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Evaluation of Antidepressant Effects of Silibinin Alone and in Combination with Sertraline and Venlafaxine in Swiss Albino Mice

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Abstract

Depression is a major psychiatric disorder characterized by persistent low mood, anhedonia, and impaired functioning. Current antidepressant therapies are often limited by delayed onset and incomplete response. The present study evaluated the antidepressant-like effects of Silibinin alone and in combination with Sertraline and Venlafaxine in a Chronic Unpredictable Mild Stress (CUMS) model in Swiss albino mice. Animals were divided into seven groups and subjected to CUMS for 6 weeks, with drug treatment administered from day 22 to day 42. Antidepressant activity was assessed using the Forced Swim Test (FST), Tail Suspension Test (TST), Sucrose Preference Test (SPT), Actophotometer, and Coat State scoring. CUMS exposure significantly increased immobility time in the FST (143.2 ± 6.18 s) and TST (155.5 ± 10 s) and reduced sucrose consumption (4.842 ± 0.5358 ml), indicating behavioral despair and anhedonia. Treatment with Silibinin (100 mg/kg), Sertraline (5 mg/kg), and Venlafaxine (16 mg/kg) significantly improved these parameters. Notably, combination therapy produced superior effects, with the Silibinin–Venlafaxine group showing the greatest reduction in immobility (68.0 ± 5.44 s in FST; 88.5 ± 4.7 s in TST) and the highest sucrose consumption (12.26 ± 0.817 ml). Combination-treated groups also demonstrated enhanced swimming and climbing behaviors. Locomotor activity analysis confirmed the absence of nonspecific CNS stimulant effects, while coat state deterioration induced by CUMS was significantly reversed in all treated groups.

Major Findings: The findings suggest that Silibinin possesses significant antidepressant-like activity and enhances the efficacy of conventional antidepressants, with the Silibinin–Venlafaxine combination showing superior outcomes.

Keywords: Antidepressant Activity, Sertraline, Silibinin, Stress, Venlafaxine

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1. Introduction

Depression is a chronic and debilitating psychiatric disorder characterized by persistent low mood, anhedonia, cognitive impairment, and disturbances in emotional and physical functioning. It represents a major contributor to the global burden of disease, affecting more than 350 million individuals worldwide and constituting the leading cause of years lived with disability[1]. Despite significant advances in understanding the neurobiology of depression and the development of multiple pharmacological classes of antidepressants, treatment outcomes remain suboptimal. Approximately 60–70% of patients respond to first-line antidepressant therapy, while 10–30% develop Treatment-Resistant Depression (TRD), which is associated with impaired social and occupational functioning, increased suicidal ideation, physical health deterioration, and greater healthcare utilisation[2,3]. These limitations highlight the urgent need for more effective and mechanistically diverse therapeutic strategies.

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The pathophysiology of depression is complex and multifactorial, involving dysregulation of monoaminergic neurotransmission, including Serotonin (5-HT), Norepinephrine (NE), and Dopamine (DA), as well as alterations in glutamatergic and GABAergic signalling[4]. In addition, hyperactivity of the Hypothalamo–Pituitary–Adrenal (HPA) axis, immune system activation, neuroinflammation, circadian rhythm disturbances, and impaired neuroplasticity contribute substantially to the development and persistence of depressive symptoms[5]. Chronic stress induces sustained activation of the HPA axis, leading to elevated cortisol levels, reduced negative feedback, and increased production of pro-inflammatory cytokines, which further disrupt monoaminergic neurotransmission and neurotrophic signalling[6,7]. Reduced Brain-Derived Neurotrophic Factor (BDNF) expression in the hippocampus and prefrontal cortex has been strongly associated with depressive states and impaired neurogenesis[4,8]. Currently prescribed antidepressants such as Selective Serotonin Reuptake Inhibitors (SSRI) and Serotonin–Norepinephrine Reuptake Inhibitors (SNRI) exert their therapeutic effects primarily by enhancing monoaminergic neurotransmission. Sertraline, a widely prescribed SSRI, increases synaptic serotonin availability by inhibiting presynaptic serotonin reuptake and has demonstrated favorable efficacy and tolerability profiles[9,10]. Venlafaxine, an SNRI, inhibits the reuptake of both serotonin and norepinephrine in a dose-dependent manner and has shown superior efficacy in certain depressive subtypes, including treatment-resistant and seasonal affective disorders[11]. However, despite their widespread use, these agents are limited by delayed onset of action, incomplete remission rates, and residual symptoms, leading to frequent use of combination or augmentation strategies in TRD.

Combination therapy has emerged as an important second-line approach in depression management, particularly for patients who fail to respond adequately to monotherapy. Combining antidepressants or adjunctive agents with complementary mechanisms of action has been shown to improve response rates, accelerate symptom relief, and enhance remission outcomes. Importantly, selecting agents with distinct pharmacodynamic targets and non-overlapping metabolic pathways reduces the risk of pharmacokinetic drug–drug interactions and improves treatment tolerability.

Natural compounds with neuroprotective and antioxidant properties have gained attention as potential antidepressant agents. Silibinin, a flavonolignan derived from milk thistle, has shown antioxidant, anti-inflammatory, and neuroprotective effects in various experimental studies. However, its antidepressant potential, particularly in combination with standard antidepressant drugs, remains insufficiently explored[12].

Silibinin, the principal bioactive flavonolignan of *Silybum marianum*, has gained increasing attention for its neuroprotective, antioxidant, and anti-inflammatory properties. Preclinical studies demonstrate that Silibinin crosses the blood–brain barrier and exhibits antidepressant-like effects in validated behavioral models, including the FST and the TST. Mechanistically, Silibinin enhances monoaminergic neurotransmission by increasing brain levels of serotonin, dopamine, and norepinephrine, stimulates 5-HT_1A receptors, and significantly upregulates BDNF expression, thereby promoting hippocampal neurogenesis and neural stem cell proliferation[13,14]. Additionally, Silibinin attenuates oxidative stress by reducing lipid peroxidation and scavenging free radicals, suppresses nitric oxide and cGMP signaling involved in glutamate release, and mitigates neuroinflammation by reducing pro-inflammatory cytokines such as Tumor Necrosis Factor (TNF-α), IL-1β, and IL-6 [15].

Given the multifaceted pathophysiology of depression and the limited success of monoaminergic monotherapy, Silibinin represents a promising candidate for adjunctive antidepressant therapy. Notably, Silibinin is primarily metabolized by CYP2C8, whereas Sertraline is metabolized mainly by CYP3A4 and CYP2B6, and Venlafaxine is biotransformed predominantly by CYP2D6. These distinct metabolic pathways minimize the likelihood of metabolic interference when used in combination.

The present study hypothesizes that Silibinin possesses antidepressant-like activity and can enhance the therapeutic effects of conventional antidepressants such as Sertraline and Venlafaxine in a chronic unpredictable mild stress model in mice.

Based on this rationale, the present study was designed to evaluate the antidepressant potential of Silibinin alone and in combination with Sertraline or Venlafaxine using a CUMS model in Swiss albino mice. Behavioral despair, anhedonia, locomotor activity, and stress-induced self-care deficits were assessed using established behavioral paradigms. This study is novel in evaluating Silibinin not only as a standalone treatment but also as an adjunct to both Sertraline and Venlafaxine in a

chronic stress-induced depression model. Furthermore, it provides comparative insights into combination therapy, highlighting the superior efficacy of the Silibinin–Venlafaxine combination in improving behavioral and anhedonia parameters without affecting locomotor activity.

2. Materials and Methods

2.1 Chemicals and Reagents

Tween 80, Dimethyl Sulfoxide (DMSO) was obtained from the laboratory stock of St. John Institute of Pharmacy and Research, Vevoor, Palghar. Sertraline, Silibinin, and Venlafaxine were purchased from Yarrow Chemicals Pvt. Ltd., Mumbai. Saline was procured from the local pharmacy store.

2.2 Dose Preparation

Regular saline (0.9% w/v NaCl) was used to dissolve Sertraline. DMSO was added one at a time until the final concentration was 5%, followed by a water solution containing 0.25% Tween 80 until the final concentration was 20%, and finally saline to reach 100% volume in order to dissolve Silibinin. Normal saline was used to dissolve Venlafaxine. Silibinin was evaluated for CNS depressant effects at intraperitoneal (i.p.) dose levels of 100 mg/kg and in combination with Sertraline and Venlafaxine at 50 mg/kg concentration.

2.3 Animals Procurement

Healthy Swiss Albino mice, sex male, weighing 25-30g, were obtained from Lascmi Biopharms Pvt. Ltd. {Registration No. 1277/PO/RcDT/09/CCSEA]. Mice were provided unrestricted access to a standard commercial diet and water. They were housed in a controlled environment with a 12-hour light/dark cycle, and ambient temperature of 22 ± 5°C, and relative humidity maintained between 50% and 60% (though not below 30% or exceeding 70%). Before beginning the experimental study, all animals were acclimatised for 7-10 days under laboratory conditions. All the experiments were conducted during the light portion between 8:00 AM and 4:00 PM. The experimental protocols and procedures used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the St. John Institute of Pharmacy and Research, Palghar, Maharashtra (SJIPR/IAEC/03/23-24).

2.3.1 Experimental Groups

A total of 42 mice were evenly distributed into seven groups (n=7/group). The groups were as follows: Control, Disease control, Standard I, Standard II, Test Group, Combination Group I, and Combination Group II.

2.3.2 Drug Administration

2.3.2.1 Dosing Regimen

The control group and the disease control group received Saline, Standard groups I received Sertraline 5 mg/kg, and group II received Venlafaxine 16 mg/kg. The Test Group was treated with Silibinin 100 mg/kg, the Combination Group I received (Sertraline 2.5 mg/kg + Silibinin 50 mg/kg), and Group II was treated with (Venlafaxine 8 mg/kg + Silibinin 50 mg/kg). Dosing started after the third week (22nd day) and lasted until the end of the 6th week (42nd day).

2.4 Chronic Unpredictable Mild Stress (CUMS) Protocol

CUMS protocol was adopted to produce stress by various methods to mimic depression by means of behavioral changes for several weeks. Except for the control group, all groups were subjected to CUMS protocol methods. Day 1: Food and water deprivation, Day 2: Overnight illumination, Day 3: Exposure to an empty bottle and foreign objects (e.g. marbles), Day 4: Cage tilt (45 degrees), Day 5: Water deprivation and Solid cage (100 ml water in 100 g bedding), Day 6: Overnight illumination and food deprivation, and on Day 7: Empty bottles and cage tilt. The aforementioned stimulations were intended to deliver one random form of stimulation per day, with no repetition of the same type of stimulation on consecutive days, ensuring the animals received the unpredictable stimulation. The process lasts for 6 weeks until the last day of the study, and behavioral tests were performed on Day 0, Day 21, and Day 42.

2.5 Evaluation of CNS Depressant Activity

2.5.1 Antidepressant Activity by Forced Swim Test (FST)

Antidepressant activity was evaluated using the FST, based on the principle of behavioral despair and reduced immobility. The individual animals from all groups were made to swim in a Plexiglas cylinder (20cm in diameter x

30cm in height) filled with water at 22-24°C, at a depth of 18cm. The mice were acclimatized and trained for 1 min one day before the test. Animals were randomly assigned to 7 groups (n = 6) and received intraperitoneal (i.p.) injections for 21 days, 30 min before the test. Animals were treated according to the dosing regimen under Drug Administration and Experimental Groups. Subsequently, each mouse was placed in the Plexiglass cylinder for 6 min on Days 0, 21, and 42. Activity scores were recorded during the last 4 minutes. An animal with immobility is referred to when animals stop struggling and stay still in water, making the slightest movements necessary to maintain its head above water[16].

2.5.2 *Antidepressant Activity by the Tail Suspension Test (TST)*

Antidepressant activity was evaluated using the TST, based on the principle of behavioral despair and reduced immobility. The individual animals from all groups were suspended on an aluminum rod using adhesive tape positioned at 1cm from the tip of the tail. Mice were suspended 58cm above the ground on an aluminum stand 58cm x 30cm. Animals were randomly assigned to 7 groups (n = 6) and received intraperitoneal (i.p.) injections for 21 days, 30 min before the test. Animals were treated according to the dosing regimen under Drug Administration and Experimental Groups. Subsequently, each mouse was suspended for 5 min for the duration of immobility on Days 0, 21, and 42. Activity scores were recorded. When mice dangle silently and motionlessly, they are said to be stationary[17,18].

2.5.3 *Antidepressant Activity by Actophotometer*

Locomotor activity was evaluated using an Actophotometer (Japson/India), based on the principle of photobeam interruption. The animal moved within an enclosed arena (30cm × 30cm × 30cm) of the Actophotometer. The mice were acclimatized before testing. Baseline activity was recorded before the administration of any treatment. Animals were randomly assigned to 7 groups (n = 6) and received intraperitoneal (i.p.) injections for 21 days, 30 min before the test. Animals were treated according to the dosing regimen under Drug Administration and Experimental Groups. Subsequently, each mouse was placed in the Actophotometer for 5 min on Days 0, 21, and 42, and activity scores were recorded[19].

2.5.4 *Antidepressant Activity by the Coat State Test*

The coat state test was performed weekly throughout the study in mice randomly allocated to 7 groups (n = 6 per group) and received intraperitoneal (i.p.) injections for 21 days. Animals were treated according to the dosing regimen under Drug Administration and Experimental Groups. The condition of seven body regions - head, neck, forepaws, hind paws, back, abdomen, and tail- was systematically evaluated. Each region was scored as 0 (excellent; smooth, shiny fur without ruffling or spiky patches), 0.5 (moderate; slightly fluffy fur with occasional spiky patches), or 1 (poor; disheveled, fluffy fur with light staining). The total coat state score for each animal was calculated by summing the scores of all seven body regions together[20] (Figure 1).

Antidepressant Activity by the Sucrose Preference Test (SPT)

Mice were randomly assigned to 7 groups (n = 6) and received intraperitoneal (i.p.) injections for 28 days. All mice were kept in individual cages and were trained to consume 4% (w/v) sucrose solution before starting CUMS protocol. The test was performed on Days 0, 14, and 28. Mice were exposed to stress for 2 weeks without

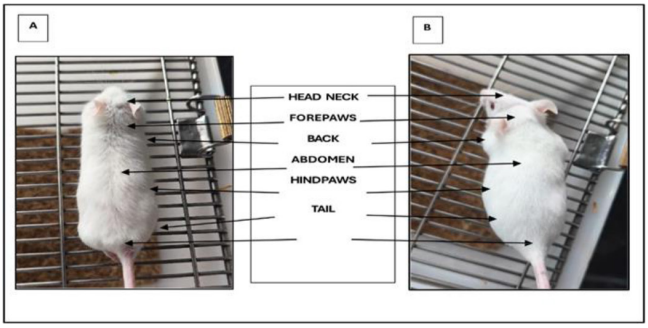


Figure 1. Evaluation of the coat states of (A) UCMS-treated mice and (B) non-UCMS control animals. The head, neck, forepaws, back, abdomen, hind paws, and tail are among the body components that are qualitatively evaluated to determine the coat state score. For each zone, the score is 0 if the fur is in good condition (smooth and shiny with no ruffled places), 0.5 if it is moderately bad (mildly fluffy with some spiky patches), and 1 if it is bad (much of the body is dirty, messy, and fluffy with minor stains). The non-UCMS control mouse in the figure would receive a score of 0.5 due to a slight degradation of the neck coat. The UCMS mouse would receive a total score of 3.5 after receiving a score of 1 for the head, 1 for the neck, 1 for the back, and 0.5 for the abdomen (which is not shown in the photo). In these images, mice were marked with picric acid.

treatment, and sucrose consumption was calculated as a baseline reading. After treatment for the next 2 weeks, sucrose consumption was calculated[21].

2.6 Statistical Analysis

The statistical evaluation was performed using the GraphPad Prism version 5.01. Groups were compared to assess the statistical significance using One-Way Analysis of Variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post hoc test. The data were represented as mean ± SD values and n = 6 per group.

3. Results

3.1 Forced Swim Test (FST)

In the FST, the disease control group showed a marked increase in immobility time (143.2 ± 6.186 s) and a significant reduction in swimming (26.17 ± 2.013 s) and climbing (45.67 ± 3.559 s) times compared to the normal control group. Treatment with Sertraline (5 mg/kg) and Venlafaxine (16 mg/kg) significantly reduced immobility duration (92.78 ± 5.845 s and 92.17 ± 6.5 s) and increased swimming (41.33 ± 2.582 s and 44.33 ± 3.266 s) and climbing (91.17 ± 3.43 s and 80.83 ± 2.858 s) times relative to disease control. Silibinin (100 mg/kg) also significantly decreased immobility time (105.5 ± 4.902 s) and improved swimming (40.17 ± 4.708 s) and climbing (83.5 ± 3.45 s) behaviours. The combination of Silibinin (2.5 mg/kg) + Sertraline (50 mg/kg) and Silibinin (8 mg/kg) + Venlafaxine (50 mg/kg) produced a greater reduction in immobility period (72 ± 5.505 s and 68 ± 5.441 s) along with a significant increase in swimming (42.5 ± 2.881 s and 48.17 ± 1.722 s) and climbing (91.67 ± 4.633 s and 107.5 ± 6.70 s) times compared to the disease control group. All the groups on Day 21 showed higher immobility time, lower swimming and climbing time when compared with the control. Day 42 shows lower immobility in both combination groups, higher swimming and climbing time when compared with the control (Figure 2-4).

3.2 Tail Suspension Test (TST)

In the TST, the disease control group showed a marked increase in immobility time (155.5 ± 10 s) compared to the normal control group. Treatment with Sertraline (5 mg/kg) and Venlafaxine (16 mg/kg) significantly reduced immobility duration (104.5 ± 3.69 s and 100.81 ± 4.07 s) relative to disease control. Silibinin (100 mg/kg) also significantly decreased immobility time (119.5 ± 4.802 s). The combination of Silibinin (2.5 mg/kg) + Sertraline (50 mg/kg) and Silibinin (8 mg/kg) + Venlafaxine (50 mg/kg) produced a greater reduction in immobility period (92.83 ± 3.4 s and 88.5 ± 4.7 s) compared to the disease

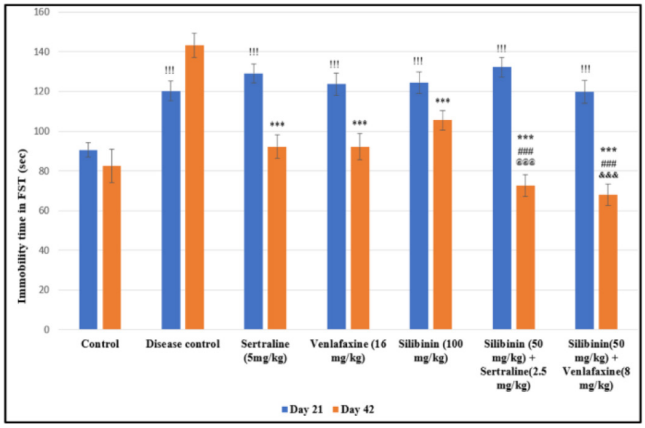


Figure 2. Immobility period in the forced swim test. Effects of Silibinin, Sertraline, and Venlafaxine treatment on immobility time in the Forced Swim Test in mice. All values given are mean ± SD. $^{***}p<0.0001$ is compared against the disease control group. $^{\&\&\&}p<0.0001$ is compared against the Venlafaxine group. $^{@@@}p<0.0001$ is compared against the Sertraline group. $^{\#\#\#}p<0.0001$ is compared against the Silibinin group. $^{!!!}p<0.0001$ is compared against the control group.

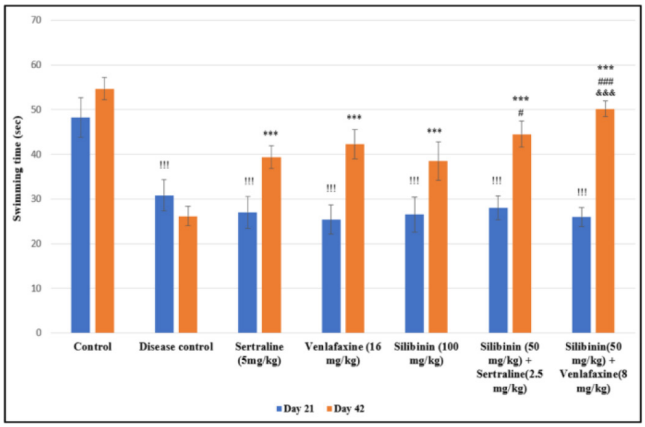


Figure 3. Swimming time in the forced swim test. Effects of Silibinin, Sertraline, and Venlafaxine treatment on swimming time in the Forced Swim Test in mice. All values given are mean ± SD. $^{***}p<0.0001$ is compared against the disease control group. $^{\&\&\&}p<0.0001$ is compared against the Venlafaxine group. $^{\#\#\#}p<0.0001$ and $^{\#}p<0.01$ are compared against Silibinin group. $^{!!!}p<0.0001$ is compared against the control group.

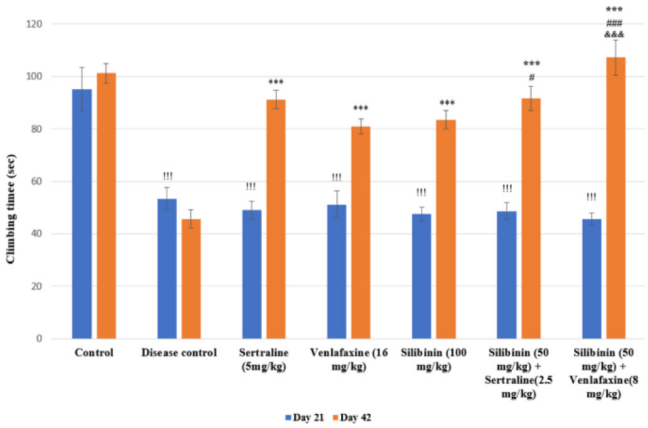


Figure 4. Climbing time in the forced swim test. Effects of Silibinin, Sertraline, and Venlafaxine treatment on climbing time in the Forced Swim Test in mice. All values given are mean ± SD. $^{***}p<0.0001$ is compared against the disease control group. $^{\&\&\&}p<0.0001$ is compared against the Venlafaxine group. $^{\#\#\#}p<0.0001$ and $^{\#}p<0.01$ are compared against Silibinin group. $^{!!!}p<0.0001$ is compared against the control group.

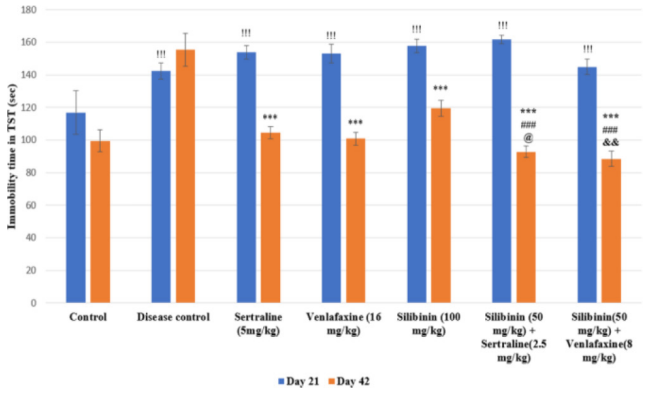


Figure 5. Immobility time in the tail suspension test. Effects of Silibinin, Sertraline, Venlafaxine treatment on immobility time in the Tail suspension test in mice. All values given are mean ± SD. $^{***}p<0.0001$ is compared against the disease control group. $^{\&\&}p<0.001$ is compared against the Venlafaxine group. $^{@}p<0.05$ is compared against the Sertraline group. $^{\#\#\#}p<0.0001$ is compared against the Silibinin group. $^{!!!}p<0.0001$ is compared against the control group.

control group. All the groups on Day 21 showed higher immobility time when compared with the control. Day 42 shows lower immobility in both combination groups when compared with the control (Figure 5).

3.3 Actophotometer

In the Actophotometer test, the disease control group showed a marked reduction in locomotor activity on day

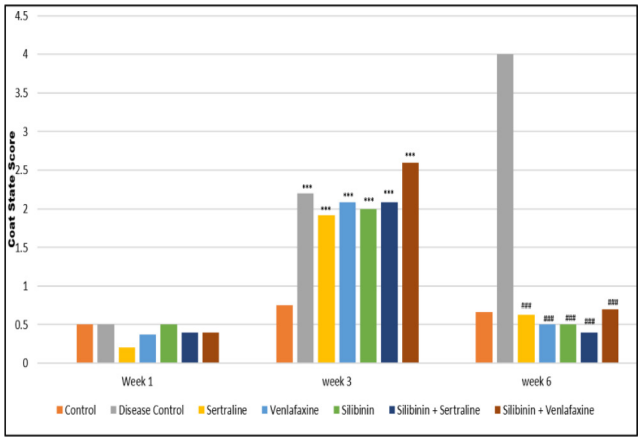


Figure 6. Total locomotor activity in the Actophotometer test. Effects of Silibinin, Sertraline, Venlafaxine treatment on locomotor activity in the Actophotometer test in mice. All values given are mean ± SD. $^{@@@}p<0.0001$ is compared against the disease control group. $^{\&}p<0.01$ is compared against the Venlafaxine group. $^{\#\#\#}p<0.0001$ is compared against the Sertraline group. $^{\$\$\$}p<0.0001$ is compared against the Silibinin group. $^{!!!}p<0.0001$ and $^{***}p<0.001$ are compared against control group.

21 (187.5 ± 8.456) and day 42 (151.5 ± 8.456) compared with the normal control group. Treatment with Sertraline (5 mg/kg) and Venlafaxine (16 mg/kg) showed comparable locomotor activity to disease control on day 21 (188.5 ± 5.431 and 185.3 ± 5.317), with a significant increase observed by day 42 (235.166 ± 5.431 and 204.5 ± 3.937). Silibinin (100 mg/kg) exhibited reduced locomotion on day 21 (171.667 ± 5.913), followed by a marked increase on day 42 (244.833 ± 5.913). The combination of Sertraline (2.5 mg/kg) + Silibinin (50 mg/kg) showed reduced locomotor counts on day 21 (164.1667 ± 4.427) and day 42 (26 ± 4.427), while Venlafaxine (8 mg/kg) + Silibinin (50 mg/kg) demonstrated lower activity on day 21 (158.5 ± 6.555) with an increase on day 42 (190.17 ± 6.555) compared with the disease control group (Figure 6).

3.4 Coat State Test

The results of the coat state test revealed that, at the start of the study, the deterioration of the coat state was negligible. At 3 weeks, all the groups that were subjected to CUMS procedure showed a significant increase in the deterioration of the coat state when compared with the control group. At the end of the 6 weeks, the groups that received the doses had significantly decreased deterioration of the coat state as compared with the Disease control group (Figure 7).

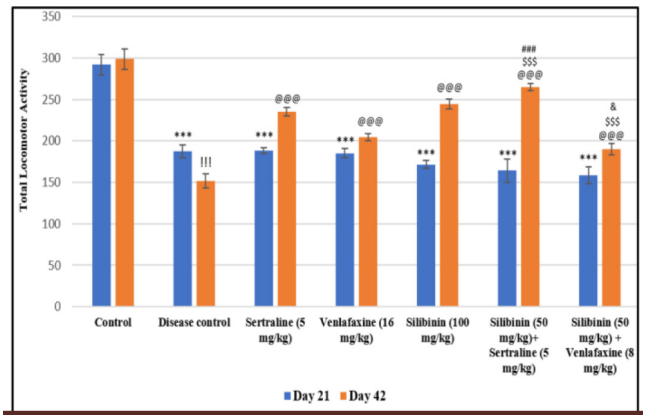


Figure 7. Coat state test score. Effects of Silibinin, Sertraline, and Venlafaxine treatment on deterioration of the coat state in mice. All values given are the mean. $^{***}p<0.0001$ is compared against the control group. $^{###}p<0.0001$ is compared against the disease control group.

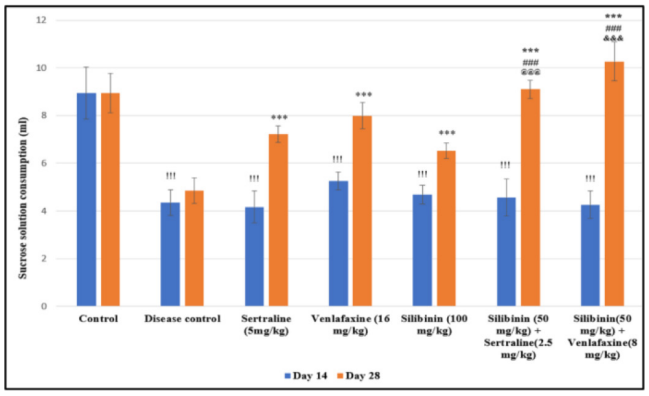


Figure 8. Sucrose consumption in the sucrose preference test. Effects of Silibinin, Sertraline, Venlafaxine treatment on sucrose consumption in the sucrose preference test in mice. All values given are mean ± SD. $^{***}p<0.0001$ is compared against the disease control group. $^{\&\&\&}p<0.0001$ is compared against the Venlafaxine group. $^{@@@}p<0.0001$ is compared against the Sertraline group. $^{###}p<0.0001$ is compared against the Silibinin group. $^{!!!}p<0.0001$ is compared against the control group.

3.5 Sucrose Preference Test (SPT)

In the SPT, the disease control group exhibited a marked reduction in sucrose consumption on day 14 (4.348 ± 0.5432 ml) and day 28 (4.842 ± 0.5358 ml) compared with the normal control group. Treatment with Sertraline (5 mg/kg), Venlafaxine (16 mg/kg), and Silibinin (100 mg/kg) showed comparable sucrose intake to disease control on day 14 (4.162 ± 0.6747, 5.254 ± 0.3785, and 4.686 ± 0.3889 ml), with a significant increase observed by day 28 (.219 ± 0.3501, 7.991 ± 0.3501, and 6.524 ± 0.3252 ml). The combination of Sertraline (2.5 mg/kg) + Silibinin (50 mg/kg) and Venlafaxine (8 mg/kg) + Silibinin (50 mg/kg) showed sucrose consumption similar to the disease control on day 14 (4.569 ± 0.7813 and 4.255 ± 0.576 ml), followed by a marked increase on day 28 (9.099 ± 0.3789 and 12.26 ± 0.817 ml) compared with the disease control group (Figure 8).

4. Discussion

The present study evaluated the antidepressant-like effects of Silibinin alone and in combination with Sertraline or Venlafaxine using the CUMS model in mice. This model reliably mimics core features of depression, including behavioral despair, anhedonia, and impaired self-care[21]. In the present study, disease control animals exhibited significant behavioral alterations across all tests, confirming successful induction of a depressive-like state.

In the FST, CUMS exposure significantly increased immobility time (143.2 ± 6.18 s) and reduced swimming and climbing behavior. Treatment with Sertraline (5 mg/kg) and Venlafaxine (16 mg/kg) significantly reduced immobility (92.78 ± 5.84 s and 92.17 ± 6.5 s, respectively), while Silibinin (100 mg/kg) also showed a notable reduction (105.5 ± 4.90 s). Importantly, combination treatments produced a greater effect, with Silibinin + Sertraline and Silibinin + Venlafaxine reducing immobility to 72 ± 5.50 s and 68 ± 5.44 s, respectively. These groups also showed marked increases in swimming and climbing times, with the Silibinin–Venlafaxine combination showing the highest climbing time (107.5 ± 6.70 s), consistent with enhanced noradrenergic activity[22].

Similarly, in the TST, disease control animals showed increased immobility (155.5 ± 10 s), which was reduced by Sertraline (104.5 ± 3.69 s), Venlafaxine (100.81 ± 4.07 s), and Silibinin (119.5 ± 4.80 s). Combination treatments further reduced immobility, with Silibinin + Venlafaxine showing the greatest reduction (88.5 ± 4.7 s), supporting enhanced antidepressant efficacy.

Locomotor activity assessed by the Actophotometer revealed that CUMS significantly reduced activity levels (151.5 ± 8.45 on day 42). Drug-treated groups showed partial recovery by day 42, such as Silibinin (244.83 ± 5.91) and Venlafaxine (204.5 ± 3.93), but without excessive stimulation. This confirms that reductions in immobility in FST and TST were due to genuine antidepressant-like effects rather than nonspecific CNS stimulation[23].

Coat state assessment demonstrated progressive deterioration after 3 weeks of CUMS exposure, indicating

impaired self-care behavior. However, all treatment groups showed significant improvement by week 6, reflecting reversal of stress-induced physical and behavioral deficits[24].

In the Sucrose Preference Test (SPT), CUMS significantly reduced sucrose intake (4.842 ± 0.5358 ml on day 28), confirming anhedonia. Monotherapy with Sertraline, Venlafaxine, and Silibinin moderately improved sucrose consumption (~6.5–8ml range), whereas combination treatments produced a marked increase, particularly Silibinin + Venlafaxine (12.26 ± 0.817 ml), indicating superior recovery from anhedonia[21].

Mechanistically, the enhanced effects observed with combination therapy may be attributed to complementary pharmacological actions. Venlafaxine's noradrenergic activity aligns with increased climbing behavior, while serotonergic modulation contributes to increased swimming behavior. Silibinin has been reported to exert antidepressant-like effects through antioxidant, anti-inflammatory, and neuroprotective mechanisms, including modulation of monoaminergic systems and BDNF pathways[8]. Additionally, the distinct metabolic pathways of Silibinin and standard antidepressants suggest a low likelihood of pharmacokinetic interaction, supporting the observed additive or synergistic effects[25]. The Silibinin–Venlafaxine combination consistently demonstrated superior efficacy across behavioral despair, anhedonia, locomotor function, and coat state assessments. These findings suggest that Silibinin may act as a promising adjunct to conventional antidepressants. However, further studies involving neurochemical markers such as monoamines, BDNF, and inflammatory mediators are required to elucidate the underlying mechanisms and support translational relevance.

5. Conclusion

Silibinin, Sertraline, Venlafaxine, and their combinations significantly reduced depression-like behaviors in the chronic unpredictable mild stress model. In the FST, immobility time was markedly decreased from 143.2 ± 6.18 s (disease control) to 105.5 ± 4.90 s (Silibinin) and further to 68.0 ± 5.44 s in the Silibinin–Venlafaxine combination group, which showed the greatest effect. Similarly, in the Tail Suspension Test, immobility was reduced from 155.5 ± 10 s (disease control) to 88.5 ± 4.70 s in the Silibinin–Venlafaxine group. Anhedonia assessment revealed a significant increase in sucrose consumption on day 28, from 4.842 ± 0.5358 ml (disease control) to 12.26 ± 0.817 ml in the Silibinin–Venlafaxine group, indicating substantial recovery. Behavioral parameters such as swimming and climbing time were also notably improved in combination-treated groups. Coat state deterioration induced by chronic stress was significantly reversed after 6 weeks of treatment across all groups. Importantly, Actophotometer results confirmed that these effects were not due to CNS stimulation, as locomotor activity remained comparable to or lower than control levels. Finally, Silibinin demonstrated significant antidepressant-like activity and enhanced the efficacy of standard antidepressants, with the Silibinin–Venlafaxine combination showing superior outcomes. Further studies involving monoamines, BDNF, and inflammatory markers are recommended to elucidate the underlying mechanisms.

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