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Morin Can Reduce Reserpine-induced Depression in Mice via Strengthening Antioxidant Defense

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Abstract

Background: Depression is one of the most common mood disorders that greatly disrupt the lives of affected individuals. Due to the numerous side effects associated with chemical antidepressants, researchers have turned their attention to natural compounds. This study investigated the effects of Morin on Reserpine-induced depression in mice.

Methods: In this study, 48 male mice were divided into six groups. The Vehicle group received normal saline, while the negative and positive control groups received Reserpine (5 mg/kg) and Reserpine (5 mg/kg) + Fluoxetine (20 mg/kg), respectively. Eighteen hours after Reserpine injection, 50, 100, and 200 mg/kg Morin were administered to treatment groups. Forced swimming test (FST), tail suspension test (TST), and light-dark box tests were done as behavioral tests. Finally, brain tissue was isolated. The activity of Superoxide Dismutase enzyme and the levels of Reduced Glutathione and Malondialdehyde in the brain were measured.

Results: Morin could significantly increase the duration of activity in FST and TST in Reserpine-treated mice. In the light and dark box tests, Morin significantly decreased the latency to the first entry to the light chamber while increasing the number of entries and total time to last in the light chamber in Reserpine-treated mice. In the brain, Morin significantly enhanced the activity of the Superoxide Dismutase enzyme and the amount of "Reduced Glutathione" while reducing the levels of Malondialdehyde.

Conclusion: The results of this study demonstrate that Morin has a dose-dependent antidepressant effect by increasing the antioxidant capacity in the brains of rats.

Keywords: Depressive disorder, Morin, Oxidative Stress, Reserpine.

Introduction

Depression is one of the most common disorders of the central nervous system (CNS) (1), leading to emotional, behavioral, and physical problems that significantly disrupt the lives of affected individuals. Those suffering from depression do not exhibit identical symptoms. Symptoms of depression vary but commonly include apathy, an inability to experience pleasure, feelings of hopelessness, social withdrawal, irritability, concentration difficulties, and indecisiveness (2).

Depression is more prevalent among women than men, particularly after childbirth (3). It is a multifactorial condition, with

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approximately 30 to 40% of cases attributed to genetic factors (3). However, environmental and non-genetic factors predominantly contribute to its onset. It is suggested that inflammatory mediators, like tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), play a role in the development of depression in humans and animals (4).

Studies indicate that the hypothalamus-pituitary-adrenal (HPA) axis is dysregulated in individuals with depression. The primary physiologic regulator of this axis is Corticotropin-releasing factor (CRF), which is elevated in depression, and increased HPA activity is considered a marker of the disorder (5). Oxidative stress is another key factor in the pathogenesis of depression (6). Oxidative stress means that free radical production exceeds antioxidant capacity in the body, leading to cellular damage and death (7). The physiological stress response is partly gender-dependent, with women generally exhibiting a more significant stress response, which correlates with a higher incidence of depression among them (7). Cognitive deficits associated with depression also play a substantial role in sustained disability during depressive episodes (8). These impairments profoundly affect treatment response, relapse risk, and overall quality of life. Therefore, addressing cognitive symptoms is crucial for reducing functional decline and relapse risk (9).

Given the side effects of chemical antidepressants and the link between depression and oxidative stress, natural compounds with antioxidant properties may offer a safer and more effective treatment option. Morin is a natural flavonoid in various medicinal plants, exhibiting significant antioxidant and anti-inflammatory properties (10). It is a flavonol (2', 4', 3, 5, 7-pentahydroxyflavone) isolated from plants in the *Moraceae* family (11). Studies have shown that morin is a bioactive compound with low cytotoxicity and many biological properties, such as anti-inflammatory, antioxidant, and free radical scavenging effects (12). It has also been studied for its potential in treating cancer (13), nephrotoxicity, diabetes, and myocardial infarction (14).

Reserpine is a plant alkaloid that induces depressive-like symptoms in laboratory rodents by inhibiting the reuptake of monoamines, leading to their depletion in the brain. Monoamines undergo spontaneous oxidation, increasing hydrogen peroxide production, which enhances reactive oxygen species (ROS) and free radical generation. This process results in mitochondrial damage and subsequent neuronal apoptosis (15).
Given these considerations, this study investigates the antidepressant effects of Morin on Reserpine-induced depression in Mice.

Materials and Methods

Chemicals

Morin and Reserpine were purchased from Sigma-Aldrich Company, USA. Kits for measuring Reduced Glutathione (GSH), Malondialdehyde (MDA), and Superoxide Dismutase (SOD) were purchased from ZellBio, GmbH (Germany).

Animals

Male mice weighing 25-33 g were obtained from the animal facility of Ahvaz Jundishapur University of Medical Sciences, Iran. They were kept under standardized conditions (22 ± 1°C) with free access to water. Food was withheld 24 hours prior to the experiment. The maintenance and care of experimental animals complied with the National Institutes of Health guidelines for the humane use of laboratory animals. The ethical code (IR.AJUMS.ABHC.REC.1402.067) was given to the project after approval by the Institutional Ethical Committee of Ahvaz Jundishapur University of Medical Sciences.

Experimental Design

The animals were randomly divided into six groups: the vehicle group (two normal saline injections), the Negative control group (normal saline + Reserpine), the Positive control group (Fluoxetine + Reserpine), and the Treatment

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groups (Morin + Reserpine 50, 100, and 200 mg/kg, intraperitoneally).

Depression was induced by administering Reserpine (5 mL/kg, IP) 18 hours before Morin administration (16). Behavioral tests were conducted 30 minutes after the Morin injection.

Behavioral Tests

Forced Swimming Test (FST)

The FST is one of the most widely used behavioral despair tests established by Porsolt et al. (17). In this test, the mice were placed for six minutes in a plexiglass cylinder (20. cm height×10 cm diameter) containing 15 cm depth of water (25±1°C). The time spent by the mouse floating in the water without struggling and only making necessary movements to keep the head above the water was considered "duration of immobility." The primary behaviors considered to be these are immobility, climbing, and swimming. Double digital cameras recorded the process, and the Digibehave system analyzed the last four min of the six min test. Antidepressant activity was measured by a decrease in the duration of immobility.

Tail suspension test (TST)

Steru et al. first performed the TST (18). Mice were hung 15 cm above the floor (1 cm from the tip of the end) individually. The mice were considered immobile only when they hung passively and were completely motionless. The Digibehave system recorded and analyzed the behavior of the suspended mice, and the duration of immobility in the last 4 min was measured and analyzed.

Dark–light box

It was first introduced by the researchers Crawley and Goodwin in 1980 (19). The dark–light box includes two plastic chambers, which are connected by a small tunnel, exposing animals to an approach-avoidance conflict and revealing anxiety-related behaviors and depression. The dark chamber was sized 20 × 15 cm^2 and was covered by a lid. The other chamber, sized 30 × 15 cm^2, was white and illuminated from above with an intensity of 600 Lux. Mice were placed into the dark chamber, and latency to the first entry, number of entries, and total time of staying in the light chamber were recorded over a 5-minute period.

Brain tissue study

Without justification following the conclusion of behavioral tests, intraperitoneal ketamine (80 mg/kg) and Xylazine (10 mg/kg) were used to anesthetize the animals. Mice were sacrificed by decapitation, and both hippocampi were removed and put into labeled tubes. Hippocampi was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenate was centrifuged at 5000 RCF for 10 min, and the supernatant was stored at −80 °C until measuring the levels of Reduced Glutathione (GSH) and Malondialdehyde (MDA), as well as the activity of Superoxide Dismutase enzyme (SOD) by commercial kits.

Statistical Analysis

Comparisons between means of different groups were analyzed by one-way ANOVA (analysis of variance) followed by Tukey's post hoc. $P< 0.05$ was considered as the level of significance. Version 8.0.2 of the Graph Pad Prism software (Graph Pad Software, Inc., San Diego, USA) was used for all statistical tests. Data were expressed as mean ± S.E.M.

Results

The effect of Morin on Behavioral tests:

Tail suspension test (TST) & Forced swimming test (FST)

Morin treatment increased the duration of mobility in FST significantly in a dose-dependent manner, particularly at the doses of 100 and 200 mg/kg ($P< 0.05$) (Figs. 1 & 2).

The same treatment with Morin at doses of 100 and 200 mg/kg also significantly increased mobility time in TST. The classic antidepressant Fluoxetine (as positive control) at a daily dose of 20 mg/kg presented a marked increase in mobility time in both TST and FST. As a result, treatment with Morin at 100 and 200 mg/kg or Fluoxetine at 20 mg/kg significantly increased mobility time in TST and FST.

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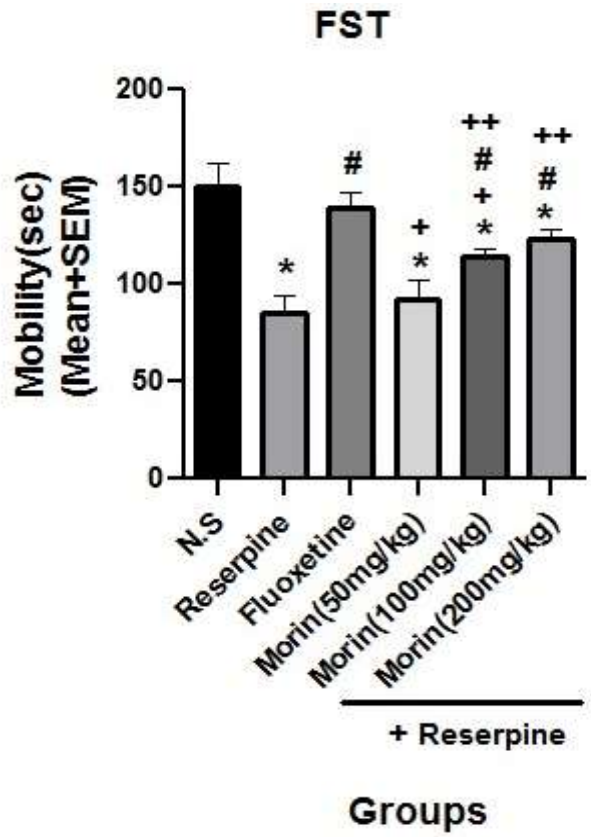

Fig. 1. The effect of Morin (50, 100, 200 mg/kg, IP) or Fluoxetine (20 mg/kg, IP) or Reserpine (5mg/kg, IP) on the mobility duration in forced swimming test. Values are as mean± SEM (n = 8). * P< 0.05 compared to normal saline (NS). # P< 0.05 compared to normal saline and Reserpine. + P< 0.05 compared to Fluoxetine. ++ P< 0.05 compared to Morin (50 mg/kg).

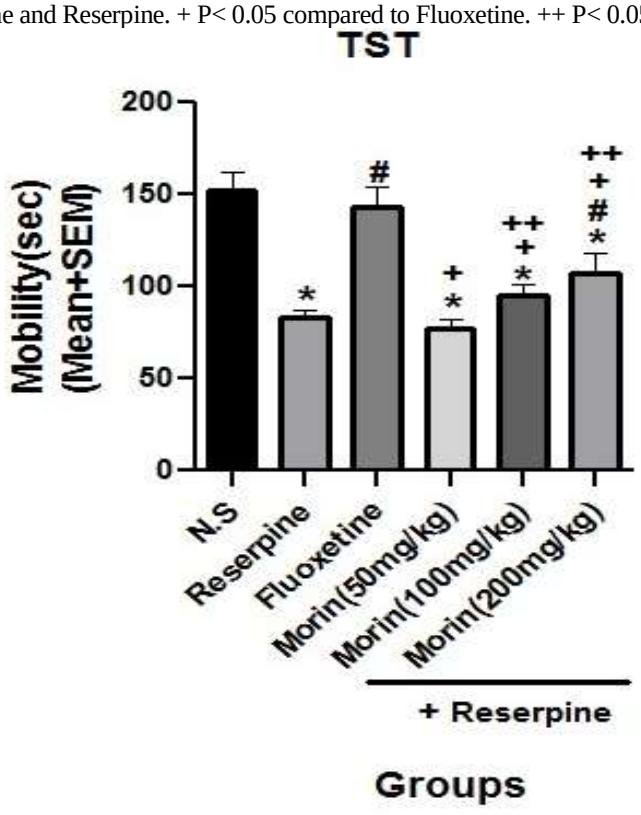

Fig. 2. The effect of Morin (50, 100, 200 mg/kg, IP) or Fluoxetine (20 mg/kg, IP) or Reserpine (5 mg/kg, IP) on mobility duration in tail suspension test. Values are as mean± SEM (n = 8). * P< 0.05 compared to normal saline (NS). # P< 0.05 compared to normal saline and Reserpine. + P< 0.05 compared to Fluoxetine. ++ P< 0.05 compared to Morin (50 mg/kg).

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The effect of Morin on the Dark –light box test: The effect of Morin on latency to first entry, number of entries, and total time of staying in the light chamber in the light and dark box were investigated (Fig. 3). Reserpine increased latency to the first entry and reduced the number of entries and total time in the light compartment compared to the regular saline group (P< 0.05). 100 and 200 mg/kg doses of Morin, similar to the Fluoxetine group, showed a significant reduction in latency to the first entry and increased the number of entries and total time of staying in the light compartment (P< 0.05).

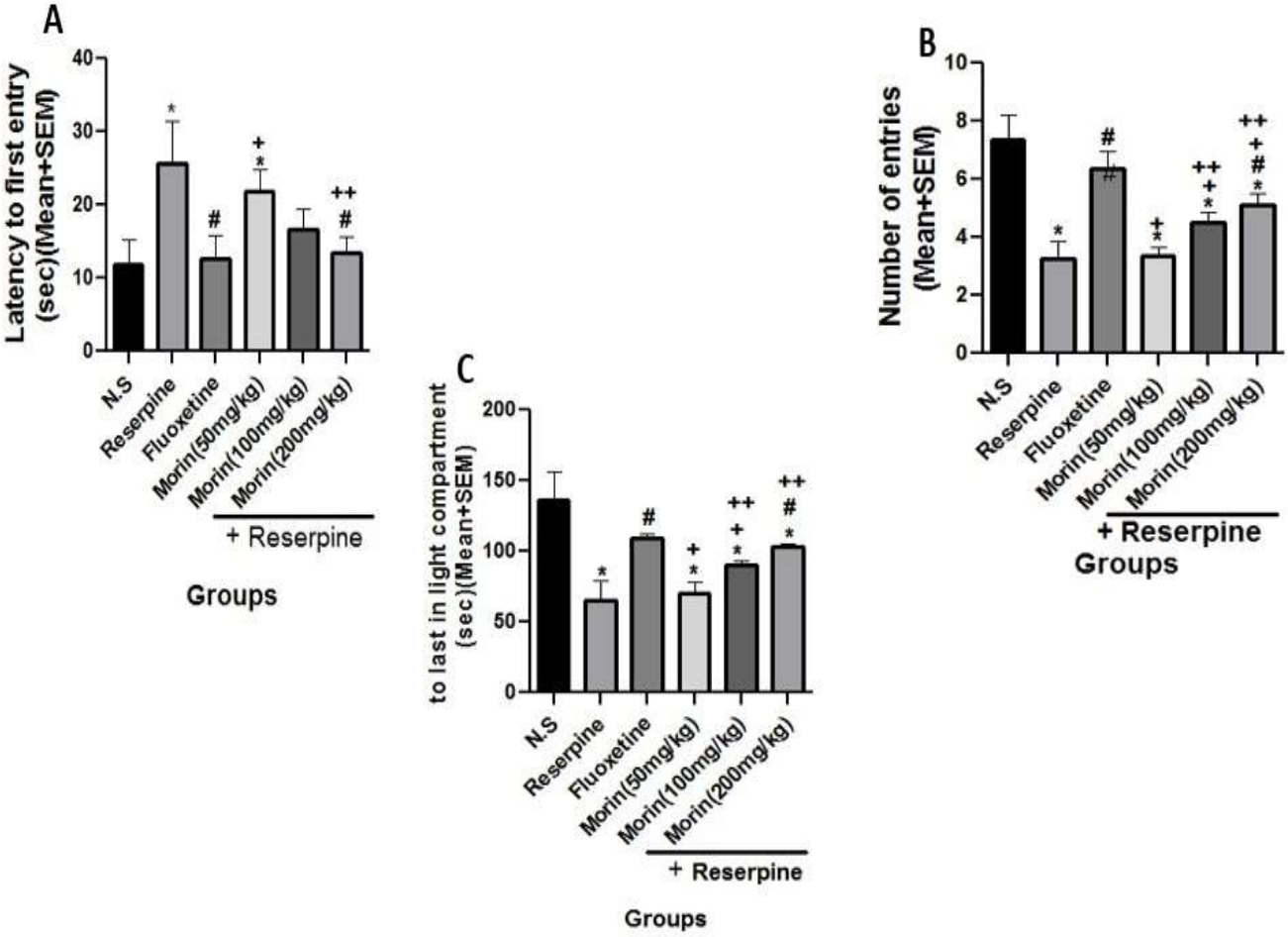

Fig. 3. The effect of Morin (50, 100, 200 mg/kg, IP) or Fluoxetine (20 mg/kg, IP) or Reserpine (5mg/kg, IP) on latency to first entry, number of entries and total time to last in the light and dark box. Values given are means± SEM (n = 8). * P< 0.05 compared to normal saline (NS). # P< 0.05 compared to normal saline and Reserpine. + P< 0.05 compared to Fluoxetine. ++ P< 0.05 compared to Morin (50 mg/kg).

Effect of Morin on SOD activity & GSH and MDA levels in hippocampus tissue: MDA levels were increased while SOD activity and GSH levels were reduced with Reserpine compared to the regular saline group (P< 0.05) (Fig. 4). Morin at the doses of 100 and 200 mg/kg, like the Fluoxetine group, resulted in a significant reduction in MDA levels and a substantial increase in SOD activity and GSH content of hippocampi tissue (P< 0.05).

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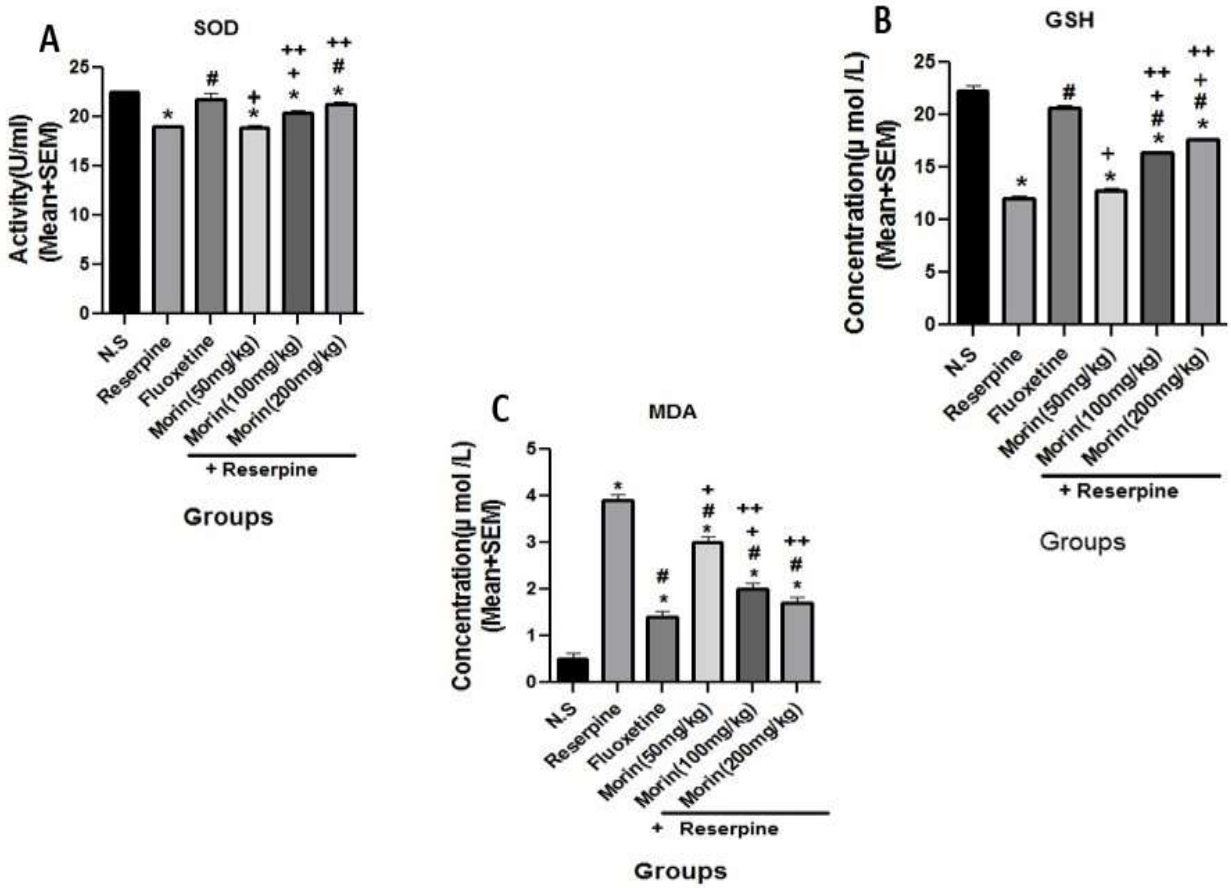

Fig. 4. Effect of Morin (50, 100, 200 mg/kg) on the level of SOD, GSH and MDA in hippocampus tissues or Fluoxetine (20 mg/kg, IP) or Reserpine (5 mg/kg, IP). Values are given as mean ±S.E.M.; (n = 8). * P< 0.05 comparing to normal saline (NS). # P< 0.05 comparing to normal saline and Reserpine. + P< 0.05 compared to Fluoxetine. ++ P< 0.05 compared to the Morin (50 mg/kg).

Discussion

Oxidative stress increases contribute to the induction of many dysregulations via increased lipid peroxidation and decreased antioxidant enzyme levels (20, 21). One of these is neuroinflammation in the brains of individuals with anxiety and depression at various stages (22).

In the present study, as expected, Reserpine injection in mice induced depressive behaviors and increased immobility duration in all three behavioral tests: Tail Suspension Test (TST), Forced Swim Test (FST), and Light-Dark Box Test, consistent with previous findings. Reserpine-induced depression significantly reduced antioxidant capacity and significantly increased the level of malondialdehyde (MDA) in the brain. However, Morin administration at doses of 50, 100, and 200 mg/kg, similar to Fluoxetine, significantly enhanced the brain's antioxidant capacity and reduced MDA levels (a marker of lipid peroxidation). Additionally, intraperitoneal injection of Fluoxetine in Reserpine-treated mice significantly reduced immobility duration in behavioral tests. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), increases 5-hydroxytryptamine (5-HT) concentrations in various brain regions without affecting other neurotransmitter receptors and possesses antioxidant properties (23).

In the FST, TST, and the light-dark box test, administration of Morin in reserpine-treated mice increased swimming duration, mobility, and the number of entries into the light compartment, as well as the time spent there, compared to mice receiving only Reserpine.

The findings of this study align with several previous investigations. For example, Lee et

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al. (2008) reported that Morin enhances the antioxidant system and exhibits hepatoprotective, nephroprotective, and neuroprotective effects (22).

Mokini et al. (2010) found that Morin, a recognized antioxidant, restores the expression and activity of antioxidant enzymes and glutathione (GSH) levels (24). Hassan et al. (2020) demonstrated that Morin suppresses neuroinflammation and protects neurons against stress and ifosfamide-induced apoptosis by inhibiting caspase-3 (25).

Chen et al. (2017) studied Morin's effects on oxidative stress, apoptosis, and inflammation in a rat model of cerebral ischemia, showing that Morin treatment reduced MDA levels and increased antioxidant defenses (GSH levels, SOD, and Glutathione peroxidase, GPx, activities). They suggested that Morin's neuroprotective effects could be beneficial in improving ischemic stroke outcomes (26).

Recent research indicates that Morin has antidepressant effects in laboratory rats exposed to chronic unpredictable mild stress (CUMS), primarily by reducing oxidative stress and neuroinflammation. It also prevents declines in key neurotransmitters such as serotonin, epinephrine, and norepinephrine, which are vital for managing depression (25).

Given these findings, there is growing interest in natural substances for developing drugs with minimal side effects. This study explored the therapeutic properties of Morin on Reserpine-induced depression in mice. The impact of Morin (50, 100, and 200 mg/kg) was examined, and the results suggest that Morin's protective effects are likely due to its ability to eliminate free radicals and provide natural cellular protection. The 100 mg/kg dose was found to be optimal for controlling various factors.

The results of this study indicate that Morin can effectively reduce depressive symptoms in male mice in a dose-dependent manner. Its antidepressant effects are attributed to its antioxidant activity, which is mediated through enhanced antioxidant capacity. Based on these findings, Morin could be considered as an adjunctive or primary treatment for depression. However, further studies in other animal models and compliance with regulatory guidelines for human trials are necessary to confirm its therapeutic potential.

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Conflict of interests

All the authors declare that they do not have any conflict of interest.

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