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Original Article

Pharmacological Evaluation of the Antidepressant and Anxiolytic Activities of the Ethanolic Extract of *Selenicereus setaceus* in Rats

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ABSTRACT

Background: Both anxiety and depression are two frequent neuropsychiatric conditions associated with oxidative stress, imbalance of neurotransmitters and even neuronal damage. In this experiment, anxiolytic and antidepressant effect of ethanolic fruit extract of *Selenicereus setaceus* extract had been tested, due to its antioxidant and neuroprotective properties. **Objectives:** The purpose of this study is to evaluate the antidepressant, anxiolytic, and neuroprotective activities of the ethanolic fruit extract of *Selenicereus setaceus* in rats subjected to Chronic Unpredictable Mild Stress (CUMS), by assessing its effects on depression- and anxiety-like behaviours, oxidative stress, dopamine levels, and stress-induced neuronal damage, as well as exploring the possible interaction of its bioactive constituent ferulic acid with depression-related molecular targets through molecular docking studies. The study aims to establish the potential of *Selenicereus setaceus* as a promising natural source for the development of novel antidepressant and anxiolytic agents. **Materials and Methods:** Fruits of *Selenicereus setaceus* had been subjected to extraction with 95% ethanolic method followed by phytochemical screening and High-Performance Liquid Chromatography analysis and by some way, it identified ferulic acid as active principle. Molecular docking study had been done for protein interaction and antidepressant, and anxiolytic activity had been studied in Chronic Unpredictable Mild Stress model, which include normal control, disease control, fluoxetine 10 mg/kg, and extract-treated groups at 5 and 10 mg/kg dose. behavioral studies involved Open field test, Elevated plus maze and Tail suspension test along with measurement of Malonaldehyde and dopamine level and histopathology of hippocampus and amygdala. **Results:** The results of the present study demonstrated that the ethanolic fruit extract of *Selenicereus setaceus* exhibited significant antidepressant, anxiolytic, antioxidant, and neuroprotective activities in Chronic Unpredictable Mild Stress model, induced rats. Treatment improved exploratory and anxiety-related behaviours, reduced immobility time in the Tail Suspension Test, decreased elevated Malonaldehyde levels, and restored reduced dopamine levels. Histopathological examination showed protection against stress-induced neuronal damage and restoration of normal neuronal architecture in the hippocampus and amygdala. Phytochemical screening revealed the presence of alkaloids, flavonoids, and phenolic compounds, while molecular docking studies demonstrated favourable binding of ferulic acid with the selected target protein, supporting its potential role in the observed therapeutic effects. **Conclusion:** In conclusion, the findings of the present study suggest that ferulic acid derived from *Selenicereus setaceus* possesses significant antidepressant and anxiolytic activity, which may be attributed to its antioxidant, neuroprotective, and neurotransmitter-modulating effects. These results highlight the potential of *Selenicereus setaceus* as a valuable natural source of bioactive compounds for the management of depression and anxiety. However, further studies are required to elucidate the precise molecular mechanisms and to explore its clinical applicability in humans.

Keywords: Antioxidant, Cums, Ferulic acid, Neuroprotection, *Selenicereus setaceus*.

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INTRODUCTION

Neuropsychiatric disorders like depression and anxiety are some of the commonest mental illnesses across the world, but they have also become one of the largest public health concerns due to their increasing prevalence as well as significant socio-economic impacts. These neuropsychiatric disorders are responsible for more than half of all cases of disability according to World Health Organisation, and they are affecting many people across the

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world, irrespective of age groups (Patel *et al*., 2013). Depression and anxiety are characterized by disturbances in mood, thought processes, emotion, and behavior resulting from a combination of various factors, including genetics, environment, psychosocial stressors, and neurobiology (Cattaneo *et al*., 2015; Cui *et al*., 2024).

Neuroplasticity is the capability of the human brain to restructure itself throughout an individual's entire lifespan through developing new neural connections and structural changes (Depression, 2017); however, the phenomenon is best observable at a young age, when processes of synaptic pruning and myelination occur during brain development (Muzio *et al*., 2016). Nevertheless, the process of neuroplasticity is never finished because humans continue to develop through neuroplasticity. The evidence of this occurs on the one hand as a result of continuous exposure to various environmental stimuli, but also as a consequence of learning and the ways adults adjust their cognitive functioning by altering existing neural networks rather than starting from scratch. According to the findings of functional neuroimaging research, neuroplasticity remains relevant in adulthood as well as the ability of the brain to reshape itself after any injury or to learn new things, which is explained mostly by the continued neuroplasticity. Moreover, depression seems to occur as a consequence of the inability of the brain to adapt itself (Cattaneo *et al*., 2015).

"In accordance with the monoamine hypothesis, low levels neurotransmitters like serotonin, norepinephrine, and dopamine (Boku *et al*., 2018) in synapse can be the cause of depression; however, modern research tends to look at depression as a multifactorial disease. In addition to dysregulation of monoaminergic neurotransmission (Taniguti *et al*., 2018) disease associated with hyperactivation of hypothalamus pituitary adrenal axis (Menke, 2024), oxidative stress a mitochondrial dysfunction (Zanetti *et al*., 2021; Yu *et al*., 2023), neuroinflammation caused by pro-inflammatory cytokines (Gałecki and Talarowska, 2018) and impairment led to neurogenesis and synaptic plasticity (Tartt *et al*., 2022). High levels of cortisol due to prolonged stress can lead to neuronal atrophy, loss of dendrites, and other negative consequences. This process is particularly obvious in the case of the hippocampus, and thus, problems with memory and emotion regulation occur (Willard *et al*., 2013).

The metabolism of neurotransmitters can be affected by inflammatory mediators like interleukin-6, TNF-α and interleukin 1β, leading to improper neuronal communication. Also, there was increase in production of Reactive oxidative species which can lead to lipid peroxidation, protein oxidation, even DNA damage. All these effects, almost forming an overall chain reaction, ultimately result in gradual neuronal degeneration (Lee and Cui, 2010). When antioxidant protection mechanisms are diminished, the effect of oxidative stress is increased further on top of what is occurring (Fries *et al*., 2023).

Anxiety problems often result in depression in many individuals. The biological mechanisms behind anxiety disorders are similar to those behind depression, such as monoamine problems, Gamma Aminobutyric acid system abnormalities (Kothari *et al*., 2010), oxidative stress (Shaker *et al*., 2013), and a specific Hypothalamic pituitary adrenal axis dysfunction, hypothalamic, pituitary, and adrenal parts (Belmaker and Agam, 2008). In terms of the approach for treatment, it usually depends on Selective serotonin reuptake inhibitors, tricyclic antidepressants or benzodiazepines, selective norepinephrine reuptake inhibitors (DeGeorge *et al*., 2022). Many patients suffer from a delayed effect on drugs, face the side effects (sexual side effects, weight gain, and insomnia), and, of course, may not use medications regularly (Rabiei *et al*., 2018). In addition, about a third of all patients do not experience full remission during the first-line treatment. Thus, there is a clear need to develop some new treatments that would be effective and safe (Baldwin *et al*., 2005).

The natural products have, well, become more important as they can target multiple pathological mechanisms, while having lesser side effects. Phytochemicals that include various types like Flavonoids, alkaloids, Phenolic acids and Polyphenols have found to possess antioxidant, anti-inflammatory, neuroprotective and neurotransmitter modulating activity (Cutler *et al*., 2023), thus making them promising candidates for neuropsychiatric disorders treatment.

Selenicereus setaceus (dragon fruit), a member of the family Cactaceae, contains several bioactive phytochemicals including ferulic acid, caffeic acid, rutin, and kaempferol Ferulic acid has demonstrated potent antioxidant, anti-inflammatory, free radical scavenging, and neuroprotective properties in experimental studies It has been shown to regulate monoaminergic neurotransmission (Adell, 2020), attenuate oxidative stress preserve neuronal architecture (Zhang *et al*., 2021), and improve cognitive function (Castrén and Monteggia, 2021). However, limited studies have systematically evaluated the antidepressant and anxiolytic potential of ferulic acid isolated specifically from *Selenicereus setaceus* using a Chronic Unpredictable Mild Stress induced animal model.

MATERIALS AND METHODS

Chemicals

Indeed, all the reagents as well as the chemicals that we utilized for conducting this experiment were basically the best possible analytical reagents available. TCA, HCl, $FeCl_3$, chloroform, concentrated H_2SO_4, acetic acid, magnesium ribbon, TBA, methanol, ethanol 95%, phosphate buffer pH 7.4, normal saline0.9% NaCl and formalin 10% were all purchased from either Yucca Enterprises or from other authorized suppliers. Indeed, the Elabscience® MDA ELISA Kit was used and it basically allowed us to quantify lipid peroxidation almost directly. As for tissue

evaluation, the hematoxylin and eosin staining reagents and some other chemicals were bought from standard retailers.

Experimental Animals

Healthy albino Wistar rats of both sexes were collected from the institutional laboratory Animal Facility. The study was conducted in accordance with the relevant institutional and ethical guidelines. All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of NITTE COLLEGE OF PHARMACEUTICAL SCIENCE under approval number [NCOPS/IAEC/22/25-26]

During entire period study of animals had been housed under the standard laboratory conditions which translates into temperatures of 22-25°C, relative humidity 55-60%, with light and dark cycle 12 hr accordingly. Prior to conducting any experiments on the animals, there was a seven-day acclimatization period which enabled them to get acquainted with their designated locations in the laboratory. During this period, rats had been unlimited access to pelleted diet, water respectively.

Collection and Authentication of Plant Material

The *Selenicereus setaceus* fruit utilized for the study was sourced from the same location, and the collection of this fruit was verified by a botanist who was qualified to do so. Prior to any drying or extraction process of the fruits, they were thoroughly washed with tap water, then distilled water. Due to fact that all the samples were obtained from the same area, there was minimized variation in terms of phytochemical makeup of the samples.

Preparation of Extract

Fresh fruits had been thoroughly washed with distilled water, and the outer peel was removed and dried. The dried material was coarsely powdered were it passed through No 40 sieve to get uniform particle size.

An accurately weighed quantity of powdered material had been subjected for extraction in Soxhlet apparatus using 95% ethanol as solvent at 60-70°C. The solvent was concentrated under reduced pressure with rotary evaporator carried at 28-30°C to avoid degradation of thermolabile constituents (Figure 1).

The dried extract had been weighed and percentage yield calculated using:

$$\text{Percentage yield (\%)} = (\text{Weight of extract} / \text{Weight of sample}) \times 100$$

Qualitative Phytochemical Screening

The ethanolic extract was analysed qualitatively for the presence of phytochemical analysis for alkaloids, flavonoids, tannins, steroids, glycosides, saponins and phenolic compounds using standard phytochemical procedures mentioned in Table 1.

In silico Molecular Docking Studies

Molecular docking studies has been performed, kind of as a way to identify or generate some predictions of the probable interactions between ferulic acid and some of the proteins which are usually associated with depression, mainly, Monoamine

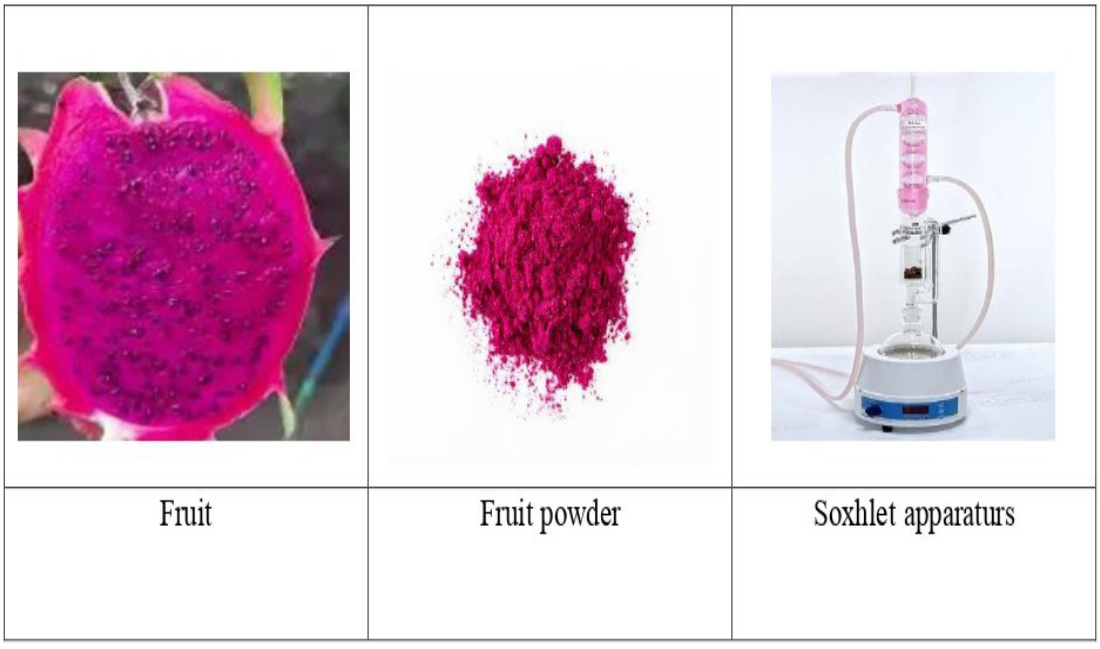


Figure 1: Extraction process.

Table 1: Quantitative phytochemical analysis screening.

Sl. No.	Phytochemicals	Test Description	Observation
1.	Alkaloids	2 mL of extract + 2 mL HCl → boil and cool → add 2 drops of Wagner's reagent	Presence of Brownish red coloration presence of alkaloid
2.	Tannin	2 mL extract + mL water + 2 or 3 drops of Ferric chloride 5%	no Green precipitate absence of tannins
3.	Flavonoids	3 mL extract → evaporated to dryness → Residue + 1-2 mL 50% CH_3CH_2OH + heat + Mg ribbon + 4-5 drops HCl	Presence of Red or orange color presence of flavonoid
4.	Steroids	5 mL extract + 2 mL $CHCl_3$ + 3 mL conc. H_2SO_4	Absence of Reddish brown precipitate at the interface
5.	Glycosides	2 mL extract + 2 mL $CHCl_3$ + 12 mL CH_3COOH	No Violet, blue, or green coloration absence of glycoside
6.	Saponins	2 mL extract → evaporated to dryness → Residue + 1 mL water → shaken vigorously	No Persistent foam (1 cm in the test tube) confirmed the absence of saponin
7.	Phenolic Compounds	2 mL extract + 2-3 drops of Ferric chloride solution (1%)	Presence of Bluish black or dark green colour indicated the presence of phenols

Oxidase A (MAO-A), TrkB, and the serotonin receptors 5-HT1A and 5-HT2A.

The three-dimensional structures of each of the target protein were downloaded from Protein Data Bank, abbreviated as PDB, while ligand itself was downloaded from PubChem. Then the ligand was prepared by energy minimization through the use of Open Babel along with Auto Dock Tools. The preparation of the protein involved removal water molecules and adding polar hydrogen wherever needed, along with charge assignment to the atoms. The docking process involved the use of Auto Dock vina, while the docking interface interactions were analysed using PyMOL and the protein-ligand interaction profiler, PLIP (Luo *et al.*, 2024).

Experimental Design

Thirty albinos Wistar had been randomly assigned to five groups were *n*=6 per group:

1. Group I: Normal (Normal saline).
2. Group II: CUMS control.
3. Group III: CUMS + Fluoxetine HCL (10 mg/kg, p.o.).
4. Group IV: CUMS + *Selenicereus setaceus* extract (100 mg/kg, p.o).
5. Group V: CUMS + *Selenicereus setaceus* extract (200 mg/kg, p.o).

Animals had been subjected to Chronic Unpredictable Mild Stress CUMS protocol for 21 period, except the normal group.

Induction of Depression by Chronic Unpredictable Mild Stress

Generally, this method implies exposing animals to variety of mild stressors in rather chaotic manner throughout the experiment period. Such stressors were neither unique nor extreme, they were selected in order to ensure exposure of the animals to different types of stress without following any predictable pattern, i.e., exposure is variable and unpredictable. Types of stressors included food deprivation, or water deprivation for 24 hr, tilting of the cage in a 45-degree angle, wet bedding, olfactory novel stressors, illumination through all-night periods, confining or restraining them for 1 hr, clamping of the tail with clothespin and also depriving them of any environmental stimuli. The use of such stressors is aimed at preventing accommodation of the animals since it results in tolerance formation and decreased the effect of the chronic stress condition. Thus, the chronic stress condition causes disruption of the hypothalamic-pituitary-adrenal axis functioning, neurotransmitter imbalance, increased oxidative stress, and impaired neuroplasticity. The symptoms, which are observed in the rats are depressed-like and anxious-like symptoms, characterized by reduced activity and exploratory behavior, reduced appetite or food interest, and hopelessness during the behavioral test procedure. They resemble symptoms of depression (Table 2 and Figure 2).

Body Weight Assessment

Body weight has been recorded before the experiment and subsequently on Days 7, 14, and 21 using a digital weighing balance to monitor physical changes during the experimental period.

Behavioral Assessment

Elevated Plus Maze (EPM)

Elevated plus maze will be utilized to measure the anxiolytic response of test agents on rats using an elevated a six-leg plus-maze circular environment made up of two open 16 X 5 cm arms and two closed 16 X 5 X 12 cm arms. Rats will receive one dose (oral) of trial agent, a vehicle, or a standard anxiolytic for each trial 1 hr prior to trial initiation. Dosing will be conducted in such a manner that the trial protocol for each rat will be initiated 1 hr post-dosing. A rat will be introduced to the EPM by placing it on one of the open arms positioned facing to the center of the EPM. An "entry" will be identified based on the placement of all four (4) of the rat's feet outside line designated that area of EPM. Number of entries and time spent in open and closed arms will be noted throughout the (5) min trial phase evaluation. During experiment, rats will be allowed socialization and all possible steps will be taken to reduce the opportunity for the introduction of other environmental components, besides the elevation associated with the EPM, that could create anxiety in the rats (Lister, 1987).

Open Field Test

This test is commonly employed behavioral assay to assess locomotion, exploration, and anxiety related responses in

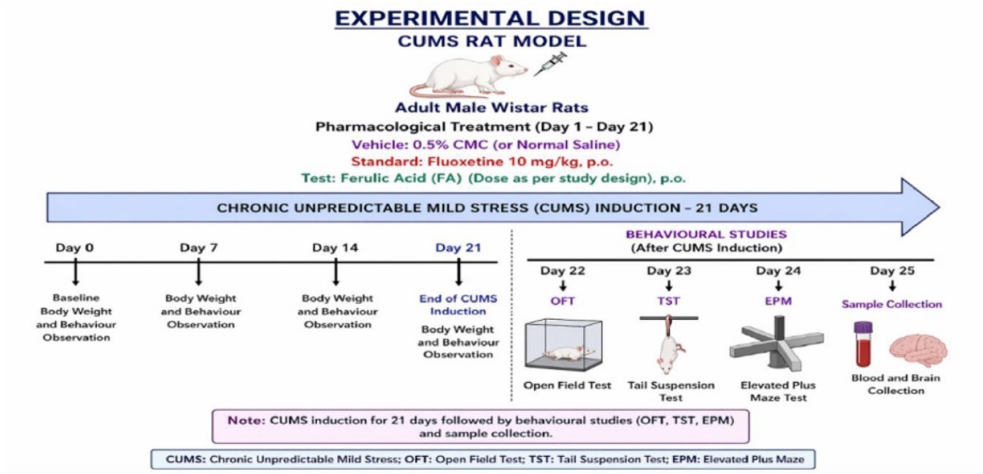


Figure 2: Experimental design.

Table 2: Experimental Stressors - Chronic Unpredictable Mild Stress Protocol.

Sl. No.	Stressors	Description
1.	Food and Water restriction	Food and water are withheld for 24 hr.
2.	Cage Tilting	Cage is tilted at approximately 45° (without bedding) for 3-4 hr to induce instability and discomfort.
3.	Damp Sawdust	About 125 mL of water is added to the cage to make the bedding maintained under moist condition for 6 hr
4.	Exposure to Novel Odour	Rats are exposed for predator or unfamiliar odour (e.g., urine or hair from another species) for 3-4 hr.
5.	Wet Cage	Bedding is replaced with water (~125 mL, 24±1°C, ~1 cm depth), and rats are kept in moist cage for 4 hr.
6.	Overnight lighting	Lights are kept on continuously 12 hr overnight, disrupting the normal light-dark cycle.
7.	Restraint Stress	Rats are placed in a ventilated restraint tube (appropriate size) for 4 hr, restricting movement.
8.	Tail Clip Stress	Approximately 1 cm from the tip of the tail is clipped briefly (~5 min) to induce mild stress.
9.	White Noise Exposure	Exposure to loud white noise (≈85 dB, e.g., predator/raptor sounds) for 10 min.
10.	No Bedding	Removal of bedding material; rats are kept in an empty cage for 1-6 hr.
11.	Empty Cage / Environmental Deprivation	Rats are housed in a cage devoid of environmental enrichment for a specified period to induce stress.

rodents. The apparatus generally consists of either square or circular arena with high walls and floor marked with grids. Rodents are assembled at center of arena and observed for 5-10 min. Parameters measured include total distance travelled, line crossings, duration spent in the center vs periphery, rearing, grooming, and fecal boli count. Increased center time suggests reduced anxiety, while decreased movement may indicate sedation or depression-like states. The test environment should be quiet and evenly illuminated (100-300 lux), and the arena must be cleaned between tests. The OFT is widely used for evaluating the effects of CNS-active drugs and behavioral phenotyping (Prut and Belzung, 2003).

Tail Suspension Test (TST)

Generally, chronic Unpredictable Mild Stress model implies exposing the rats to a variety of mild stressors in chaotic manner throughout the experiment period. Such stressors were neither unique nor extreme, they were selected in order to ensure exposure of the animals to different types of stress without following any predictable pattern, i.e., exposure is variable and unpredictable. Types of stressors included food deprivation, or water deprivation for 24 hr, tilting of the cage in a 45-degree angle, wet bedding, olfactory novel stressors, illumination through all-night periods, confining or restraining them for 1 hr and also depriving them of any environmental stimuli. The use of such stressors is aimed at preventing accommodation of the animals since it results in tolerance formation which reduces the effect of the chronic stress condition. Thus, the chronic stress condition causes disruption of the hypothalamic-pituitary-adrenal axis functioning, neurotransmitter imbalance, increased oxidative stress, and impaired neuroplasticity. The symptoms, which are observed in the rats are depressed-like and anxious-like symptoms, characterized by reduced activity and exploratory behavior, reduced appetite or food interest, and hopelessness during the behavioral test procedure. They resemble symptoms of depression (Zhang *et al*., 2021).

Biochemical Assessment

Estimation of Malondialdehyde (MDA)

The determination of the 2-thiobarbituric acid amount in the brain homogenate (10 g/100 mL, made 0.1 M phosphate buffer, pH 7.4) had been done by mixing brain homogenate with TBA reagent (2 mL) and the acidic reagent, which contained 0.25 N HCl solution, 15% TCA solution, and 0.37% TBA solution (the proportion of 1:1:1). The solutions were heated to 90°C for 40 min, cooled, centrifuged at 4°C, 5000 rpm during 10 min, then absorbance of TBA had been measured at 535 nm against the reagent blank (Dhangar *et al*., 2025).

The concentration of malondialdehyde in sample calculated according to following formula:

$A/EC \times DF = MDA\mu mol \times 1000 = MDA$ nmol/mg protein

Where, A = Absorbance, EC = Extinction coefficient, DF = Dilution factor.

Dopamine Estimation by ELISA

Serum dopamine concentration (DA; 4-(2-aminoethyl) benzene-1,2-diol) was determined using a competitive ELISA as previously reported by (Kim *et al*. 2008) Blood was collected and subsequently processed into blood serum in plain vacutainers (in about 20 to 30 min). Blood sera were subjected to centrifugation at 3000(±) Xg in 10 to 15 min. The serum was then incubated together with standards in dopamine (DAO)-specific antibody-coated wells in 1:1 ratio. Incubation was then followed by addition of HRP labelled secondary antibodies which had been incubated in presence of standards for 60 min at 37°C. The HRP incubation process the wells were then washed using phosphate buffered saline and the addition of tetramethylbenzidine to the wells to generate color development. Color development was terminated up on addition of stop solution and the OD of TMB had been measured using microplate reader at 450 nm with range 630 to 720 nm. Dopamine concentrations were determined from the standard curve and expressed in pg/mL.

Histopathological Examination of Brain

Brain tissue samples had been fixed with 10% formalin and then dehydrated using increasing levels of alcohol. The brain tissue samples were embedded in paraffin and sliced into 3-5 µm using the rotary microtome. The paraffin was then stripped off the sliced sections, followed by hydration and staining of the slides using hematoxylin and eosin and cresyl violet stains. The pathological changes and presence of Nissl substance and neurons were determined microscopically Olympus using Mag Vision software at 40× and 100× magnifications.

Lesions had been graded as:

- Minimal: <1%.
- Mild: 1-25%.
- Moderate: 26-50%.
- Moderately severe: 51-75%.
- Severe: 76-100%.

Statistical Analysis

All behavioral, biochemical data had been expressed as Mean±Standard Error of Mean (Mean±SEM). Statistical analysis had been performed using SPSS version 19.0. Comparisons among groups were carried out using one-way Analysis of Variance (ANOVA) followed by Bonferroni post hoc test. A p-value<0.01 had been considered statistically significant (Ekeanyanwu *et al*., 2021).

RESULTS

Extraction

Fruits of *Selenicereus setaceus* were successfully processed and soxhlet extraction subjected to 95% ethanol as solvent. extraction had been carried at 60-70°C under continuous reflux conditions, followed by concentration of the extract under reduced pressure to remove the solvent. A dark brown, semisolid ethanolic extract was obtained with a characteristic Odor. The extract was collected, weighed, and the percentage yield had been calculated with reference to initial weight of dried fruit powder. obtained extract had been stored at airtight container under refrigerated conditions until further phytochemical, HPLC, and pharmacological studies. The successful extraction indicated the efficient recovery of bioactive phytoconstituents from the fruit, which were subsequently used for the evaluation of antidepressant and anxiolytic activities.

Phytochemical Analysis

Preliminary phytochemical screening of the ethanolic fruit of *Selenicereus setaceus* revealed presence of alkaloids, flavonoids, and phenolic compounds (+), while tannins, steroids, glycosides, and saponins (–) are absent. The observed presence of flavonoids and phenolic compounds is indicative of substantial antioxidant activity, whereas alkaloids may contribute to the observed neuroprotective, antidepressant, and anxiolytic activities of the extract (Table 3).

Table 3: Indication of presence or absence of phytochemical constituents.

Sl. No.	Phytochemical Constituent	Results
1.	Alkaloids	+
2.	Tannins	–
3.	Flavonoids	+
4.	Steroids	–
5.	Glycosides	–
6.	Saponins	–
7.	Phenolic Compounds	++

Key: (+) Present, (–) Absent.

Molecular Docking

Docking Analysis of Ligand with Target Protein

Molecular docking studies had been conducted to assess binding affinity and interaction profile of selected ligand ferulic acid with the target protein TrkB kinase (PDB ID: 4ASZ). docking results had been analysed binding energy kcal/mol and root mean square deviation values. The docking study revealed multiple conformations of ligand within active site of target protein. Among all docking poses, the best binding affinity was observed at -5.9 kcal/mol, which indicates a moderate binding interaction between the ligand and protein. The RMSD values for the best pose were 0.000 Å, suggesting that this conformation is the most stable and serves as the reference structure. Other conformations showed increasing RMSD values, indicating deviations from the optimal binding pose. Binding affinities ranges of -5.2 to -5.9 kcal/mol suggest that ligand exhibits moderate binding strength, which may contribute to its biological activity (Table 4 and Figure 3).

Body Weight Assessment

Body weight of rats after exposure to CUMS has decreased consistently over time. Their body weight was normal on Day 0 and had already started to decrease at Day 7, with this decrease becoming even more severe by Day 14. By Day 21, this was a clear and obvious decline. overall decline of body weight in these rats is an indication of chronic stress being induced most likely

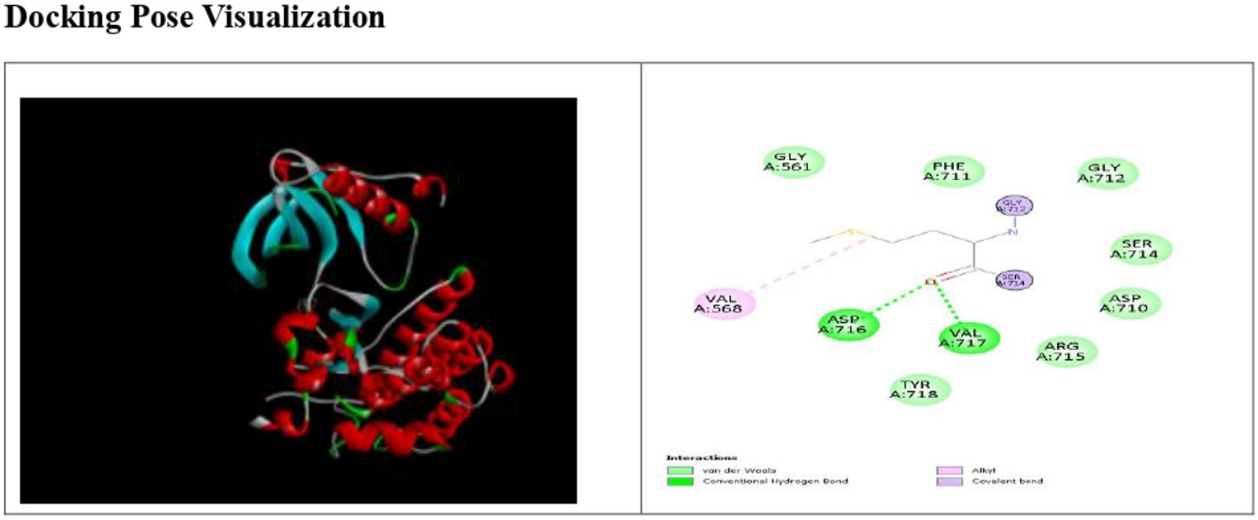


Figure 3: 3D Structure of Ligand-Protein Complex, 2D structure of ligand protein complex.

Table 4: Molecular Docking analysis of phytochemical of *Selenicereus setaceus* against targeted protein - TrkB kinase and target ligand - Ferulic acid.

Ligands	Binding Affinity	RMSD/ub	RMSD/lb
4ASZ_445858_uff_E=177.42	-5.9	0	0
4ASZ_445858_uff_E=177.42	-5.8	2.558	1.235
4ASZ_445858_uff_E=177.42	-5.8	5.454	2.333
4ASZ_445858_uff_E=177.42	-5.6	18.069	16.815
4ASZ_445858_uff_E=177.42	-5.6	25.919	24.561
4ASZ_445858_uff_E=177.42	-5.5	23.157	21.22
4ASZ_445858_uff_E=177.42	-5.4	29.15	26.993
4ASZ_445858_uff_E=177.42	-5.3	21.602	21.384
4ASZ_445858_uff_E=177.42	-5.2	13.396	11.242

due to reductions in dietary intake, changes to depressive like behaviours.

Behavioral Assessment

Open Field Test

a) In inner surface assessment test had been performed to examine exploratory behavior and locomotor activity in rats. control group CUMS showed lower inner surface activity compared to the normal group, indicating stress-induced behavioral impairment. Treatment with *Selenicereus setaceus* (100 mg/kg and 200 mg/kg) increased the time spent in inner surface at dose-dependent manner. 200 mg/kg dose produced a greater improvement than the 100 mg/kg dose and approached the effect of standard drug Fluoxetine HCL (10 mg/kg). These findings suggest that *Selenicereus setaceus* enhances exploratory behavior and reduces anxiety- and depression-like symptoms in CUMS-induced rats (Figure 4).

b) In outer surface assessment, the control group spent more time in the peripheral area, reflecting increased anxiety-like behavior. Administration of *Selenicereus setaceus* reduced time spent in outer surface when compared with control group, indicating reduced anxiety and improved behavioral performance. Effect was more pronounced in the 200 mg/kg dose group, which showed results comparable to the standard treatment. These results demonstrate the anxiolytic and antidepressant potential of Selenicereus setaceus in stressed animals (Figure 4).

Plus Maze

a) Control group exhibited a marked decrease in open arm activity, indicating enhanced anxiety-like behavior following CUMS exposure. Treatment with *Selenicereus setaceus* (100 mg/kg and 200 mg/kg) significantly increased open arm activity compared to control group and standard group. The higher dose produced a greater effect and showed improvement toward normal values. The increased open arm activity suggests a significant anxiolytic effect of *Selenicereus setaceus* (Figure 5).

b) Control group showed increased time spent in the closed arms, reflecting heightened anxiety. *Selenicereus setaceus* treatment reduced closed arm activity in both dose groups, with the 200 mg/kg dose producing a more pronounced effect. The reduction in closed arm preference indicates decreased anxiety and improved behavioral responses in treated animals (Figure 5).

Tail Suspension

In Tail Suspension Test, the control group exhibited increased immobility time, indicating depression-like behavior induced by chronic stress. Treatment with *Selenicereus setaceus* (100 mg/kg and 200 mg/kg there was significant reduction in immobility time relative to the control and standard group. antidepressant effect was more evident at 10 mg/kg, which showed results closer to those of Fluoxetine (10 mg/kg). These findings indicate that Selenicereus setaceus possesses significant antidepressant activity against CUMS-induced depressive behavior in rats (Figure 6).

Biochemical Assessment

Malondialdehyde

Level of Malondialdehyde reflecting lipid peroxidation and oxidative stress, had been significantly increased in CUMS control group when compared to normal, indicating enhanced oxidative damage due to chronic stress. Treatment with *Selenicereus setaceus* (5 mg/kg and 10 mg/kg) decreased MDA levels compared to the standard and control group, with 10 mg/kg dose showing a greater reduction. These findings suggest *Selenicereus setaceus* possesses antioxidant activity and protects against stress-induced oxidative damage at the brain (Figures 7 and 8).

Dopamine

The dopamine level was decreased in CUMS control group compared to normal group, indicating impairment of dopaminergic system associated with depressive behavior. Administration of *Selenicereus setaceus* (100 mg/kg and 200 mg/kg) increased dopamine levels relative to control group, with higher dose producing more pronounced effect. The improvement

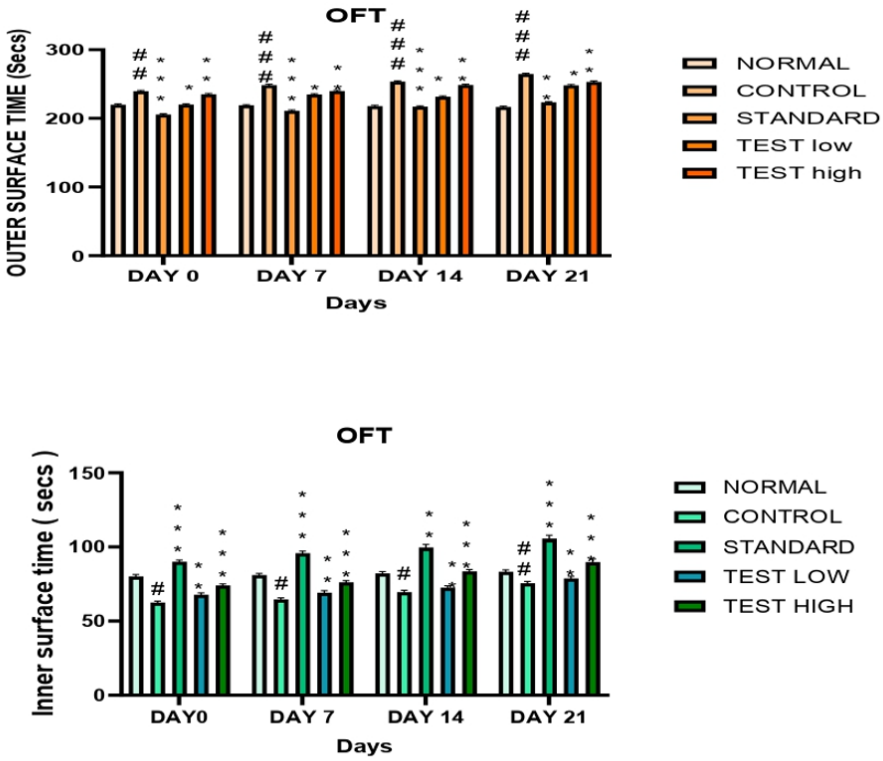


Figure 4: Open field test inner surface time and outer surface. Data expressed as Mean±SEM (n=6); significance values are ***p<0.001, **p<0.01, *p<0.05, normal vs standard, standard vs test groups using one way ANOVA followed Tukey kramer Multiple Comparison test by Graph pad prism software.

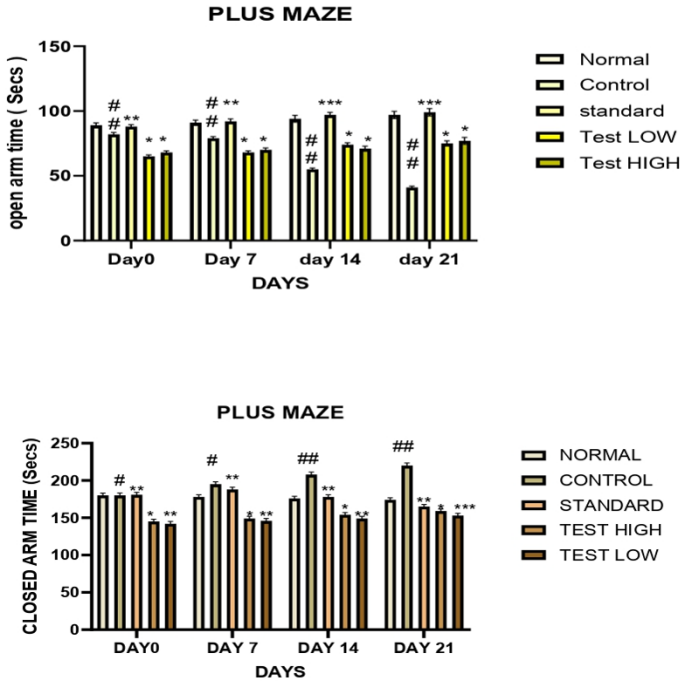


Figure 5: Plus maze closed arm parameter and open arm parameter. Data has been expressed as Mean ± SEM (n=6); significance values are ***p<0.001, **p<0.01, *p<0.05,. normal vs standard, standard vs test groups using one way ANOVA followed Tukey kramer Multiple Comparison test by Graphpad prism software.

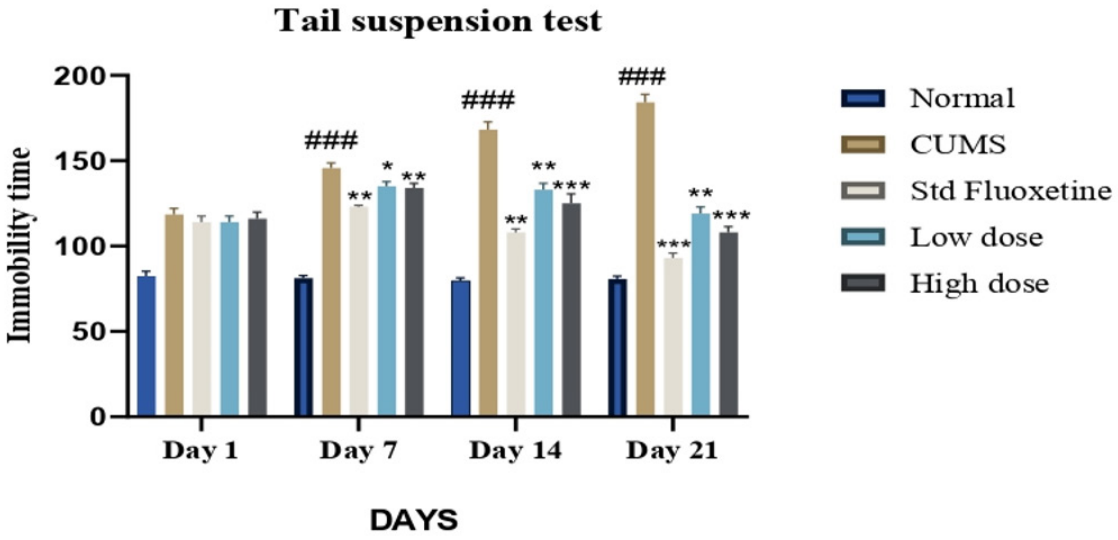


Figure 6: Tail suspension immobility time parameter. Data has been expressed as Mean±SEM (n=6); significance values are ***p<0.001, **p<0.01, *p<0.05,. normal vs standard, standard vs test groups using one way ANOVA followed Tukey kramer Multiple Comparison test by Graphpad prism software.

in dopamine concentration suggests that *Selenicereus setaceus* may exert antidepressant activity by enhancing neurotransmitter levels and restoring normal brain function (Figures 9 and 10).

Histopathology

Histopathological Evaluation and Neuroprotective Mechanism

Stress-induced depression is characterized by chronic disruption of HPA axis, associated with elevated cortisol levels, neuroinflammation, oxidative stress, reduced Brain derived neurotropic factor expression, decreased p11 levels of protein, enhanced glutamatergic signaling, impaired synaptic plasticity. These alterations lead to neuronal atrophy and loss of synaptic connectivity, particularly in the hippocampus, and amygdala. The amygdala plays a crucial role in depression by exhibiting hyperactivity thereby promoting persistent negative emotions and anxiety. Fluoxetine hydrochloride, a selective serotonin reuptake inhibitor SSRI, exerts antidepressant effects by inhibiting the serotonin transporter, increasing synaptic serotonin levels, promoting hippocampal neurogenesis, enhancing synaptic plasticity, and reducing neuroinflammation and oxidative stress. *Selenicereus setaceus*, a naturally occurring phenolic antioxidant, possesses potent neuroprotective, anti-inflammatory, and anti-apoptotic properties. It protects neuronal tissues by scavenging reactive oxygen species, modulating signaling pathways such as Toll Like Receptor 4/MyD88 and Sirtuin1, inhibiting glutamate-mediated excitotoxicity, and reducing neuronal apoptosis. Histopathological examination of the stress-induced control group revealed moderate degenerative changes, pyknotic nuclei with neuronal hyperplasia in the hippocampus, and gliosis with neuronal hyperplasia in the amygdala (Grade 3+). In contrast, the fluoxetine-treated group demonstrated normal neuronal morphology in all examined brain regions. *Selenicereus setaceus* treatment at both 100 mg/kg and 200 mg/kg restored normal histological architecture, with the high-dose group showing marked reduction of stress-induced lesions and near-normal morphology of the hippocampus, and amygdala. These findings suggest that *Selenicereus setaceus* exerts significant neuroprotective effects against stress induced brain damage, primarily through its antioxidant and anti-inflammatory activities, thereby preserving neuronal integrity and reducing neuroinflammation mentioned.

DISCUSSION

The current study examined the antidepressant and anxiolytic of ethanolic fruit extract of *Selenicereus setaceus* using a Chronic Unpredictable Mild Stress CUMS model in rats. Preliminary Phytochemical screening analysed presence of alkaloids, flavonoids, and phenolic compounds, these phytochemicals are well known for their antioxidant and neuroprotective properties and may contribute to the observed pharmacological effects. Exposure to chronic stress produced significant anxiety- and depression like behaviors in animals. Open field test and elevated plus maze, *Selenicereus setaceus* treated groups showed improved exploratory activity and reduced anxiety compared to stress control group. Similarly, in Tail Suspension Test, *Selenicereus setaceus* significantly reduced immobility time, indicating antidepressant activity. These findings had agreement with previous reports demonstrating that *Selenicereus setaceus* can improve mood-related behaviors through modulation of

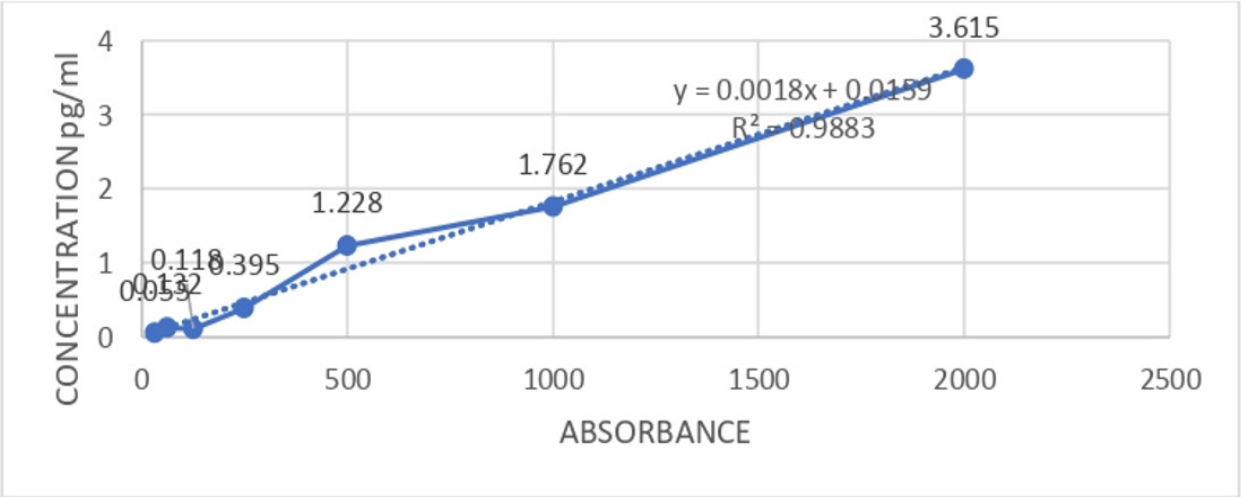


Figure 7: Standard Results.

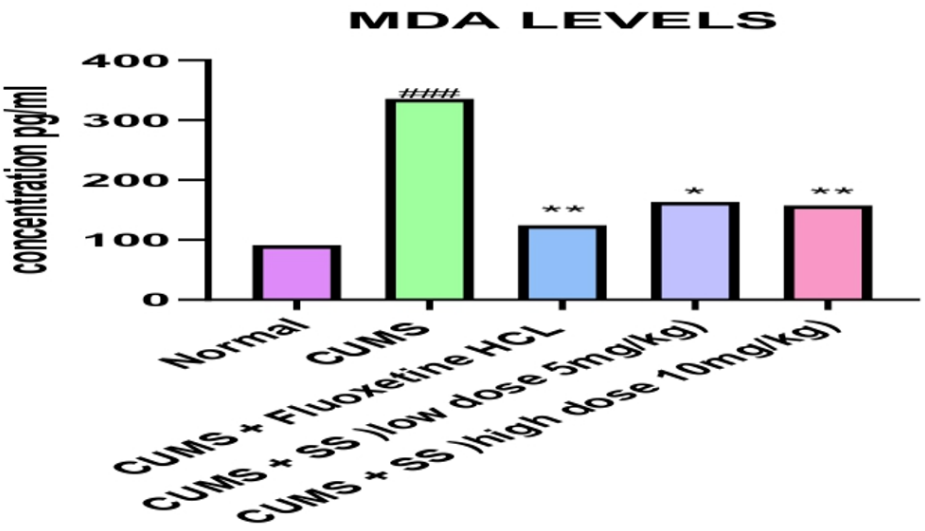


Figure 8: Test Mda Results.

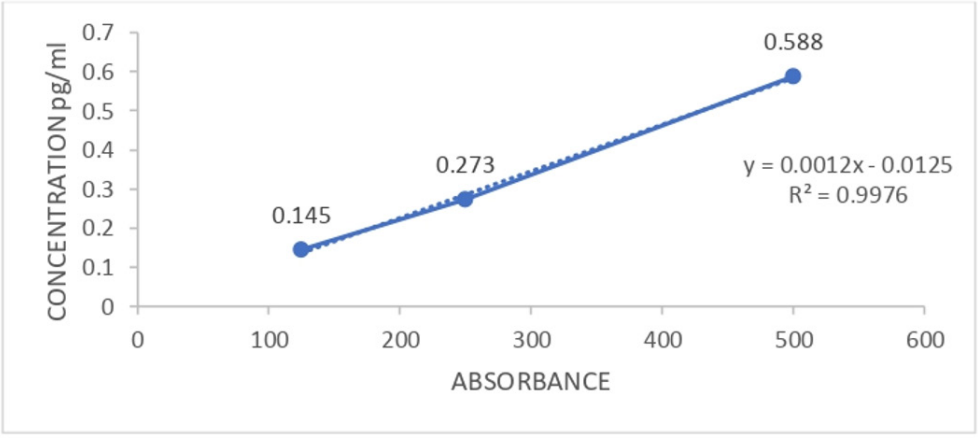


Figure 9: Standard Results.

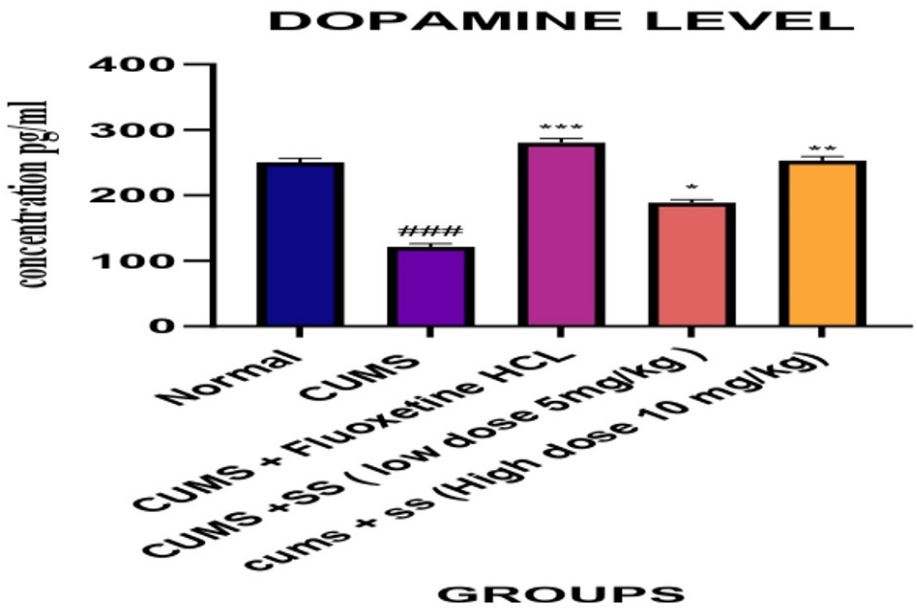
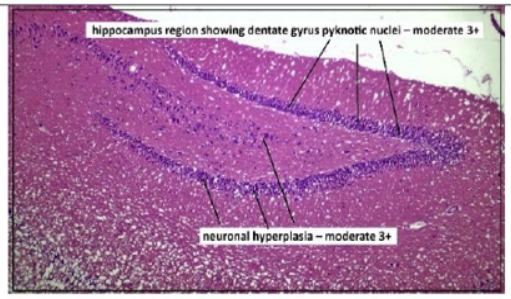
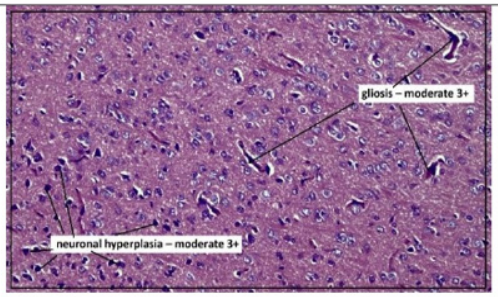


Figure 10: Test Dopamine Results.



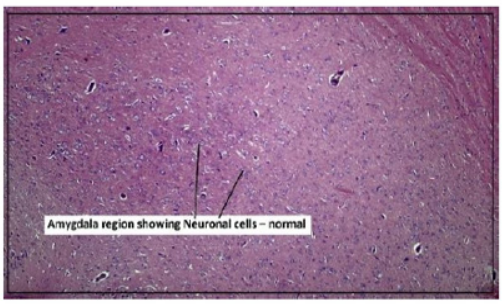
DC Rat Brain: hippocampus dentate gyrus region normal Morphology – NAD+ (X40)



DC Brain: Amygdala region showing Neuronal cells – normal – NAD+ (X40)



STANDARD Rat Brain: hippocampus dentate gyrus region normal Morphology – NAD+ (X40)



Standard Rat Brain: Amygdala region showing Neuronal cells – normal – NAD+ (X40)

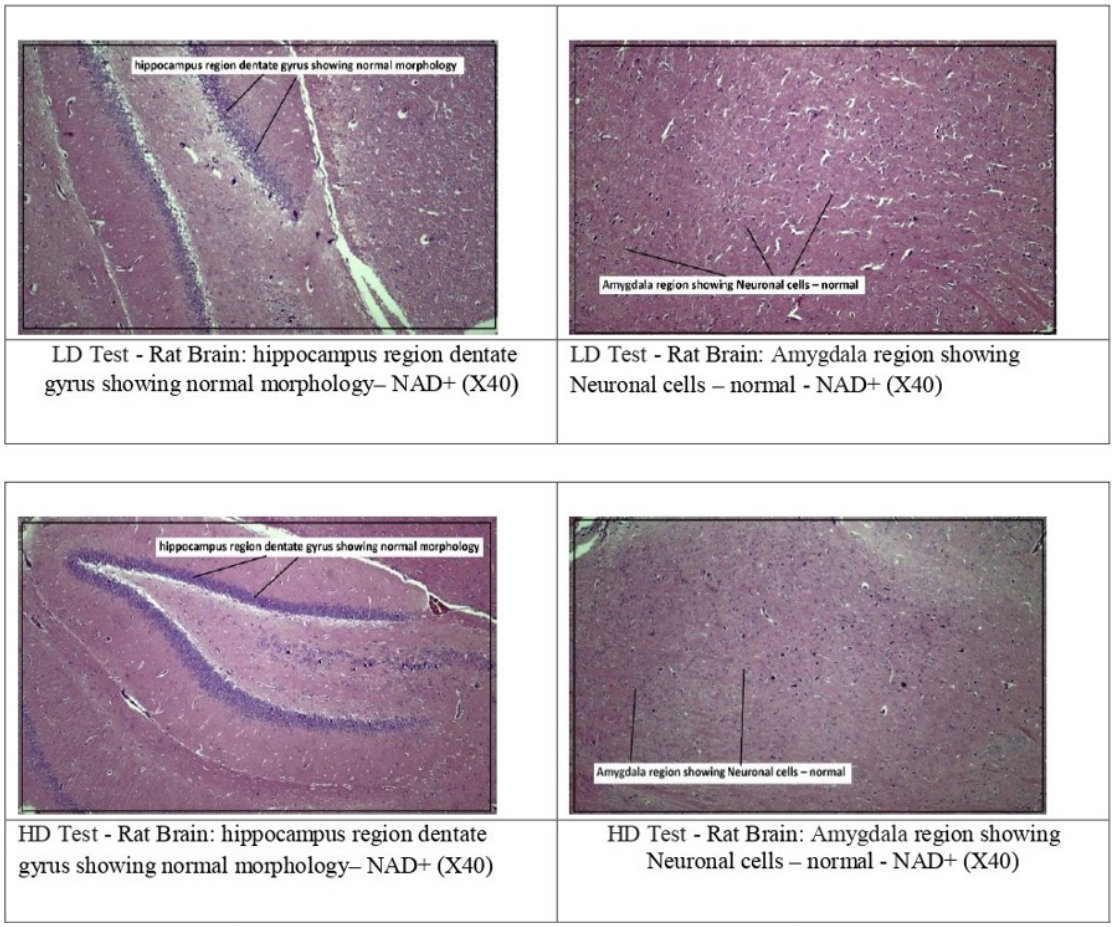


Figure 11: Histopathology of depression and anxiety activity.

monoaminergic neurotransmitters and reduction of oxidative stress. Biochemical evaluation revealed elevated MDA levels and decreased dopamine levels in CUMS-induced rats, indicating increased oxidative stress and impaired neurotransmission. Treatment with ferulic acid significantly reduced lipid peroxidation and restored dopamine levels towards normal. This suggests that the antidepressant and anxiolytic effects of ferulic acid may be mediated through its antioxidant activity and its ability to maintain neurotransmitter balance. Histopathological examination further supported the behavioral and biochemical findings. Stress-induced rats exhibited neuronal degeneration, inflammatory changes, gliosis, and pyknotic nuclei in the hippocampus, and amygdala. *Selenicereus setaceus* treatment markedly restored normal neuronal architecture and reduced stress-induced pathological alterations, indicating significant neuroprotective activity. Molecular docking studies also demonstrated favourable binding of ferulic acid with the selected target protein, supporting its potential role in modulating pathways associated with depression and anxiety.

CONCLUSION

Results of present study indicate that ethanolic fruit extract of *Selenicereus setaceus* exhibits significant antidepressant, anxiolytic, and neuroprotective activities in Chronic Unpredictable Mild Stress induced rat model. Phytochemical screening confirmed the presence of bioactive phenolic compounds. Behavioral assessments showed improved exploratory behavior and reduced depressive-like symptoms, while biochemical studies revealed decreased lipid peroxidation (MDA) and restored dopamine levels. Histopathological examination further confirmed protection against stress-induced neuronal damage in the hippocampus, and amygdala. Molecular docking studies supported these findings by demonstrating favorable interactions of ferulic acid with the selected target protein. Overall, the results suggest that *Selenicereus setaceus* exerts its therapeutic effects through antioxidant, neuroprotective, and neurotransmitter-modulating mechanisms, highlighting *Selenicereus setaceus* as promising natural source for development of novel antidepressant and anxiolytic agents. Further studies warranted to elucidate its molecular mechanisms and clinical potential.

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ABBREVIATIONS

ANOVA: Analysis of Variance; **CUMS:** Chronic Unpredictable Mild Stress; **ELISA:** Enzyme-Linked Immunosorbent Assay; **EPM:** Elevated Plus Maze; **HPA:** Hypothalamic-Pituitary-Adrenal; **IAEC:** Institutional Animal Ethics Committee; **MDA:** Malondialdehyde; **OFT:** Open Field Test; **PDB:** Protein Data Bank; **RMSD:** Root Mean Square Deviation; **ROS:** Reactive Oxygen Species; SEM: Standard Error of the Mean; **SPSS:** Statistical Package for the Social Sciences; **TST:** Tail Suspension Test; **TrkB:** Tropomyosin Receptor Kinase B.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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