Journal of Integrative Neuroscience

*J. Integr. Neurosci.* **2023**; 22(3): 60
https://doi.org/10.31083/j.jin2203060

*Original Research*

# Salvianolic Acids Alleviate Chronic Mild Stress-Induced Depressive-Like Behaviors in Rats

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Academic Editor: Woo-Yang Kim
Submitted: 21 August 2022 Revised: 30 September 2022 Accepted: 11 October 2022 Published: 8 May 2023

**Abstract**

**Background**: Salvianolic acids possess anti-inflammatory properties. This study investigated the therapeutic effect of salvianolic acids on chronic mild stress (CMS)-induced depressive-like behaviors in rats and the involvement of toll-like receptor 4 (*TLR4*) and myeloid differentiation factor 88 (*MyD88*). **Methods**: Eighty male Sprague-Dawley rats were randomly subjected to CMS or non-CMS protocol for 6 weeks. Starting 3 weeks after CMS exposure, the rats in each group were administered saline, fluoxetine (positive control), salvianolic acids, or salvianolic acids + fluoxetine daily for 3 weeks. The body weight change, sucrose preference, and immobility duration in forced swimming were examined before and after drug treatment. The rats were sacrificed at 3 weeks after drug treatment. Quantitative real-time PCR was performed to measure the mRNA levels of *TLR4* and *MyD88* in the prefrontal cortex and hippocampus of rats. **Results**: Compared with non-CMS rats, CMS rats had significantly reduced weight gains and sucrose preference, along with significantly increased immobility durations and elevated mRNA levels of *TLR4* and *MyD88* in both the prefrontal cortex and hippocampus. Treatment with fluoxetine and salvianolic acids, alone or in combination, facilitated weight gains, alleviated depressive-like behaviors, and reduced cerebral *TLR4/MyD88* mRNA levels in CMS rats. Besides, fluoxetine and salvianolic acids additively suppressed *TLR4/MyD88* mRNA expression in the prefrontal cortex of rats. Furthermore, *TLR4* mRNA levels in both hippocampus and prefrontal cortex positively correlated with *MyD88* mRNA expression, inflammatory cytokine secretion, and immobility duration but negatively correlated with sucrose preference. **Conclusions**: Thus, salvianolic acids alleviate depressive-like behaviors, possibly by suppressing *TLR4/MyD88*-mediated inflammatory signaling in the brain.

**Keywords:** chronic mild stress; depression; *Salvia miltiorrhiza*; salvianolic acids; toll-like receptor 4; myeloid differentiation factor 88

## 1. Introduction

Depression is a mood disorder characterized by a persistent feeling of sadness and loss of interest, representing one of the leading causes of disability worldwide [1]. Major depressive disorder (MDD) is a highly prevalent type of depression that has been projected by WHO as the first cause of the burden of disease globally by 2030 [2]. Approximately 30% of patients with MDD are resistant to conventional treatment for depression [3]. Therefore, it is urgently needed to better understand the development of depression to identify a more effective treatment.

Accumulating evidence has suggested that inflammation contributes to the pathogenesis of depression [4–6]. Numerous studies have shown that microglia mediate inflammatory signaling that regulates mood and that microglial activation is responsible for depression symptoms [7–9]. The innate immune receptor Toll-like receptor 4 (*TLR4*) is highly expressed on the surface of microglia, serving as a first-line defense against invading microbes [10]. Together with its signaling adaptor myeloid differentiation factor 88 (*MyD88*), *TLR4* plays a critical role in microglial activation [11]. Hines *et al.* [12] have reported that inflammatory stimulus lipopolysaccharide (LPS) activates *TLR4/MyD88* signaling and triggers cytokine production in mouse microglia, resulting in depressive behaviors in mice. Blocking *TLR4/MyD88* interaction may prevent LPS-induced microglial activation, cytokine production, and depressive behaviors. Besides, *TLR4* signaling is upregulated in peripheral blood mononuclear cells of untreated patients with MDD. Psychotherapy reduces the expression of *TLR4* and inflammatory markers, displaying a positive correlation with the improvement of depressive symptoms [13]. Thus, targeting *TLR4/MyD88* signaling represents a promising therapeutic strategy in depression treatment.

**Table 1. Chronic mild stress protocol.**

| Stressor | Details |
|---|---|
| Cage shaking | Rats were subjected to cage shaking for 1 h |
| Water deprivation | Rats were subjected to water deprivation for 24 h |
| Reversed day/night cycle | Rats were under a 12:12 h light: dark cycle |
| Food deprivation | Rats were subjected to food deprivation for 24 h |
| Tilted cage | Rats were subjected to cage tilting (about 45°) along the vertical axis for 24 h |
| Hot water swimming | Rats were forced to swim in 45 °C water for 5 min |
| Wet bedding | Rats were subjected to wet bedding for 24 h |
| Wrap restraint | Rats were individually restrained for 1 h at 4 °C |
| Tail clamping | Rat's tail was clamped for 1 min |

*Salvia miltiorrhiza* (Danshen) is a traditional Chinese medicine that is widely used in patients with cardiovascular diseases and acute ischemic stroke due to its function in promoting blood circulation. Salvianolic acids are the most abundant water-soluble components in *S. miltiorrhiza*, including salvianolic acid A and salvianolic acid B [14]. Salvianolic acid B can cross the blood-brain barrier and alleviate chronic mild stress (CMS)-induced depressive behaviors in animal models [15–18]. However, the underlying mechanism remains unclear. Recent studies have shown that salvianolic acid B suppresses *TLR4/MyD88* signaling in primary cortical neurons and white adipose tissue in rodents [19,20]. Thus, we hypothesized that the suppression of *TLR4/MyD88* signaling is involved in the antidepressant effect of salvianolic acids.

To test our hypothesis, we examined the antidepressant effect of salvianolic acids and the involvement of *TLR4/MyD88* signaling in a CMS rat model that is widely used in depression research [21]. FDA-approved antidepressant drug fluoxetine was used as a positive control [22]. Our results suggest that the antidepressant effect of salvianolic acids is associated with the disturbance of *TLR4/MyD88*-mediated inflammatory signaling in the brain.

## 2. Materials and Methods

### *2.1 Animals and CMS Model*

Eighty Sprague-Dawley male rats weighing 180–220 g were obtained from the Experimental Animal Center of Hebei Medical University (Shijiazhuang, Hebei, China; Certificate No. 1411040). The rats were housed at 23 ± 2 °C under a 12: 12-h light: dark cycle, with free access to food and water. All experiments were conducted following the guidelines of the Animal Care and Use Committee of Hebei Medical University (#2014116).

The rats were randomly divided into CMS and non-CMS groups (n = 40/group). Rats in the CMS group were exposed to mild stressors daily for 6 weeks, as previously described [23]. The mild stressors included cage shaking for 1 h, water deprivation for 24 h, soiled cage for 24 h, reverse of light/dark cycle, food deprivation for 24 h, cage tilting for 24 h, swimming in 45 °C water for 5 min, wrap restraint at 4 °C for 1 h, tail clamping for 1 min, and wrap restraint for 1 h (Table 1). Rats in the non-CMS group were housed in a separate room under identical conditions without stress.

### *2.2 Drug Treatment*

Fluoxetine was purchased from Tokyo Chemical Industry Co., Ltd. (BODF0-DQ, Tokyo Chemical Industry Co., Ltd, Tokyo, Japan). Salvianolic acids were obtained from Tasly Pharmaceutical Co., Ltd. (Z20110011, Tasly Pharmaceutical Co., Ltd, Tianjin, China). The drugs were dissolved in 0.9% saline solution on the day of treatment. At 3 weeks after CMS exposure, both CMS and non-CMS rats were randomized into 4 subgroups (n = 10 rats/subgroup), respectively, and treated with 0.9% saline solution (10 mL/kg), fluoxetine (20 mg/kg) [24], salvianolic acids (40 mg/kg) [25], or fluoxetine + salvianolic acids daily for 3 weeks via intraperitoneal injection. The body weights were measured before CMS exposure (baseline), 3 weeks post-stress (before drug treatment), and 3 weeks post-treatment. The weight gain rate post-stress was calculated as [(body weight post-stress – baseline body weight)/body weight post-stress] × 100%. The weight gain rate post-treatment was calculated as [(body weight post-treatment – body weight post-stress)/body weight post-treatment] × 100%.

### *2.3 Sucrose Preference Test*

The sucrose preference test was carried out in the animal's home cage as previously described [26,27]. The rats were given 1% sucrose (13-201-00107, Tianjin Baishi Chemical Co. Ltd, Tianjin, China) solution for acclimation for 1 day, and the sucrose water was replaced with pure water on the second day. On the day of the test, the rats were fasted with no water for 10 h, provided with 1% sucrose solution and pure water bottles, and the consumption of sucrose was measured after 2 h. Preference % = [consumption of sucrose water/(consumption of sucrose water + consumption of pure water) × 100%].

**Table 2. Primers used in quantitative real-time PCR.**

| Gene | Sense Primer (5′-3′) | Antisense Primer (5′-3′) |
|---|---|---|
| *TLR4* | TCCACAAGAGCCGGAAAGTT | TGAAGATGATGCCAGAGCGG |
| *MyD88* | AGTTTGGCTTCACCCCACAA | GCAAAGAGGCCTCCATTCCT |
| $\beta$-Actin | GGAGATTACTGCCCTGGCTCCTA | GACTCATCGTACTCCTGCTTGCTG |

*TLR4*, toll-like receptor 4; *MyD88*, myeloid differentiation factor 88.

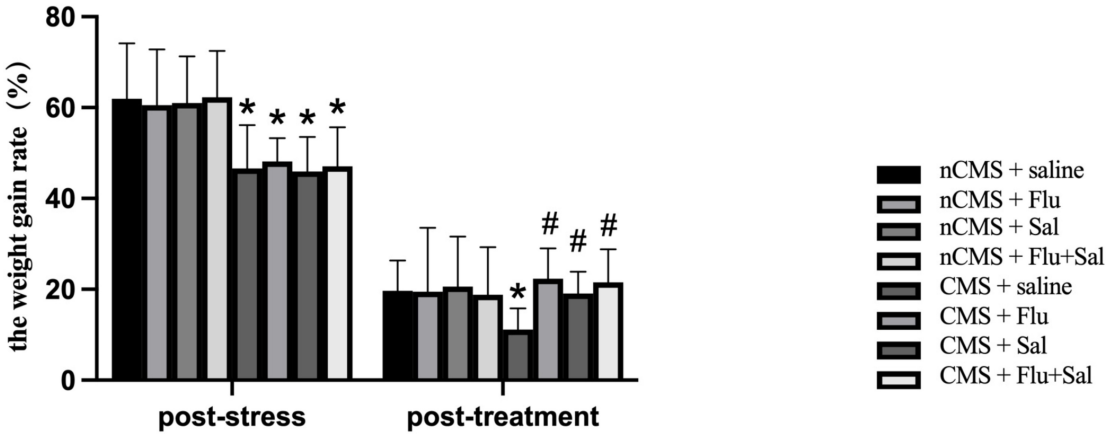


**Fig. 1. Salvianolic acids increased body weight gain rates in rats exposed to chronic mild stress (CMS).** The body weights were measured before CMS exposure (baseline), 3 weeks post-stress (before drug treatment), and 3 weeks post-treatment. The weight gain rate post-stress was calculated as [(body weight post-stress – baseline body weight)/body weight post-stress] $\times$ 100%. The weight gain rate post-treatment was calculated as [(body weight post-treatment – body weight post-stresss)/body weight post-treatment] $\times$ 100%. Data are expressed as the mean $\pm$ standard deviation (SD). *$p < 0.05$ vs. nCMS + saline; $^{\#}p < 0.05$ vs. CMS + saline. n = 10. CMS, chronic mild stress; nCMS, non-exposed to chronic mild stress; Sal, salvianolic acids; Flu, fluoxetine.

### *2.4 Forced Swimming Test*

The forced swimming test was performed as previously described using a glass cylinder (20 cm in diameter and 50 cm high) (XR-XQ202, Shanghai Xinruan Information Technology Co. Ltd, Shanghai, China) filled with tap water (20 cm deep) at 25 $\pm$ 1 °C [28]. Briefly, each rat was placed in the cylinder for 5 min, and the duration of immobility was recorded during the last 4 min. The water was refreshed between each test.

### *2.5 Quantitative Real-Time PCR*

Rats were sacrificed at 3 weeks after drug treatment (24 h after the final stressor exposure). The whole prefrontal cortex and hippocampus of each rat were immediately harvested and snapped frozen in liquid nitrogen. Quantitative real-time PCR was performed to measure the mRNA levels of *TLR-4* and *MyD88* in the prefrontal cortex and hippocampus tissue samples. Total RNA was isolated using TRIzol (DP405-02, Tiangen Biotech Co. Ltd., Beijing, China), followed by cDNA synthesis (RR047B, TaKaRa, Tokyo, Japan). PCR was performed using SYBR green (RR82LR, TaKaRa, Tokyo, Japan) and the primers (Table 2). $\beta$-actin was used as an internal reference. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

### *2.6 Statistical Analysis*

Data were expressed as the mean $\pm$ standard deviation. Statistical analysis was performed using Prism 5 (GraphPad Software Inc., San Jose, CA, USA) and SPSS 21.0 (IBM, Armonk, NY, USA). The treatment/time interaction was assessed using two-way ANOVA. The intergroup comparison was conducted using a one-way analysis of variance. A pairwise comparison was performed using the LSD method. The correlation of *TLR4* expression with *MyD88* expression, inflammatory cytokine secretion, or behavioral performance was evaluated using Spearman correlation analysis. $p < 0.05$ was considered statistically significant.

## 3. Results

### *3.1 Salvianolic Acids Facilitate Weight Gains in CMS Rats*

Because body weight changes reflect the overall impact of a chronic stressful situation [29], we examined the effect of salvianolic acids treatment on body weight changes. As shown in Fig. 1, at 3 weeks post-stress, CMS rats had reduced weight gain rates compared with non-CMS rats (all $p < 0.05$), suggesting that the animal model was successfully generated. At 3 weeks post-treatment, the weight gain rates of CMS rats treated with fluoxetine and salvianolic acids, alone or in combination, were higher than

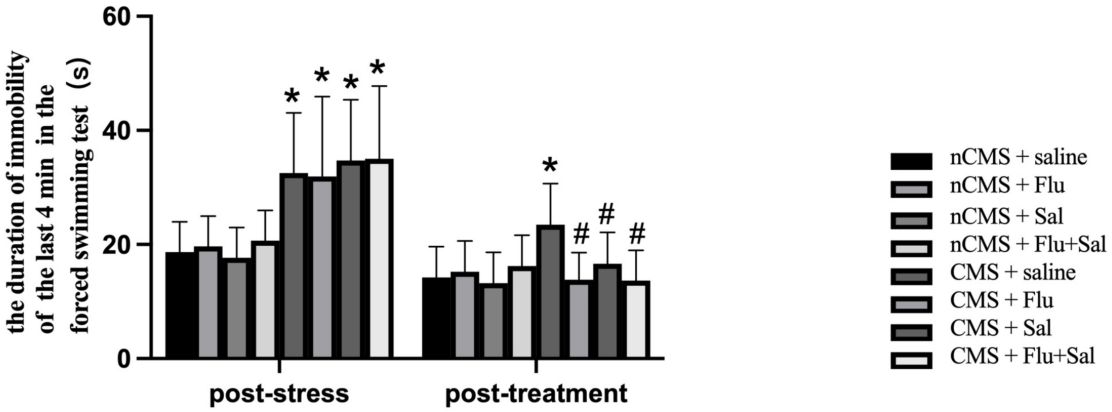


**Fig. 2. Salvianolic acids alleviated immobility in CMS rats.** The forced swimming test was performed before and after drug treatment. The duration of immobility was recorded during the last 4 min of the test. Data are expressed as the mean ± (SD). *$p < 0.05$ vs. nCMS + saline; $^{\#}p < 0.05$ vs. CMS + saline. n = 10.

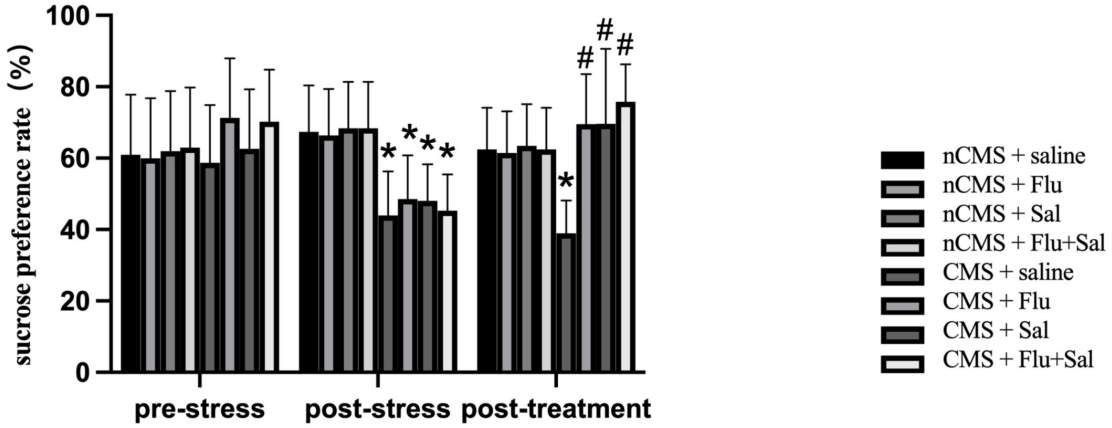


**Fig. 3. Salvianolic acids improved sucrose preference in CMS rats.** Sucrose preference test was performed before and after drug treatment. The sucrose preference was calculated as the percentage of the sucrose solution intake of the total fluid intake. Data were expressed as the mean ± SD. *$p < 0.05$ vs. nCMS + saline; $^{\#}p < 0.05$ vs. CMS + saline. n = 10.

those treated with saline (all $p < 0.05$). No significant difference was observed in the weight gain rates among the drug-treated CMS rats or among all four subgroups of non-CMS rats (all $p > 0.05$). Two-way ANOVA revealed significant treatment/time interaction ($F_{(14,144)}$=10.258, $p < 0.001$) and treatment effect ($F_{(7,72)}$ = 29.013, $p < 0.001$).

### *3.2 Salvianolic Acids Alleviate Immobility in CMS Rats*

The forced swimming test is commonly used to evaluate behavioral despair in animal models [30]. As shown in Fig. 2, before treatment, CMS rats showed significantly increased immobility durations compared with non-CMS rats (all $p < 0.05$). After 3 weeks of treatment, the immobility durations of CMS rats treated with saline remained significantly longer than those of non-CMS rats. However, compared with CMS rats administered saline, CMS rats administered fluoxetine and salvianolic acids, alone or in combination, exhibited decreased immobility durations (all $p < 0.05$). No significant difference was observed in the immobility durations among the drug-treated CMS rats or among all four subgroups of non-CMS rats (all $p > 0.05$). Two-way ANOVA revealed significant treatment/time interaction ($F_{(7,70)}$ = 5.390, $p < 0.001$) and treatment effect ($F_{(7,70)}$ = 33.251, $p < 0.005$).

### *3.3 Salvianolic Acids Improve Sucrose Preferences in CMS Rats*

To examine the effect of salvianolic acids on anhedonia, we performed the sucrose preference test [26]. As shown in Fig. 3, sucrose preference was reduced in CMS rats compared with that in non-CMS rats before drug treatment (all $p < 0.001$), suggesting that CMS induces anhedonia in model rats. After 3 weeks of treatment, the sucrose preferences of CMS rats administered fluoxetine and salvianolic acids, alone or in combination, were elevated to comparable levels of non-CMS rats (all $p > 0.05$), whereas the sucrose preferences of CMS rats treated with saline remained lower than non-CMS rats treated with saline ($p < 0.001$). No significant difference was observed in the sucrose preferences among the drug-treated CMS rats or

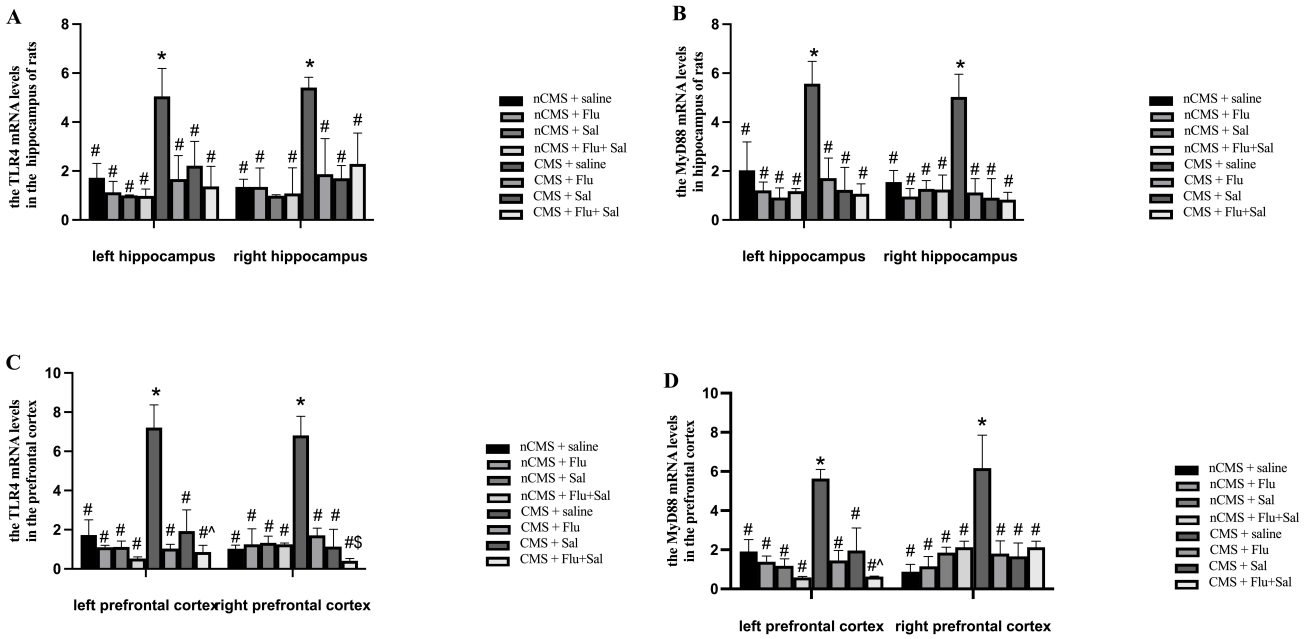


**Fig. 4. Salvianolic acids reversed CMS-induced upregulation of toll-like receptor 4 (*TLR4*) and of myeloid differentiation factor 88 (*MyD88*) mRNA expression in rat brain.** Rats were sacrificed at 3 weeks after drug treatment. Quantitative real-time PCR was performed to measure the mRNA expression of *TLR4* in the hippocampus (A) and the mRNA expression of *TLR4* in the prefrontal cortex (C). Quantitative real-time PCR was performed to measure the mRNA expression of *MyD88* in the hippocampus (B) and the mRNA expression of *MyD88* in the prefrontal cortex (D). $^{*}p < 0.05$ vs. nCMS + saline; $^{\#}p < 0.05$ vs. CMS + saline; $^{\wedge}p < 0.05$ vs. CMS + Sal; $^{\$}p < 0.05$ vs. CMS + Flu; n = 10.

**Table 3. Spearman's correlation analysis of cerebral *TLR4* mRNA expression with *MyD88* mRNA expression, inflammatory cytokine secretion, and behavioral test (*r*-value).**

| *TLR4* mRNA level | Sucrose preference | Forced swimming test | Interleukin-1$\beta$ (IL-1$\beta$) | Interleukin-2 (IL-2) | Interferon-$\gamma$ (IFN-$\gamma$) | Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) | *MyD88* mRNA level |
|---|---|---|---|---|---|---|---|
| *TLR4* mRNA level in the hippocampus | −0.153$^{a}$ | 0.011$^{a}$ | 0.001 | 0.061 | 0.274 | 0.070$^{a}$ | 0.810$^{a}$ |
| *TLR4* mRNA level in the prefrontal cortex | −0.178$^{a}$ | 0.027$^{a}$ | 0.028$^{a}$ | 0.033 | 0.194 | 0.041$^{a}$ | 0.915$^{a}$ |

$^{a}p < 0.05$.

among the 4 subgroups of non-CMS rats (all $p > 0.05$). Two-way ANOVA revealed significant treatment/time interaction ($F_{(14,152)} = 3.360$, $p < 0.001$) and treatment effect ($F_{(7,76)} = 30.123$, $p < 0.005$).

### *3.4 Salvianolic Acids Reverse CMS-Induced Upregulation of TLR4 and MyD88 mRNA Expression in Rat Brain*

Considering the involvement of *TLR4* and *MyD88* in the development of depression [12], we determined the mRNA levels of *TLR4* and *MyD88* in the hippocampus and prefrontal cortex of rats. As shown in Fig. 4A,B, before treatment, the mRNA levels of *TLR4* and *MyD88* in both the left and right hippocampus of saline-treated CMS rats were higher than those of saline-treated non-CMS rats, suggesting that CMS induces upregulation of *TLR4/MyD88* expression in the brain. Compared with saline treatment, 3 weeks of treatment with fluoxetine and salvianolic acids, alone or in combination, reduced *TLR4* and *MyD88* mRNA in CMS rats (all $p < 0.05$) at comparable levels of those in non-CMS rats (all $p > 0.05$). Similar results were observed in both left and right prefrontal cortexes of rats (Fig. 4C,D).

Of note, fluoxetine and salvianolic acids cotreatment outperformed fluoxetine alone in suppressing *TLR4* mRNA expression in the right prefrontal cortex of rats ($p < 0.05$). Fluoxetine and salvianolic acids cotreatment also outperformed salvianolic acids alone in suppressing *TLR4* and *MyD88* mRNA expression in the left prefrontal cortex of rats (both $p < 0.05$).

### *3.5 TLR4 mRNA Expression in the Brain is Correlated with MyD88 Expression, Inflammatory Cytokine Secretion, and Behavioral Performance of CMS Rats*

Our previous data have shown that CMS enhances interleukin-1$\beta$ (IL-1$\beta$) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) production in the hippocampus and prefrontal cortex of rats [31]. Compared with saline treatment, 3 weeks of treatment with fluoxetine and salvianolic acids, alone or in combination, reduced IL-1$\beta$ and TNF-$\alpha$ secretion in the

brain of CMS rats at comparable levels of non-CMS rats. Thus, we further assessed the correlation of *TLR4* mRNA expression with *MyD88* mRNA expression, inflammatory cytokine secretion, and behavioral performance. Our results showed that *TLR4* mRNA levels in both hippocampus and prefrontal cortex were positively correlated with *MyD88* mRNA expression, TNF-$\alpha$ secretion, and immobility duration but negatively correlated with sucrose preference. *TLR4* mRNA expression was also positively correlated with IL-1$\beta$ secretion in the prefrontal cortex of rats (all $p < 0.05$; Table 3).

## 4. Discussion

Depression is characterized by persistent sadness and loss of interest, along with cognitive and physical symptoms such as feelings of worthlessness, sleep disturbances, and lack of energy [1]. In this study, we used a well-established CMS protocol to induce depressive symptoms in rats [21]. By monitoring the body weight changes and assessing the results of forced swimming and sucrose preference tests that are common behavioral tests in depression-like behavior in rodents [32], we found that three weeks of CMS exposure significantly reduced weight gains and sucrose preference while increasing immobility duration in rats. This is consistent with the results of previous studies [33,34], suggesting that the CMS rat model is successfully generated. We further found that treatment with fluoxetine and salvianolic acids, alone or in combination, effectively facilitated weight gains and alleviated depressive-like behaviors in CMS rats. Importantly, treatment with fluoxetine and salvianolic acids, alone or in combination, significantly reduced the mRNA levels of *TLR4* and *MyD88* in the hippocampus and prefrontal cortex of CMS rats. Moreover, *TLR4* mRNA expression in the brain positively correlated with *MyD88* expression, inflammatory cytokine production, and behavioral performance in CMS rats. These results suggest that salvianolic acids exhibited a comparable antidepressant effect to fluoxetine possibly by suppressing *TLR4/MyD88* signaling in the brain.

Microglial activation is involved in the development of depression. Significant changes are noted in the number, morphology, and activity of microglia in depression [35–37]. Kreisel *et al.* [38] have demonstrated that in a chronic unpredictable stress mouse model, following an initial 2–3 days of stress-induced microglial proliferation and activation, some microglia underwent apoptosis, dystrophy, and decline in numbers within the hippocampus, but not in other brain regions. Pharmaceutical blockade of the initial stress-induced microglial activation rescued the microglial disturbance as well as the depressive-like behavior, suggesting that the dynamic microglial alteration has an etiological role in chronic stress-induced depression. *TLR4* expressed in the prefrontal cortex and hippocampus plays an important role in stress-induced depression [39]. Upon recognizing specific pathogen-associated molecular patterns, *TLR4* initiates innate immune responses through *MyD88* or TRIF to activate the transcription factor nuclear factor $\kappa$B (NF-$\kappa$B), leading to neuroinflammatory responses [40]. It is well accepted that activation of *TLR4/MyD88* signaling induces phosphorylation of NF-$\kappa$B and subsequent expression of inflammatory mediators, such as IL-1$\beta$, IL-6, and TNF-$\alpha$ in the brain, contributing to the development of depression [31,41]. Consistently, our results showed that CMS exposure resulted in significant upregulation of *TLR4* and *MyD88* mRNA expression in the frontal cortex and the hippocampus of rats. Of note, *TLR4 mRNA* expression in the brain positively correlated with *MyD88* expression, inflammatory cytokine secretion, and behavioral performance in CMS rats. These findings suggest that CMS activates *TLR4/MyD88* signaling and enhances the production of inflammatory cytokines, leading to the development of depressive-like behavior.

Nonsteroidal anti-inflammatory drugs, alone or in combination with other antidepressants, are promising therapeutic agents for depression [42,43]. Studies have shown that salvianolic acids attenuate inflammation in different organs and tissues, including the brain [19,44]. A recent study has shown that salvianolic acid B ameliorates CMS-induced depressive-like behaviors and inhibits CMS-induced neural apoptosis and microglial activation in the hippocampus and cortex of mice [18]. Consistent with these reports, our results showed that salvianolic acids alleviated depressive-like behaviors in CMS rats, comparable to antidepressant fluoxetine. No additive or synergistic effect was observed in depressive-like behavior alleviation when fluoxetine and salvianolic acids were administered in combination. Furthermore, salvianolic acids reversed CMS-induced upregulation of *TLR4* and *MyD88* mRNA expression in the hippocampus and front cortex of rats, consistent with a previous study [19]. Interestingly, fluoxetine and salvianolic acids cotreatment outperformed fluoxetine alone in suppressing *TLR4* mRNA expression in the right prefrontal cortex of rats and outperformed salvianolic acids alone in suppressing *TLR4* and *MyD88* mRNA expression in the left prefrontal cortex of rats, suggesting that fluoxetine and salvianolic acids have an additive effect on disturbing cerebral *TLR4/MyD88* signaling. However, the underlying mechanism remains unknown and needs further investigation.

This study has several limitations. First, we only examined the mRNA levels of *TLR4* and *MyD88* in rat brains. The protein expression of these two genes and other components of *TLR4* signaling will be investigated in future studies. Second, we did not examine the cerebral production of inflammatory cytokines in this study. The correlation assay was based on the results of our previous study. Third, further investigation is required to establish a causal link between salvianolic acid treatment and the suppression of *TLR4* signaling in microglia.

## 5. Conclusions

In conclusion, we demonstrated that salvianolic acids were comparable to fluoxetine in alleviating CMS-induced depressive-like behaviors in rats while suppressing mRNA expression of *TLR4* and *MyD88* in the prefrontal cortex and hippocampus of rats. Salvianolic acids and fluoxetine showed an additive effect on suppressing *TLR4/MyD88* signaling in the prefrontal cortex of rats. Our results suggest that salvianolic acids are promising antidepressants targeting *TLR4/MyD88* signaling in the brain.

## Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Abbreviations

CMS, chronic mild stress; *TLR4*, toll-like receptor 4; *MyD88*, myeloid differentiation factor 88; MDD, Major depressive disorder; LPS, lipopolysaccharide.

## Author Contributions

FZ and LY conceived and coordinated the study, designed, performed and analyzed the experiments, wrote the paper. LW, WL, SW, XW and CA carried out the data collection, data analysis, and revised the paper. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

All experimental protocols were carried out in accordance with the guidelines of the Animal Care and Use Committee of The First Hospital of Hebei Medical University (#2014116).


## Acknowledgment

The authors thank Dr. Shuang Chen who significantly assisted us during different stages of the research.

## Funding

This work was supported by the National Natural Science Foundation of China [grant number 81271489], the Natural Science Foundation of Hebei Province [grant number H2022206544], Hebei Medical University "14th Five-Year" Clinical Medicine Innovation Research Team Support Program [grant number 2022LCTD-A1], The Hebei Province Government-funded Clinical Medicine Outstanding Talent Training Project [grant number LS202009].


## Conflict of Interest

The authors declare no conflict of interest.

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