, 15^{th,} and 60th day of dosing by a 1000mg/kg dose as compared to a 500mg/kg dose. Similarly, 1000mg/kg showed a more substantial decline in peripheral square crossings as compared to 500mg/kg.

Table III displays the effect of *B. vulgaris* powder on the light and dark apparatus in the rat. Both doses showed more substantial time spent in the light area as compared to the control throughout the treatment period. Comparison among the treated groups showed a more noteworthy incline in time spent in the light area on day 7th and 45 by the 500mg/kg dose as compared to 1000mg/kg. However, on day 30th and day 60^{th,} the time spent in the light area was substantially raised by a 1000mg/kg dose as compared to a 500mg/kg dose.

4. Discussion

Anxiety is the feeling of worry, which is a common symptom of many diseases. For the evaluation and analysis of this anxiolytic behavior, many types of animal models are available. One of them is the cage crossing for evaluation and analysis of anxiety and locomotor behavior (Munawwar et al. 2024). The results of our study have shown a persistent decrease in cage crossings with the

Table I: Effect on Central Square Crossings in Open Field Activity on Rats by *B. vulgaris* at different doses.

Drugs	0 Day	7 Days	15 days	30 Days	45 Days	60 days
Control 0.1ml Distilled water	4.0±0.81	5.0±0.82	4.6±1.17	4.4±1.17	4.4±0.699	3.9±0.87
***B. vulgaris*, 500mg/kg**	3.5±0.52	5.8±0.42**	6.5±0.52***	5.5±0.53**	5.9±0.74***	5.9±0.57***
***B. vulgaris*, 1000mg/kg**	6.0±0.47	9.6±0.51***!!!	9.3±0.48***!!!	6.0±0.81***	6.1±0.87***	7.9±0.88***!!!

Table II Effect on Peripheral Squares in Open Field on Rats Activity by *B. vulgaris* at different doses.

Drugs	0 Day	7 Days	15 days	30 Days	45 Days	60 days
Control 0.1ml Distilled water	147.7±1.05	146.6±1.26	146.8±1.47	146.4±1.34	147.9±0.87	146.2±1.22
***B. vulgaris*, 500mg/kg**	106.9±0.87	56.1±0.87***	33.8±1.03***	52.5±1.43***	30.9±0.99***	27.1±0.87***
***B. vulgaris*, 1000mg/kg**	67.9±0.87	38.0±0.81***!!!	63.5±0.70***!!!	28.0 ±0.66***!!!	23.7±0.82***!!!	18.1±0.87***!!!

Values are represented as X ± SD, where ***, * represent p≤ 0.001 and p≤ 0.05 as compared to control, ###, # represent p≤ 0.001 and p≤ 0.05 as compared to standard, and !!! represent p ≤ 0.001 when compared among treated groups. (n=6).

doses of 500mg/kg of *B. vulgaris* powder as compared to the control group, thus indicating the anxiolytic potential of *B. vulgaris* powder. *B. vulgaris* consists of caffeic acid, which is a significant constituent liable for producing anxiolytic properties in low doses. The claimed mechanism of the anxiolytic property of caffeic acid is through the GABAergic mechanism (Pereira et al. 2006). The anxiolytic effect is mediated by GABA binding to its receptors, causing hyperpolarization. This hyperpolarization occurs due to the opening of chloride ions; thus, this mechanism reduces the firing rate of brain cells that are neurons (Mamun et al. 2016). Another model is the head dip, which is also called the hole board method, and is used in the study for the assessment and analysis of the exploratory and anxiety behavior (Campos et al. 2013). The results of our study indicated that there is a continuous decline in the head dip as compared to the control group. Head dip model results are matched with the results of cage crossing. It is also observed that *B. vulgaris* at a 500mg/kg dose doesn't have a sedative effect compared to lorazepam, which is known as the standard for producing an anxiolytic effect. Based on the literature evaluation, we can claim that the starting 500mg/kg dose of *B. vulgaris* powder binds to the $GABA_A$ ionotropic receptor and produces a remarkable decrease in head dip and cage crossing, but because of negative feedback on the 15th day, GABA levels were reduced, which increases head dip and cage crossing readings. Later on, both the readings of cage crossing and head dip again reduced; this may be because of binding GABA with the $GABA_B$ metabotropic receptor. Literature evidence confirmed that $GABA_B$ receptors are positive modulators of novel class anxiolytics, which do not have side effects as compared to $GABA_A$ receptors, and since $GABA_B$ full agonists, for example, baclofen (Mombereau et al. 2004).

One more test for the evaluation of anxiety, exploratory, and locomotor behavior in animals is the open field test. The results of our study indicate a continuous increase in central crossings with the comparison to the control group. We also observed that the peripheral crossings were

Table III: Effect of Time Spent in the Light Area in Light and Dark Model by *B. vulgaris* at different doses.

Drugs	0 Day	7 Days	15 days	30 Days	45 Days	60 days
Control 0.1ml Distilled water	27.2±1.47	30.0±0.81	25.8±1.54	25.4±1.17	28.3±0.67	29.8±1.03
B. vulgaris, 500mg/kg	39.4±1.26	64.9±0.87***	126.2±0.78***	101.9±0.87***	148.5±0.52***	157.5±0.53***
B. vulgaris, 1000mg/kg	33.5±0.52	60.9±0.99***!!!	126.2±0.63***	160.4±0.51***!!!	121.2±0.91***!!!	208.7±0.67***!!!

Values are represented as X ± S.D, where ***, * represent p≤ 0.001 and p≤ 0.05 as compared to control, and !!! represent p ≤ 0.001 when compared among treated groups. (n=6)

reduced constantly with the treatment of a 500mg/kg dose of *B. vulgaris,* with the comparison of the control group; thus, the *B. vulgaris* 500mg/kg dose reduced the overall locomotor activity and produced anxiolytic effects on rodents.

Another important test is the light and dark experiment. Rodents are naturally repulsive to bright light and develop anxiety, but anxiolytic agents increase the time duration of rodents in the light area (Yadav et al. 2008). Our study indicated that the time spent in light increased till day 15th, but then it reduced on the 30th day and again rose till the end of the treatment. The dose–response relationship was clarified, showing that the 1000 mg/kg dose produced more pronounced effects at several time-points, though not uniformly across the study duration.

A high concentration of oxygen is used by the brain to possess an antioxidant effect (Ng et al. 2008). Due to oxidative stress, the brain starts to undergo lipid composition that favors lipid peroxidation. This results in proteins and enzymes damage as well as reduction and inactivation of ion channels and receptors (Valko et al. 2007). Oxidative stress also reduces the membrane fluidity of brain cells, so it may alter brain activity, neuronal function, and neurotransmission. This oxidative stress of the brain is susceptible to cause many neurodegenerative, neuropsychiatric, and neurological problems, which include depression, anxiety, and schizophrenia (Månsson et al. 2019). *B. vulgaris* has betalain, which has a high concentration of water-soluble nitrogen components. Betalains are of two types. One is betacyanins, which are responsible for the red coloration of *B. vulgaris*. The second is betacyanins, which mainly possess the antioxidant property (Escribano et al. 1998). Due to this property, *B. vulgaris* shows anxiolytic activity. Our results in both open field and light and dark tests, respectively, showed anxiolytic activity after 30 days of dosing, and this might be because of the antioxidant property of *B. vulgaris* after chronic dosing.

Although the results of the study were promising, the study's limitations should be mentioned. The study did not highlight the exact mechanism of action, as molecular assays were not done. The dose-response curve was not linear, suggesting the need for intermediate dose inclusion and pharmacokinetic evaluation. Finally, the study used only behavioral endpoints, which limits mechanistic interpretation.

5. Conclusion

Although preliminary, the findings support the potential anxiolytic properties of *B. vulgaris* and provide a foundation for more detailed mechanistic and translational research. Future research should incorporate neurochemical analyses (e.g., GABA levels, oxidative stress biomarkers), receptor-binding studies, and electrophysiological assessments to confirm the mechanistic basis of *B. vulgaris* 's anxiolytic activity.

Data Availability

All the data related to the available manuscript is available to the Authors.

Conflict of Interest

The Authors declare that they have no competing interests.

Funding Statement

This research did not receive any funding.

Consent Forms

Not applicable

Study Approval

Mentioned in the methodology section.

Author's Contributions

SS and **RI** conceptualized the study, **SS, RM,** and **HR** performed the experimental work, **SS** and **HR** did the statistical analysis, **SS, RM,** and **HA** wrote the manuscript, and **RI** reviewed the manuscript.

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