Chinese Journal of Health Management
Volume 20 Issue 4, Year of Publication 2026, Page 1061-1066 | DoI-10.564220/1674-0815.2026.1033

ISSN: 1674-0815

Chinese Journal of Health Management

Chinese Medical Association

Behavioral Evaluation of the Antidepressant Effects of Combined Liquiritin and Cyanocobalamin in a Reserpine-Induced Rat Model of Depression

Mrs. Hina Deepak Mehta*[1], Dr. Darshan Telange[1], Dr. Deepak Khobragade[1], Dr. Anil Pethe[1]
[1]Datta Meghe College of Pharmacy, DMIHER Deemed to be University, Sawangi (Meghe), Wardha, Maharashtra, India.

Article Information
Received: 16-03-2026
Revised: 02-04-2026
Accepted: 22-04-2026
Published: 26-05-2026

Keywords
Depression; Liquiritin; Cyanocobalamin; Reserpine; Fluoxetine; Tail Suspension Test; Open Field Test; Sucrose Preference Test.

ABSTRACT:

Background: Depression is a multifactorial neuropsychiatric disorder associated with substantial social and economic burdens. Despite the availability of antidepressant therapies, their limitations in efficacy and adverse effects necessitate the exploration of novel therapeutic approaches. The present study investigated the antidepressant potential of liquiritin, cyanocobalamin, and their combination in a reserpine-induced rat model of depression. **Methods:** Depression-like behavior was induced in rats by oral administration of reserpine (0.5 mg/kg) for three days. The animals received liquiritin (25 mg/kg), cyanocobalamin (100 µg/kg), a combination of liquiritin and cyanocobalamin, or fluoxetine (10 mg/kg) as a reference standard. Antidepressant-like activity was assessed using the Tail Suspension Test (TST), Open Field Test (OFT), and Sucrose Preference Test (SPT). **Results:** Reserpine-treated rats exhibited significant behavioral alterations characterized by increased immobility time, reduced locomotor and exploratory activity, and decreased sucrose preference compared with normal controls, confirming the induction of depression-like behavior in rats. Treatment with liquiritin and cyanocobalamin individually improved these behavioral deficits. Notably, combination treatment produced greater antidepressant-like effects than either monotherapy, as evidenced by reduced immobility time, increased locomotor and exploratory behavior, and restoration of sucrose preference. The behavioral improvements observed with combination treatment were comparable to those achieved with fluoxetine. **Conclusion:** Combined administration of liquiritin and cyanocobalamin demonstrated significant antidepressant-like activity in a reserpine-induced rat model of depression. These findings suggest that this combination may represent a promising therapeutic strategy for the management of depressive disorders and warrant further mechanistic and translational investigations.

INTRODUCTION:

Depression is a prevalent neuropsychiatric disorder characterized by persistent low mood, anhedonia, and cognitive impairment, imposing significant social and economic burdens worldwide. (1,2) Conventional antidepressant therapies predominantly target monoaminergic systems but are often limited by delayed therapeutic onset and adverse side effects. (3) This has spurred interest in alternative and adjunctive treatments derived from natural compounds possessing neuroprotective and antioxidant properties. (3,4)

Liquiritin, a flavonoid isolated from Glycyrrhiza glabra, has garnered attention because of its demonstrated neuroprotective and antidepressant-like activities in preclinical models. Its mechanisms are thought to involve antioxidative effects and modulation of neuroinflammatory pathways, which may counteract the neurochemical imbalances underlying depression. (5, 6) Similarly, cyanocobalamin (vitamin B12) plays a critical role in neurotrophic support and monoaminergic regulation, with deficiencies linked to depressive symptoms. Supplementation with vitamin B12 has been associated with improved mood and cognitive function, suggesting its potential as a complementary antidepressant agent. (7,8)

Experimental models of depression, such as reserpine-induced behavioral despair in rodents, provide a validated platform for evaluating antidepressant efficacy. (9) Reserpine depletes monoamines, inducing depression-like symptoms, including increased immobility, reduced locomotion, and anhedonia, measurable via behavioral assays such as the Tail Suspension Test (TST), Open Field Test (OFT), and Sucrose Preference Test (SPT). (10) This study investigated the individual and combined antidepressant effects of these compounds using a validated reserpine-induced depression model. (11)

2. MATERIALS AND METHODS:

2.1 Animals:

Adult male Wistar albino rats (180–200 g) (n=6 per group) were housed under standard laboratory conditions with ad libitum access to food and water. All procedures confirmed to the institutional ethical guidelines Protocol No. DMIHER/IAEC/25-26/83.

2.2 Experimental Design and Drug:

Depression was induced by oral reserpine (0.5 mg/kg) once daily for three days, based on dose standardization, confirming significant depression-like behavior without toxicity. The groups included Normal Control, Disease Control (reserpine), fluoxetine (10 mg/kg), liquiritin (25 mg/kg), cyanocobalamin (100 µg/kg), and combination (Liquiritin + Cyanocobalamin). Treatments were administered for 14 days after reserpine induction.

2.3 Behavioral Assessments:

Tail Suspension Test (TST): Immobility time recorded as an index of behavioral despair.
Open Field Test (OFT): Assessed locomotor activity (line crossings), exploratory behavior (rearing frequency), and anxiety-like behavior (time spent in center).
Sucrose Preference Test (SPT): Measured preference for sucrose solution over water as an indicator of anhedonia.

3. RESULTS:

3.1 Dose Standardization:

Reserpine doses of 0.2 and 0.5 mg/kg were tested to identify the optimal dose of reserpine required to induce consistent depression-like behavior. The 0.5 mg/kg dose produced a significantly greater increase in immobility time ($p < 0.001$) without toxicity and was selected for the main experiment.

Oral administration of reserpine at doses of 0.2 and 0.5 mg/kg significantly increased the immobility time in the Tail Suspension Test compared to the normal control group. The increase was dose-dependent, and the 0.5 mg/kg dose produced significantly greater immobility than the 0.2 mg/kg dose ($p < 0.05$). One-way ANOVA demonstrated a highly significant difference between the groups ($p < 0.001$).

Table 1. Effect of Oral Reserpine on Immobility Time in Tail Suspension Test

Group	Immobility Time (sec)
Normal Control	89.33 ± 1.28
Reserpine (0.2 mg/kg)	128.50 ± 2.11***
Reserpine (0.5 mg/kg)	170.33 ± 2.46***#

***p < 0.001 vs Normal Control; #p < 0.05 vs Reserpine (0.2 mg/kg).

Reserpine administration produced a dose-dependent increase in the duration of immobility in rats. The normal control group exhibited an immobility time of 89.33 ± 1.28 s, whereas treatment with reserpine at doses of 0.2 mg/kg and 0.5 mg/kg increased the immobility time to 128.50 ± 2.11 s and 170.33 ± 2.46 s, respectively. One-way ANOVA revealed significant differences among the groups ($p < 0.001$). The higher dose produced significantly greater immobility compared to the lower dose

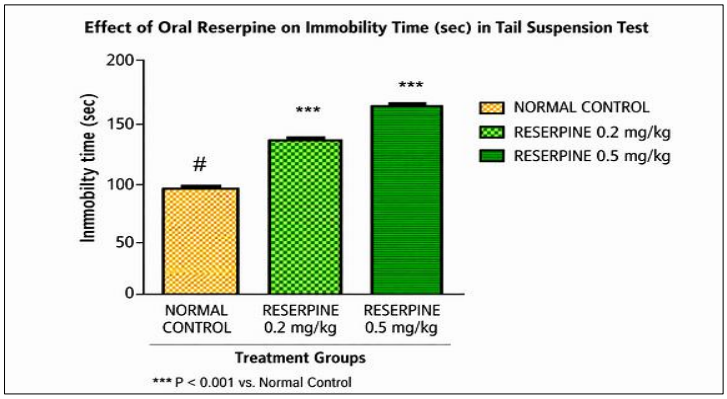

Figure 1: Effect of oral reserpine (0.2 and 0.5 mg/kg) on the immobility time in the Tail Suspension Test.

Reserpine induces depression-like behavior through the irreversible depletion of central monoamines, including serotonin, dopamine, and norepinephrine. The observed dose-dependent increase in immobility time confirmed the successful induction of behavioral despair. The 0.5 mg/kg dose produced a more pronounced depressive phenotype without evidence of toxicity or mortality and was therefore selected for the induction of depression in subsequent experiments. These findings are consistent with previous reports demonstrating that reserpine-induced monoamine depletion produces robust and reproducible depression-like behavior in rodents.

3.2 Tail Suspension Test:

The Tail Suspension Test was used to evaluate behavioral despair. The disease control group exhibited a marked increase in immobility time compared to the normal control group, confirming the successful induction of depression. Fluoxetine treatment significantly reduced the duration of immobility, validating the responsiveness of the experimental model.

Treatment with Liquiritin and Cyanocobalamin individually produced significant reductions in the immobility time compared with the disease control group ($p < 0.001$). Liquiritin exhibited greater antidepressant activity than cyanocobalamin. Notably, the combination of Liquiritin and Cyanocobalamin produced a significantly greater reduction in the immobility time than either monotherapy and achieved values statistically comparable to fluoxetine ($p > 0.05$).

The disease control group exhibited a marked increase in immobility time (216.33 ± 2.18 s) compared with the normal control group (88.67 ± 1.12 s), confirming the successful induction of depression. Treatment with Liquiritin and Vitamin B12 significantly reduced the immobility time to 149.33 ± 1.54 s and 159.17 ± 1.62 s, respectively. The combination treatment produced a pronounced reduction in the immobility time (106.17 ± 1.36 s), approaching the effect observed with fluoxetine (96.33 ± 1.28 s).

Chinese Journal of Health Management
Volume 20 Issue 4, Year of Publication 2026, Page 1061-1066 | DoI-10.564220/1674-0815.2026.1033

Table 2: Effect of treatments on immobility time in Tail Suspension Test.

Group	Immobility Time (sec)
Normal Control	88.67 ± 1.12
Disease Control	216.33 ± 2.18
Liquiritin	149.33 ± 1.54
(Cyanocobalamin) Vitamin B12	159.17 ± 1.62
Combination	106.17 ± 1.36
Fluoxetine	96.33 ± 1.28

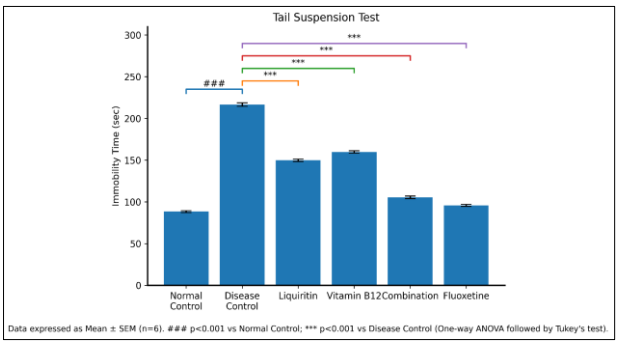

Figure 2: Effect of Liquiritin, Cyanocobalamin, and Combination Treatment on the immobility time.

Increased immobility time is considered a hallmark of behavioral despair and depression-like behavior. The marked elevation observed in reserpine-treated animals confirmed the successful establishment of the depression model. Fluoxetine significantly reversed this behavioral deficit by enhancing serotonergic neurotransmission.

Liquiritin significantly reduced the immobility time, suggesting potent antidepressant activity. This effect may be attributed to the modulation of monoaminergic pathways, antioxidant activity, suppression of neuroinflammation, and enhancement of neurotrophic factors such as BDNF. Cyanocobalamin also reduced the immobility time, likely by improving neurotransmitter synthesis and neuronal function.

Combination therapy exhibited superior efficacy compared to monotherapy, indicating a synergistic interaction between Liquiritin and Cyanocobalamin. The behavioral improvement observed with the combination approached that of fluoxetine, suggesting that simultaneous neuroprotection, antioxidant activity, and restoration of monoaminergic neurotransmission contributed to the enhanced antidepressant response.

3.3 Open Field Test:

Reserpine markedly reduced line crossings, rearing frequency, and center time ($p < 0.001$ vs. Normal Control). Fluoxetine and combination treatments restored these parameters to near normal levels ($p < 0.001$ vs. Disease Control). Liquiritin and cyanocobalamin monotherapies significantly improved these behaviors ($p < 0.01$), but to a lesser extent than combination therapy.

Table 3: Effect of treatments on Open Field Test.

Group	Line Crossings	Rearing Frequency	Center Time (sec)
Normal Control	78.33 ± 2.14	18.16 ± 1.02	32.50 ± 1.24
Reserpine Control	31.50 ± 1.68***	6.33 ± 0.66***	10.83 ± 0.79***
Fluoxetine	70.16 ± 2.01###	16.50 ± 0.84###	28.16 ± 1.12###
Liquiritin	55.66 ± 1.87###	12.83 ± 0.60###	21.33 ± 0.94###
Cyanocobalamin	50.50 ± 1.55###	11.66 ± 0.49###	19.50 ± 0.88###
Combination	74.83 ± 2.26###	17.33 ± 0.71###	30.66 ± 1.08###

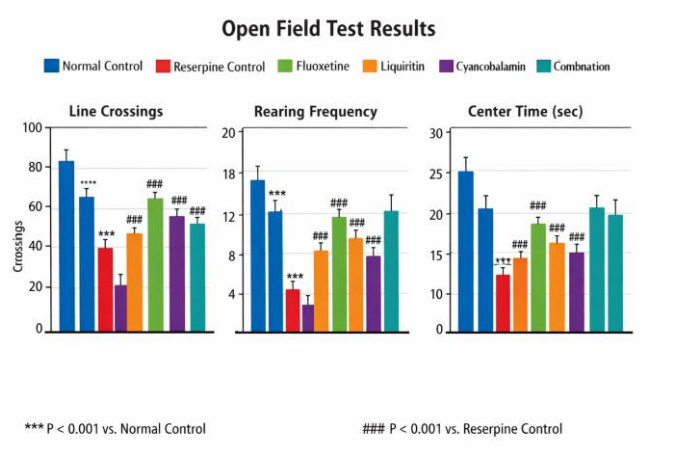

Figure 3: Open Field Test behavioral parameters in rats. Values are expressed as mean ± SEM (n = 6). ***p < 0.001 vs. Normal Control; ###p < 0.001 vs. Reserpine Control. The combination of Liquiritin + Cyanocobalamin restored locomotor activity, exploratory behavior, and center time to nearly normal levels, comparable to fluoxetine.

3.4 Sucrose Preference Test:

Reserpine significantly decreased sucrose preference to 41.36 ± 0.92% ($p < 0.001$ vs. Normal Control). Fluoxetine and combination treatments restored preference to above 80% ($p < 0.001$ vs. Disease Control). Liquiritin and cyanocobalamin monotherapies significantly improved sucrose preference ($p < 0.01$) but did not fully restore it to normal levels.

Table 4: Effect of treatments on Open Field Test.

Group	Sucrose Preference (%)
Normal Control	87.42 ± 1.18
Reserpine Control	41.36 ± 0.92###
Fluoxetine	80.54 ± 1.04***
Liquiritin	67.28 ± 0.88**
Cyanocobalamin	63.14 ± 0.81**
Combination	84.16 ± 1.09***

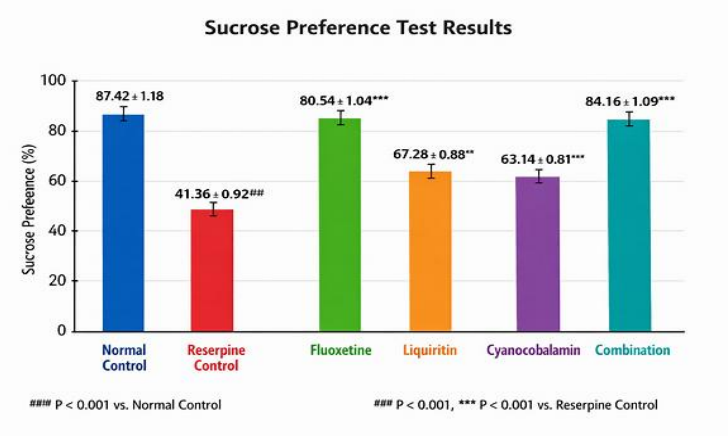

Figure 4: Sucrose Preference (%) across groups (mean ± SEM).

4. Statistical Analysis:

Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed using GraphPad Prism. Significance was set at $p < 0.05$.

5. DISCUSSION:

The reserpine model effectively induced depressive- and anxiety-like behaviors, as demonstrated by increased immobility and reduced locomotion, exploratory activity, and anhedonia. Liquiritin and cyanocobalamin exerted significant antidepressant-like effects, likely via flavonoid-mediated neuroprotection and vitamin B12's neurotrophic and monoaminergic modulation. Their combination showed synergistic efficacy, producing behavioral improvements comparable to those of fluoxetine. This supports the potential clinical utility of combined therapy to enhance antidepressant outcomes while possibly reducing side effects.

5. CONCLUSION:

Co-administration of liquiritin and cyanocobalamin significantly ameliorated depressive behaviors in reserpine-induced depression, surpassing the effects of individual treatments and matching fluoxetine efficacy. These findings justify further mechanistic and clinical investigations of this combination as a novel antidepressant strategy

REFERENCES:

1. Delgado, P. L. (2004). How antidepressants help with depression: mechanisms of action and clinical response. *The Journal of Clinical Psychiatry*, *Suppl 65, 4* (4), 25–30.
2. Elias, E., Zhang, A. Y., & Manners, M. T. (2022). Novel Pharmacological Approaches to the Treatment of Depression. *Life (Basel, Switzerland)*,*12* (2), 196. https://doi.org/10.3390/life12020196
3. Fan, Z.-Z., Zhao, W.-H., Guo, J., Cheng, R.-F., Zhao, J.-Y., Yang, W.-D., Wang, Y.-H., Li, W., & Peng, X.-D. (2012). Antidepressant activities of flavonoids from Glycyrrhiza uralensis and their neuroprotective effects in rats. *Acta Pharmaceutica Sinica*, *47* (12), 1612–7.
4. Kedzierska, E., & Wach, I. (2016). Tests and models were used to assess antidepressant-like activity in rodents. *Current Issues in Pharmacy and Medical Sciences*, *29* (2), 61–65. https://doi.org/10.1515/cipms-2016-0013
5. Liao, L., & Zhang, Z. (2022). Liquiritin Relieves Oxygen-Glucose Reperfusion-Induced Neuronal Injury via Inhibition of the p38MAPK/NF-\\u03baB Signaling Pathway. *Revista Brasileira de Farmacognosia*, *32* (2), 221–229. https://doi.org/10.1007/s43450-022-00233-1
6. Richard, I. H., & Lyness, J. M. (2006). *An Overview of Depression* (pp. 33–41). Humana. https://doi.org/10.1007/978-1-59259-960-8_4
7. Thulasingam, M., & Vellapandian, C. (2025). Exploring New Frontiers in Pharmacological Treatment of Depression: A Review of Recent Advances.*Current MedicinalChemistry*,*32*(6),1121–1135. https://doi.org/10.2174/0109298673342524250109181220
8. Mehta HD, Kasimedu S, Raj BKC, and Kiran V. Optimizing Orphan Drug Rucaparib Transdermal Patches for Ovarian Cancer: A Design Expert-Based Strategy for Prolonged Drug Release. International Journal of Drug Delivery Technology. 2024;14(3):1441-1449.
9. DR Telange, SP Jain, AM Pethe, VS Dave Phytosomes and phytosomal nanoparticles: A promising drug delivery system for flavonoids and nutraceuticals Nutraceutical Delivery Systems, 2022; 153-171
10. Mehta HD International Journal of Phytopharmacology. a review on griffonia simplicifollia a natural anti-depressant 6(2), 2015, 76-79. e- ISSN 0975 – 9328 Print ISSN 2229 – 7472
11. Mehta HD, Telange D. Combination Therapy of Phytochemicals and Vitamins in the Treatment of Depression: Translational Potential of Liquiritin and Cyanocobalamin. Int J Drug Deliv Technol. 2026;16(50s): 1081-1085. DOI: 10.25258/ijddt.16.50s.112