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ORIGINAL ARTICLE

Paeoniflorin Prevents Depression Like Behavior in Rats by Suppressing Mitophagy Mediated NOD like Receptor Protein 3 Inflammasome Signaling

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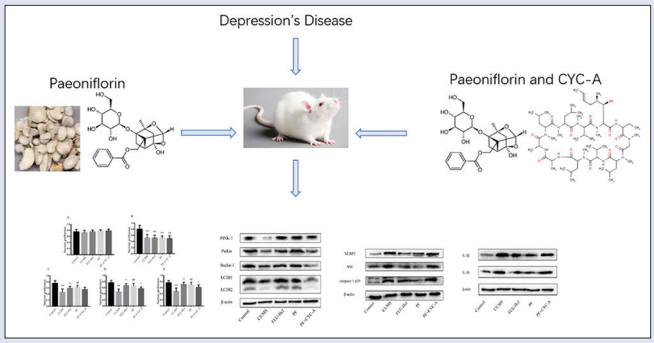
ABSTRACT

Background: The pathogenesis of depression is related to the NOD-like receptor protein 3 (NLRP3) inflammasome activation and low level of mitophagy. In traditional Chinese medicine, *Paeonia lactiflora* Pall is a common herb as a possible treatment for depression. As a main and active constituent of *P. lactiflora* Pall, paeoniflorin (PF)'s mechanisms of antidepression effects are not evidently unstated. Therefore, this study intended to explore whether PF can prevent depression-like behavior by conquering NLRP3 inflammasome activation and whether PF prevents NLRP3 inflammasome activation via upregulating mitophagy. **Materials and Methods:** Ten of a total of 50 rats were selected randomly as the control group. After establishment of the chronic unpredictable mild stress (CUMS) model, CUMS rats were randomly divided into four groups: CUMS group, fluoxetine hydrochloride group, PF group, and PF + cyclosporine A group. After 3 weeks of drug involvement, behavioral tests were measured. The protein expressions of PINK-1, Parkin, Beclin-1, LC3B, NLRP3, apoptosis-associated speck-like protein containing CARD (ASC), caspase-1 p20, interleukin-1β (IL-1β), and IL-18 were spotted with Western Blot method. **Results:** PF upturned stress persuaded behavioral changes and PF treatment augmented sucrose consumption rates ($P < 0.01$) in sucrose preference test and reduced the immobility time ($P < 0.01$) in forced swimming test of CUMS rats. PF also improved the levels of mitophagy-related proteins PINK-1, Parkin, Beclin-1, and LC3B ($P < 0.01$) in the hippocampus. Moreover, PF decreased the levels of NLRP3 inflammasome-related proteins (NLRP3, ASC, caspase-1 p20 antibody, IL-1β, and IL-18 [$P < 0.01$]) tempted by stress. **Conclusion:** PF advances depression in CUMS rats, reduced the inflammatory injury in the hippocampus of CUMS rats. Hence, based on those facts, NLRP3 inflammasome activation is accomplished by inhibiting its effect on mitophagy.

Key words: Antidepressant-like activity, depression, mitophagy, NOD-like receptor protein 3 inflammasome, paeoniflorin

SUMMARY

- Paeoniflorin employs antidepressant-like effects in chronic unpredictable mild stress rats and thus expands rats' behaviors
- Paeoniflorin not only improves the activity of mitophagy but also prevents the NOD-like receptor protein 3 inflammasome activation in the hippocampus of chronic unpredictable mild stress rats. Thus, paeoniflorin hinders NOD-like receptor protein 3 inflammasome activation in depression context by modifiable mitophagy.



Abbreviations used: ASC: Apoptosis-associated speck-like protein containing CARD; CUMS: Chronic unpredictable mild stress; CYC-A: Cyclosporine A; FLU-HCl: Fluoxetine hydrochloride; FST: Forced swimming test; IL-1β: Interleukin-1β; NLRP3: NOD-like receptor protein 3; PF: Paeoniflorin; SPT: Sucrose preference test.

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INTRODUCTION

Depression represents a wide range of mental illnesses with multifactorial psychopathologies. It is one of the most common mental ailments and a major public health problem in the modern society.[1] Although many treatments are available for depression, more than 30% of patients with depression do not effect reasonable recovery.[2] Therefore, the development of safer and effective drugs for this illness is highly obligatory.

In a recent report, it has been familiar that depression is strongly associated with inflammation.[3] NOD-like receptor protein 3 (NLRP3) inflammasome activation activates inflammation and depression.[4] It has been specified that the components of NLRP3 inflammasome in the serum of depressed rats were augmented. Application of NLRP3 inflammasome inhibitor reduced Interleukin-1β (IL-1β) levels in the serum and hippocampus tissues and lessened the depression-associated behaviors in rats.[5,6] NLRP3 gene knockout mice did not demonstrate depressive behavior under chronic stress.[7] As a downstream effector

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of NLRP3 inflammasome, IL-1 β can precisely imitate the activation level of NLRP3 inflammasome. IL-1 β composes the first step of pro-inflammatory response to psychological stress and when unduly produced, it causes cell injury in stress-related diseases comprising depression.[8] It was found that patients with severe depression and suicidal tendencies have severe neuroinflammatory responses, IL-1 β is augmented in their frontal cortex.[9] Therefore, the NLRP3 inflammasome may be a key mark to the treatment of depression.

Mitophagy is a kind of autophagy in which damaged mitochondria are familiar by the autophagosomes to the lysosomes for degradation.[10] Mitophagy destroys damaged mitochondria and inhibits NLRP3 inflammasome activation via mitochondrial reactive oxygen species (mtROS) and mitochondrial DNA (mtDNA). In this way, mitophagy defends cells against damage.[11,12] There is an indication that the mitophagy level is reduced in the hippocampus of depressed rats and some antidepression drugs could improve mitophagy expressively.[13,14]

In traditional Chinese medicine, *Paeonia lactiflora* Pall has the function of "shuganjieyu." Paeoniflorin (PF), a monoterpene glycoside compound (the main active component of *P. lactiflora* Pall), is frequently used to treat depressive-like disorder.[15] For instance, PF eased cell injury caused by depression-related neurotoxicity.[16] An earlier study displayed that PF reduced the serum level of inflammatory mediators such as IL-1 β, tumor necrosis factor-α, and IL-6 and decreased the expression of neuroinflammatory factors in the prefrontal cortex, ventral hippocampus, and amygdala.[17] A study on menopause-induced depression showed that application of PF at a dose of 10 mg/kg diminished the stress hormone levels.[18] The anti-inflammatory effect of PF in the nervous system has also been detected in many models of neuroinflammation.[17,19] However, whether PF moves the NLRP3 inflammasome and mitophagy remains indescribable.

In this study, chronic unpredictable mild stress (CUMS) rats were employed as a depression model. PF was used as an intervention for CUMS rats. Fluoxetine hydrochloride (FLU-HCl) was used as a positive control drug for CUMS rats. Cyclosporine A (CYC-A), a mitophagy inhibitor, was employed combined with PF. Levels of the proteins (PINK-1, Parkin, Beclin-1, and LC3B) related to mitophagy and proteins (NLRP3, apoptosis-associated speck-like protein containing CARD [ASC], caspase-1 p20, IL-1 β, and IL-18) related to NLRP3 inflammasome in the hippocampus were distinguished.

MATERIALS AND METHODS

Reagents and antibodies

PF (CAS No. 23180-57-6, purity was higher than 98%) was purchased from Chenguang Biotechnology Co. Ltd., Baoji, China. FLU-HCl (CAS No. 56296-78-7) was purchased from Sigma-Aldrich Co. Ltd., USA. CYC-A (CAS No. z130495) was procured from National Institutes for Food and Drug Control (Beijing, China). BCA Protein Assay Kit (Cat # PC 0020) was obtained from Solarbio Science and Technology Co. Ltd., Beijing, China. β-actin antibody was bought from Bioss Biotechnology Co. Ltd., Beijing, China. Anti-TMS1 (ASC, ab175449), anti-caspase-1 (ab1872), anti-NLRP3 (ab214185), anti-IL-18 (ab191860), anti-IL-1 β (ab9722), anti-PINK-1 (ab23707), anti-Parkin (ab77924), anti-LC3B1/2 (ab48934), anti-SQSTM1/p62 (ab91526), and anti-Beclin-1 (ab62557) antibodies were acquired from Abcam Biotechnology Co. Ltd., Cambridge, UK.

Model and treatment

Animals

Seventy male SD rats (180 ± 20 g) were provided by the Animal Centre of Xi'an Jiaotong University (P. R. China, Certificate No. SCXK [Shan] 2017-003). Animals were accommodated at the conservative dwelling unit under standard conditions (a controlled room temperature [25°C ± 2°C], relative humidity [65% ± 10%], and 12 h light/dark cycle). This research was permitted by the Animal Ethics Committee of Shaanxi University of Traditional Chinese Medicine. The experimental design is revealed in Figure 1.

Chronic unpredictable mild stress model

After 5 days of adaptation, the rats were given sucrose drinking training and sucrose preference rate baseline test. In the first 24 h, the rats were given two bottles of 1% sucrose solution (500 mL). In the second 24 h, the rats were given 1 bottle of 1% sucrose solution (500 mL) and 1 bottle of water (500 mL). Additional, baseline assessment of sucrose preference (SP) of rats was verified. After water prohibition for 24 h, the rats were given 1% sucrose solution and 1 bottle of water. After calculating the SP rate, the rats with low sucrose intake (SP < 0.5, SP = weight of sucrose solution intake/weight of total intake) were detached to prevent low-level sweetness in rats from distressing the results. Each rat was exposed to 1 stressor per day for 3 weeks except those in the control group (n = 10). The CUMS modeling method was achieved as described earlier.[20] A series of stressors in the CUMS group comprised swimming in cold water (4°C) for 5 min, cage tilting at a 45° angle (12 h), overnight lighting for 36 h, wet pad for 15 h (per 100 g sawdust with 200 mL water), food deprivation for 24 h, clamping tail for 5 min (tail of rats were clamped gently for 5 times, 50s each time), water deprivation for 24 h, crowding for 3 h (every eight rats were located in a small cage with a size of 25 cm × 15 cm × 13 cm), cage shaking for 5 min (each cage was shaken to the extent that rats could not stand), and vinegar stimulation for 24 h (20 mL vinegar was scattered in each cage). All stressors were pragmatic randomly and continuously at any time of the day and were given 1 or 2 times within the CUMS procedure. SP and weight were planned after CUMS treatment again. When the rats in the control and model group had noteworthy differences in SP, the CUMS model was measured effective.

Grouping and treatment

After the CUMS model was recognized successfully, the SP of rats was tested again and sucrose predilections of each rat before and after modeling were likened. Rats which botched during the modeling steps were detached from the experiment. Successfully stressed rats with reliable weight and SP were aimlessly divided into four groups: CUMS group (n = 10), FLU-HCl group (n = 10), PF group (n = 10), and PF + CYC-A group (n = 10). Rats in the control group, CUMS group, FLU-HCl group, and PF group received intraperitoneal (i. p.) injection of 0.9% sodium chloride (10 mL/kg) every day. Rats in PF + CYC-A group received i. p. injection of CYC-A (10 mg/kg) every day. Rats in the control

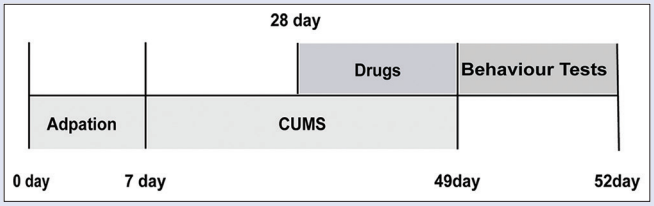

Figure 1: The experimental design. The chronic unpredictable mild stress protocol lasted for 6 weeks, during which rats were stimulated by a variety of mild stressors. After chronic unpredictable mild stress procedure for 3 weeks, drugs for rats in each group were administered. Sucrose preference test and forced swimming test were implemented at the final stage of animal experiments

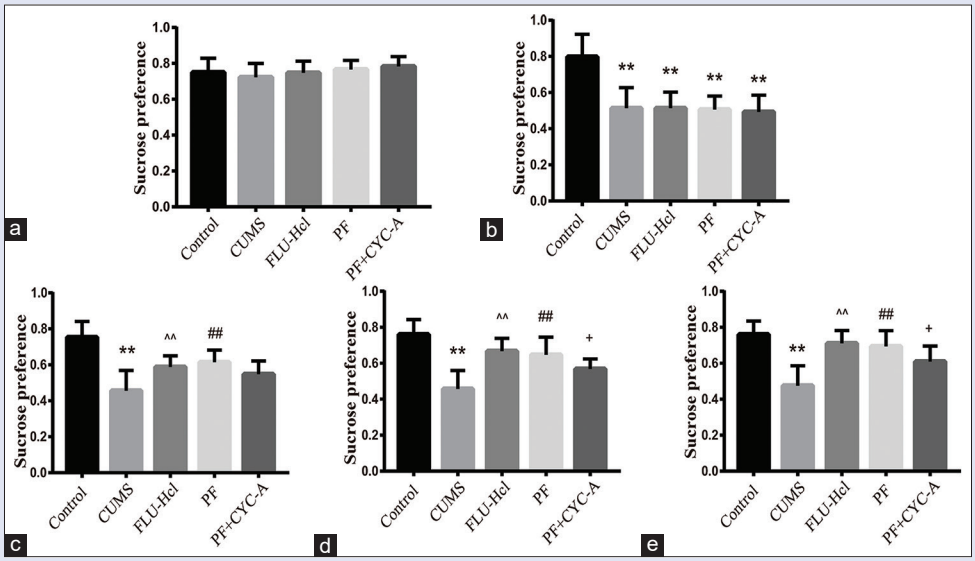

Figure 2: Sucrose preference rate. Before modeling, there was no difference in sucrose preference test among all groups (a). After 3-week chronic unpredictable mild stress procedure (b), $^{**}P < 0.01$ indicates comparison with control group. After 1-week treatments (c). $^{**}P < 0.01$ indicates comparison with control group, $^{\wedge\wedge}P < 0.01$ indicates comparison with the chronic unpredictable mild stress group, $^{\#\#}P < 0.01$ indicates comparison with chronic unpredictable mild stress group. After 2- and 3-week treatments (d and e). $^{**}P < 0.01$ indicates comparison with control group, $^{\wedge\wedge}P < 0.01$ and $^{\#\#}P < 0.01$ indicate comparison with chronic unpredictable mild stress group, $^{+}P < 0.05$ indicates comparison with PF group

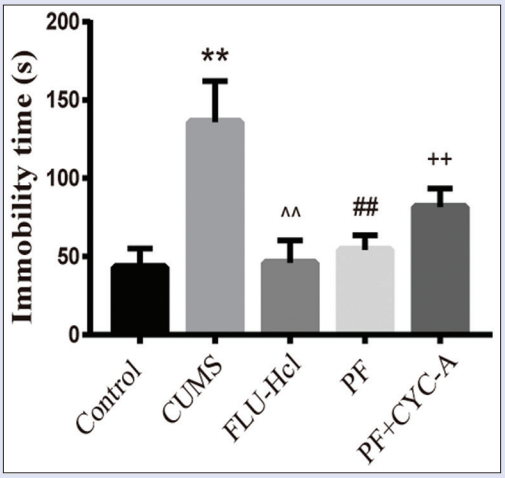

Figure 3: Immobility time of forced swimming test. After 3 weeks of treatments, the immobility time of rats during forced swimming was tested. $^{**}P < 0.01$ indicates comparison with the control group, $^{\wedge\wedge}P < 0.01$ and $^{\#\#}P < 0.01$ indicate comparison with the chronic unpredictable mild stress group, $^{+}P < 0.05$ indicates comparison with the PF group

group and CUMS group received intragastric (i. g.) administration of 0.9% sodium chloride (10 mL/kg) every day. Rats in the FLU-HCl group received i. g. administration of FLU-HCl (10 mg/kg) every day and rats in PF group and PF + CYC-A group received i. g. administration of PF (10 mg/kg) every day. Rats in each group were interfered at 10 a. m. for 3 weeks. During the treatments, all the rats excluding those in the control group continued to receive stressors.

Behavioral tests

After 3-weeks' treatment, behavioral tests were accomplished. Behavioral tests involved sucrose consumption test and forced swimming test (FST). To evade that one behavioral test might have restricted with behavior in the subsequent test, the behavioral tests were carried out at an interval of 2 days. FST was carried out at 3 pm and SP test (SPT) was achieved at 8 pm. In our study, SPT was performed weekly. On the day of behavior test, drug interferences were still carried out on rats.

Sucrose preference test

SPT is often used to measure the anhedonia behaviors of rodents.[21] To remove the effect of circadian rhythm on water drinking in rats, the test was achieved at night (8 p. m.) in a quiet room with controlled temperature (25°C ± 2°C) and relative humidity (65% ± 10%). Before SPT, the rats were deprived of food and water for 24 h. Then, the rats were given 200 mL water and 200 mL 1% sucrose solution for 1 h. In the middle of SPT, the bottle position was transformed. Before and after SPT, the drinking tubes were assessed to calculate SP.[22] The SP percentage was designed according to the following formula: SP = weight of sucrose solution intake/weight of total intake. When the SP of rats in the CUMS group was decreased compared with control group, which represented the anhedonia of rats, indicating the CUMS depression model was effective.[23]

Forced swimming test

The rats were placed in a glass cylinder with a size of 20 cm (diameter) ×40 cm (height), filled with water to a 30 cm depth. In this test, the rats were mandatory to swim in a glass cylinder for 6 min. The duration of immobility was logged during the last 4 min. For each test, water with a controlled temperature (24°C ± 1°C) was substituted for each rat. After the test, the rats were dehydrated and placed in a thermostat for 5 min.

Western blot analysis

After treatments and behavioral tests, the rats were surrendered for Western blot tissue collection. After saline infusion, the rats were anatomized and decapitated immediately on ice. Hippocampus tissues were carefully isolated on the ice and preserved at −80°C. Hippocampus tissues (100 mg) were evaluated, placed in a centrifugal tube, and homogenized by 1 mL hippocampus tissue

lysis buffer solution. After incubation on ice for 30 min (the protein was blended every 10 min), the protein supernatants were centrifuged with a high-speed centrifuge (12,000 g, 4°C) for 15 min. BCA assay kit was employed to assess protein concentrations. After the addition of protein sample buffer, the proteins were denatured in water (100°C) for 5 min, then separated by SDS-PAGE gel electrophoresis and transferred to a 0.45 μm polyvinylidene fluoride membrane. The membrane was congested in TBST containing 5% skimmed milk for 2 h at room temperature and then incubated with primary antibodies for 10 h at 4°C. After washing four times (5 min each time), the membrane was protected with an anti-rabbit secondary antibody (room temperature for 2 h). Finally, the membrane was imagined by the chemiluminescence detection system.

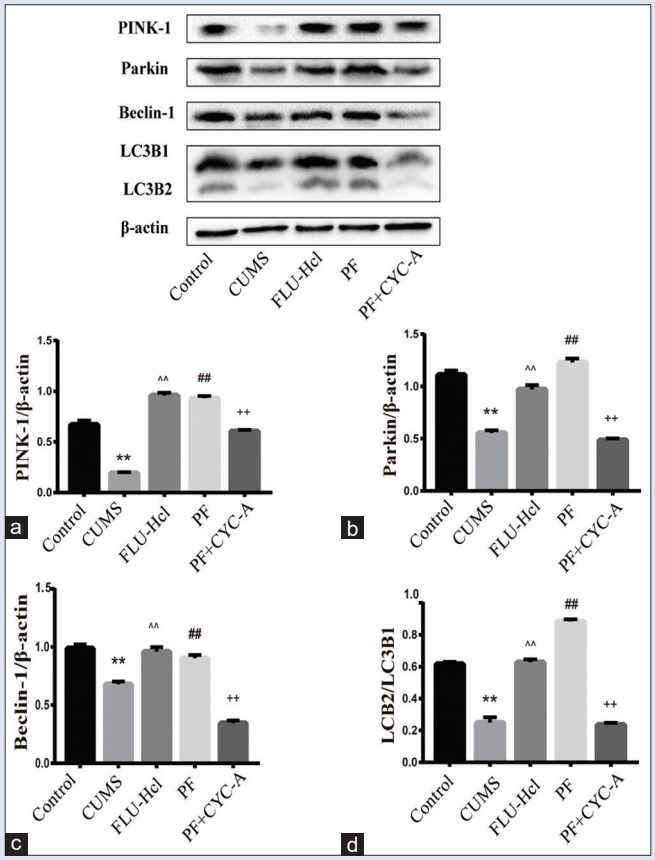

Figure 4: The level of mitophagy. After 3 weeks of treatments, the expressions of PINK-1 (a), Parkin (b), Beclin-1 (c), and LC3B (d) of rats were tested. $^{**}P < 0.01$ indicates comparison with the control group, $^{\wedge\wedge}P < 0.01$ and $^{\#\#}P < 0.01$ indicate comparison with the chronic unpredictable mild stress group, $^{++}P < 0.01$ indicates comparison with the PF group

Data analysis

Data were presented as mean ± standard deviation and analyzed by ANOVA. Dunnett's *t*-test and LSD *t*-test were also used. Statistical differences between the groups were measured significant when the probability level of $P < 0.05$.

RESULTS

Paeoniflorin improves the stress state of chronic unpredictable mild stress rats

After CUMS model was recognized, the rats were in a state of depression such as clustering, weight loss, appetite loss, hair loss, irritability, and low spirits. PF could suggestively advance the state of depression of rats.

Paeoniflorin increases sucrose consumption rate

As shown in Figure 2a, the baseline of SP for rats was confirmed before the establishment of CUMS, there was no alteration in SP rate among the five groups. After 3-week modeling, sucrose consumption rates for CUMS rats were diminished when compared with the control group [$P < 0.01$ Figure 2b]. After FLU or PF treatment for 1 week, 2 weeks, and 3 weeks, the sucrose consumption rates were augmented [$P < 0.01$, Figure 2c-e]. In addition, after PF treatment for 2 and 3 weeks, rats in the PF group showed a significant surge in the sucrose consumption rate as compared with those in the PF + CYC-A group [$P < 0.05$, Figure 2d and e].

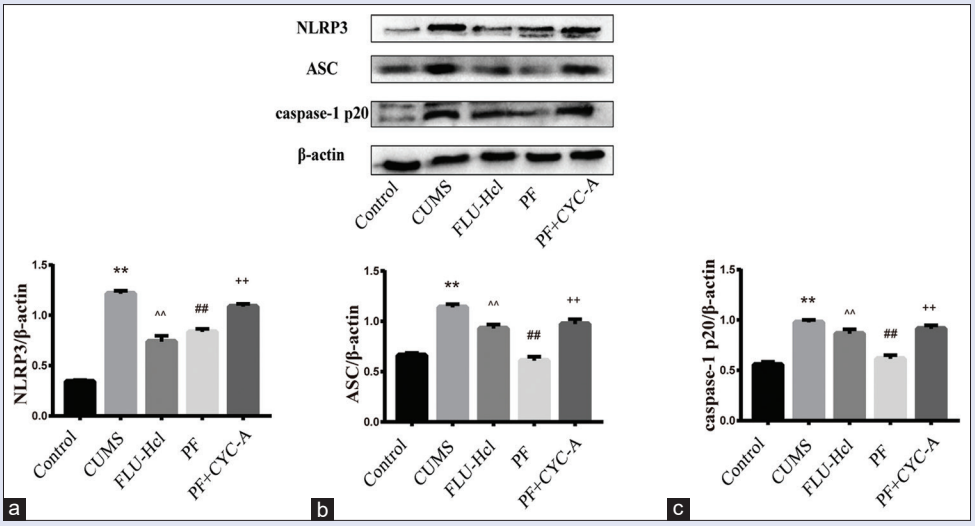

Figure 5: NOD-like receptor protein 3 inflammasome activation. After 3 weeks of treatments, the expression of NOD-like receptor protein 3 (a), Apoptosis-associated speck-like protein containing CARD (b), and Caspase-1 p20 (c) of rats were tested. $^{**}P < 0.01$ indicates comparison with the control group, $^{\wedge\wedge}P < 0.01$ and $^{\#\#}P < 0.01$ indicate comparison with the chronic unpredictable mild stress group, $^{++}P < 0.01$ indicates comparison with the PF group

Paeoniflorin decreases immobility time in forced swimming test

In CUMS group, the immobility time of rats was suggestively greater than the control group [$P < 0.01$, Figure 3]. In contrast, the immobility time of rats in FLU group and PF group was strikingly reduced after 3-week treatment [$P < 0.01$ Figure 3]. As expected, PF treatment abridged the immobility time compared with PF + CYC-A treatment [$P < 0.01$, Figure 3].

Paeoniflorin increases the level of mitophagy

The expressions of mitophagy-related proteins PINK-1, Parkin, Beclin-1, and LC3B in hippocampal tissues are exposed in Figure 4a-d. The CUMS procedure reduced the protein expression of PINK-1 [$P < 0.01$, Figure 4a], Parkin [$P < 0.01$, Figure 4b], Beclin-1 [$P < 0.01$, Figure 4c], and LC3B [$P < 0.01$, Figure 4d]. PF competently augmented the levels of these proteins [$P < 0.01$, Figure 4a-d]. Application of mitophagy inhibitor, CYC-A, eliminated the effects of PF on the protein expression, as showed by the importantly reduced protein expression [$P < 0.01$, Figure 4a-d].

Paeoniflorin blocks activation of NOD-like receptor protein 3 inflammasome

The expressions of proteins involved in NLRP3 inflammasome activation are shown in Figure 5a-c. The result shows that the CUMS procedure improved NLRP3 [$P < 0.01$, Figure 5a], ASC [$P < 0.01$, Figure 5b], and caspase-1 p20 [$P < 0.01$, Figure 5c] in the hippocampus. PF or FLU-HCl treatment reduced these proteins in CUMS rats [$P < 0.01$, Figure 5a-c]. Application of mitophagy inhibitor, CYC-A, expressively augmented the expression levels of NLRP3 [$P < 0.01$, Figure 5a], ASC [$P < 0.01$, Figure 5b], and caspase-1p20 [$P < 0.01$, Figure 5c].

Paeoniflorin decreases the levels of interleukin-1 β and interleukin-18

Variations in protein levels of IL-1 β and IL-18 in the hippocampus of rats are shown in Figure 6a and b. Reliably, the CUMS procedure augmented the protein levels of IL-1β [$P < 0.01$, Figure 6a] and IL-18 [$P < 0.01$, Figure 6b] suggestively, which were diminished by PF [$P < 0.01$, $P < 0.01$, Figure 6a and b] and FLU-HCl treatment [$P < 0.01$, Figure 6a and b]. Application of mitophagy inhibitor, CYC-A, meaningfully augmented the levels of IL-1β [$P < 0.01$, Figure 6a] and IL-18 [$P < 0.01$, Figure 6b].

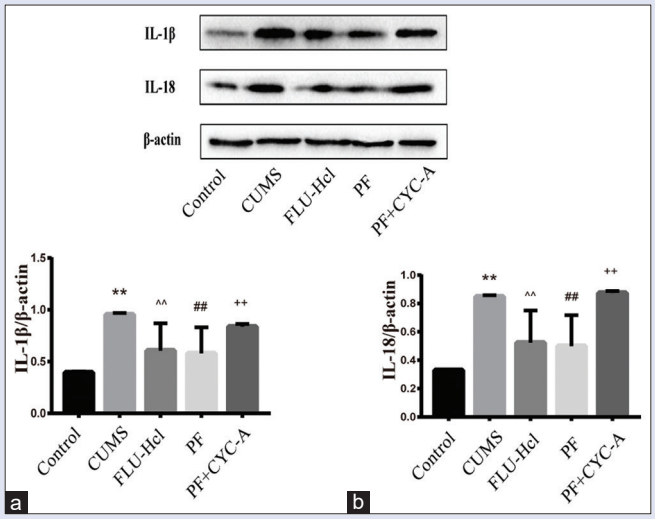

Figure 6: Analysis of pro-inflammatory cytokines. After 3 weeks of treatments, the expression of IL-1 β (a) and IL-18 (b) of rats were tested. $^{**}P < 0.01$ indicates comparison with the control group, $^{\wedge\wedge}P < 0.01$ and $^{\#\#}P < 0.01$ indicate comparison with the chronic unpredictable mild stress group, $^{++}P < 0.01$ indicates comparison with the PF group

DISCUSSION

Depression designates a group of mental disorders with multifactorial psychopathologies. CUMS is demarcated as a chronic impulsive mild stress-induced depression model, which is a typical modeling method for depression. The etiology and pathological mechanism of CUMS model are similar to those of human depression.[24] Thus, this work purposes to study the mechanism of depression.[25] PF is a major essential of the root part of *P. lactiflora* Pall and has several biological activities.[26] The antidepressant-like effects of PF have been reported earlier.[27] There were indications recommended that the antidepressant mechanisms of PF are thoroughly related to its inhibition of neuroinflammatory response.[17,19] Thus, we explored the pharmacological mechanism of PF and examined whether NLRP3 inflammasome and mitophagy mediate the antidepressant-like effects of PF.

SPT is frequently employed to measure the state of absence of delight. FST is often used to evaluate the success of depression models as well as the effectiveness of antidepressant agents.[28] After CUMS procedure, FST established that rats precisely replicated the depressive state. The depressive state of rats was recognized in the decrease of sucrose consumption in SPT and the increase of immobility time in FST. Application of PF upturned the stress-induced behavioral changes, demonstrating that PF exerts antidepressant effects.

Inflammation is concerned in depression pathologic process. Studies have shown that chronic stress and systemic inflammation happen in CUMS rats.[29] NLRP3 inflammasome activation has been detected in the hippocampus of depressed mice and NLRP3 inflammasome intercedes depression through neuroinflammation.[8] The antidepressant effect of fluoxetine was linked to its inhibition of the NLRP3 inflammasome.[30] NLRP3 inflammasome is composed of NLRP3, ASC, and procaspase-1. After NLRP3 inflammasome activation, procaspase-1 is cleaved into mature caspase-1 through MAPK and NF-kappa B signaling pathways and the pro-IL-1 β and pro-IL-18 are cleaved into mature IL-1 β and IL-18.[31]

In this study, the protein levels of NLRP3, ASC, caspase-1 p20, IL-1 β, and IL-18 in the hippocampus of rats were augmented after the CUMS procedure, representing incidence of a notable inflammatory injury in the hippocampus. Particularly, the stress-induced inflammation injury was improved by treatment with PF or FLU-HCl, signifying that PF employs antidepressant effects by inhibiting NLRP3 inflammasome activation.

Accumulating evidence proposes that mitochondria are convoluted in the NLRP3 inflammasome activation.[32] The release or acquaintance of mitochondrial ROS and DNA after mitochondrial injury causes the assembly of the NLRP3 inflammasome activation.[33] Thus, the oxidation of mtDNA activates NLRP3 inflammasome.[34] Mitophagy abolishes damaged mitochondria and averts mtROS and mtDNA from being released into the cytoplasm, thereby limiting the NLRP3 inflammasome activation.[35] Stimulating macrophages knockout genes LC3B or Beclin-1 with ATP, resulted in an increase in damaged mitochondria and activation of NLRP3 inflammasome.[36] Mitophagy prevents the NLRP3 inflammasome activation.[37] PINK-1-Parkin pathway is a key regulator of mitophagy. Protein kinase (PINK-1) is amalgamated in the cytoplasm, after which it enters the mitochondria through the molecular channel of the mitochondrial membrane. It is finally degraded by proteolytic enzymes in the mitochondria.[38] Mitochondrial injury leads to the

accumulation of PINK-1 on extracorporeal membrane of mitochondria. Parkin, an E3 ubiquitin-protein ligase, is activated by PINK-1. This ligase can ubiquitinate dented mitochondrial membrane proteins, which interact with signal junction proteins and autophagy-related proteins on the phagocytic membrane. In this way, it pledges the phagocytosis and finally reduces damaged mitochondria.[39,40]

In this study, the levels of PINK-1, Parkin, Beclin-1, and LC3B proteins in the hippocampus of rats were reduced after the CUMS procedure, representing that the level of mitophagy was diminished in the hippocampus. The level of mitophagy in the hippocampus was augmented after treatment with PF or FLU-HCl, signifying that the PF antidepressant consequence may comprise enhancement of mitophagy. We also found that the mitophagy inhibitor, CYC-A, could partially deteriorate the downregulation effect of PF on NLRP3 inflammasome activation, thereby blocking the antidepressant effects of PF.

CONCLUSION

PF improves depressive symptoms in CUMS rats. This is accomplished by inhibiting the NLRP3 inflammasome activation. In addition, it averts inflammation temperately by enhancing mitophagy.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kupfer DJ, Frank E, Phillips ML. Major depressive disorder: New clinical, neurobiological and treatment perspectives. Lancet 2012;379:1045-55.
2. Chang T, Fava M. The future of psychopharmacology of depression. J Clin Psychiatry 2010;71:971-5.
3. Michael M. Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:664-75.
4. Wong ML, Inserra A, Lewis MD, Mastronardi CA, Leong L, Choo J, *et al.* Wesselingh, Inflammasome signaling affects anxiety-and depressive-like behavior and gut microbiome composition. Mol Psychiatry 2016;21:797-805.
5. Zhang Y, Liu L, Peng YL, Liu YZ, Wu TY, Shen XL, *et al.* Involvement of inflammasome activation in lipopolysaccharide-induced mice depressive-like behaviors. CNS Neurosci Ther 2014;20:119-24.
6. Zhang Y, Liu L, Liu YZ, Shen XL, Wu TY, Zhang T, *et al.* NLRP3 inflammasome mediates chronic mild stress-induced depression in mice via neuroinflammation. Int J Neuropsychopharmacol 2015;18:1-8.
7. Su WJ, Zhang Y, Chen Y, Gong H, Lian YJ, Peng W, *et al.* NLRP3 gene knockout blocks NF-κB and MAPK signaling pathway in CUMS-induced depression mouse model. Behav Brain Res 2017;322:1-8.
8. Elísabet AG, Cristina UM, Fabiola MA, Tatyana R, Nieves CB, Jesús RC, *et al.* Stress-induced depressive behaviors require a functional nlrp3 inflammasome. Mol Neurobiol 2016;53:4874-82.
9. Pandey GN, Rizavi HS, Ren X, Fareed J, Hoppensteadt DA, Roberts RC, *et al.* Proinflammatory cytokines in the prefrontal cortex of teenage suicide victims. J Psychiatr Res 2012;46:57-63.
10. Yoo SM, Jung YK. A molecular approach to mitophagy and mitochondrial dynamics. Mol Cells 2018;41:18-26.
11. Zhong ZY, Umemura A, Sanchez-Lopez E, Liang S, Shalapour S, Wong J, *et al.* Nf-κb restricts inflammasome activation via elimination of damaged mitochondria. Cell 2016;164:896-910.
12. Zhou RB, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature 2011;475:122-221.
13. Li DM, Zheng J, Wang MY, Feng L, Liu YY, Yang N, *et al.* Wuling powder prevents the depression-like behavior in learned helplessness mice model through improving the TSPO mediated-mitophagy. J Ethnopharmacol 2016;186:181-8.
14. Búrigo M, Roza CA, Bassani C, Fagundes DA, Rezin GT, Feier G, *et al.* Effect of electroconvulsive shock on mitochondrial respiratory chain in rat brain. Neurochem Res 2006;31:1375-9.
15. Mao QQ, Ip SP, Tsai SH, Che CT. Antidepressant-like effect of peony glycosides in mice. J Ethnopharmacol 2008;119:272-5.
16. Mao QQ, Zhong XM, Feng CR, Pan AJ, Li ZY, Huang Z. Protective effects of paeoniflorin against glutamate-induced neurotoxicity in pc12 cells via antioxidant mechanisms and Ca2+antagonism. Cell Mol Neurobiol 2010;30:1059-66.
17. Li J, Huang S, Huang W, Wang W, Wen G, Gao L, *et al.* Paeoniflorin ameliorates interferon-alpha-induced neuroinflammation and depressive-like behaviors in mice. Oncotarget 2017;8:8264-82.
18. Huang H, Zhao J, Jiang L, Xie Y, Xia Y, Lv R, *et al.* Paeoniflorin improves menopause depression in ovariectomized rats under chronic unpredictable mild stress. Int J Clin Exp Med 2015;8:5103-11.
19. Tang NY, Liu CH, Hsieh CT, Hsieh CL. The anti-inflammatory effect of paeoniflorin on cerebral infarction induced by ischemia-reperfusion injury in sprague-dawley rats. Am J Chin Med 2010;38:51-64.
20. Li R, Wang X, Qin T, Qu R, Ma S. Apigenin ameliorates chronic mild stress-induced depressive behavior by inhibiting interleukin-1β production and nlrp3 inflammasome activation in the rat brain. Behav Brain Res 2016;296:318-25.
21. Strekalova T, Gorenkova N, Schunk E, Dolgov O, Bartsch D. Selective effects of citalopram in a mouse model of stress-induced anhedonia with a control for chronic stress. Behav Pharmacol 2006;17:271-87.
22. Bai M, Zhu X, Zhang Y, Zhang S, Zhang L, Xue L, *et al.* Abnormal hippocampal BDNF and miR-16 expression is associated with depression-like behaviors induced by stress during early life. PLoS One 2012;7:1-8.
23. Liu MY, Yin CY, Zhu LJ, Zhu XH, Xu C, Luo CX, *et al.* Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat Protoc 2018;13:1686-98.
24. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress and its restoration by a tricyclic antidepressant. Psychopharmacology 1987;93:358-64.
25. Liu YM, Shen JD, Xu LP, Li HB, Li YC, Yi LT. Ferulic acid inhibits neuro-inflammation in mice exposed to chronic unpredictable mild stress. Int Immunopharmacol 2017;45:128-34.
26. Ji Y, Wang T, Wei ZF, Lu GX, Jiang SD, Xia YF, *et al.* Paeoniflorin, the main active constituent of *Paeonia lactiflora* roots, attenuates bleomycin-induced pulmonary fibrosis in mice by suppressing the synthesis of type I collagen. J Ethnopharmacol 2013;149:825-32.
27. Qiu F, Zhong X, Mao Q, Huang Z. The antidepressant-like effects of paeoniflorin in mouse models. Exp Ther Med 2013;5:1113-6.
28. Cryan JF, Holmes A. Model organisms: The ascent of mouse: Advances in modelling human depression and anxiety. Nat Rev Drug Discov 2015;4:775-90.
29. Lópezlópez AL, Jaime HB, Mdc EV, Padilla MB, Palacios GV, Fja A. Chronic unpredictable mild stress generates oxidative stress and systemic inflammation in rats. Physiol Behav 2016;161:15-23.
30. Du RH, Tan J, Sun XY, Lu M, Ding JH, Hu J. Fluoxetine inhibits NLRP3 inflammasome activation: Implication in depression. Int J Neuropsychopharmacol 2016;19:1-9.
31. Lin KM, Hu W, Troutman TD, Jennings M, Brewer T, Li XX, *et al.* Irak-1 bypasses priming and directly links tlrs to rapid nlrp3 inflammasome activation. Proc Natl Acad Sci U S A 2014;111:775-80.
32. Jo EK, Kim JK, Shin DM, Sasakawa C. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 2016;13:148-59.
33. Subramanian N, Natarajan K, Clatworthy MR, Wang Z, Germain RN. Mitochondria play a central role in nlrp3 inflammasome activation. Mitochondrion 2015;24:S15-6.
34. Zhong Z, Shuang L, Sanchezlopez E, Feng H, Shalapour S, Lin XJ, *et al.* New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. Nature 2018;560:198-203.
35. Azad MB, Chen Y, Gibson SB. Regulation of autophagy by reactive oxygen species (ROS): Implications for cancer progression and treatment. Antioxid Redox Signal 2009;11:777-90.

36. Shimada K, Crother T, Karlin J, Dagvadorj J, Chiba N, Shuang C, *et al.* Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. Immunity 2012;36:401-14.
37. Wu J, Li X, Zhu G, Zhang Y, He M, Zhang J. The role of Resveratrol-induced mitophagy/autophagy in peritoneal mesothelial cells inflammatory injury via NLRP3 inflammasome activation triggered by mitochondrial ROS. Exp Cell Res 2016;341:42-53.
38. Harper JW, Ordureau A, Heo JM. Building and decoding ubiquitin chains for mitophagy. Nat Rev Mol Cell Biol 2018;19:93-108.
39. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, *et al.* PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol 2010;189:211-21.
40. Callegari S, Oeljeklaus S, Warscheid B, Dennerlein S, Thumm M, Rehling P, *et al.* Phospho-ubiquitin-PARK2 complex as a marker for mitophagy defects. Autophagy 2017;13:201-11.