Int. J. Morphol.,
42(2):470-478, 2024.

Acteoside Attenuates Recognition Memory Impairment in CUMS Rats via Regulating Synaptic Plasticity and mTOR Signaling Pathway

El Acteoside Atenúa el Deterioro de la Memoria de Reconocimiento en Ratas ELCI Mediante la Regulación de la Plasticidad Sináptica y la Vía de Señalización mTOR

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SUN, M.; FAN, W.; WANG, X.; MENG, J.; ZHANG, F.; CHANG, Q. & DENG, H. Acteoside attenuates recognition memory impairment in CUMS rats via regulating synaptic plasticity and mTOR signaling pathway. *Int. J. Morphol., 42(2)*:470-478, 2024.

SUMMARY: We evaluated the role and mechanism of acteoside in the regulation of memory impairment induced by chronic unpredictable mild stress (CUMS). CUMS was used to induce depression in rats and the successful establishment of CUMS model were verified by forced swimming test and sucrose preference test. The Y-maze test and novel object recognition test assessed memory functions. The structural changes in the cortex and hippocampus were observed by hematoxylin and eosin (HE) staining. Immunofluorescence staining and western blotting determined the protein levels. Y-maze test and novel object recognition test showed that there was memory performance impairment in rats of CUMS group, which was improved by the acteoside treatment. HE staining showed that CUMS exposure damaged the structure in the cortex and hippocampus, while the acteoside treatment alleviated the structural changes. Compared with the control group, the levels of BNDF and CREB in the cortex and hippocampus of the CUMS group were significantly decreased. Acteoside significantly reversed the expressions of these proteins in CUMS rats. Meanwhile, compared with the control group, the levels of p-mTOR and p-P70S6K in the cortex and hippocampus of the CUMS group were significantly increased, and these changes were significantly reversed by acteoside. Nevertheless, the effect of acteoside on mTOR signaling was markedly blocked by rapamycin, a specific inhibitor of mTOR signaling. Acteoside can attenuate memory impairment and ameliorate neuronal damage and synaptic plasticity in depression rats probably via inhibiting the mTOR signaling pathway. Acteoside may serve as a novel reagent for the prevention of depression.

KEY WORDS: Acteoside; Chronic unpredictable mild stress (CUMS); Depression; Memory impairment. Synaptic plasticity; mTOR signaling.

INTRODUCTION

Depression is a serious mental illness caused by multiple stress factors and is characterized by high morbidity, high disability rate, high suicide rate, and easy relapse (Cuijpers *et al*., 2019). It can bring serious harm to human survival and health (Dobrek & Glowacka, 2023). Due to the complex pathogenesis of depression, there is still no specific therapeutic regimen in the clinic at present (Cipriani *et al*., 2018). In recent years, the number of depression patients has been increasing gradually with the change in the social environment and the increase in competitive pressure. The World Health Organization predicts that depression will become one of the most common chronic diseases in clinics, second only to hypertension, by around the year 2030 (Kolovos *et al*., 2017). Accordingly, there is an urgent need to develop new antidepressant drugs with better efficacies and fewer side effects.

Acteoside is one of the main active components of the Genus acteoside (Cheimonidi *et al*., 2018). It has antioxidant, anti-inflammatory, and anti-apoptosis effects and can effectively protect the central nervous system (Gan *et al*., 2018; Li *et al*., 2018; Chen *et al*., 2019b). Recently, it has been reported that Pedicularis resupinata extract, in

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FUNDING. This study was supported by Henan Province Key Research and Development and Promotion Project (Science and Technology Tackling Key Problems) (232102310501), Key Scientific Research Projects of Higher Education Institutions in Henan Province (19A310001), Luohe Youth Top-notch Talent Project (2018QNBJRC01002), and Development and Improvement Project of Innovation and Entrepreneurship Ability in Rehabilitation Medicine of Luohe Medical College (2021LYZKJXM008).

Received: 2023-12-08 Accepted: 2024-01-13

which acteoside is the main component, had antidepressant-like effects in depression model mice (Lim *et al*., 2022). Similarly, our previous study has shown that acteoside improved depression-like behavior in rats induced by chronic unpredictable mild stress (CUMS) (Deng *et al*., 2018). In addition to depression-like behavior, cognitive impairment is also an important symptom induced by stress (Li *et al*., 2016). Studies have found that rats exposed to CUMS displayed task-dependent cognitive impairment (Yan *et al*., 2020; Alqurashi *et al*., 2022). However, there have been no reports focusing on the effects of acteoside on learning and memory.

Learning and memory are advanced functions of the brain, and the underlying principles have received much attention. So far, it has been widely accepted that synaptic plasticity is the biological basis of learning and memory function (Yan *et al*., 2020). Synaptic plasticity is associated with the encoding and storage of memory function, which requires a series of orderly changes in the number and/or structures of synapses (Diering & Huganir, 2018). It is widely known that the cortex and hippocampus are two key brain regions for cognitive development and emotion regulation (Saleh *et al*., 2017). Therefore, we selected the cortex and hippocampus as the target regions in this study.

In this study, the rat model of depression was induced by CUMS. The effects and possible underlying mechanisms of acteoside on the learning and memory functions in depression rats were investigated. Moreover, the cognitive changes during the development of depression were also studied to reveal their association with the disease pathogenesis. Our findings support that acteoside may serve as a novel reagent for the prevention and treatment of depression.

MATERIAL AND METHOD

Study animals. The Sprague Dawley rats, weighing 200-240 g, were obtained from the Hunan SJA Laboratory Animal Co., Ltd. (No.: SCXK-Xiang 2011-0003). These rats were maintained under a 12-h light/dark cycle (lights on/off at 07:00/19:00) with ad libitum access to food and water. This study was conducted in strict accordance with the recommendations of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The research protocol with animal experimentation was approved by the Animal Care and Use Committee of the Luohe Medical College. All efforts were made to minimize the pain and numbers of the animals used in this study.

CUMS depression model establishment. After acclimatization for 1 week, the rats were subjected to CUMS for 4 weeks (Wang *et al*., 2021a). Stress factors included fasting (for 24 h), water deprivation (for 24 h), reversal of day and night (for 24 h), swimming in hot water (45°C, for 5 min), swimming in ice-cold water (4 °C, for 5 min), tail suspension (for 20 min), tail clipping (for 2 min), and wet bedding (for 24 h). Different stress factors were given every 2 days and the stress factors given each time were random and unpredictable. Control rats were kept at standard conditions. The model establishment was verified with the forced swimming (FS) test and sucrose preference (SF) test.

Animal treatment and grouping. For assessment of memory performance, rats were randomly divided into control, CUMS, CUMS+Fluoxetine (CUMS+Flu), and CUMS+Acteoside (CUMS+Act) groups (n=12 each group). Fluoxetine (20 mg/kg; positive control; Eli Lilly Co., Ltd., Suzhou, China) or acteoside (60 mg/kg; Cat. No. 133-131216; purity ≥ 98 %; Nanjing Guang Run Biotechnology Co., Ltd., China) was intraperitoneally administrated to the rats in the CUMS+Flu or CUMS+Act group, respectively, once every two days for 3 weeks. The dose of acteoside was determined based on our previous study (Deng *et al*., 2018). The same amount of saline was given to the rats in the CUMS and control groups. The Y-maze test and novel object recognition (NOR) test were performed at 24 h after the last drug administration in a blinded manner.

To evaluate the involvement of mTOR signaling in the improving effects of acteoside on learning and memory, rats were randomly divided into control, CUMS, CUMS+Acteoside (CUMS+Act), and CUMS+ Acteoside+Rapamycin (CUMS+Act+Rap) groups (n=6 each group). Acteoside was given as above described. Rapamycin (8 mg/kg; Beyotime Biotechnology Co., Ltd., Shanghai, China) was intraperitoneally given to the rats in the CUMS+Act+Rap group. The drug administration was performed once every two days for 3 weeks. The dose of rapamycin was determined based on a previous study (Johnson *et al*., 2013). The normal saline was given to the rats in the CUMS and control groups at equal volumes.

Behavioral tests

FS test. The FS test was carried out 24 h after CUMS model establishment using a transparent plastic container with an upper diameter of 40 cm and a lower diameter of 33 cm. The water depth was 30 cm and the water temperature was (24±1)°C. The rats were slowly placed into the plastic container with their heads facing the container wall and allowed to swim freely. The immobility time of the rats within 5 min was recorded. The animals

were considered immobile if they floated on the water without moving their limbs, breathed with their heads above the water, or paddled occasionally to keep their bodies from sinking.

SP test. The SP test was conducted 24 h after the FS test. Briefly, the rats were caged individually. First, rats underwent adaptive training, with two bottles containing 200 mL of 1 % sucrose solution in each cage for 24 h. Then, fasting and water deprivation were performed immediately for 24 h following the sucrose adaptive training. After that, one bottle containing 200 mL of pure water and one bottle containing 200 mL of 1 % sucrose solution were placed in each case. The positions of the two bottles were changed 0.5 h later. The sucrose consumption and pure water consumption were recorded. The SP of each rat was calculated using the formula of SP (%)=[sucrose consumption/(sucrose consumption + pure water consumption)]×100 %.

Y-maze test. The Y-maze consisted of three identical arms (35 cm length × 8 cm height × 15 cm width) positioned 120 degrees apart from each other. The rats were gently placed at the end of one arm. The times and sequences of each rat entering the three arms within 5 min were recorded. An alternation was defined as consecutive entries to three arms without overlapping triplets. The alternation percentage was calculated with the following formulation: Alternation (%)=[(alternation times)/(total times of arm entries - 2)]×100 %.

NOR test. The NOR test was performed in a black opaque plastic box (80 cm × 60 cm × 40 cm). The NOR test consisted of two phases: the sampling phase and the retention test phase with a 24 h interval. Rats were acclimated to the environment at 24 h before the sampling phase. In the sampling phase, the rats were allowed to explore the two identical objects for 10 min and the total time spent on exploring both objects was recorded. In the retention test phase, the rats were allowed to explore two different objects (one was familiar, while the other one was novel) for 5 min. The time spent on exploring each object, and the total time spent on exploring both objects, were recorded. The recognition index was calculated by the formula of [time spent on exploring the novel object / (time spent on exploring the novel object + time spent on exploring the familiar object)]× 100 %.

Sampling. At 24 h after the behavioral test, half of the rats in each group were anesthetized and perfused with 4 % paraformaldehyde (PFA). The other half of the rats in each group were sacrificed by decapitation. Then, the brain tissues, including the cortex and hippocampus, were collected.

Hematoxylin and Eosin (HE) staining. The morphology of neurons in the cortex and hippocampus was detected with the HE staining Kit (Beyotime) following the manufacturer's protocol. Briefly, the cortex and hippocampus tissues were fixed with 4 % PFA overnight and sliced into 20 μm sections. After that, the sections were incubated successively with hematoxylin and eosin staining solution. Finally, the sections were mounted and observed with a microscope (Olympus, Tokyo, Japan).

Immunofluorescence staining. The tissue sections were permeabilized with 0.3 % Triton X-100 for 2 h, followed by blocking with 10 % BSA for 1 h. Subsequently, the sections were incubated with the rabbit anti-brain derived neurotrophic factor (BDNF) (1:500 dilution; Abcam) or mouse anti-cAMP-response element binding protein (CREB) (1:500 dilution; Abcam) polyclonal primary antibody overnight at 4 °C. After washing with PBS, the sections were incubated with a secondary antibody at room temperature for 2 h. The nuclei were stained with DAPI (Beyotime) for 5 min. Fluorescence was detected and observed under a fluorescence microscope (Olympus). Three discontinuous brain slices were randomly selected from each rat, and three fields were randomly selected from each slice under a 200 × microscope. The average number of fluorescence-positive cells was calculated with the Image Pro Plus 6.0 software (Media Cybernetics, USA).

Western blotting. The cortex and hippocampal tissues were lyzed with RIPA lysis buffer (Beyotime). Subsequently, protein concentration was tested by a BCA Protein Assay Kit (Beyotime). After that, proteins were subjected to SDS-PAGE and transferred to PVDF membranes. The membranes were blocked for 15 min at room temperature with blocking buffer (Beyotime) and then incubated with primary antibodies overnight at 4 °C. The primary antibodies included rabbit anti-BDNF (1:1000 dilution; Abcam), mouse anti-CREB (1:1000 dilution; Abcam), rabbit anti-p-mTOR (1:1000 dilution; CST), rabbit anti-mTOR (1:1000 dilution; CST), rabbit anti-p-P70S6K (1:1000 dilution; CST), rabbit anti-P70S6K (1:1000 dilution; CST), and rabbit anti-b-actin (1:5000 dilution; Gene Tex). The membranes were then incubated with secondary antibodies for 1 h at room temperature. b-actin was used as an internal reference to analyze the target protein levels.

Statistical analyses. The SPSS 20.0 statistical software and GraphPad Prism 6.0 statistical software were used for statistical analysis. Data are expressed as mean ± SD. Statistical analyses were performed using the student's t-test or one-way ANOVA followed by SNK-q post hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

Depression model is established by CUMS in rats. To determine the establishment of CUMS depression model in rats, we first performed the FS test, a classic test to assess depression in rats. As shown in Figure 1A, after 4 weeks of CUMS exposure, there were increased immobility times in the CUMS group as compared to the control group ($P<0.01$). This response reflected behavioral hopelessness, a central symptom of depression. We further performed the SP test, another classic test to evaluate the state of depression. We found that, after 4 weeks of CUMS exposure, significant decreases in sucrose intake were observed in CUMS rats as compared with control rats ($P<0.01$) (Fig. 1B). The decrease in sucrose intake suggested that CUMS rats were experiencing anhedonia, i.e. the loss of pleasure associated with sucrose intake. Hence, the results of these behavioral tests demonstrated that 4 weeks of CUMS exposure could indeed induce depression-like behaviors in the rats, indicating the successful establishment of the depression model.

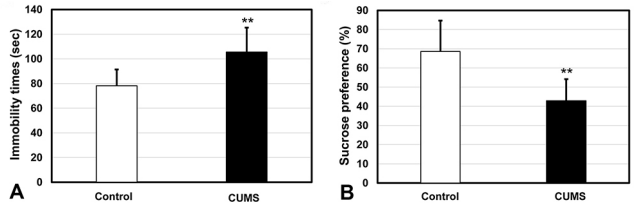

Fig. 1. Detection of depression-like behaviors with FS test and SP test. The depression-like behaviors of the control and CUMS groups were detected with the FS test and SP test. Comparisons of the immobility times (A) and the sucrose preference (B). Compared with the control group, ** $P<0.01$.

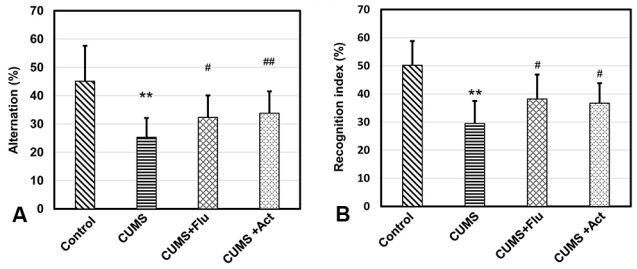

Fig. 2. Detection of learning and memory functions with the Y-maze test and NOR test. The learning and memory functions of the control, CUMS, CUMS+Flu, and CUMS+Act groups were detected with the Y-maze test and NOR test. Comparisons of the alternation percentage (A) and the recognition index (B). Compared with the control group, ** $P<0.05$; compared with the CUMS group, # $P<0.05$, ## $P<0.01$.

Acteoside attenuates recognition memory impairment in CUMS rats. We next determined whether acteoside affects the recognition memory performance of CUMS rats by using the Y-maze test and NOR test, which are generally used to assess learning and memory capacity. As shown in Figure 2A, CUMS caused a significant decrease in the alternation percentage ($P<0.01$), which was reversed by the fluoxetine and acteoside treatments ($P<0.05$, $P<0.01$). Similarly, the NOR test showed that CUMS led to a significant reduction in the recognition index ($P<0.01$), and this reduction was reversed by the fluoxetine and acteoside treatments, respectively (both $P<0.05$) (Fig. 2B). Thus, these results indicate that CUMS could impair the learning and memory functions of rats, while acteoside could attenuate the recognition memory impairment in CUMS rats.

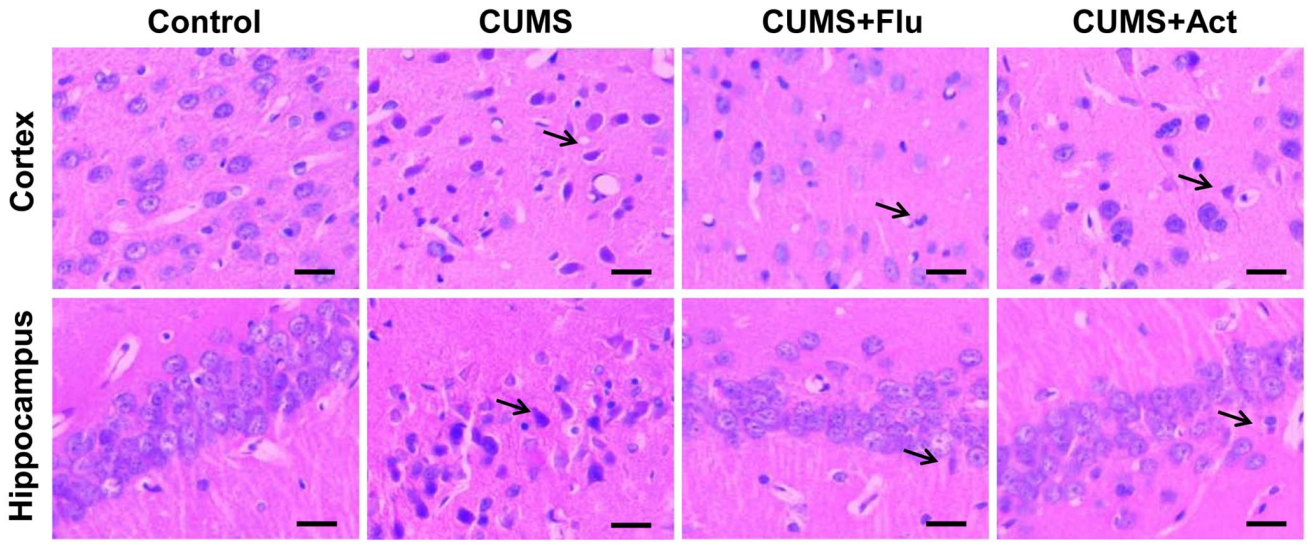

Fig. 3. Detection of the morphology of neurons in the cortex and hippocampus with HE staining. Representative images were shown. Arrows indicate neuronal pyknosis. Scale bar, 50 μm.

Acteoside modifies hippocampal structural damage in CUMS rats. It is well known that the cortex and hippocampus are the key brain regions for cognitive development and emotion regulation (Fuchsberger & Paulsen, 2022; Guo *et al*., 2022). Thus, we investigated the effect of acteoside on the morphology of neurons in the cortex and hippocampus in the CUMS rats by HE staining. As shown in Figure 3, in the control group, the structure of neurons was intact, with clear boundaries and visible nucleoli in the cortex. The neurons were arranged closely and neatly in the hippocampus. In the CUMS group, the structure of neurons was denatured. There were vacuoles and some spindle- or triangle-shaped pyknosis. The nucleoli disappeared. The hippocampal neurons were arranged loosely. Compared with CUMS group, the structures of neurons in the CUMS+Flu and CUMS+Act groups were improved. Only a few cells were pyknotic and hippocampal neurons were arranged neatly. The results indicate that acteoside, to some extent, could repair neuronal morphology.

Acteoside improves synaptic plasticity in CUMS rats. Synaptic plasticity is the neurobiological basis of learning and memory (Fuchsberger & Paulsen, 2022; Wang *et al*., 2022). To determine the involvement of synaptic plasticity in the attenuating effects of acteoside on recognition memory impairment in CUMS rats, we then detected the levels of BDNF and CREB, which are related to the neuronal development and synaptic function, by immunofluorescence staining. As shown in Figures 4A-4E, BDNF and CREB levels in the cortex and hippocampus of CUMS rats were reduced compared with that in the control group ($P<0.01$). In contrast, their levels were elevated after acteoside and fluoxetine treatment ($P<0.05$, $P<0.01$). These results imply that acteoside may ameliorate neuronal and synaptic damage in the CUMS rat brain by regulating the expression of BDNF and CREB. Furthermore, western blotting results also demonstrated elevated levels of BDNF and CREB after acteoside treatment, confirming the effect of acteoside on synaptic plasticity (Fig. 5A-5C).

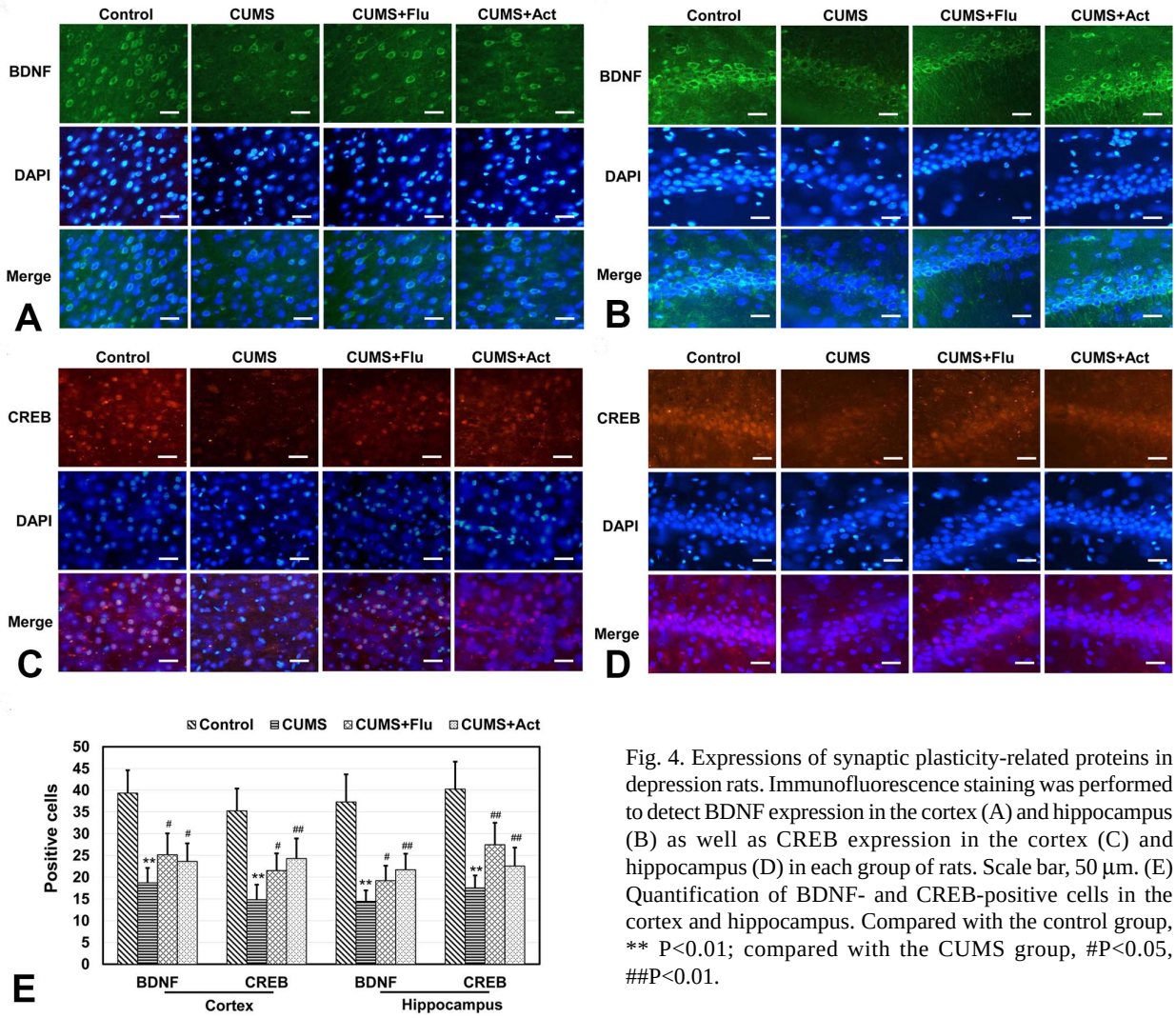

Fig. 4. Expressions of synaptic plasticity-related proteins in depression rats. Immunofluorescence staining was performed to detect BDNF expression in the cortex (A) and hippocampus (B) as well as CREB expression in the cortex (C) and hippocampus (D) in each group of rats. Scale bar, 50 μm. (E) Quantification of BDNF- and CREB-positive cells in the cortex and hippocampus. Compared with the control group, ** $P<0.01$; compared with the CUMS group, #$P<0.05$, ##$P<0.01$.

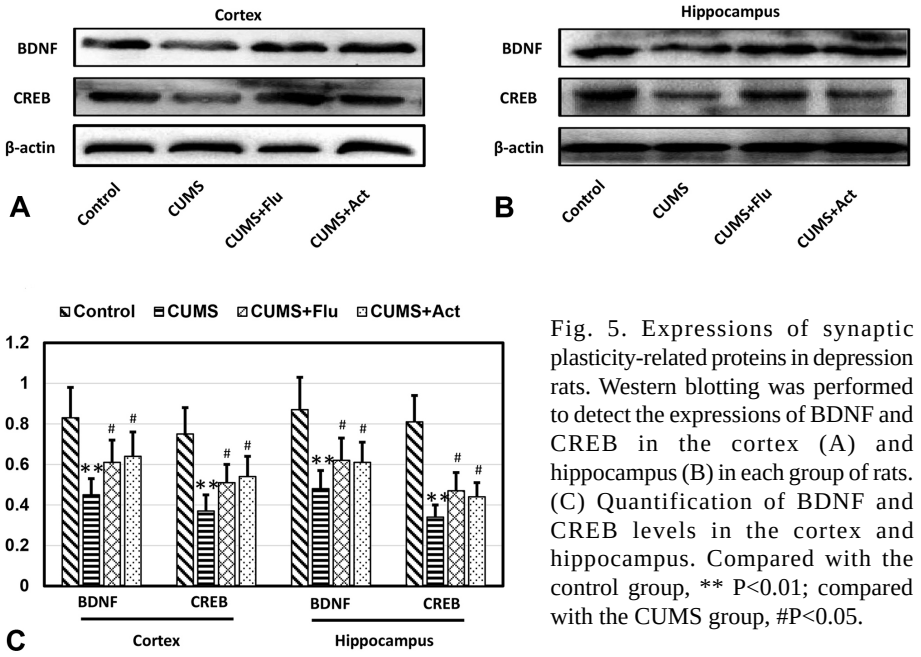

Fig. 5. Expressions of synaptic plasticity-related proteins in depression rats. Western blotting was performed to detect the expressions of BDNF and CREB in the cortex (A) and hippocampus (B) in each group of rats. (C) Quantification of BDNF and CREB levels in the cortex and hippocampus. Compared with the control group, ** P<0.01; compared with the CUMS group, #P<0.05.

Acteoside inhibits mTOR signaling in CUMS rats. We next explored the mechanism by which acteoside improved synaptic plasticity in CUMS rats. The mTOR is a well-documented molecule in regulating the BDNF signaling pathway, and it has been reported that acteoside was related to the inhibition of mTOR signaling in H2O2-induced nerve damage (Qu *et al*., 2022). Therefore, we hypothesize that acteoside may inhibit the mTOR signaling, thus activating the BDNF signaling and improving synaptic plasticity. As expected, western blotting showed that acteoside treatment decreased the phosphorylation levels of mTOR and P70S6K both in the cortex and hippocampus of CUMS rats significantly (P<0.05, Fig. 6A-6C). Furthermore, rapamycin, a specific inhibitor of mTOR signaling, was used to verify the effect of acteoside on mTOR signaling in CUMS rats. It was found that the inhibition effect of acteoside on mTOR signaling was markedly relieved by rapamycin, confirming the important role of mTOR signaling in mediating the effect of acteoside on CUMS. Therefore, acteoside might improve synaptic plasticity in CUMS rats by inhibiting the mtor signaling pathway.

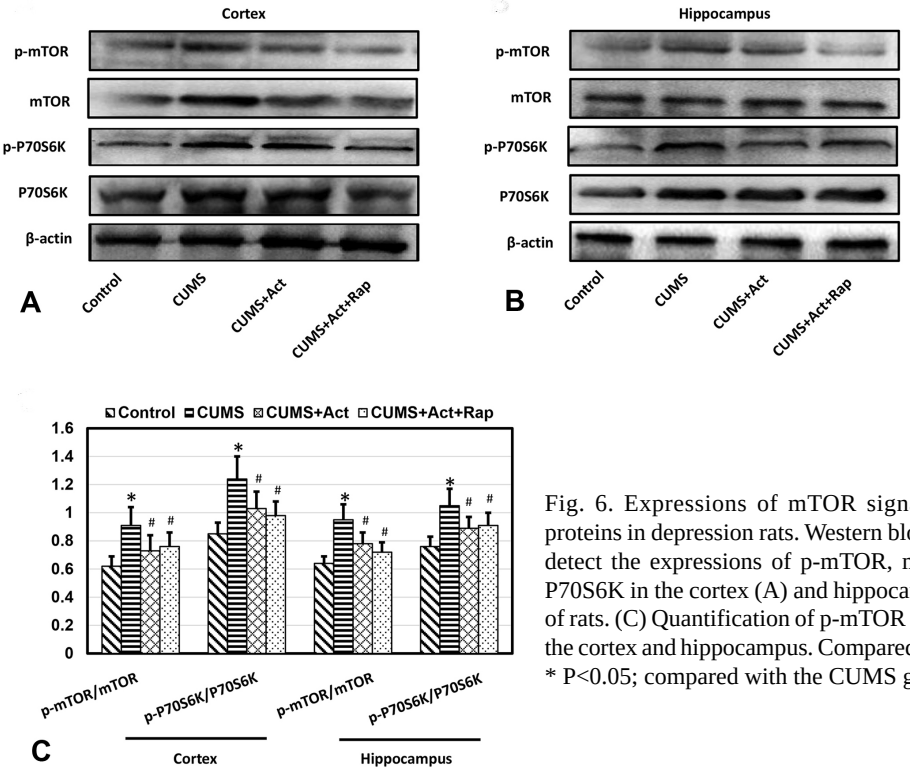

Fig. 6. Expressions of mTOR signaling pathway-related proteins in depression rats. Western blotting was performed to detect the expressions of p-mTOR, mTOR, p-P70S6K, and P70S6K in the cortex (A) and hippocampus (B) in each group of rats. (C) Quantification of p-mTOR and p-P70S6K levels in the cortex and hippocampus. Compared with the control group, * P<0.05; compared with the CUMS group, #P<0.05.

DISCUSSION

During CUMS, experimental animals are separated from their companions, and subjected to a variety of unpredictable mild stresses for a long time, realistically simulating various unexpected difficulties that people would face independently in daily life (Xiang *et al*., 2020). CUMS can simulate symptoms of depression, including motor ability, exploratory behavior, and decreased social communication ability (Qiao *et al*., 2016). Therefore, it has been generally believed that the CUMS depression model is highly effective and is recognized as one of the widely used depression models. In this study, we confirmed that CUMS could indeed induce depression-like behaviors in the rats via the FS test and SP test, indicating the successful establishment of the depression model.

The Y-maze and NOR behavioral tests have been widely used to assess animal learning and memory functions, the reliabilities of which have also been confirmed (Lu *et al*., 2019; Luvuno *et al*., 2020). Our previous study has shown that acteoside could improve depression-like behaviors in rats (Deng *et al*., 2018). In addition to depression-like behavior, cognitive impairment represents another important symptom induced by stress (Li *et al*., 2016). In this study, the learning and memory functions of CUMS depression rats were detected with the Y-maze and NOR tests. Our results showed that rats in the CUMS group had a significant decrease in the alternation percentage than those in the control group. Moreover, rats in the CUMS group had a significantly decreased recognition index compared with the control rats. These results indicate that CUMS could impair the learning and memory functions of model rats.

Acteoside, one of the main active components in the Genus acteoside, has been reported to reduce blood glucose and lipid levels and exert anti-aging and anti-tumor effects (Shiao *et al*., 2017). In the present study, our results showed that acteoside administration significantly improved the learning and memory functions of CUMS depression rats. The effects were similar to those of fluoxetine, which is a widely used antidepressant (Hojo *et al*., 2017) and was used as a positive control drug herein.

Synaptic plasticity is the neurobiological basis of learning and memory, and the morphology and number of neurons are the important basis to measure synaptic plasticity (Duman *et al*., 2016). Meanwhile, the cortex and hippocampus play crucial roles in the development of cognitive abilities and the regulation of emotions (Ge *et al*., 2021). In the present study, we investigated the effect of acteoside on the morphology of neurons in the cortex and hippocampus of CUMS rats. The results indicated that acteoside, to some extent, could repair the neuronal morphology. Subsequently, we found that acteoside could enhance synaptic plasticity by up-regulating the levels of BDNF and CREB.

BDNF, a marker for new synapses, is associated with the promotion of neuronal survival, development, growth, and differentiation, as well as the regulation of synaptic plasticity and nerve regeneration (Kowian´ski *et al*., 2018). It has been shown that the decreased BDNF expression levels reduced learning and memory functions (El Hayek *et al*., 2019). BDNF binds to its specific receptor TrkB, which induces the coupling and self-phosphorylation of TrkB. Meanwhile, phosphorylated TrkB is known to activate the transcription factor CREB, further activating BDNF expression (Nadimi *et al*., 2020). In this study, acteoside was first shown to affect learning and memory, implying its positive role in the regulation of the BDNF signaling pathway. Subsequently, immunofluorescence staining and western blotting results revealed that acteoside treatment upregulated the expressions of BDNF and CREB in the CUMS rats, confirming the activation of the BDNF signaling pathway.

It is worth exploring how acteoside stimulates the BDNF signaling pathway and improves synaptic plasticity. The mTOR signaling is involved in a variety of pathological and physiological processes (Blackwell *et al*., 2019). In addition to protein kinase functions, mTOR is an important signal transduction molecule that plays a key role in the formation and development of the brain, and the generation of synapses (Carabulea *et al*., 2023). P70S6K is a major downstream effector of Mtor (Eda *et al*., 2022). However, whether the activation of the mTOR/P70S6K signaling pathway plays a neuroprotective role or aggravates nerve tissue injury in brain injury remains controversial. It has been reported that selective activation of mTOR could improve learning and memory (Chen *et al*., 2019a; Tian *et al*., 2020). However, other studies have shown that memory deficits could be alleviated by inhibiting the mTOR signaling pathway (Chen *et al*., 2018; Pascual *et al*., 2021). Given that mTOR is both the upstream signal and the downstream signal of the BDNF pathway (Pascual *et al*., 2021; Wang *et al*., 2021b), the interaction between the mTOR signaling and the BDNF pathway is involved in synaptic plasticity, affecting learning and memory. In this study, we found that proteins of the mTOR signaling were decreased in the CUMS rats treated with acteoside, implying the possibility that acteoside suppresses the mTOR signaling. Therefore, it can be concluded that the effect of acteoside on synaptic plasticity may be closely related to the inhibition of the mTOR signaling pathway.

CONCLUSION

In conclusion, our results demonstrated that acteoside attenuated the CUMS-induced memory impairment in CUMS depression model rats. These effects could be attributed to improved neuron structure in the cortex and hippocampus regions. The potential mechanism may be the improvement of synaptic plasticity by inhibiting mTOR signaling. Our study suggests that acteoside may become a promising new reagent for both the prevention and the treatment of depression.

Ethics Approval. This study was conducted in strict accordance with the recommendations of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The research protocol with animal experimentation was approved by the Animal Care and Use Committee of the Luohe Medical College.

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RESUMEN: Evaluamos el papel y el mecanismo del acteoside en la regulación del deterioro de la memoria inducido por estrés leve crónico impredecible (ELCI). Se utilizó ELCI para inducir depresión en ratas y el establecimiento exitoso del modelo ELCI se verificó mediante una prueba de natación forzada y una prueba de preferencia de sacarosa. La prueba del laberinto en Y y la prueba de reconocimiento de objetos novedosos evaluaron las funciones de la memoria. Los cambios estructurales en la corteza y el hipocampo se observaron mediante tinción con hematoxilina y eosina (HE). La tinción por inmunofluorescencia y la transferencia Western determinaron los niveles de proteína. La prueba del laberinto en Y y la prueba de reconocimiento de objetos novedosos mostraron que había un deterioro del rendimiento de la memoria en ratas del grupo ELCI, que mejoró con el tratamiento con acteósidos. La tinción con HE mostró que la exposición a ELCI dañó la estructura de la corteza y el hipocampo, mientras que el tratamiento con actósidos alivió los cambios estructurales. En comparación con el grupo de control, los niveles de BNDF y CREB en la corteza y el hipocampo del grupo ELCI disminuyeron significativamente. Acteoside revirtió significativamente las expresiones de estas proteínas en ratas ELCI. Mientras tanto, en comparación con el grupo control, los niveles de p-mTOR y p-P70S6K en la corteza y el hipocampo del grupo ELCI aumentaron significativamente, y estos cambios fueron revertidos significativamente ELCI por el acteoside. Sin embargo, el efecto del acteoside sobre la señalización de mTOR fue notablemente bloqueado por la rapamicina, un inhibidor específico de la señalización de mTOR. El acteoside puede atenuar el deterioro de la memoria y mejorar el daño neuronal y la plasticidad sináptica en ratas con depresión, probablemente mediante la inhibición de la vía de señalización mTOR. Acteoside puede servir como un reactivo novedoso para la prevención de la depresión.

PALABRAS CLAVE: Acteoside; Estrés leve crónico impredecible (ELCI); Depresión; Deterioro de la memoria. Plasticidad sinaptica; Señalización mTOR.

REFERENCES

Alqurashi, G. K.; Hindi, E. A.; Zayed, M. A.; Abd El-Aziz, G. S.; Alturkistani, H. A.; Ibrahim, R. F.; Al-Thepyani, M. A.; Bakhlgi, R.; Alzahrani, N. A.; Ashraf, G. M.; *et al*. The impact of chronic unpredictable mild stress-induced depression on spatial, recognition and reference memory tasks in mice: behavioral and histological study. *Behav. Sci. (Basel), 12(6)*:166, 2022.

Blackwell, T. K.; Sewell, A. K.; Wu, Z. & Han, M. TOR signaling in Caenorhabditis elegans development, metabolism, and aging. *Genetics, 213(2)*:329-60, 2019.

Carabulea, A. L.; Janeski, J. D.; Naik, V. D.; Chen, K.; Mor, G. & Ramadoss, J. A multi-organ analysis of the role of mTOR in fetal alcohol spectrum disorders. *FASEB J., 37(5)*:e22897, 2023.

Cheimonidi, C.; Samara, P.; Polychronopoulos, P.; Tsakiri, E. N.; Nikou, T.; Myrianthopoulos, V.; Sakellaropoulos, T.; Zoumpourlis, V.; Mikros, E.; Papassideri, I.; *et al*. Selective cytotoxicity of the herbal substance acteoside against tumor cells and its mechanistic insights. *Redox Biol., 16*:169-78, 2018.

Chen, K.; Zheng, Y.; Wei, J. A.; Ouyang, H.; Huang, X.; Zhang, F.; Lai, C. S. W.; Ren, C.; So, K. F. & Zhang, L. Exercise training improves motor skill learning via selective activation of mTOR. *Sci. Adv., 5(7)*:eaaw1888, 2019a.

Chen, L.; Yao, H.; Chen, X.; Wang, Z.; Xiang, Y.; Xia, J.; Liu, Y. & Wang, Y. Ginsenoside Rg1 decreases oxidative stress and down-regulates Akt/mTOR signalling to attenuate cognitive impairment in mice and senescence of neural stem cells induced by D-Galactose. *Neurochem. Res., 43(2)*:430-40, 2018.

Chen, Q.; Xi, X.; Zeng, Y.; He, Z.; Zhao, J. & Li, Y. Acteoside inhibits autophagic apoptosis of retinal ganglion cells to rescue glaucoma-induced optic atrophy. *J. Cell. Biochem., 120(8)*:13133-40, 2019b.

Cipriani, A.; Furukawa, T. A.; Salanti, G.; Chaimani, A.; Atkinson, L. Z.; Ogawa, Y.; Leucht, S.; Ruhe, H. G.; Turner, E. H.; Higgins, J. P. T.; *et al*. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *Lancet, 391(10128)*:1357-66, 2018.

Cuijpers, P.; Quero, S.; Dowrick, C. & Arroll, B. Psychological treatment of depression in primary care: recent developments. *Curr. Psychiatry Rep., 21(12)*:129, 2019.

Deng, H.; Sun, M.; Wu, Q. & Chang, Q. Acteoside improves depression-like behavior in CUMS rats by regulating the BDNF-TrkB signaling pathway. *Chin. J. Pathophysiol., 34(09)*:1633-7, 2018.

Diering, G. H. & Huganir, R. L. The AMPA receptor code of synaptic plasticity. Neuron, 100(2):314-29, 2018.

Dobrek, L. & Glowacka, K. Depression and its phytopharmacotherapy-A narrative review. *Int. J. Mol. Sci., 24(5)*:4772, 2023.

Duman, R. S.; Aghajanian, G. K.; Sanacora, G. & Krystal, J. H. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat. Med., 22(3)*:238-49, 2016.

Eda, T.; Okada, M.; Ogura, R.; Tsukamoto, Y.; Kanemaru, Y.; Watanabe, J.; On, J.; Aoki, H.; Oishi, M.; Takei, N.; *et al*. Novel repositioning therapy for drug-resistant glioblastoma: in vivo validation study of clindamycin treatment targeting the mTOR pathway and combination therapy with temozolomide. *Cancers (Basel), 14(3)*:770, 2022.

El Hayek, L.; Khalifeh, M.; Zibara, V.; Abi Assaad, R.; Emmanuel, N.; Karnib, N.; El-Ghandour, R.; Nasrallah, P.; Bilen, M.; Ibrahim, P.; *et al*. Lactate mediates the effects of exercise on learning and memory through SIRT1-dependent activation of hippocampal Brain-Derived Neurotrophic Factor (BDNF). *J. Neurosci., 39(13)*:2369-82, 2019.

Fuchsberger, T. & Paulsen, O. Modulation of hippocampal plasticity in learning and memory. *Curr. Opin. Neurobiol., 75*:102558, 2022.

Gan, L.; Li, X.; Zhu, M.; Chen, C.; Luo, H. & Zhou, Q. Acteoside relieves mesangial cell injury by regulating Th22 cell chemotaxis and proliferation in IgA nephropathy. *Ren. Fail., 40(1)*:364-70, 2018.

Ge, X.; Guo, F.; Fan, J.; Chen, B. T.; Yu, L.; Ren, J.; Li, J. Q.; Lu, C. L.; Mo, J. W.; Li, S. J.; *et al*. Chaihu Guizhi decoction produces antidepressant-like effects via sirt1-p53 signaling pathway. *Nan Fang Yi Ke Da Xue Xue Bao, 41(3)*:399-405, 2021.

Guo, Z.; Ruan, Z.; Zhang, D.; Liu, X.; Hou, L. & Wang, Q. Rotenone impairs learning and memory in mice through microglia-mediated blood brain barrier disruption and neuronal apoptosis. *Chemosphere, 291(Pt. 2)*:132982, 2022.

Hojo, M.; Nagahara, A.; Asaoka, D.; Shimada, Y.; Sasaki, H.; Matsumoto, K.; Takeda, T.; Ueyama, H.; Matsumoto, K. & Watanabe, S. A Systematic review of the effectiveness of antianxiety and antidepressive agents for functional dyspepsia. *Intern. Med., 56(23)*:3127-33, 2017.

Johnson, S. C.; Yanos, M. E.; Kayser, E. B.; Quintana, A.; Sangesland, M.; Castanza, A.; Uhde, L.; Hui, J.; Wall, V. Z.; Gagnidze, A.; *et al*. mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. *Science, 342(6165)*:1524-8, 2013.

Kolovos, S.; Bosmans, J. E.; Riper, H.; Chevreul, K.; Coupé, V. M. H. & van Tulder, M. W. Model-based economic evaluation of treatments for depression: a systematic literature review. *Pharmacoecon. Open, 1(3)*:149-65, 2017.

Kowian´ski, P.; Lietzau, G.; Czuba, E.; Was´kow, M.; Steliga, A. & Morys´, J. BDNF: a key factor with multipotent impact on brain signaling and synaptic plasticity. *Cell. Mol. Neurobiol., 38(3)*:579-93, 2018.

Li, E.; Deng, H.; Wang, B.; Fu, W.; You, Y. & Tian, S. Apelin-13 exerts antidepressant-like and recognition memory improving activities in stressed rats. *Eur. Neuropsychopharmacol., 26(3)*:420-30, 2016.

Li, M.; Zhou, F.; Xu, T.; Song, H. & Lu, B. Acteoside protects against 6-OHDA-induced dopaminergic neuron damage via Nrf2-ARE signaling pathway. *Food Chem. Toxicol., 119*:6-13, 2018.

Lim, D. W.; Han, D. & Lee, C. Pedicularis resupinata extract prevents depressive-like behavior in repeated corticosterone-induced depression in mice: a preliminary study. *Molecules, 27(11)*:3434, 2022.

Lu, Y.; Sareddy, G. R.; Wang, J.; Wang, R.; Li, Y.; Dong, Y.; Zhang, Q.; Liu, J.; O'Connor, J. C.; Xu, J.; *et al*. Neuron-derived estrogen regulates synaptic plasticity and memory. *J. Neurosci., 39(15)*:2792-809, 2019.

Luvuno, M.; Khathi, A. & Mabandla, M. V. The effects of exercise treatment on learning and memory ability, and cognitive performance in diet-induced prediabetes animals. *Sci. Rep., 10(1)*:15048, 2020.

Nadimi, H.; Djazayery, A.; Javanbakht, M. H.; Dehpour, A.; Ghaedi, E.; Derakhshanian, H.; Mohammadi, H.; Mousavi, S. N. & Djalali, M. Effect of vitamin D supplementation on CREB-TrkB-BDNF pathway in the hippocampus of diabetic rats. *Iran. J. Basic Med. Sci., 23(1)*:117-23, 2020.

Pascual, M.; Lopez-Hidalgo, R.; Montagud-Romero, S.; Urena-Peralta, J.R.; Rodriguez-Arias, M. & Guerri, C. Role of mTOR-regulated autophagy in spine pruning defects and memory impairments induced by binge-like ethanol treatment in adolescent mice. *Brain Pathol., 31(1)*:174-88, 2021.

Qiao, H.; Li, M. X.; Xu, C.; Chen, H. B.; An, S. C. & Ma, X. M. Dendritic spines in depression: what we learned from animal models. *Neural Plast., 2016*:8056370, 2016.

Qu, Y. J.; Ding, M. R.; Gu, C.; Zhang, L. M.; Zhen, R. R.; Chen, J. F.; Hu, B. & An, H. M. Acteoside and ursolic acid synergistically protects H2O2-induced neurotrosis by regulation of AKT/mTOR signalling: from network pharmacology to experimental validation. *Pharm. Biol., 60(1)*:1751-61, 2022.

Saleh, A.; Potter, G. G.; McQuoid, D. R.; Boyd, B.; Turner, R.; MacFall, J. R. & Taylor, W. D. Effects of early life stress on depression, cognitive performance and brain morphology. *Psychol. Med., 47(1)*:171-81, 2017.

Shiao, Y. J.; Su, M. H.; Lin, H. C. & Wu, C. R. Acteoside and isoacteoside protect amyloid b peptide induced cytotoxicity, cognitive deficit and neurochemical disturbances in vitro and in vivo. *Int. J. Mol. Sci., 18(4)*:895, 2017.

Tian, A.; Ma, X.; Li, H. & Zhang, R. Dl-3n-butylphthalide improves spatial learning and memory in rats with vascular dementia by reducing autophagy via regulation of the mTOR signaling pathway. *Exp. Ther. Med., 19(3)*:1940-6, 2020.

Wang, J. Y.; Zhang, Y.; Chen, Y.; Wang, Y.; Li, S. Y.; Wang, Y. F.; Zhang, Z. X.; Zhang, J. & Rong, P. Mechanisms underlying antidepressant effect of transcutaneous auricular vagus nerve stimulation on CUMS model rats based on hippocampal alpha7nAchR/NF-kappaB signal pathway. *J. Neuroinflammation, 18(1)*:291, 2021a.

Wang, Y.; Fu, A. K. Y. & Ip, N. Y. Instructive roles of astrocytes in hippocampal synaptic plasticity: neuronal activity-dependent regulatory mechanisms. *FEBS J., 289(8)*:2202-18, 2022.

Wang, Y.; Wang, Q.; Chen, J.; Yao, L. H.; Tang, N.; Jiang, Z. X. & Luo, Y. Protective effect of hydroxysafflor yellow a against chronic mild stress-induced memory impairments by suppressing Tau phosphorylation in mice. *Curr. Med. Sci., 41(3)*:555-64, 2021b.

Xiang, D.; Xiao, J.; Sun, S.; Fu, L.; Yao, L.; Wang, G. & Liu, Z. Differential regulation of DNA methylation at the CRMP2 promoter region between the hippocampus and prefrontal cortex in a CUMS depression model. *Front. Psychiatry, 11*:141, 2020.

Yan, L.; Xu, X.; He, Z.; Wang, S.; Zhao, L.; Qiu, J.; Wang, D.; Gong, Z.; Qiu, X. & Huang, H. Antidepressant-like effects and cognitive enhancement of coadministration of Chaihu Shugan San and Fluoxetine: dependent on the BDNF-ERK-CREB signaling pathway in the hippocampus and frontal cortex. *Biomed. Res. Int., 2020*:2794263, 2020.

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