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A Chinese herbal medicine production of anxiolytic and cognitive enhancing effects in a rat model of posttraumatic stress disorder

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Free and Easy Wanderer Plus (FEWP) is a traditional Chinese medicine that has been used to treat stress- and depression-related diseases in China. To study the effects of FEWP on post-traumatic stress disorder (PTSD), the present study investigated how chronic treatment with FEWP affected rodent behaviors in a modified single-prolonged stress and shock (SPS and S) model, which induces certain behavioral manifestations similar to those seen in PTSD patients. Locomotor activities (LMA), elevated Plus Maze (EPM), and Morris Water Maze (MWM) tests were used to determine its effects on general locomotion, anxiety and cognitive functions following traumatic exposure. The results showed that SPS impaired performance in MWM and produced anxiogenic-like effects in EPM, both of which were significantly ameliorated by chronic treatment of FEWP at doses of 5 and 10 mg/kg/d. The effects of FEWP were not due to changes in locomotion. The current findings provide strong evidence that FEWP may be a useful drug for treating PTSD.

Key words: Single prolonged stress and shock, post-traumatic stress disorder, Morris water maze, open field, elevated plus maze.

INTRODUCTION

PTSD is a severe, recurring and debilitating anxiety disorder that develops after exposure to a traumatic event. A variety of both pharmacological and psychological interventions for PTSD have been developed. For example, two types of commonly used antidepressants, the selective serotonin re-uptake inhibitors (SSRIs) and serotonin norepinephrine re-uptake inhibitors (SNRIs) are used as the first-line pharmacotherapeutic treatments for PTSD. These available pharmacological agents generally produce meaningful therapeutic results and have the advantage of treating depression and other co-morbid disorders, but their actions are far from ideal due to limited responses, high remission rates, and tolerability issues (Zhang and Davidson, 2007). It is reported that less than one third of the patients are responsive to these drugs (Gelenberg and Hopkins, 2007). In addition, a large number of individuals cannot tolerate the side effects of SSRIs (Berger et al., 2007). Furthermore, it is well established that PTSD can induce cognitive defects (Bremner, 2006; Falconer et al., 2008; Leskin and White, 2007; Morgan et al., 2006; Rauch et al., 2009). Due to current limitations of antidepressants and the desire to treat cognitive deficiencies in those with PTSD, it is imperative to develop novel PTSD pharmacotherapies combining key features of antidepressants and cognitive enhancers. Because of aforementioned issues, a large proportion of Chinese PTSD patients have sought alternative therapy, especially traditional Chinese medicine.

FEWP, known as Shu-gan-jie-yu in China is a well-known herbal medicine that has long been used to alleviate mood symptoms. FEWP has a remarkably wide range of beneficial properties (Kuang et al., 2008; Li et al.,

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2008), particularly for treating mood swings, irritability, premenstrual tension and menopausal syndromes. A recent placebo-controlled clinical study has demonstrated adjunctive use FEWP with carbamazepine has additive beneficial effects in bipolar patients, especially during the depressive phase (Zhang et al., 2007). FEWP monotherapy has also been considered an effective alternative treatment for depression (Mao et al., 2010). Based on its successful application in treating mood disorders, we hypothesized that FEWP might also be an effective treatment for PTSD. The single prolonged stress (SPS) model is widely used to mimic some core symptoms observed in PTSD patients (Iwamoto et al., 2007; Liebsch et al., 1998; Takahashi et al., 2006; Wang et al., 2008). SPS can impair spatial learning and memory and produce anxiogenic effects. In the current study, we assessed the effects of chronically administration of FEWP in a modified SPS paradigm (SPS and shock, SPS and S) using LMA, EPM and MWM tests. We aimed to establish a dose response curve of FEWP (2.5, 5 and 10 mg/kg/d) for this PTSD model and the doses selected were based on clinical observations and our preliminary experiments. The behavioral indices included (I) general locomotor activity assessed by OF; (II) anxiogenic-like behaviors assessed by EPM; (III) learning and memory assessed by MWM.

MATERIALS AND METHODS

Subjects

All the experiments were conducted in accordance with Guidelines for the Care and Use of Animals in Neuroscience and Behavioral Research and approved by the Committee of Animal Care and Use for Research of the Fourth Military Medical University. A total of 48 adult male Sprague-Dawley rats (8-week old) were used in the present study. The rats were group-housed and maintained with a 12 h light/dark cycle (lights on at 06:00 a.m) with ad libitum food and water. To reduce the confounding effect of corticosterone fluctuation of with the circadian rhythm, we carried out the experiments over a fixed period of time. After 10 days of acclimatization to the colony, the rats were randomly divided into four groups: the Sham + Veh group receiving sham treatment (no SPS and S) followed by vehicle administration; the SPS and S + Veh group receiving SPS and S treatment followed by vehicle administration; the SPS and S + FEWP group receiving SPS and S treatment followed by chronic FEWP administration; and the SPS and S + Paroxetine group as a control with effective drugs for PTSD, Paroxetine (GlaxoSmithKline K.K., Suzhou, China) was dissolved in water and kept in light roof drinking bottles, administered in a dose of 20 mg/(kg/day). The SPS and S + FEWP group was divided into three sub-groups with different doses (2.5, 5 and 10 mg/kg/d).

Single prolonged stress and shock (SPS and S)

The SPS and S treatment was adopted from previous reports (Wang et al., 2008), briefly stated that rats were first restrained for 2 h. Each rat was immobilized by placing them inside a disposable clear polyethylene rodent restraint cone with only the tail protruding out. The wide end of the cone was sealed with tape at the base of the tail. The size of the bag was adjusted according to the size of the rat in order to achieve complete immobilization. A hole in the small end of the cone allowed the rats to breathe freely. The immobilization was immediately followed by a 20 min forced swim in 24°C water. A clear acrylic cylinder (24 cm in diameter, 50 cm in height) was used for the forced swim. The cylinder was filled to two-thirds of its volume with water. After recuperating for 15 min, the rats were exposed to diethyl ether until loss of consciousness. The rats were then maintained in a cubic shock chamber until recovery (about 30 min), and subsequently were treated with an electric foot shock (1 mA for 4 s) via the metal grid.

Administration of FEWP

FEWP was provided by the Institute of Pharmacology at the Fourth Military Medical University. The formulation of FEWP was described in detail by a previous study (Iwamoto et al., 2007). Briefly, it comprised of the medicinal herbs of Bupleurum chinense DC. (12.5%), Scutellaria baicalensis Georgii (12.5%), Zingiber officinale Rosc. (11.2%), Angelica sinensis (Oliv.) Diels. (9.7%), Gardenia jasminoides Ellis. (9.7%), Paonia suffruticosa Andr. (9.7%), Paonia lactiflora Pall. (9.7%), Atractylodes macrocephala Koidz. (8.3%), Poriae cocos (Schw.) Wolf (6.9%), Mentha haplocalyx Briq. (5.6%) and Glycyrrhiza uralensis Fisch. (4.2%). The herbal powder prepared was directly dissolved into drinking water contained in a lightproof, scaled bottle, at a dose of 2.5, 5, or 10 mg in crude herbal weight/kg per day for 14 days following SPS and S procedures. The doses were based on our pilot study (data not shown) and were adjusted according to the group means of weight and water intake in the study. Although we were fully aware that the drinking delivery approach could not accurately reflect the real dose of FEWP the animals received, intraperitoneal (i.p.) injection or intragastric administration was not used because of the extra stress to the rats.

Behavioral tests

Locomotor activities (LMA)

On the fifteenth day after SPS and S, the rat was placed at the center of a cubic chamber (470 × 470 × 470 mm) and allowed to freely move around. The animal’s locomotor activities (horizontal distance) were recorded for 15 min. All animals were habituated to the testing room for 20 min before the start of the session. The test room was dimly illuminated with indirect white lighting. The chamber was cleaned with 70% ethanol before a new session started.

Elevated Plus Maze test (EPM)

On the same day of the LMA (1 h after LMA), the EPM was conducted to measure the anxiety levels. The paradigm used in the present study was adopted from well-established protocols (Katagiri et al., 2004).

Briefly, the Plexiglas apparatus consisted of a plus-shaped platform elevated 50 cm from the floor. Two of the opposing arms (50 × 10 cm) were enclosed by 40-cm-high side and end walls (closed arms), whereas the other two arms had no walls (open arms). At the beginning of the test, the rat was placed in the central area (10 × 10 cm) of the maze facing a closed arm. Standard parameters were recorded by a video camera during the 5 min exposure and scored offline for: (1) percentage of open-arm entries to the total number of entries into all arms and (2) percentage of time spent in open arms to total time spent in all arms.
Table 1. The results of LMA and EPM.

<table>
<thead>
<tr>
<th></th>
<th>Horizontal distance (mm)</th>
<th>Percentage of open armes (%)</th>
<th>Time spent in open armes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham+Veh</td>
<td>10129.00 ± 873.20</td>
<td>47.63 ± 3.28**</td>
<td>21.86 ± 2.77*</td>
</tr>
<tr>
<td>SPS and S+Veh</td>
<td>10176.67 ± 1310.36</td>
<td>22.72 ± 2.71</td>
<td>14.33 ± 2.28</td>
</tr>
<tr>
<td>FEWP1</td>
<td>9646.00 ± 1322.11</td>
<td>46.86 ± 3.04**</td>
<td>16.16 ± 1.85</td>
</tr>
<tr>
<td>FEWP2</td>
<td>8375.12 ± 1262.67</td>
<td>46.86 ± 3.34**</td>
<td>18.59 ± 1.61*</td>
</tr>
<tr>
<td>FEWP3</td>
<td>9457.13 ± 972.63</td>
<td>40.22 ± 2.96**</td>
<td>16.67 ± 2.01</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>9756.25 ± 1008.58</td>
<td>47.05 ± 4.56**</td>
<td>20.22 ± 3.15*</td>
</tr>
</tbody>
</table>

Data represent means ± SEM. FEWP 1, 2 and 3 represent doses of 2.5, 5 and 10 mg/kg/d. Paroxetine administered in a dose of 20 mg/(kg/day). *p<0.05, ** p<0.01 compared to SPS and S+Veh.

**Morris water maze (MWM)**

Spatial learning and memory performance were measured by MWM according to the classical Morris protocol. All animals (except for SPS and S + Paroxetine group) were subjected to MWM for six consecutive days after EPM. The apparatus consisted of a black colored pool, 160 cm in diameter and 55 cm in height, constructed from a sturdy plastic material. The pool was housed in a temperature-controlled room, which maintained water temperature at 20 to 23°C. The pool was filled with approximately 23 cm of water made dark with black ink and divided into four quadrants, labeled North (N) –South (S) –East (E) –West (W). A cylindrical dark olive-green colored platform, 21 cm in height and 10 cm in diameter, was placed in one of the quadrants (the target quadrant). The platform was submerged approximately 2 cm below the surface of the water during the spatial learning trials. The apparatus was surrounded by 3 pre-set extra-maze cues on the wall. A digital video camera was positioned directly above the pool enabling full collection of swimming activity in the different quadrants and connected to a computer with the Dig Behave System (Jiliang Software, China). The latency and the trace of each animal’s swimming path were automatically obtained for each trial.

Spatial learning was evaluated by a navigation test (without cue) initiated on the sixteenth day after SPS and S (between 8:00 and 14:00) and lasted for a consecutive of five days. Video recording began with the animal placed in a quadrant (not containing the platform) with its head facing the wall. The animal was allowed to swim for 60 s. If it failed to locate the escape platform within 60 s, it was gently assisted onto the platform and allowed to remain there for an additional 20 s. The animals received four learning trials each day, utilizing a randomized entering point and an intra-trial interval (ITI) of 20 s, which began by the time the animal reached the platform. The time it took for the rat to find the platform was recorded as escape latency. Spatial learning ability was evaluated based on averaged escape latencies on each day. On the sixth day of the MWM test, spatial memory was evaluated using the probe test. For a 60-s probe trial, the platform was removed and the rat was initially placed at a novel position that bisected two of the cardinal start positions. The ratio of time spent in the target quadrant (without platform) was recorded as an index of spatial memory.

**Statistical analyses**

All results were expressed as mean ± S.E.M. A P value less than or equal to 0.05 was considered statistically significant. All data were analyzed by analysis of variance (ANOVA). The LMA and EPM data were analyzed using a one-way ANOVA. The MWM data were analyzed by a two-way ANOVA (Groups × Days) with repeated measures on Trials and Days. One-way ANOVA was used to evaluate the spatial memory data obtained via the probe test. As a measure of procedural impairment, swimming speed was analyzed in the experiment by one-way ANOVA. The Fisher’s least significant difference (LSD) post hoc test was used to make group-wise comparisons.

**RESULTS**

**Locomotor activities**

One-way ANOVA showed no significant effect of SPS and S and FEWP administration on horizontal distance, and no interaction among six groups (Table 1), indicating that neither SPS and S nor chronic FEWP intervention significantly affected spontaneous locomotor activity.

**Anxiogenic-like behaviors**

Differences among % open arm entries were significant among the six groups [F (5, 43) = 15.64, P < 0.01]. The LSD post hoc test showed that % open arm entries for the SPS and S + Veh group was significantly lower than that in the non-stressed group (P < 0.01). On the other hand, three FEWP treatment groups were significantly increased compared to the stressed + vehicle group, and were not different from the sham group and SPS and S + Paroxetine group [F (5, 43) = 13.61] (Table 1). As for the percentage of time spent in the open arms, one-way ANOVA also showed significant effects of SPS and S [F (5, 43) = 14.25], FEWP [F (5, 43) = 15.66] and the interaction between SPS and S and FEWP [F (5, 43) = 12.09]. Post hoc analysis revealed that the percentages of open arm time in the Sham+Veh, SPS and S + Paroxetine group and FEWP groups were higher than that obtained for the SPS and S+Veh group (P < 0.05) (Table 1).

**Spatial learning and memory**

Two-way ANOVA indicated that group, day and trial odd
significant effect on the escape latency (Figure 1A, $F (4, 36) = 14.01$) and the pathlength to the platform, $F (4, 36) = 15.31$). The designed post hoc test showed that the escape latency of the SPS and S+Veh group increased significantly ($P < 0.01$, versus Sham+Veh), while all three administrations of FEWP reversed this effect ($P < 0.01$) on day 2 and 3. There were no significant differences among the FEWP1, FEWP2, FEWP3 and Sham+Veh groups (Figure 1A). The swimming speed during the test was also evaluated and analyzed by one-way ANOVA. Neither the group nor the trial had effects on swimming speed [group*trial, $F (4, 36) = 8.01$]. Thus, we concluded that the increased escape latency was due to impairment of spatial acquisition ability and FEWP can reverse such effect. The present results also showed that SPS and S significantly impaired spatial memory (Figure 1B). One-way ANOVA was used to analyze the percentage of time spent in the target quadrant. The Fisher’s LSD post hoc test showed that the two higher dosages of FEWP treatment markedly ameliorated SPS and S-induced decrease in the time spent in the target quadrant ($P < 0.05$, FEWP2 versus SPS and S + Veh; $P < 0.01$, FEWP3 versus SPS and S + Veh) (Figure 1B).

**DISCUSSION**

Rodent behavioral model studies play an important role in the evaluation of novel drugs used for the treatment of mental disorders. SPS and S is a behavioral model that can induce anxiogenic-like behaviors and spatial learning and memory impairments, which mimics the anxiety and cognitive disorders observed in patients with PTSD. To our knowledge, the current study is the first to demonstrate that FEWP can produce significant anxiolytic effects and attenuate cognitive impairment in this animal model. Thus, the present findings highlight the potential clinical application of the Chinese herb FEWP in treating PTSD patients. Our results demonstrate that FEWP significantly affects the emotional responses to stressful external stimuli, which reflect anxiety levels in rats. In the EPM test, chronic administrations of FEWP (2.5, 5 and 10 mg/kg/d) rescued SPS and S-induced decrease of open arm entries and time spent in the open arm, indicating FEWP might be effective for the treatment of the abnormal emotional responses after a traumatic event. In previous studies, FEWP displays antidepressant-like actions (Li et al., 2008). The present study showed that FEWP did not affect locomotor activities. Therefore, both the anxiogenic and antidepressant like effects of FEWP are not due to its effects on general locomotion but rather its reactivity to stress.

The current results also demonstrated that the two higher doses of FEWP (5 and 10 mg/kg/d) could prevent the SPS and S-induced impairment of spatial learning and memory. In the MWM test, the curve of escape latency reflects the learning process and the time spent in the target quadrant in the probe test reflects memory ability. Our data showed that FEWP shortened the escape latency and increased the time spent in the target quadrant in SPS and S-treated animals. Learning and memory are commonly used indices of cognitive function. Stress-induced cognitive deficits may constitute part of PTSD symptoms or the secondary clinical manifestation of these symptoms. Therefore, preventing the onset and/or progression of cognitive deficits is critical to PTSD.
treatment. The present preclinical study provides strong evidence to support a cognitive enhancing role of FEWP in treating PTSD patients. Previous studies reveal that adjunctive administration of FEWP with CBZ results in markedly better clinical outcomes and response rates in terms of depression measures in bipolar patients when compared to the CBZ monotherapy and placebo treatment. Additionally, adjunctive use of FEWP improves the tolerability to long-term CBZ treatment. It has also been suggested that FEWP monotherapy may be an effective alternative treatment for depressed patients.

The data obtained in the present study show that FEWP may also be considered as a medication for anxiety disorders. FEWP is actually a mixture of several Chinese herbs, so its effect may stem from its individual components. *Bupleurum chinense DC*, one of the major components of FEWP, has hepatoprotective effect and adjuvant potentials on the immune responses to stress (Cheng et al., 2004; Chin et al., 1996; Sun, 2006; Yen et al., 2005). The relationship between the immune system and psychiatric disorders, especially anxiety and depression, has been well documented. Animals and humans exposed to environmental stressors usually have abnormal activation of the immune systems, manifested by increased expression of cytokines (Herbert and Cohen, 1993; Kook et al., 1995; Stein et al., 1991). *Scutellaria baicalensis* Georgi, another component of FEWP, has been used to treat cerebral ischemia in addition to bacterial infection and inflammatory diseases. Neuronal cells immunoreactive to the choline acetyltransferase (ChAT), a marker for cholinergic neurons, are significantly increased in the hippocampus after *S. baicalensis* administration. Furthermore, *S. baicalensis* extracts enhance the survival of the hippocampal progenitor cell line, HiB5 and its differentiation to ChAT immunoreactive neurons.

Finally, it increased levels of neurotransmitters in the hippocampus and the protective effect against neurotoxicity is also observed with the treatment of *S. baicalensis* extracts (Cheng et al., 2008; Heo et al., 2009). Li et al. (2003) assessed some other traditional Chinese medicine for mental disorders and found Zingiber might be useful for some stress disorders. Topic et al. (2002) assessed the effects of Zingiber officinalis (ZC), a mixture of *Zingiber officinale* and *Ginkgo biloba* extracts, on learning and memory as well as on markers of oxidative stress in aged rats. ZC can improve inhibitory avoidance learning and produce anxiolytic effects in adult animals. Chronic administration of ZC can also facilitate spatial learning in aged animals and reduce the expression of oxidative stress markers in brain tissue. Katagiri et al. (2004) examined the effects of some Chinese medicines, including *Z. officinale*, on the plasma levels of adrenocorticotropic hormone (ACTH) and cortisol under stress conditions by repeated blood sampling.

The results showed that these medicines modulate the function of the hypothalamo-pituitary-adrenal (HPA) axis and autonomic nervous system. *Angelica sinensis* has been used to treat cardiovascular and cerebrovascular diseases in traditional Chinese medicine for thousands of years. Modern phytochemical studies showed that *Z-ligustilide* (Z-LIG) is the main lipophilic component of *A. sinensis*. Pretreatment of the PC12 cells with Z-LIG significantly attenuated H2O2-induced cell death and decreased Bax expression (Crispo et al., 2010). Furthermore, Z-LIG improved cellular tricarboxylic acid cycle and concentration-dependently up-regulated Bcl-2 expression (Zhou et al., 2011). These findings suggest that *A. sinensis* may be useful in the treatment of some stress disorders in which oxidative stress and apoptosis are main pathological mechanisms (Kuang et al., 2006; Yang et al., 2007; Yu et al., 2008). It has been demonstrated that *Paeonol* significantly improves the learning and memory ability in MWM test and step-down passive avoidance test in d-galactose (D-gal)-treated mice (Lu et al., 2010). Further investigation showed that the effect of *Paeonol* on the improvement of cognitive deficit was related to its ability to increase acetylcholine (Ach) and glutathione (GSH) levels, restore superoxide dismutase (SOD), and Na+, K+-adenosinetriphosphatase activities and decrease cholinesterase Ache activity and malondialdehyde (MDA) levels in D-gal-treated mice. Finally, *Paeonol* ameliorated neuronal damage in both the hippocampus and temporal cortex in these mice (Zhong et al., 2009).

In conclusion, the different components of FEWP may play different roles in its therapeutic action. FEWP overall can produce robust anxiolytic and cognitive enhancing effects in the SPS and S animal model of PTSD. There are certain limitations of the current study. Tests to assess fear responses to traumatic cues were not performed. In addition, other responses to the conditioning stimuli were not evaluated. Further studies are needed to elucidate the mechanisms of PTSD-like behavioral alterations in the SPS and S model and how each individual component of FEWP contributes to its overall behavioral actions.

REFERENCES


