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# The anxiolytic-like effects of ginsenoside Rg2 on an animal model of PTSD

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ABSTRACT

Post traumatic stress disorder (PTSD) is one of the mental illness. The antidepressant-like properties of ginsenoside Rg2 (GRg2) have been shown, while little is known about its anti-PTSD-like effects. In the present study, the PTSD-associated behavioral deficits in rats were induced following exposure to single prolonged stress (SPS). The results showed that the decreased time and entries in the open arms in elevated plus maze test (EPMT) and increased freezing duration in contextual fear paradigm (CFP) were reversed by GRg2 (10 and 20 mg/kg) without affecting the locomotor activity. In addition, GRg2 (10 and 20 mg/kg) could block the decreased levels of progesterone, allopregnanolone, serotonin (5-HT), 5-Hydroxyindoleacetic acid (5-HIAA), corticotropin releasing hormone (CRH), corticosterone (Cort) and adrenocorticotropic hormone (ACTH) in the brain or serum. In summary, GRg2 alleviated the PTSD-associated behavioral deficits with biosynthesis of neurosteroids, normalization of serotonergic system and HPA axis dysfunction.

## 1. Introduction

Post traumatic stress disorder (PTSD) is a serious mental illness, with an estimated lifetime prevalence rate of 6.8% in the general population in the United States, and an estimated 23% among post-9/11 US Veterans (Akiki et al., 2017). So far, there are no major breakthroughs to prevent PTSD. A number of treatments are considered to ameliorate the PTSD-associated behavior. For instance, selective serotonin reuptake inhibitors (SSRIs), like sertraline and paroxetine, are the usual options (Bentefour et al., 2016; Jerud et al., 2016). However, various inevitable side effects may be induced by SSRIs, i,e withdrawal, sedation, dependence as well as cognitive dysfunction. Consequently, considerable effort has been invested to search better prevention of PTSD.

More researches focus on the plant preparations and natural extracts to against PTSD (Zhang, 2014). Ginsenoside Rg2 (GRg2), one of the major active components in the root and stem leaves of ginseng (Fig. 1) (Ren et al., 2017), has been proposed with a range of pharmacological effects. In neurological study, GRg2 exerts the potential effects on attenuating neurotoxicity and memory impairments (Zhang et al., 2008). For instance, GRg2 exerted the neuroprotection on cerebral ischemia-reperfusion, which induced impairment of neurological responses, memory and caudate-putamen neuronal apoptosis in a vascular dementia (VD) rodent model (Zhang et al., 2008). In addition, PC12 cells were protected by GRg2 to against β-amyloid$_{25\text{-}35}$-induced apoptosis via the phosphoinositide 3-kinase/Akt pathway (Cui et al., 2017). Also, GRg2 defended the formation of A$\beta_{1\text{-}40}$ suggesting that GRg2 might also considered as a potential treatment strategy for Alzheimer's disease (AD) (Li et al., 2007). Moreover, depressive-like behavior in rodents after exposure to chronic mild stress could be ameliorated by GRg2 (Ren et al., 2017). Although GRg2 possess the neuroprotection and antidepressant-like effects, anti-PTSD-like effects of GRg2 are still explored.

Researches into the underlying neurobiology of PTSD implicates the regulation of various neurotransmitters and neuroendocrine systems, such as dysregulation of neurosteroids biosynthesis, hypothalamic-pituitary-adrenal (HPA) axis or monoaminergic neurotransmission (Jin et al., 2016; Locci and Pinna, 2017). PTSD may be developed by dysregulation of HPA axis, showing that enhanced negative feedback inhibition of HPA axis may be one of the negative factors to PTSD (Burbiel, 2015). The changed stress hormones in HPA axis, e.g corticosterone (Cort), corticotrophin releasing hormone (CRH), adrenocorticotropic hormone (ACTH), are closely associated with the pathology of PTSD (Danan et al., 2018; Feng et al., 2014; Jin et al., 2016). The release and dysregulation of glucocorticoids could be induced by the stress hormones above in patients or animal models with PTSD

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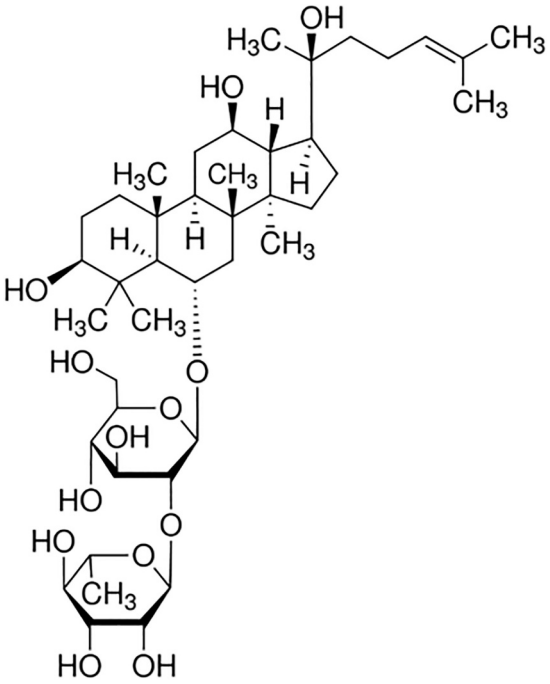

**Fig. 1.** The chemical structure of ginsenoside Rg2 (GRg2).

(Rasmusson et al., 2017).

Moreover, disturbed monoaminergic neurotransmission is also an involvement in PTSD (Strawn et al., 2010). The monoaminergic hypothesis indicated that monoamines are important neurotransmitters relevant to the etiology of PTSD. Previous study also indicated that the elevated levels of monoamine neurotransmitters in brain after the treatments of PTSD versus that of controls. In fact, most of the treatments of PTSD elicited more than one mechanism based on the monoaminergic hypothesis, such as inhibition of the reuptake of 5-HT and its metabolites.

Furthermore, the down-regulation of neurosteroid biosynthesis has been implicated as one of the possible inductions of PTSD (Pinna and Rasmusson, 2012). Neuroactive steroids (e.g progesterone and allopregnanolone) have been shown to elicit the anti-PTSD-like properties (Nin et al., 2011; Zhang et al., 2017). For instance, normalization of allopregnanolone levels in the brain may induce the pharmacological profile of PTSD (Nin et al., 2011).

The present study evaluates the anti-PTSD-like effects of GRg2. The animal model (single prolonged stress, SPS) is selected. This model can be useful for modeling PTSD-like symptoms based on the facts that those who subject to multiple traumas, or a trauma early in life, are more susceptible to developing PTSD following a traumatic event (Souza et al., 2017). The SPS rat has an impairment in the recall of fear extinction learning may be an underlying cause of PTSD symptoms (Milad et al., 2008; Souza et al., 2017). Following the preparation of SPS model (the classical PTSD model in rodent), the anti-PTSD-like effects of GRg2 were assessed by various behavioral tests. After the behavioral tests, the significance of neurosteroids biosynthesis, HPA stress hormones and monoamines in the anti-PTSD-like activities of GRg2 was also evaluated.

## 1. Materials and methods

### 2.1. Drugs

Sertraline (Sigma-Aldrich, U.S.A.) (15 mg/kg, i.g.) was intragastric gavage (i.g.) as a positive control in the behavioral tests according to its anti-PTSD-like effects on the SPS model (Miao et al., 2014). The drug was prepared in 0.9% normal saline with the treatment based on the previous regimens (Miao et al., 2014). GRg2 (purity ≥ 98%) was also prepared in 0.9% normal saline and administrated once daily at doses of 5, 10 and 20 mg/kg (i.g) for 14 days (from day 2 to 15, 8:00–9:00 a.m.) after the SPS procedures (Fig. 2). The doses of GRg2 were based on its antidepressant-like effects (Ren et al., 2017). The behavioral assessments were performed 1 h after drugs treatment (start at 9:00–10:00 a.m.). The control group was administrated by 0.9% saline (i.g.).

### 2.2. Animals

Sprague-Dawley rats (180–200 g) were maintained in the temperature (22–24 °C) - and humidity (45–55%)- controlled room. The rodents were housed in a 12h- light/dark cycle environment with food and water available freely. All the protocols were carried out in accordance with National Institute of Health Guide for the care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996).

### 2.3. The preparation of SPS model

SPS is a widely used animal model in the PTSD researches (Miao et al., 2014). After the acclimatization period, each rat was placed in a restrainer with the tail-gate for 2 h. The size was adjusted based on the size of individual to achieve complete immobilization. Following the restraint, each rat was placed individually into a clear acrylic cylinder (diameter 20 cm, height 45 cm, and contained about 25 cm of water at the temperature of 23–24 °C) to perform a 20-min forced swim. After the 15-min recuperation, each one was subjected to

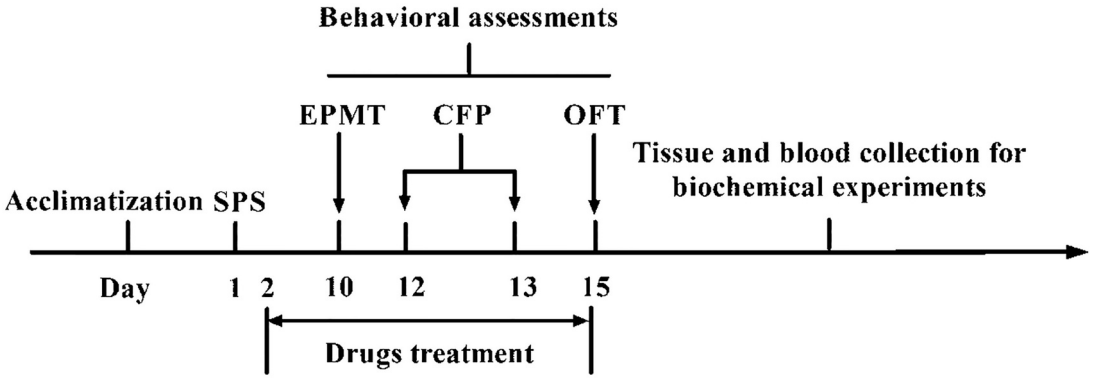


**Fig. 2.** Treatment and behavioral test schedules. Animals were subjected to SPS on day 1. From day 10 through 15, various behavioral tests were performed: elevated plus maze test (EPMT), contextual fear paradigm (CFP), and open field test (OFT). GRg2 (at doses of 5, 10 and 20 mg/kg, i.g.) and sertraline (at a dose 15 mg/kg, i.g.) were administered daily from day 2 to 15 and 1 h before testing.

ether vapors until loss of consciousness. The control group remained in a room adjacent to SPS rats and was handled.

### 2.4. Behavioral paradigms

Nine days after exposure to SPS protocols, the behavioral assessments were performed, including the elevated plus-maze test (EPMT) (day 10), contextual fear paradigm (CFP) (day 12 and 13), and open-field test (OFT) (day 15).

#### 2.4.1. Elevated plus maze test (EPMT)

The EPMT is a classical assessment for evaluating the PTSD-associated anxiogenic behavior in rodents (Zhao et al., 2016). The apparatus consisted of four arms (60 × 12 cm): two closed arms with dark walls (40 cm hight) and two open arms. The arms were connected by a central platform (12 × 12 cm), and the maze was 50 cm above the ground. Each one was placed in the platform facing the closed arms and scored when all four paws passed over the dividing line. Time and numbers of entries into open arms were obtained as PTSD-associated anxiogenic indices by observers who were blind to treatment conditions.

#### 2.4.2. Contextual fear paradigm (CFP)

The freezing response on re-experience to traumatic context is considered as a evaluation of PTSD-associated fear memory (Miao et al., 2014). Each rat was subjected to a 180-s conditioned context without stimulation. Then, a foot electric shock (0.8 mA, for 4 s) through the stainless steel grid floor was given. The rats remained in the chamber for an additional 1 min before being returned to their home cages. Each rat was placed in the conditioning chamber where it was exposed to the foot shock previously after 24 h. The contextual fear response was considered as the time of freezing-like behavior by observers blinded to the treatment groups during 5-min interval. PTSD-associated freezing behavior was defined as a total absence of head/body movement except for that associated with breathing.

#### 2.4.3. Open field test (OFT)

To evaluate the significance of locomotor activity in the anti-PTSD-like effects of GRg2, crossings, rears, and fecal pallets was assessed. The rats were placed in the corner of a plastic box (dimensions: 76 × 76 × 46 cm) which the base was divided into equal sectors for the 5-min acclimation. After that, crossings (with four paws placed into a new square), rears (with both front paws raised from the floor), and fecal pallets were scored for 5 min.

### 2.5. Levels of neurosteroids measurement

Dysfunction of neurosteroids biosynthesis (e.g progesterone and allopregnanolone) is considered as one of the possible causes to the development of PTSD (Nin et al., 2011; Rasmusson et al., 2017). The levels of progesterone and allopregnanolone were evaluated by enzyme linked immunosorbent assay (ELISA) (Zhang et al., 2017). The prefrontal cortex and hippocampus were dissected at the end of OFT in 24 h. Both regions were extracted by 1 mL buffer per 100 mg tissue and then homogenized in the ice-cold lysis buffer. The tissue homogenate solutions were centrifuged at 9000 g for 30 min at 4 °C. The supernatants were collected. The levels of progesterone and allopregnanolone were quantified by Enzyme Immunoassay kit and optical density values were determined at 450 nm in ELISA plate reader (Beckman, USA).

### 2.6. Levels of Cort, CRH and ACTH measurement

The blood was sampled and collected at the end of behavioral tests in 24 h. The samples were centrifuged (3000 g, 30 min) at 4 °C and stored at −80 °C until further analyses. The levels of Cort , CRH and ACTH in serum were determined by ELISA kits (Cort: R&D Systems, USA; CRH: Dldevelop, China; ACTH: Dldevelop, China) based on the instructions. A sample/standard and conjugate were added to each well, and the plate was incubated for 1 h at room temperature. The optical density values were read at 450 nm by an ELISA plate reader (Beckman, USA) after several washes and proper color development.

### 2.7. High-performance liquid chromatography with electrochemica detection (HPLC-ECD)

To further evaluate the monoaminergic neurotransmission in the anti-PTSD-like effect of GRg2, the levels of monoamines and their metabolites in the prefrontal cortex and hippocampus were detected by HPLC-ECD (Wang et al., 2016). The rats were decapitated at the end of behavioral tests in 24 h. The brain regions were dissected on ice by a binocular dissection microscope and homogenized in the ice-cold tissue lysis buffer. The samples were centrifuged at 10,000 × g for 30 min at 4 °C and then filtered through a 0.45 μm pore membrane. The sample or standard solution was injected into the reversed-phase SunFire™ $C_{18}$ column (250 mm × 4.6 mm, 5 μm). Separation was performed in an isocratic elution mode. The monoamine neurotransmitters and metabolites (5-HT, 5-HIAA, DOPAC, DA, AD, HVA and NE) were calculated as ng/g weight of the brain tissues.

### 2.8. Statistical analysis

The data were analyzed by GraphPad Prism 5.0 (Version 2.0; GraphPad Software Inc., San Diego, CA) and presented as the mean ± S.E.M. The statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison tests. Differences at an alpha value ($p < 0.05$) were considered statistically significant.

## 3. Results

### 3.1. Anti-PTSD-like effects of GRg2 in EPMT

As shown in Fig. 3, the percentage of time ($F$ (5,54) = 7.310, $p < 0.05$; Fig. 3C) and entries ($F$ (5,54) = 8.588, $p < 0.05$; Fig. 3D) into open arms was reduced significantly after exposure to SPS in rodents. Similar to effects of sertraline (15 mg/kg), both decreased time and entries were blocked by GRg2 (10 and 20 mg/kg). There was no significant difference in total time ($F$ (5,54) = 1.101, $p > 0.05$; Fig. 3A) and entries ($F$ (5,54) = 0.06923, $p > 0.05$; Fig. 3B) in arms among groups. These results indicated that the PTSD-associated anxiogenic behavior was ameliorated by GRg2 in EPMT.

### 3.2. Anti-PTSD-like effects of GRg2 in CPF

The effects of GRg2 on the PTSD-like associated contextual freezing behavior in rats were shown in Fig. 4. The freezing time was significantly elevated after exposure to SPS. In line with the effects of sertraline (15 mg/kg), the increased freezing time was markedly reversed by GRg2 (10 and 20 mg/kg) ($F$ (5,54) = 10.28, $p < 0.05$). These results indicated that the PTSD-associated contextual freezing behavior was alleviated by GRg2 treatment in CPF.

### 3.3. Effects of GRg2 on locomotor activity in OFT

The effects of GRg2 on the locomotor activity were shown in Fig. 5. There was no significant difference on the number of line crossings ($F$ (5,54) = 0.9304, $p > 0.05$, Fig. 5A), rears ($F$ (5,54) = 1.669, $p > 0.05$, Fig. 5B), or fecal pallets ($F$ (5,54) = 0.07124, $p > 0.05$, Fig. 5C) among the groups. These results indicated that the locomotor activity was not affected by GRg2 treatment in rats.

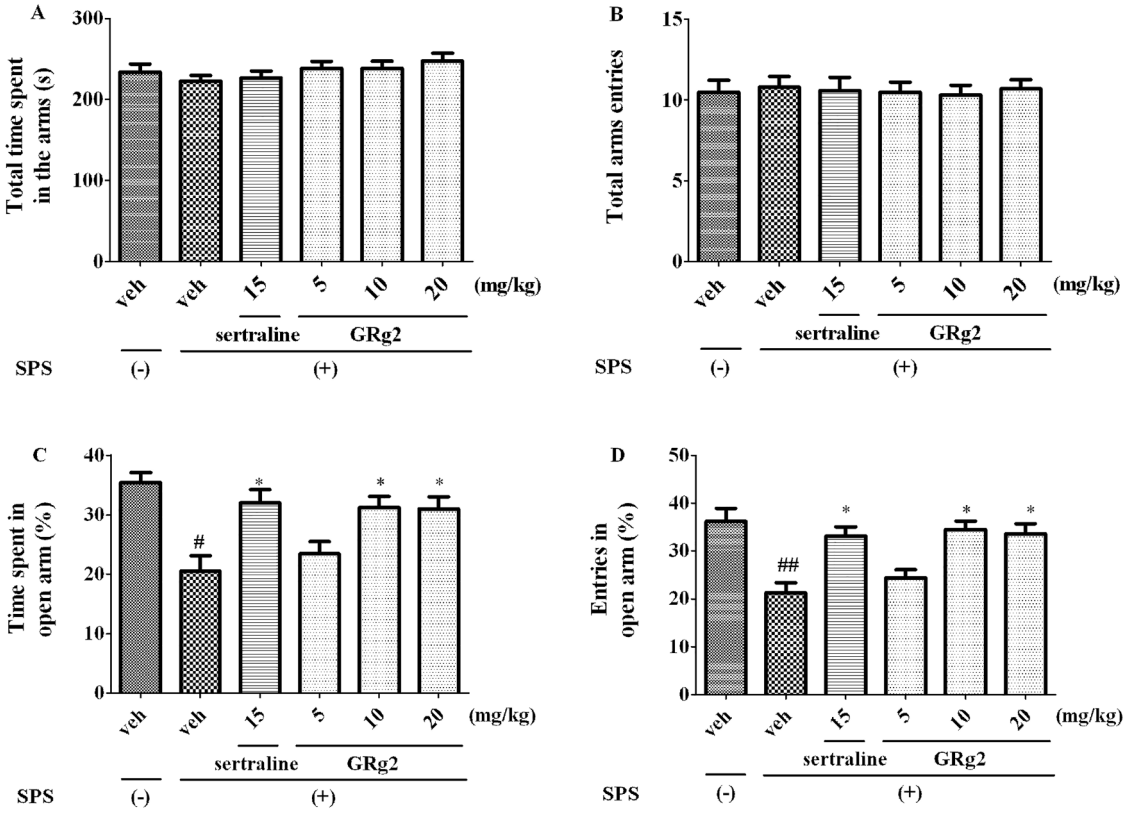


**Fig. 3.** The anti-PTSD-like effects of GRg2 treatment following exposure to SPS in rats. The behavior was presented by percentages of time (C) and entries (D) into open arms, as well as total time (A) and entries (B) in the arms. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. vehicle-treated SPS (−) group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle treated SPS (+) group ($n = 10$).

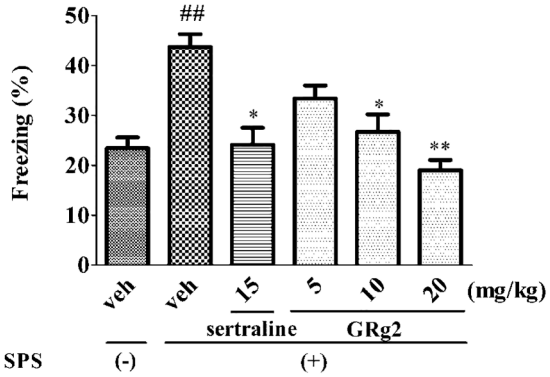


**Fig. 4.** The anti-PTSD-like effects of GRg2 on the freezing behavior in rats. $^{\#\#}p < 0.01$ vs. vehicle-treated SPS (−) group; $^{*}p < 0.05$, and $^{**}p < 0.01$ vs. vehicle-treated SPS (+) group ($n = 10$).

### 3.4. Anti-PTSD-like of GRg2 on the levels of neurosteroids

The effects of GRg2 on the levels of neurosteroids in rats were shown in Fig. 6. The levels of progesterone and allopregnanolone in the prefrontal cortex and hippocampus were significantly decreased after the exposure to SPS, respectively. Consistent with the effects of sertraline (15 mg/kg), both decreased levels of neurosteroids were significantly reversed by GRg2 (10 and 20 mg/kg) in the prefrontal cortex ($F$ (5,30) = 3.039, $p < 0.05$, for progesterone, Fig. 6A; $F$ (5,30) = 5.326, $p < 0.05$, for allopregnanolone, Fig. 6B) and hippocampus ($F$ (5,30) = 4.353, $p < 0.05$, for progesterone, Fig. 6C; $F$ (5,30) = 5.822, $p < 0.05$, for allopregnanolone, Fig. 6D), respectively. These results indicated that the anti-PTSD-like effects of GRg2 were associated with the biosynthesis of progesterone and allopregnanolone in the brain.

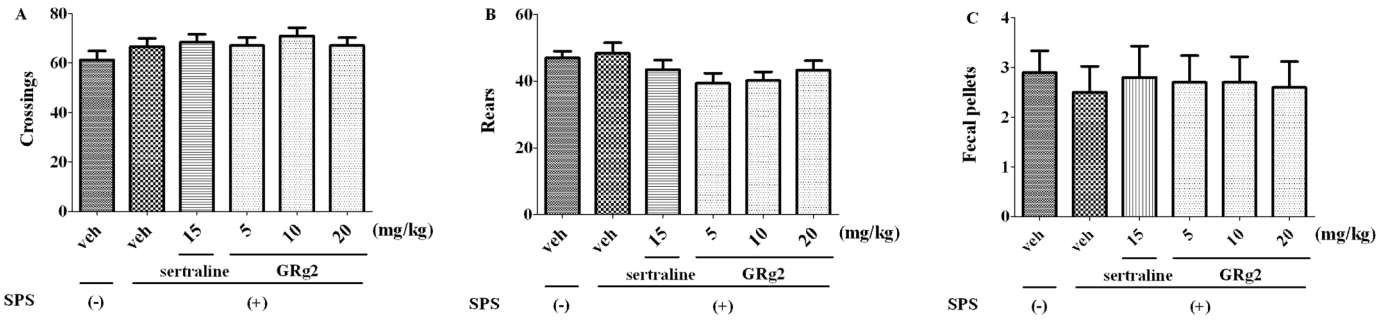


**Fig. 5.** The anti-PTSD-like effects of GRg2 on locomotor activity. None of the treatments altered the number of line crossings (A), rears (B), and fecal pallets (C) in the OFT ($n = 10$).

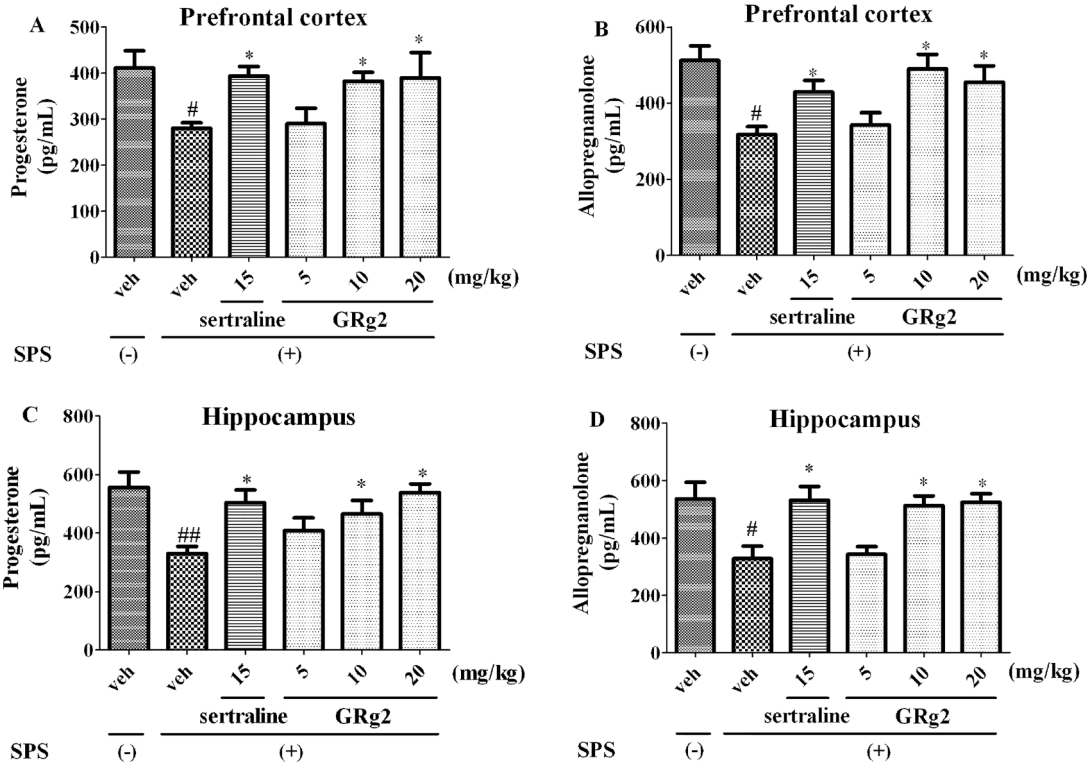


**Fig. 6.** The effects of GRg2 on levels of progesterone and allopregnanolone in the prefrontal cortex (A, B) and hippocampus (C, D), respectively. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. vehicle-treated SPS (−) group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle-treated SPS (+) group ($n = 6$).

### 3.5. Anti-PTSD-like of GRg2 on the HPA axis changes

The effects of GRg2 on the levels of Cort, CRH and ACTH in rats were shown in Fig. 7. Following exposure to SPS, the levels of Cort ($F$ (5,30) = 5.445, $p < 0.05$; Fig. 7A), CRH ($F$ (5,30) = 7.782, $p < 0.05$; Fig. 7B) and ACTH ($F$ (5,30) = 3.950, $p < 0.05$; Fig. 7C) in serum were significantly increased. In accordance with sertraline (15 mg/kg), these effects were markedly blocked by GRg2 (10 and 20 mg/kg), respectively. These results indicated that the anti-PTSD-like effects of GRg2 were associated with decreased levels of HPA stress hormones (Cort, CRH and ACTH).

### 3.6. Anti-PTSD-like of GRg2 on the levels of monoamine neurotransmitters in the brain

As shown in table (Tables 1 and 2), the levels of 5-HT ($F$ (5,30) = 2.882, $p < 0.05$; Table 1) and 5-HIAA ($F$ (5,30) = 2.641, $p < 0.05$; Table 1) in the prefrontal cortex were significantly decreased after exposure to SPS. Similar to the effects of sertraline (15 mg/kg), the decreased levels of 5-HT and 5-HIAA were reversed by GRg2 (10 and 20 mg/kg), respectively. In line with the results of the prefrontal cortex, the decreased levels of 5-HT ($F$ (5,30) = 2.660, $p < 0.05$; Table 2) and 5-HIAA ($F$ (5,30) = 2.552, $p < 0.05$; Table 2) in the hippocampus were also significantly reversed by GRg2 (10 and 20 mg/kg), respectively. However, NE ($F$ (5,30) = 0.4580, $p > 0.05$, for prefrontal cortex, Table 1; $F$ (5,30) = 0.5222, $p > 0.05$, for hippocampus , Table 2) , AD ($F$ (5,30) = 0.3082, $p > 0.05$, for prefrontal cortex, Table 1; $F$ (5,30) = 0.4471, $p > 0.05$, for hippocampus , Table 2), HVA ($F$ (5,30) = 0.3352, $p > 0.05$, for prefrontal cortex, Table 1; $F$ (5,30) = 0.1354, $p > 0.05$, for hippocampus , Table 2), DA ($F$ (5,30) = 0.6141, $p > 0.05$, for prefrontal cortex, Table 1; $F$ (5,30) = 0.2099, $p > 0.05$, for hippocampus, Table 2), DOPAC ($F$ (5,30) = 0.07659, $p > 0.05$, for prefrontal cortex, Table 1; $F$ (5,30) = 0.4704, $p > 0.05$, for hippocampus, Table 2) in both brain regions were not significantly affected by GRg2. These results indicated that the anti-PTSD-like effects of GRg2 were associated with the normalized levels of 5-HT and 5-HIAA.

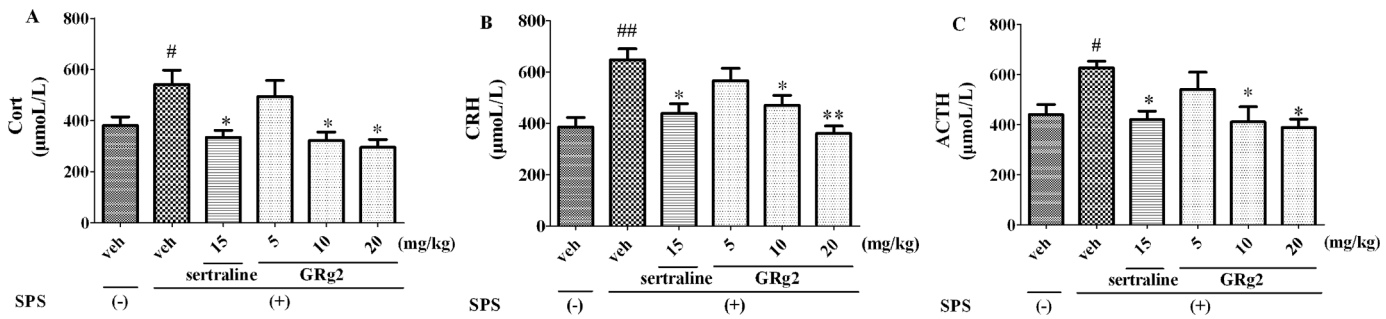


**Fig. 7.** The anti-PTSD-like effects of GRg2 treatments on Cort (A), CRH (B), ACTH (C) in serum. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. vehicle-treated SPS (−) group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle-treated SPS (+) group ($n = 6$).

**Table 1**
The effects of GRg2 on prefrontal cortex monoamine neurotransmitter levels in SPS rats.

| Groups | 5-HT | 5-HIAA | NE | AD | HVA | DA | DOPAC |
|---|---|---|---|---|---|---|---|
| SPS (−) | 310.3 ± 37.24 | 293.3 ± 38.34 | 197.7 ± 14.16 | 153.7 ± 20.57 | 144.8 ± 25.56 | 94.00 ± 9.539 | 159.7 ± 18.59 |
| SPS (+) | 164.7 ± 22.95## | 168.8 ± 24.78# | 222.0 ± 34.88 | 165.0 ± 22.84 | 173.8 ± 31.23 | 108.7 ± 6.791 | 171.5 ± 33.98 |
| sertraline 15 mg/kg | 264.2 ± 28.70* | 267.8 ± 35.09* | 168.8 ± 22.97 | 162.0 ± 22.89 | 138.7 ± 18.91 | 108.7 ± 13.60 | 170.7 ± 34.23 |
| GRg2 5 mg/kg | 197.3 ± 27.82 | 218.0 ± 25.81 | 192.3 ± 20.79 | 186.3 ± 21.84 | 145.7 ± 24.96 | 109.8 ± 13.71 | 177.0 ± 39.89 |
| GRg2 10 mg/kg | 249.0 ± 24.73* | 282.5 ± 32.33* | 206.7 ± 37.61 | 180.0 ± 25.41 | 137.8 ± 18.03 | 109.5 ± 9.902 | 171.7 ± 36.58 |
| GRg2 20 mg/kg | 283.0 ± 45.25* | 300.8 ± 31.78* | 216.8 ± 32.25 | 176.0 ± 18.89 | 148.2 ± 13.86 | 121.7 ± 12.40 | 153.2 ± 25.85 |

# $p < 0.05$,
## $p < 0.01$ vs. vehicle-treated SPS (−) group;
* $p < 0.05$ vs. vehicle-treated SPS (+) group ($n = 6$).

## 4. Discussion

The pharmacological profile and possible involvement of GRg2 in an animal PTSD model were evaluated. Similar to the effects of sertraline, significant suppression of enhanced anxiety and contextual fear in rats was produced by GRg2. Moreover, the anti-PTSD-like effects of GRg2 were associated with biosynthesis of neurosteroids, preventing HPA axis dysfunction and normalization of serotonergic system.

The PTSD behavior is a serious mental problem and has been increased worldwide (Vogt et al., 2017). SPS has been defined as a valid PTSD animal model based on the fact that enhanced negative feedback of HPA axis would be induced by SPS (Kohda et al., 2007). The presents study indicated that a sustained PTSD-associated contextual fear and anxiogenic-like behavioral deficits was induced by SPS, as the elevated freezing duration and decreased exploration into the open arms. One of the explanations was that the acquisition of conditioned fear was induced by SPS (Souza et al., 2017), which were consistent with the PTSD symptoms. In the PTSD study, the rodents were subjected to traumatization which may generated the stress-induced anxiogenic effects (Frewen and Lanius, 2014). Moreover, the locomotor activity was not affected by SPS (Miao et al., 2014; Qiu et al., 2016). The PTSD-associated freezing behavior to the context associated with aversive stress was not generated by affecting the locomotor activity in rodents.

Interestingly, the PTSD-associated aversive behavior was successfully blocked by GRg2 (10 and 20 mg/kg) with the increased exploration in the open arms and the decreased freezing time. The doses ranges of GRg2 were almost confirmed between CFP and EPMT, and were also concordant with its antidepressant-like effects (Ren et al., 2017). In addition, the fear and anxiogenic-like behavior deficits in stressed animals were alleviated by GRg2 without affecting the locomotor activity, which was consistent with the antidepressant-like effects of GRg2 (Ren et al., 2017).

Hyperactivity of the HPA axis is also commonly observed in patients with PTSD (Jovanovic et al., 2010). For instance, altering levels of stress hormones in HPA axis are the possible involvements in the treatments of PTSD (Jin et al., 2016). The HPA axis composes a feedback loop, including the hypothalamus, pituitary and adrenal glands (Andersen et al., 2013). The HPA stress response is regulated by neural mechanisms originally, invoking CRH release from hypothalamic paraventricular nucleus (PVN) neurons (Herman et al., 2016). The arginine vasopressin and CRH are released by the hypothalamus in response to the stressor, and then induces the ACTH secretion from the pituitary, which stimulates the release of Cort from the adrenal cortex (Jin et al., 2016). As observed in our study, the increased levels of CRH, Cort and ACTH in rats were induced by SPS, which was accompanied by conditioning anxiogenic- and fearful- like behavior. The findings were supported by that elevated levels of HPA stress hormones (e.g Cort and ACTH) in serum following the time-dependent sensitization (TDS) (Jin et al., 2016). Bssides PTSD, the increased levels of CRH, Cort and ACTH were also observed in rats with depression (Huang et al., 2015). In line with another PTSD study, elevated levels of CRH, Cort and ACTH in serum were also blocked by SSRIs (e.g sertraline) (Jin et al., 2016). Not only in PTSD study, the antidepressant-like effects of sertraline were associated with the normalized levels of CRH, Cort and ACTH (Qiu et al., 2017), suggesting that levels of these stress hormones could be regulated by sertraline.

It was reported that dysfunction of prefrontal cortex or hippocampus is implicated in the neuropathogenesis of PTSD (van Rooij et al., 2017; Wen et al., 2017). Fear regulation is tightly controlled and this is thought to depend on the prefrontal cortex. The prefrontaL cortex is thought to regulate fear expression and mediates fear suppression (Giustino and Maren, 2015). Moreover, the hippocampus has also been identified as a key mediator of learned fear. Given the role of the hippocampus in encoding contextual and spatial information it is not surprising this region plays a substantial role in the fear circuit. Numerous studies have shown that hippocampal lesions dampen fear to a context previously associated with a shock unconditioned stimulus (Kim and Fanselow, 1992; Selden et al., al.,1991). In fact, the SPS procedure may induce contextual fear and freezing behavior that may represent as the severity of anxiety due to the dysfunction of prefrontal cortex and hippocampus (Qiu et al., 2016). Thus, to evaluate the role of neurosteroids in the anti-PTSD-like effects of GRg2, the levels of endogenous neurosteroids and monoaminergic neurotransmitters were assessed in the above brain regions.

More findings indicate that biosynthesis of neurosteroids (e.g. progesterone and allopregnanolone) has been implicated as one of the involved factors to the development of PTSD (Nin et al., 2011; Zhang et al., 2017). The presents study showed that the anti-PTSD-like effects

**Table 2**
The effects of GRg2 on hippocampal monoamine neurotransmitter levels in SPS rats.

| Groups | 5-HT | 5-HIAA | NE | AD | HVA | DA | DOPAC |
|---|---|---|---|---|---|---|---|
| SPS (−) | 263.7 ± 55.54 | 318.8 ± 41.28 | 199.7 ± 37.04 | 192.0 ± 12.56 | 158.0 ± 24.87 | 183.5 ± 24.15 | 198.0 ± 35.03 |
| SPS (+) | 155.5 ± 20.62## | 178.3 ± 21.14## | 182.0 ± 41.21 | 232.7 ± 32.36 | 172.3 ± 28.44 | 156.8 ± 31.13 | 185.7 ± 35.94 |
| sertraline 15 mg/kg | 262.2 ± 39.79* | 304.5 ± 28.10* | 246.3 ± 32.46 | 183.5 ± 35.65 | 176.5 ± 39.44 | 157.8 ± 17.07 | 176.0 ± 17.88 |
| GRg2 5 mg/kg | 172.5 ± 19.42 | 208.5 ± 41.53 | 178.3 ± 38.62 | 204.0 ± 30.88 | 181.7 ± 22.72 | 186.2 ± 51.67 | 155.2 ± 21.94 |
| GRg2 10 mg/kg | 260.2 ± 30.00* | 286.5 ± 43.51* | 179.2 ± 36.49 | 205.0 ± 29.13 | 170.3 ± 30.85 | 168.3 ± 33.61 | 159.7 ± 17.65 |
| GRg2 20 mg/kg | 287.3 ± 21.49** | 289.8 ± 32.84* | 181.2 ± 34.59 | 182.0 ± 21.62 | 153.0 ± 30.28 | 194.0 ± 36.90 | 191.8 ± 14.95 |

## $p < 0.01$ vs. vehicle-treated SPS (−) group;
* $p < 0.05$,
** $p < 0.01$ vs. vehicle-treated SPS (+) group ($n = 6$).

of sertraline were also involved in the increased levels of progesterone and allopregnanolone in the prefrontal cortex and hippocampus. Other observations support our findings showing that SSRIs reversed the decreased neurosteroid levels (e.g allopregnanolone) in brain and normalized the PTSD-like behavioral deficits in rodents (Pinna and Rasmusson, 2012). Besisdes PTSD researches, SSRIs exerted their antidepressant-like effects via the increased levels of allopregnanolone in rodents without affecting the levels of pregnenolone or progesterone (Pinna et al., 2009). Like sertraline, both reduced neurosteroids biosynthsis in the prefrontal cortex and hippocampus were significantly blocked by GRg2, respectively. The results indicated that the anti-PTSD-like effects of GRg2 were associated with the biosynthesis of progesterone and allopregnanolone in the brain. These effects were similar to selective brain steroidogenic stimulants, which the PTSD-associated contextual fear response was normalized by the elevated levels of allopregnanolone (Pinna, 2014; Rasmusson et al., 2017). Allopregnanolone actives the neurosteroid recognition sites at the GABAA receptors, such as α4, α5, α1/α2 and γ2, to exert the anxiolytic-, anti-PTSD-, and/or anticonvulsant- like effects (Puia et al., 2015; Rasmusson et al., 2017).

The monoaminergic system closely interacts in the central nervous system (CNS) (particularly in the prefrontal cortex and hippocampus) in mammals and are involved in stress-related disorders (e.g PTSD) (Ebenezer et al., 2016; Récamier-Carballo et al., 2017). Based on these findings, the role of monoamines in the anti-PTSD-like effects of GRg2 was also assessed. The levels of 5-HT and 5-HIAA in the prefrontal cortex and hippocampus were significantly decreased. The findings were supported by the reduction in levels of 5-HT and 5-HIAA after exposure to SPS in rodents. The decreased levels of 5-HT have been associated with impulsivity, aggression, fear, and sadness/depression (Wang et al., 2016; Wu et al., 2016). In addition, the improvement of symptoms following treatment with SSRIs supports the role of serotonergic dysfunction in PTSD (Ariel et al., 2017). The anti-PTSD-like effects of SSRIs may be mediated by improvement of serotonergic function and modulation of anxiety, anger, mood, and impulsivity (Echiverri-Cohen et al., 2016). In the present study, decreased levels of 5-HT and 5-HIAA in SPS rats were reversed by sertraline (15 mg/kg.). Inhibition of 5-HT uptake by sertraline in higher extracellular levels of 5-HT and this is regarded as the basis of its activity, although the exact antidepressant mechanism has yet to be elucidated (Sanchez et al., 2014). Like sertraline (15 mg/kg.), the decreased levels of 5-HT and 5-HIAA were also significantly antagonized by GRg2 (10 and 20 mg/kg), respectively. The present study also confirmed with the possibility that the 5-HT-induced inward was blocked by pretreatment of GRg2 (Choi et al., 2003). Taking together, the serotonergic action of GRg2 might be similar to the effects of SSRIs. Further evaluation on the presumptive adaptive changes, such as 5-HT receptors in prefrontal cortex and hippocampus, is still needed to elucidate the precise role of 5-HT in the anti-PTSD-like effects of GRg2.

Generally, our findings indicate that the anti-PTSD-like effects of GRg2 were accompanied by (or associated with) modulation of neurosteroids biosynthesis, HPA axis and serotonergic activation. Further researches are needed to clarify the exact molecular mechanisms underlying the anti-PTSD-like effects of GRg2 and to better understand the neuropathological changes in PTSD.

## Authors' contributions

All authors participated in the preparation of the manuscript, and read and approved the final manuscript.

## Statement of interest

The authors have declared that no competing interests exist.

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