Behavioural Brain Research 301 (2016) 55–62

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

# Sulforaphane produces antidepressant- and anxiolytic-like effects in adult mice

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## HIGHLIGHTS

- Sulforaphane exerts antidepressant- and anxiolytic-like activities in mice.
- Sulforaphane exerts anxiolytic-like activities in stress-induced depressed mice.
- Sulforaphane inhibits HPA axis activity in stress-induced depressed mice.
- Sulforaphane inhibits inflammatory response in stress-induced depressed mice.

## ARTICLE INFO

*Article history:*
Received 29 October 2015
Received in revised form
16 December 2015
Accepted 17 December 2015
Available online 22 December 2015

*Keywords:*
Sulforaphane
Stress
Depression
Inflammatory response
Anxiety
Hypothalamic-pituitary-adrenal axis

## ABSTRACT

Increasing evidence suggests that depression is accompanied by dysregulation of neuroimmune system. Sulforaphane (SFN) is a natural compound with antioxidative, anti-inflammatory and neuroprotective activities. The present study aims to investigate the effects of SFN on depressive- and anxiety-like behaviors as well as potential neuroimmune mechanisms in mice. Repeated SFN administration (10 mg/kg, i.p.) significantly decreased the immobility time in the forced swimming test (FST), tail suspension test (TST), and latency time to feeding in the novelty suppressed feeding test (NSF), and increased the time in the central zone in the open field test (OPT). Using the chronic mild stress (CMS) paradigm, we confirmed that repeated SFN (10 mg/kg, i.p.) administration significantly increased sucrose preference in the sucrose preference test (SPT), and immobility time in the FST and TST of mice subjected to CMS. Also, SFN treatment significantly reversed anxiety-like behaviors (assessed by the OPT and NSF) of chronically stressed mice. Finally, ELISA analysis showed that SFN administration blocked the increase in the serum levels of corticosterone (CORT), adrenocorticotropic hormone (ACTH), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in chronically stressed mice. In summary, these findings demonstrated that SFN has antidepressant- and anxiolytic-like activities in stressed mice model of depression, which likely occurs by inhibiting the hypothalamic-pituitary-adrenal (HPA) axis and inflammatory response to stress. These data support further exploration for developing SFN as a novel agent to treat depression and anxiety disorders.



*Abbreviations:* ACTH, adrenocorticotropic hormone; CMS, chronic mild stress; CNS, central nervous system; CORT, corticosterone; ELISA, enzyme-linked immunosorbent assay; FST, forced swimming test; GR, glucocorticoid receptor; HPA axis, hypothalamic-pituitary-adrenal axis; IL-1β, interleukin-1β; IL-6, interleukin-6; LAT, locomotor activity test; LPS, lipopolysaccharide; Nrf2, nuclear factor E2-related factor 2; NSF, novelty suppressed feeding test; OPT, open field test; SFN, Sulforaphane; SPT, sucrose preference test; TNF-α, tumor necrosis factor-α; TST, tail suspension test.

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http://dx.doi.org/10.1016/j.bbr.2015.12.030
0166-4328/

## 1. Introduction

Major depression, one of the most devastating mental illnesses affects 17% of individuals worldwide [1]. It is estimated that depression causes approximately 1 million people to commit suicide annually, imposing a major burden on the society. Although antidepressants have been clinically available for several decades, only 33% of depressed patients are sensitive to the first antidepressant medication [2]. In addition, the current antidepressants are associated with serious adverse effects. Therefore, natural products have attracted increasing attention for preventing and treating neurodegenerative and psychiatric disorders, including depression. Many types of natural products have comparable efficiency to prescription medications with no or reduced side effects [3–6].

Extensive evidence has shown that stress, especially chronic stress, is one of the most important factors responsible for depressive disorders [7–9]. Maladaptive response to stress causes hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis by stimulating adrenocorticotropic hormone (ACTH) release and subsequent peripheral release of steroids/cortisol from the adrenal grand, while antidepressants could reverse depressive-like behaviors and inhibit the activation of the HPA axis in animal models and patients with depression [10–12]. Depression-related disruptions in a neuroimmune axis control depressive-like behaviors by interfering the immune system and the CNS. It has been found that circulating cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6(IL-6), increased in the patients with depression [13,14]. A recent study showed that peripheral inflammation predates the occurrence of depression [15]. Children with higher circulating levels of IL-6 at age 9 are at a 10% greater risk for developing MDD by age 18 than the general population or children with low levels of IL-6 [15]. Collectively, these studies suggest that aberrant periphery immune responses to stress can lead to exaggerated risks for developing disorders in CNS via amplifying the initial inflammatory signal that can directly or indirectly act on neuronal plasticity, which is contributed to stress susceptibility and depression-like behavioral phenotypes [16,17].

Sulforaphane (SFN: 1-isothiocyanato-4-methylsulfinylbutane) is an organosulfur compound found in broccoli and other cruciferous vegetables (chemical structure is shown in (Fig. 1). As a dietary phytochemical with low toxicity, sulforaphane is widely consumed and has qualified for consideration as food, dietary supplement, or drug, depending on its intended use. Sulforaphane has multiple health benefits, such as anticancer, antioxidant, anti-inflammatory and neuroprotective effects [18–21]. A recent study found that the induction of nuclear factor E2-related factor 2 (Nrf2) by SFN could reverse LPS-induced depressive-like behaviors in mice, indicating the potential antidepressant-like effects of sulforaphane [22].

Therefore, the present study aimed to examine whether repeated SFN administration produces antidepressant-like effects in a validated mouse models of depression. In addition, we assessed the HPA activity and immune response by measuring the serum levels of corticosterone (CORT), ACTH, IL-6 and TNF-α to elucidate the potential mechanism of SFN action.

## 2. Materials and methods

### 2.1. Animals

One hundred and twenty male ICR mice (with a 5% attrition rate), weighing 22–24 g, were individually housed at a constant temperature (23 ± 2 °C) with 12 h/12 h light/dark cycles and free access to food and water. All mice were transferred to the experimental room 1 h before behavioral tests, and all drug administration and behavioral tests were performed in the dark phase. All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and they were approved by the Local Animal Use Committee of Hebei Medical University.

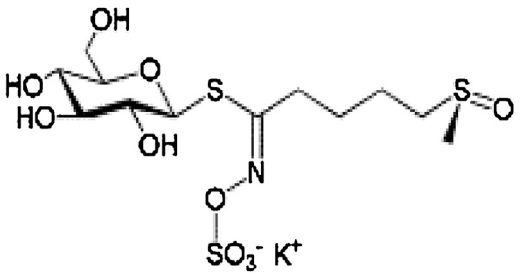


**Fig. 1.** Chemical structure of sulforaphane.

### 2.2. Drugs

DL-Sulforaphane (SFN) [Sigma–Aldrich (Shanghai) Trading Co., Ltd.] was dissolved in saline and administered by the intraperitoneal (i.p.) route; SFN was injected once daily for 14 continuous days within the dose range of 1 to 10 mg/kg (i.p.) [23]. Fluoxetine hydrochloride [Sigma–Aldrich (Shanghai) Trading Co., Ltd.], acting as a positive control drug, was dissolved in saline and paralleled injected (10 mg/kg, i.p.) for 14 consecutive days.

### 2.3. Tail suspension test

The tail suspension test was performed according to previous reports [24,25]. Briefly, mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail for 6 min. Immobility was defined as the absence of limb or body movements, except for those caused by respiration when the mice hung passively and were completely motionless. During the test, mice were separated from each other to prevent possible visual and acoustical associations. The results were expressed as the time (in s) that animals spent immobile in the last 4 min of the 6 min session.

### 2.4. Forced swimming test

The forced swimming test was performed as previously described [24,26]. Mice were placed into a 20-cm diameter × 35-cm high plastic cylinder filled to a depth of 20 cm with23–25 °C water for 6 min. This session was videotaped, and the floating time was measured. Immobility was defined as the absence of movement, except for motion that was required to maintain the animal's head above the water. The results were expressed as the time (in seconds) that animals spent immobile in the last 4 min of the 6 min session.

### 2.5. Open field test

The apparatus consisted of a (40 cm × 40 cm × 35 cm) square arena that was divided into 25 equal squares on the floor of the arena. Mice were individually placed in the center of the cage, and the number that crossed to adjacent squares was counted as horizontal locomotor activity for 5 min. The time in the central zone was recorded to reflect the anxiety-like behaviors of mice [27].

[1] These authors contributed equally to this work.

### 2.6. Novelty-suppressed feeding

The novelty-suppressed feeding test (NSF) was adapted from previous studies [26,28]. The mice were deprived of food for 24 h before the test in their home cages. On the test day, mice were individually placed in an open field arena (40 cm × 40 cm × 35 cm) with small pellets of food placed in the center. Each mouse was first placed in a corner of the cage. The latency to approach the food and begin eating was recorded (in seconds) as the main test parameter (maximum time, 5 min). Immediately after each mouse was taken back to its home cage, food consumption during the first 5 min was quantified to exclude the possibility that stress affected normal appetite and feeding. A more 'anxious' animal will take more time to begin eating in a novel environment.

### 2.7. Chronic mild stress

The chronic mild stress (CMS) protocol was adapted from previous reports, with minor modifications [4,26]. Briefly, mice were exposed to a variable sequence of mild, unpredictable stressors for 28 days. A total of 10 different stressors were used; two stressors were used per day. The stressors included restraint for 3 h, cold for 1 h at 4 °C, water deprivation for 24 h, vibration for 1 h, tilted cages (45°) for24 h, forced cold swim for 5 min, crowding for 24 h, soiled bedding for 24 h, light/dark cycle reversal for 36 h, food deprivation for 24 h, and tail clamp for 1 min. Control mice were handled daily without any stress in the housing room.

### 2.8. Sucrose preference test

The sucrose preference test was performed as previous studies [4,26], the mice were trained to adapt to a 1% sucrose solution (w/v) for 48 h at the beginning of the experiment; two bottles of a 1% sucrose solution were placed in each cage. After adaptation, the mice were deprived of water for 24 h, which was followed by the sucrose preference test (SPT), during which mice were housed in individual cages for 24 h with exposure to two identical bottles; one was filled with a 1% sucrose solution, and the other was filled with water. Sucrose and water consumption (in g) were measured. Sucrose preference (%) = consumption × 100/(sucrose consumption + water consumption).

### 2.9. Enzyme-linked immunosorbent assay

We detected the serum levels of CORT, ACTH, IL-6 and TNF-α by enzyme-linked immunosorbent assay (ELISA) according to our previous study [25]. Briefly, 1 ml of blood was collected from decapitation bleeding. Blood samples were kept at room temperature for 1 h, and centrifuged at 3000 rpm for 10 min. The serum (supernatant fraction) was transferred into a new tube for subsequent assays. Serum CORT, ACTH, IL-6 and TNF-α levels were measured with commercially available ELISA kits (CORT, ml001959; ACTH, ml001895, IL-6, ml002293; TNF-α, ml002095, mlbio, China) according to the manufacturer's instructions. To exclude the potential impact of diurnal rhythm on mouse hormone levels, blood samples were collected in the same time window of 4:00–6:00 pm.

### 2.10. Experimental design

#### 2.10.1. Experiment 1: Effects of repeated sulforaphane administration on depressive- and anxiety-like behaviors in acutely stressed mice

As shown in Fig. 2A, experiment 1 was aimed to determine the effects of SFN on depressive- and anxiety-like behaviors in mice responded to acute stress. After a 5-day habituation, mice were randomly divided into 5 groups ($n$ = 10–12 per group) and were injected (i.p.) with saline, SFN (1, 3, or 10 mg/kg) or fluoxetine (10 mg/kg) daily for 14 consecutive days. Behavioral tests, including NSF, OPT, TST and FST, were conducted 24 h after the last SFN treatment.

#### 2.10.2. Experiment 2: Effects of repeated sulforaphane administration on depressive- and anxiety-like behaviors in chronically stressed mice

To further assess the effects of SFN on depressive- and anxiety-like behaviors in mice after chronic stress. CMS procedure was used in this experiment. Mice were divided into 4 groups ($n$ = 9–12 per group): Control + saline, CMS + saline, CMS + SFN, and CMS + fluoxetine. After a 5-day habituation, mice in CMS groups were treated whit a consecutive 28-day chronic stress procedure. Since the 14th day during CMS procedure, CMS-treated mice were randomly divided into 3 subgroups and were injected with saline (10 ml/kg, i.p.), SFN (10 mg/kg, i.p.) or fluoxetine (10 mg/kg, i.p.) daily for 14 days. Mice in control group were left in their homecages only with saline injections daily for 14 days. Behavioral tests, including SPT, NSF, OPT, TST and FST, were conducted 24 h after the last drug treatment (see Fig. 3A).

#### 2.10.3. Experiment 3: Effects of repeated sulforaphane administration on serum levels of CORT, ACTH, IL-6 and TNF-α in chronically stressed mice

Experiment 3 was aimed to investigate whether the antidepressant-like effects of SFN are associated with inhibition the HPA axis activity and/or regulation on immune system, twenty mice were used and were divided into four groups ($n$ = 5–6 per groups) with CMS and drug treatments similar to those in the experiment 2. Mice were decapitated 24 h after the last drug treatment, without any behavioral tests, and the blood was collected to detect the serum concentration of CORT, ACTH, IL-6 and TNF-α by ELISA analysis.

### 2.11. Data analysis

Data are expressed as the mean ± SEM. Statistical analysis of the data from acute and chronically stressed mice was performed by one-way analysis of variance (ANOVA), respectively, which was followed by a post hoc Dunnett's test. (For details, see Section 3). $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Repeated sulforaphane administration exerted antidepressant and anxiolytic-like effects in acutely stressed mice

We firstly tested the effects of repeated sulforaphane administration on depressive-like behaviors in acutely stressed mice. Mice were randomly divided into 5 groups ($n$ = 10–12 per group) and were injected (i.p.) with saline, SFN (1, 3, or 10 mg/kg) or fluoxetine (10 mg/kg) daily for 14 consecutive days. NSF, OPT, TST and FST, were conducted 24 h after the last SFN treatment.

One-way ANOVA of the TST data revealed a significant effect of SFN dose [$F_{3,41} = 3.476$, $P = 0.025$]. Post hoc analyzes showed that repeated SFN treatment for 14 days at dose of 10 mg/kg ($p < 0.05$) significantly reduced immobility time, but the 1 or 3 mg/kg doses had no effects on the immobility time compared with saline-treated control mice. The positive control fluoxetine (10 mg/kg) significantly reduced immobility time in the TST ($p < 0.01$) compared with control mice (Fig. 2B).

One-way ANOVA of the FST data showed a significant effect of SFN dose [$F_{3,41} = 3.862$, $P = 0.017$]. Post hoc analyzes showed that repeated SFN treatment at doses of 3 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.01$) significantly reduced floating time, but the 1 mg/kg dose

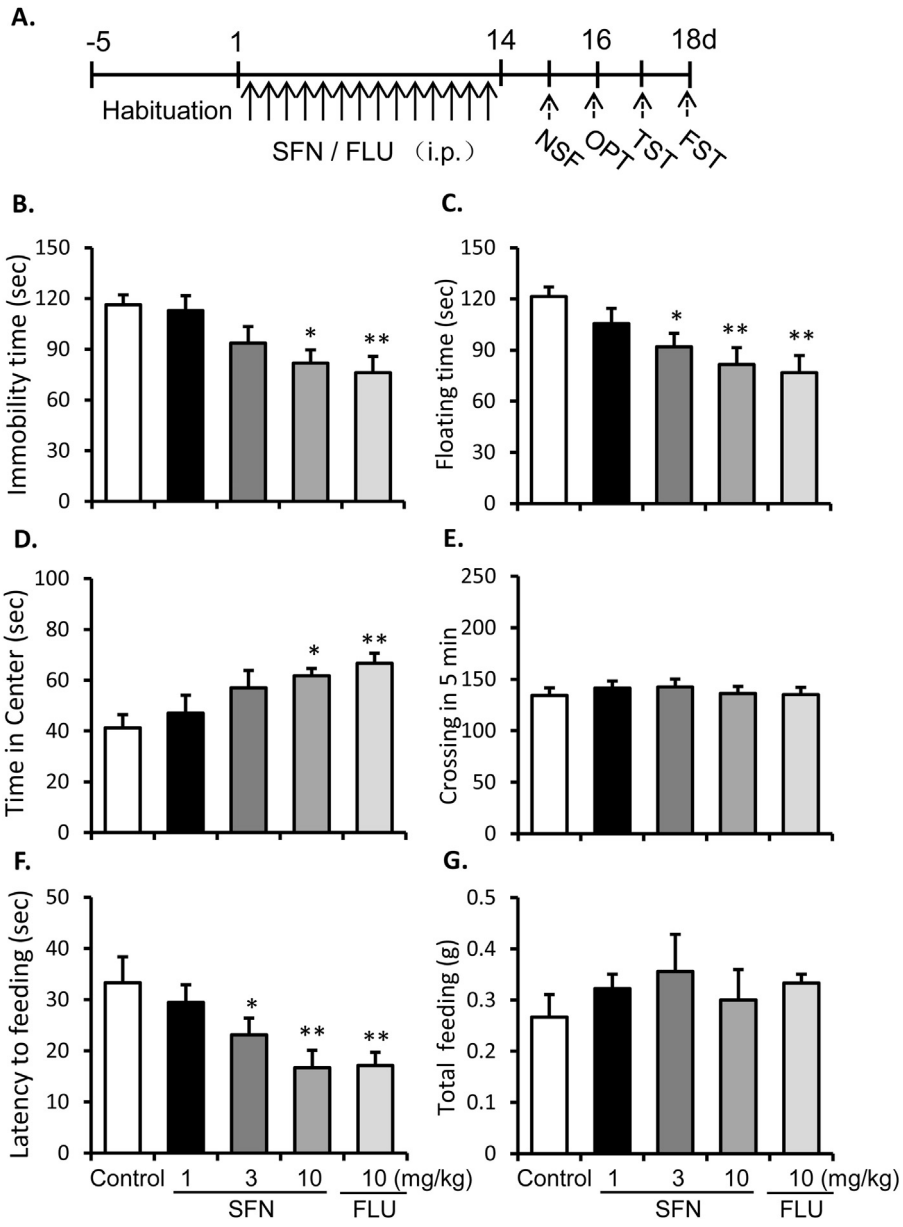


**Fig. 2.** Sulforaphane produced antidepressant- and anxiolytic-like behaviors in mice. (A) Experimental procedure. After a 5-day adaptation period, the mice were given daily administration of saline, sulforaphane (1, 3, and 10 mg/kg, i.p.) or fluoxetine (10 mg/kg, i.p.) for 14 days. Beginning on day 15, behavioral tests were conducted to assess the depressive- and anxiety-like behaviors. Sulforaphane significantly decreased the immobility time of mice in the TST (B) and FST (C) and increased the time spent in the central zone (D) without affecting the crossing activities (E) in the OPT. Sulforaphane significantly decreased the latency to feeding (F) without affecting the total feeding in homecages (G) during the NSF test. $^{*}P<0.05$, $^{**}P<0.01$ versus the saline-treated control group. $n=10$–12 per group.

had no effects on the floating time compared with saline-treated control mice. The positive control fluoxetine (10 mg/kg) significantly reduced immobility time in the TST ($p<0.01$) compared with control mice (Fig. 2C). The results elicited that sulforaphane is able to produce antidepressant-like effects in the FST and TST.

Next, the potential anxiolytic effects of SFN were also evaluated. One-way ANOVA of the data revealed showed a significant effect of SFN dose in the OPT [$F_{3,41}=2.964$, $P=0.044$] and in the NSF [$F_{3,41}=4.020$, $P=0.014$]. Post hoc analyzes showed that repeated SFN treatment at the dose of 10 mg/kg ($p<0.05$) and fluoxetine administration ($p<0.01$) significantly increased the time spent in the central zone in the OPT (Fig. 2D). SFN treatment at the doses of 3 mg/kg ($p<0.05$) and 10 mg/kg ($p<0.01$) and fluoxetine administration ($p<0.01$) significantly decreased the latency to feeding in the NSF (Fig. 2F), compared with saline-treated control mice. Repeated fluoxetine and SFN treatment at all doses had no significant effects on the crossing activities in the OPT (Fig. 2E, $P>0.05$) and total food intake in homecages (Fig. 2G, $P>0.05$). The results elicited that sulforaphane is able to produce antidepressant- and anxiolytic-like effects in mice responded to acute stress.

### 3.2. Repeated sulforaphane administration reversed depressive-like and anxiety-like behaviors in chronically stressed mice

We further assessed the antidepressant-like effects of SFN in the chronic mild stress (CMS) paradigms (Fig. 3A), one of the most valid models of depression. The mice subjected to CMS exhibited key depressive-like phenotypes(i.e., ahedonia, despair), reflected by a decreased in sucrose preference in SPT ($F_{1,16}=13.437$, $p<0.01$, Fig. 3B), increased floating time in the FST($F_{1,16}=6.617$, $p<0.05$, Fig. 3D) and increased immobility time in the TST

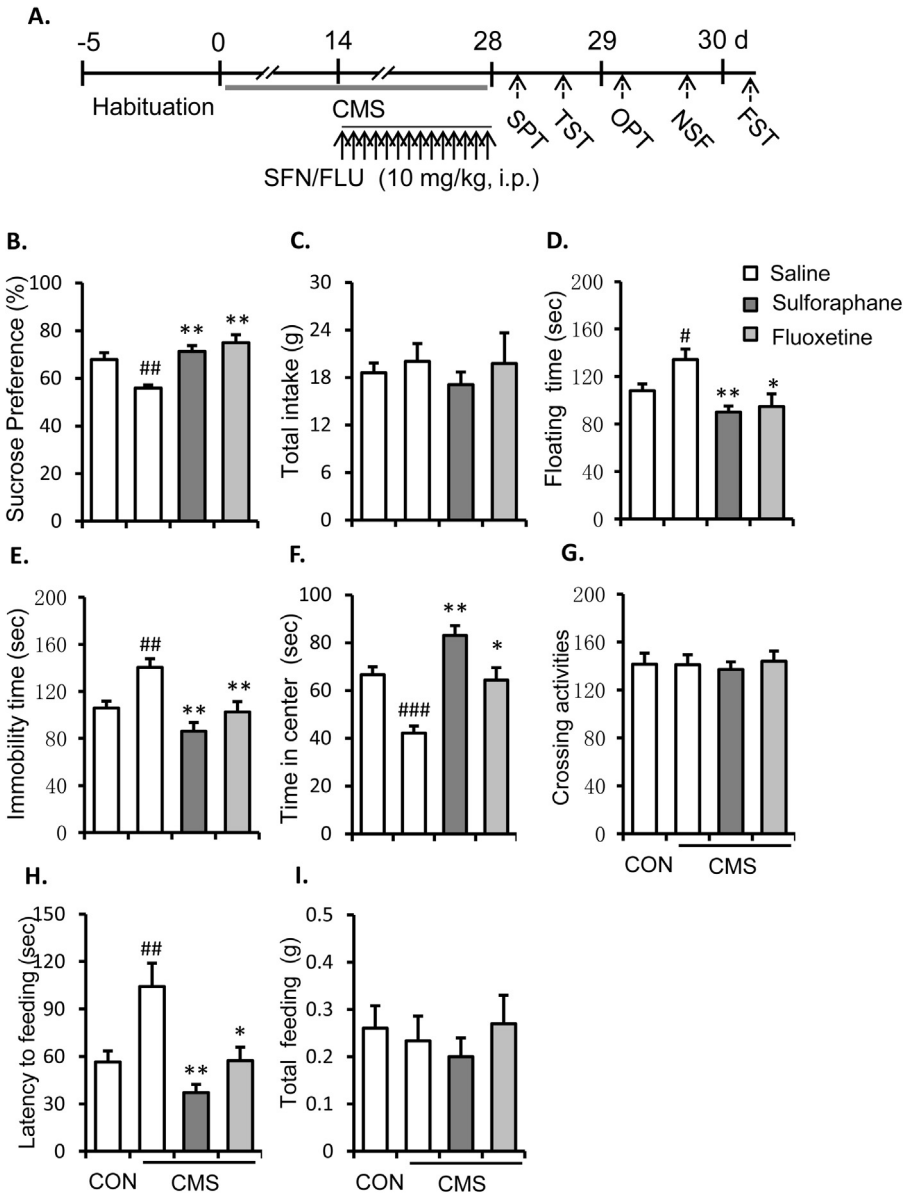


**Fig. 3.** Sulforaphane reversed the depressive- and anxiety-like behaviors in chronically stressed mice. (A) Experimental procedure. After a 5-day adaptation period, mice were treated by chronic stress for 28 days. On day 14, mice were injected with sulforaphane (10 mg/kg, i.p.) or Flu daily 0.5 h before stress for 14 days. During day 28–30, behavioral tests were conducted to assess the depressive- and anxiety-like behaviors. Sulforaphane significantly reversed the chronic stress-induced decrease of sucrose preference (B) without affecting the total intake (C), the increase of immobility in the FST (D) and TST (E). Sulforaphane significantly increased the time spent in the central zone (F) without affecting the crossing activities (G) of chronically stressed mice during the OPT. Sulforaphane significantly decreased the latency to feeding (H) without affecting the total feeding in homecages (I) of chronically stressed mice during the NSF test. $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.005$ versus the saline-treated control group; $^{*}P<0.05$ and $^{**}P<0.01$ versus the saline-treated CMS group. $n=9$–12 per group.

($F_{1,16}=13.617$, $p<0.01$, Fig. 3E). Repeated SFN administration significantly increased sucrose preference ($F_{1,17}=25.883$, $p<0.01$, Fig. 3B), reduced the floating time in the FST ($F_{1,17}=21.267$, $p<0.01$, Fig. 3D) and immobility time in the TST ($F_{1,17}=26.281$, $p<0.01$, Fig. 3E), consistent with the effect of fluoxetine. SFN or fluoxetine treatment had no significant effects on the total water intake (Fig. 3C, $P>0.05$).

The OPT and NSF were conducted to investigate the anxiolytic effects of SFN. One-way ANOVA analysis showed that the mice subjected to CMS exerted significant anxiety-like phenotypes, reflected by a decreased time spent in the central zone in the OPT ($F_{1,17}=28.685$, $p<0.001$, Fig. 3F) and a prolonged latency to feeding in the NSF ($F_{1,17}=9.678$, $p<0.01$, Fig. 3H) compared with control mice. Similar to the positive control fluoxetine treatment (10 mg/kg), The repeated SFN (10 mg/kg, i.p.) administration reversed the anxiety-like behaviors of mice induced by CMS, including increasing the time spent in the central zone in the OPT ($F_{1,17}=59.354$, $p<0.01$, Fig. 3F) and decreasing the prolonged latency to feeding in the NSF ($F_{1,17}=21.759$, $p<0.05$, Fig. 3H), without having any significant effects on the locomotion activity ($P>0.05$, Fig. 3G) or total feeding in homecages ($p>0.05$, Fig. 3I).

### 3.3. Repeated sulforaphane administration decreased the serum levels of CORT, ACTH, IL-6 and TNF-α in chronic stress treated mice

The effects of SFN on inflammatory mediators were evaluated using ELISA analysis (Fig. 4A). The results showed that four weeks of CMS significantly increased the serum IL-6 ($F_{1,9}=13.025$, $p<0.05$, Fig. 4B) and TNF-α ($F_{1,9}=34.437$, $p<0.01$, Fig. 4C) levels, compared with the control group. Similar to the effects of fluoxetine

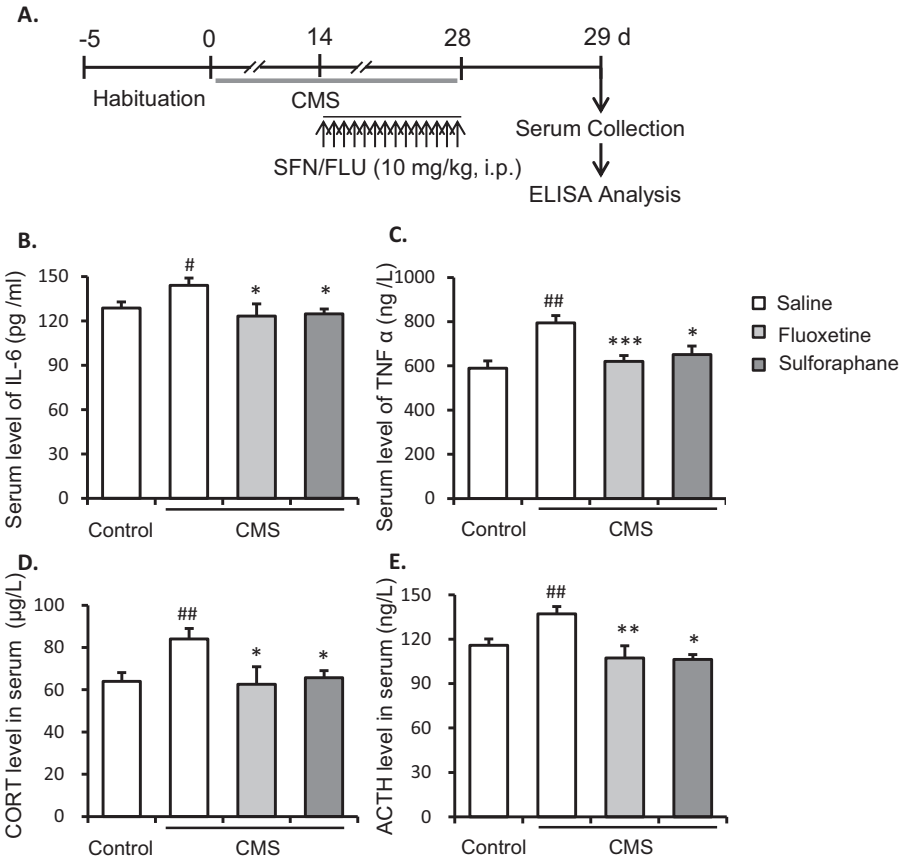


**Fig. 4.** Sulforaphane reversed the chronic stress-induced increase of serum levels of CORT, ACTH, IL-6, and TNF-α. (A) Experimental procedure. After a 5-day adaptation period, mice were treated with chronic stress procedure for 28 days. On day 14, sulforaphane (10 mg/kg, i.p.) was administered daily 0.5 h before stress for 14 days. On day 29, mice were decapitated and serum samples were collected for ELISA analysis. Sulforaphane (10 mg/kg, i.p.) significantly decreased the IL-6 (B), TNF-α (C), CORT (D), and ACTH (E) levels in chronically stressed mice. $^{\#}P<0.05$ and $^{\#\#}P<0.01$ versus the saline-treated control group; $^{*}P<0.05$ and $^{**}P<0.01$ versus the saline-treated CMS group. $n=5$-6 per group.

administration, SFN administration significantly alleviated the CMS-induced increased serum IL-6 ($F_{1,9}=15.5.02$, $p<0.05$, Fig. 4B) and TNF-α ($F_{1,9}=20.383$, $p<0.005$, Fig. 4C) levels.

The effects of SFN on HPA activity were determined by analysing the serum CORT and ACTH levels. The results showed that the mice subjected to CMS had significant higher serum levels of CORT($F_{1,9}=23.805$, $p<0.01$, Fig. 4D) and ACTH ($F_{1,9}=11.621$, $p<0.01$, Fig. 4E), compared with the control group. SFN administration during the CMS process significantly alleviated the CMS-induced increased serum CORT ($F_{1,9}=18.031$, $p<0.05$, Fig. 4D) and ACTH ($F_{1,9}=15.79$, $p<0.01$, Fig. 4E) levels.

## 4. Discussion

In the current study, using mice acute and chronic stress model, we explored whether repeated SFN administration has antidepressant- and anxiolytic-like effects in adult mice. We found that repeated SFN administration has antidepressant- and anxiolytic-like effects in the mice subjected to acute or chronic stress. In addition, we found that repeated SFN administration inhibited hyperactivity of the HPA axis and the inflammatory response in CMS mice, indicating that the neuroprotective effects on depression and anxiety may be associated with modulation on HPA axis activity and immune system. Interestingly, the antidepressant-like efficiency of SFN equals to fluoxetine, a widely used antidepressant in clinic. Considering the comparable efficiency to prescription medication with no reported side effects, SFN should be well investigated as a potential new antidepressant in the future.

Many evidence revealed that stress, especially chronic stress is the key point to induce depression in animals and human [9,12,13,29]. Chronic stress could induce significant depressive- and anxiety-like phenotypes in rodents, including ahedonia, despair, and anxiety behaviors, which can be reversed by classic antidepressants(i.e., fluoxetine, venlafaxine) and potential antidepressants (i.e., curcumin, green tea polyphenols) [5,26,30,31]. Sucrose preference test was the most widely used parameter to assess the ahedonia of depressed animals, the lower sucrose preference, the higher level of depression of animals [26]. The FST and TST are two standard models that are used for assessing depressive-like behaviors and the putative antidepressant-like activity of compounds. The immobility time in these paradigms reflects the antidepressant-like activity [4,5,26]. In the present study, we found that repeated daily administration of SFN for consecutive 14 days (10 mg/kg, i.p.) significantly decreased the immobility time in the FST and TST in the mice responded to acute stress, indicating that SFN has potential antidepressant-like effect. The antidepressant-like effect of SFN was comparable to fluoxetine, a widely used classical antidepressant with anti-inflammatory and inhibitory effects on HPA axis activity [32]. Simultaneously, potential anxiolytic effects of SFN also was observed by OPT and NSF procedures. The results showed that the administration of SFN at the present doses did not affect the locomotor activity, assessed by the crossing activities, excluding the possibility that the antidepressant- and anxiolytic effects of SFN were resulted from alteration of the

locomotion activity of mice. The CMS procedure, a well-evaluated chronic stress animal model, was used to further investigate the antidepressant-like and anxiolytic effects of repeated administration of SFN. Depressive- and anxiety-like behaviors in the mice with CMS treatment were reversed by repeated fluoxetine or SFN administration, indicating that SFN have antidepressant- and anxiolytic-like effects in the chronic mild stress mice model.

Chronic mild stress leads to HPA axis dysfunction, including the increase in the circulating glucocorticoids (CORT in rodents and cortisol in humans), which contributes to the development of depression in animals and humans [29,33–35]. Chronic CORT administration could induce depressive- and anxiety-like behaviors in rodents [36,37]. Most antidepressants and antidepressive intervene methods could reverse the depressive-like behaviors and hyperactivity of the HPA axis that is induced by stress [38]. To investigate the potential mechanism of the antidepressant-like activities of SFN, we measured the serum CORT and ACTH levels in chronically stressed mice. As shown above, SFN blocked the increase in serum CORT and ACTH levels in mice subjected to CMS, indicating that the antidepressant effect of SFN is mediated by regulating the HPA axis homeostasis to increase the ability of mice to cope with stressful conditions. It has been shown that hyperactivity of the HPA axis decreases the function of the glucocorticoid receptor (GR), particularly in the hippocampus, impairing glucocorticoid feedback inhibition [39]. Dysfunction of the GR reduces neurogenesis and impairs neuroplasticity, leading to the development of depressive performance. Therapeutic effect of antidepressants is mediated by modulating the expression of GR to ameliorate many of the disturbances in depressive-like behaviors [40]. Given that GR plays a key role in the development and treatment of depression, the regulatory effect of SFN on GR expression and the target genes involved in antidepressant-like effects needs to be further addressed in further studies.

The causative relationship between inflammation and depression is gradually becoming more consistent [13]. A recent study showed that infliximab, a monoclonal antibody against TNF-α, was found to be effective in modulating the mood of depressed patients, indicating that anti-inflammation would be an efficient therapy for depression. Using a stress-induced depression mouse model, the present study confirmed the antidepressant- and anxiolytic effect of SFN is accompanied by the inhibition of inflammatory response to chronic stress. The potential neuronal mechanism (i.e., neuroinflammation) is needed for further investigation. Neuroprotective effect of SFN has been reported in various experimental animal models and human, including neurodegeneration, neuroinflammation [20,41–44], which are mainly mediated by Nrf2, a potential therapeutic target against brain inflammation [21,45–47]. SFN could increase Nrf2 DNA-binding activity and up-regulate Nrf2 target genes in BV2 microglia, while reduce LPS-induced interleukin (IL-) 1β, IL-6, and inducible nitric oxide synthase in primary microglia in adult and aged mice, indicating that SFN is a potential beneficial supplement that may be useful for reducing microglial mediated neuroinflammation and oxidative stress associated with aging [48]. In addition, SFN effectively attenuates 3-NP-induced striatal toxicity by activating the Keap1-Nrf2-ARE pathway and inhibiting the mitogen-activated protein kinases (MAPKs) and NF-kappa B pathways. Additionally, SFN-mediated generation of reactive oxygen species (ROS) induces autophagy via ERK activation, independent of Nrf2 activity in neuronal cells [46,49]. A recent study reported that chronic fluoxetine treatment restored CORT-induced decreases in Nrf2 protein levels and its target genes in the hippocampus and cortex of mice [50]. Chronic inflammation induced by deletion of Nrf2 leads to a depressive-like phenotype, while induction of Nrf2 by sulforaphane has antidepressant-like effects in an inflammatory model of depression elicited by LPS[22]. Nrf2 signaling was reported to participate the antidepressant-like effect produced by agmatine and contribute to fluoxetine-induced neuroprotection via an unexpected mechanism involving 5-HT transporter system [22,51]. It has been reported that systemic SFN administration reduced LPS-induced hippocampus inflammation indicating the neuronal mechanism mediating the effects of SFN on neuroinflammation [52]. The specific molecular mechanisms underlying the antidepressant-like effects of SFN in CMS-induced depression, including the potential role of Nrf2 pathway, should be further investigated.

In summary, this study demonstrated that sulforaphane has antidepressant-like effects in stressed mice models of depression, as well as potential anxiolytic activities without having apparent adverse effects (motor alteration). The antidepressant-like effects of SFN are associated with a normalization of the stress-induced HPA axis dysfunction and the inhibitory effects on the inflammatory response to stress, which highlights the neuroimmune mechanism of depression. Further research is needed to determine the potential neuronal mechanism underlying the antidepressant effects and whether the antidepressant effects of SFN in mice are applicable to depressed patients. Furthermore, SFN as a natural compound has significant antidepressive and anxiolytic effect that is comparable to fluoxetine, which may provide a new potential agent for depression treatment.

## Author contributions

S.H and M.Y. conceived and designed the experiments. G.Q., X.Y., W. X., and L.Y. performed the behavioral Tests. W.S., S.H., and Y.G. analyzed the behavioral data and prepared the figures. W.S., Z.P., and G.Y. conducted the ELISA assays and analyzed the data. W.S., S.H., Z.P., and G.Q. wrote and revised the paper.

## Acknowledgments

This work was supported in part by the National Natural Science Foundation of China (81201038, 31371140), Special Foundation for Excellent Youth Investigator from Hebei Province of China (To H-S Shi), China Postdoctoral Science Foundation (2012m521924, 2013t60963), Natural Science Foundation of Hebei Province of China (15275517), Special Foundation for Excellent Undergraduate Students of Hebei Province (201410089042, 201410089042). All of the authors hereby declare that they have no competing financial interests.

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