JEBHM Journal of Evidence-Based Herbal Medicine

Research Article

Effects of ethanolic extract of *Eclipta prostrata* L. on cognitive dysfunction of MK-801-induced schizophrenia model in mice

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Abstract: Schizophrenia is a chronic and severe mental disorder with a prevalence of approximately 1% worldwide. Schizophrenia is a heterogeneous disease with positive symptoms (hallucinations and delusions), negative symptoms (lack of sociability, depression, and apathy), and cognitive impairment. In the present study, we investigated whether the ethanolic extract of *Eclipta prostrata* L. (EEEP) ameliorates the cognitive impairment on MK-801-induced schizophrenia animal model. EEEP (50, 100 mg/kg, p.o.) and clozapine (1 mg/kg, i.p.) was administrated 1 h before the test. MK-801 (0.2 mg/kg, i.p.) was administrated 30 min before the test to induce schizophrenia-like behaviors in mice. EEEP (50, 100 mg/kg, p.o.) significantly ameliorated MK-801-induced cognitive dysfunction in a novel object recognition test. In addition, EEEP (100 mg/kg) alleviated the impaired prepulse inhibition (PPI) induced by MK-801 in the acoustic startle response test. These behavioral changes may be related with normalized ERK signaling in prefrontal cortex of MK-801 treated mice. These results suggested that EEEP might be contributed to as a treatment for schizophrenia, especially in ameliorating cognitive dysfunction.

Keywords: Schizophrenia, Cognitive impairment, Eclipta prostrata L., MK-801, Clozapine

1. Introduction

Schizophrenia is a chronic and multi-complex psychiatric disorder with a worldwide incidence of approximately 0.5-1% [1]. Positive, negative symptoms and cognitive dysfunction characterized in schizophrenia. Positive symptoms, including delusions and hallucinations, are caused by difficulty in recognizing reality. In contrast, negative symptoms including lack of social interaction develop into depression and anhedonia [2, 3]. Cognitive dysfunction is one of the core symptoms chronically present in patients with schizophrenia and has received increasing attention as it is associated with the pathophysiology of schizophrenia [4, 5]. Cognitive dysfunction is an essential part of treating of schizophrenia because it is characterized by difficulty concentrating and memory problems [6]. Other than positive or negative symptoms of schizophrenia, cognitive dysfunction has suggested predominant and longitudinal observation in schizophrenia patients [7]. Additionally, Kraepelin who first coined schizophrenia as 'dementia praecox', defined schizophrenia from other psychiatric disorders, such as bipolar disorder, and characterized cognitive impairment as the disease progressed in schizophrenia patients [8, 9]. Therefore, in recent years, many researchers have suggested that cognitive impairment may play a key role in the development and treatment of schizophrenia, with studies led by the National Institutes of Health (NIH) [10].

Typical and atypical anti-psychotics, which are currently used in clinical practice, are known to be effective for the positive symptoms of schizophrenia (delusion, hallucination, auditory disturbance, etc.) and negative symptoms (anhedonia, social withdrawal, disorganized behavior, etc.). However, they have reported limited effects on cognitive function [11]. Furthermore, side effects such as metabolism syndrome, intestinal obstruction, and epilepsy have been reported, along with a 1-2% probability of agranulocytosis [3, 11-13]. In addition, only about 40-50% of patients who taking anti-psychotic medication demonstrate efficacy and 10-30% of patients exhibit incomplete drug responses [14]. Therefore, growing needs for developing novel treatment options that are not only effective for various symptoms of schizophrenia but also have fewer side effects and improved drug resistance.

Eclipta prostrata Linné, belonging to the Asteraceae family, is a plant that is widely grown mainly in tropical and subtropical regions. In Korea, it grows in the central area and southern parts of Jeju. Traditionally, *E. prostrata* has been used for its protective effects on the kidneys and liver, and anti-inflammatory activities have been recognized [15]. In addition, EEEP has been shown to be effective on cognitive dysfunction in animal models of scopolamine-induced

memory impairment using the passive avoidance test (PAT), Y-maze test (YMT), and Morris water maze (MWM) test [16]. However, the effects of EEEP in an animal model of MK-801-induced schizophrenia are unknown. If EEEP could improve cognitive dysfunction in the MK-801, N-Methyl-D-aspartate (NMDA) receptor antagonist, -induced schizophrenia-like behaviors in mice, it could be potential candidate treatment for schizophrenia. Therefore, in this study, we aimed to examine the effect of EEEP in MK-801-induced schizophrenia-like behavior in mice by performing the novel object recognition test, the acoustic startle response test, open field test and identifying related signaling transduction.

2. Materials and Methods

2.1. Animals

Male ICR mice (5 weeks old, weighing 25-27 g) were obtained from Orient Bio Inc. (Gapyeong Korea) and were housed in groups of four per cage at the Animal Center of Kangwon National University. They were fed animal solid chow (2018S; Envigo, Madison, WI). During the experimental period, animals were maintained at a room temperature of 23 ± 2 ℃, with a humidity level of 40 ± 10%, and a 12-hour light-dark cycle (06:00-18:00) was maintained. All animal experiments were approved by the Institutional Animal Care and Use Committee of Kangwon National University (KW-211115-1).

2.2. Materials

MK-801 (dizocilpine) and clozapine (CLZ) were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO). Anti-ERK and anti-phosphorylated ERK (pERK) antibodies were obtained from Cell Signaling Technology Inc. (Danvers, MA). Other reagents not mentioned were of the highest available grade and were purchased from commercial sources. Dried Leaf E. prostrata was finely ground and mixed with 1000 mL of 70% ethanol per 100 g of E. prostrata and then ultrasonically extracted twice at 70 ℃ for 2 h. After the extraction, the solution was vacuum-filtered and concentrated using a rotary evaporator. The concentrated EEEP was freeze-dried and stored at -20 ℃. The yield of the extract was 15.71 ± 0.05%.

2.3. Treatment

MK-801 and EEEP were dissolved in 0.9% saline solution. Clozapine, dopamine D2 receptor antagonist as a the positive control substance, was dissolved on 10 mL of 0.9% saline containing 10 μL of HCl. EEEP (50 or 100 mg/kg) was administered per oral (p.o.) to the animals, and clozapine (1 mg/kg) was administered intraperitoneally (i.p.) 1 h before the behavioral experiments [17-20]. Thirty min after the sample treatments, MK-801 (0.2 mg/kg, i.p.) was administered to induce schizophrenia-like behaviors in mice. The experimental groups were classified as shown in Table 1. The dose of EEEP was based on data from previous studies [16]. The administration route of MK-801, clozapine and EEEP were decided by the previous studies.

Table 1. Information of groups

Group		Administration		
Control	(n=8)	0.9 % Saline (p.o.)		
MK-801 only	(n=8)	EEEP 0 mg/kg (p.o)	+	MK-801 0.2 mg/kg (i.p.)
EEEP (50 mg/kg)	(n=8)	EEEP 50 mg/kg (p.o)	+	MK-801 0.2 mg/kg (i.p.)
EEEP (100 mg/kg)	(n=8)	EEEP 100 mg/kg (p.o)	+	MK-801 0.2 mg/kg (i.p.)
Clozapine	(n=8)	Clozapine (CLZ) 1 mg/kg (i.p.)	+	MK-801 0.2 mg/kg (i.p.)

2.4. Novel object recognition test

To acclimate the animals to the testing environment, they were allowed to freely explore a black acryl box measuring 40 × 40 × 40 cm^3 for 10 min per day over a period of 2 days. After 24 h, two identical objects were placed inside the box, and the animals were given 10 min to explore them. After 2 h, one of the objects was replaced with a novel object that was different in shape from the familiar object and allowed to be explored again for 5 min. The exploration time for both the familiar and novel objects was measured, and the exploration time ratio, discrimination index, and total exploration time were calculated. The discrimination index was calculated as follows; Discrimination index (%) = [(Tnovel - Tfamiliar) / (Tnovel + Tfamiliar)] × 100

2.5. Acoustic startle response test

The acoustic startle response test was conducted using the SR-LAB startle chamber (San Diego Instrument, San Diego, CA). A platform is placed inside a ventilated startle chamber. On top of the platform, a Plexiglas cylinder, which served as a confinement device for the animals, is placed. When the animals were startled by the sound stimulus, their startle response, manifested as whole-body tremors, caused vibrations in the confinement device. These vibrations were digitized using a computer program. A high-frequency speaker was installed inside the chamber to deliver various auditory stimuli to the animals or provide a continuous background noise of 70 dB. The animals were placed within the confinement device and acclimated for 5 min at 70 dB, followed by the presentation of a 120 dB auditory stimulus in 5 consecutive trials 40 ms each. To measure the response of the animals to auditory stimuli, various sound stimuli of 80, 90, 100, 110 or 120 were presented and response to each stimulus was measured. Subsequently, the animals' responses were measured under three different conditions: no stimulus was presented (no stimulus), only a 120 dB sound was presented (pulse alone) and a pre-stimulus was presented followed by a 120 dB sound after a 100 ms interval (prepulse + pulse). After measuring the startle response of the animals to each stimulus as described above, we calculated the pre-stimulus suppression rate by measuring the ratio of the response to the 120 dB stimulus alone and the 120 dB after the pre-stimulus. The percentage of prepulse inhibition was calculated as followed; Prepulse inhibition (PPI; %) = 100 - [(prepulse + pulse) / (pulse alone)] × 100

2.6. Open field test

To measure spontaneous activity, the animals were allowed to freely explore in a black acrylic box of 40 × 40 × 40 cm^3 for 30 min. The distance moved and total distance moved were determined every 5 min using the video tracking program viewer 3.0 software (Biobserve, Bonn, Germany).

2.7. Western blot analysis

Thirty min after administering EEEP (100 mg/kg, p.o.) followed by MK-801 (0.2 mg/kg, i.p.), the prefrontal cortex of the brain tissues were isolated. The tissues were homogenized in a cold lysis buffer containing protease and phosphatase inhibitors. Thereafter, crude proteins from homogeneous were collected by centrifugation at 15,000 rpm for 30 min at -4 ℃. The protein samples were prepared to contain 20 μg of protein and separated by electrophoresis on a 10% SDS PAGE gel. Subsequently, the proteins were transferred onto a PVDF membrane at 190 mA for 70 min. To prevent non-specific binding of antibodies, blocking was performed with 5% skim milk at room temperature for 2 h. The samples were then treated with primary antibody (ERK, 1:1000; pERK, 1:1000) and incubated overnight at 4 ℃. Subsequently, the samples were incubated with a secondary antibody conjugated with horseradish peroxidase (HRP) at room temperature for 2 h. To enhance chemiluminescent signal, the membrane was thoroughly washed with tris-buffered saline/tween 20 (TBS-T) after each step. After that, the membrane was incubated with ECL western blotting reagent and the bands were detected and visualized using the bio-imaging program of LAS-500 imager (GE Healthcare Life Science, Marlborough, MA). Quantification of the bands was performed using Image J software (LOCI, University of Wisconsin, Madison, WI, USA). The level of phosphorylation was calculated as the ratio of phosphorylated protein to total protein on the same membrane.

2.8. Data analysis and statistics

All experimental values are expressed as means ± standard error of the means (S.E.M.). Discrimination index in the novel object recognition test, the total distance moved in the open field test, and Western blot data were compared among groups using one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls test for *post hoc* analysis of significant differences between experimental groups. The exploration time ratio in the novel object recognition test and acoustic startle amplitude and PPI of acoustic startle response test were analyzed using two-way ANOVA,

followed by Bonferroni test for post hoc comparisons and analysis of the experimental values. Statistical significance was considered when the p-value was equal to or less than 0.05.

3. Results

3.1. EEEP ameliorated MK-801-induced cognitive dysfunctions in mice

Cognitive impairments such as decreased attention, spatial and long-term memory deficits in animal models of schizophrenia that can mimic cognitive dysfunction observed in patients with schizophrenia [21, 22]. Therefore, we carried out to evaluate whether EEEP (50 or 100 mg/kg) could ameliorate MK-801-induced cognitive dysfunction on the novel object recognition test. There were significant differences between groups in the object exploration ratio (two-way ANOVA, treatment, $F_{4, 66} = 0$, $p > 0.05$; object, $F_{1, 66} = 66.2$, $p < 0.001$; interaction treatment x object, $F_{4, 66} = 12.4$, $p < 0.001$, Fig. 1A) and discrimination ratio (one-way ANOVA, $F_{4, 33} = 6.223$, $p < 0.001$, Fig. 1B). We observed the control group preferred novel object more than familiar object. However, the MK-801-treated group did not differ in the ratio of exploration time between the two objects, which means that cognitive function was impaired. In comparison to the MK-801-treated group, the administration of EEEP (50 or 100 mg/kg) and clozapine (1 mg/kg) group significantly improved the preference for the novel object (Fig. 1A). Similarly, the discrimination index decreased by MK-801 was significantly increased by EEEP and clozapine (Fig. 1B). Therefore, our findings suggest that EEEP ameliorates cognitive functions on MK-801-induced schizophrenia-like behavior in mice.

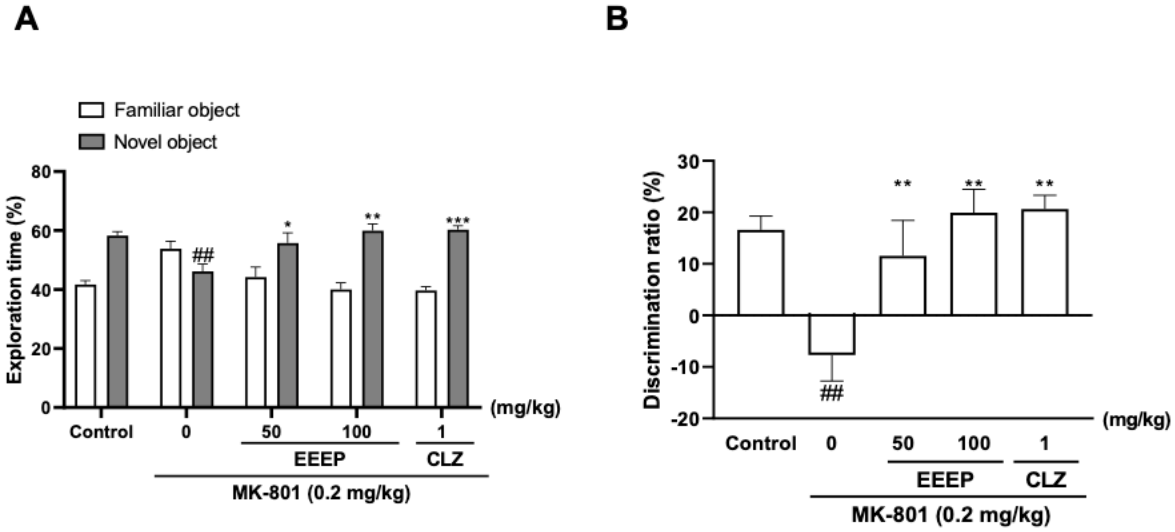

Figure 1. Effects of EEEP on the MK-801-induced objects recognition impairments in the novel object recognition test. (A) the percentage of exploration time spent on the novel or the familiar objects, (B) the rate of discrimination ratio. Data represent Means ± S.E.M (n=7-8 / Group) (A) $^{\#\#}P < 0.01$ versus the control group, $^{*}P < 0.05$, $^{**}P < 0.01$ versus the MK-801-treated group (two-way ANOVA), (B) $^{\#\#}P < 0.01$ versus the control group, $^{**}P < 0.01$ versus the MK-801-treated group. EEEP, ethanolic extract of *Eclipta prostrata* L.; CLZ, clozapine.

3.2. EEEP ameliorated MK-801-induced prepulse inhibition deficits in mice

Prepulse inhibition (PPI) is the suppression of the startle response when a pre-stimulus is given compared to the main stimulus alone, which reflect on sensorimotor gating and it is known to be impaired in schizophrenia patient [3, 21]. In animal models, administration of MK-801 has been shown to robust startle responses and impair prepulse inhibition [21, 23]. Based on these findings, we aimed to investigate whether EEEP could ameliorate sensorimotor deficits and prepulse inhibition induced by MK-801 in the acoustic startle response test.

Two-way ANOVA revealed significant group effects on the startle amplitude (treatment, $F_{3,140} = 41.9$, $p < 0.001$; pulse intensity, $F_{4,140} = 123$, $p < 0.001$; interaction treatment x pulse intensity, $F_{12,140} = 4.11$, $p < 0.001$, Fig. 2A) and PPI (treatment, $F_{3,112} = 95.8$, $p < 0.001$; prepulse intensity, $F_{3,112} = 68.9$, $p < 0.001$; interaction treatment x prepulse intensity, $F_{9,112} = 11.8$, $p < 0.001$, Fig. 2B). We observed significantly increased amplitudes in startle responses at 90, 110, 120

dB compared to the control group following administration of MK-801, indicating abnormal startle responses. The clozapine group significantly ameliorated the abnormally increased amplitude. At the same time, there was no effect by EEEP treatment (Fig. 2A). These results suggest that sensorimotor deficits induced by MK-801 resulted in inappropriate responses to environmental stimuli. In PPI test, when a prepulse higher than the background level of 70 dB by 3, 6, 12, 16 dB was provided followed by a maximal 120 dB level stimulus, the MK-801-treated group exhibited a significant reduction in PPI compared to the control group (Fig 2B). This indicates that the prepulse inhibited the control group's response to the main stimulus, while the MK-801-treated group remained sensitive to the stimulus despite the prepulse. The PPI reduction by MK-801 was significantly enhanced by EEEP (100 mg/kg) and clozapine (1 mg/kg) at 82 and 86 dB. This finding suggests the potential to ameliorate deficient PPI in individuals with schizophrenia.

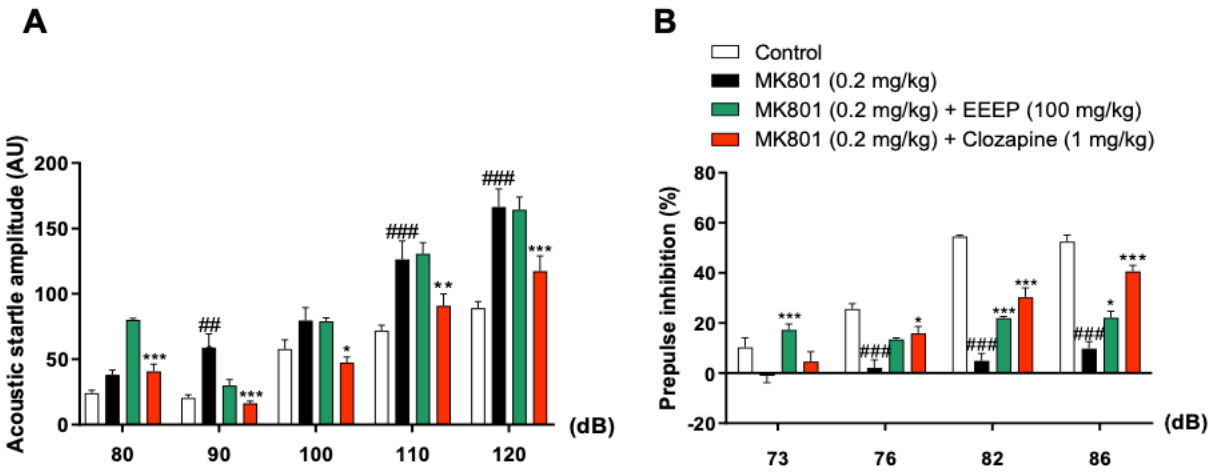

Figure 2. Effects of EEEP on the MK-801-induced prepulse inhibition deficits in the acoustic startle response test. (A) acoustic startle amplitude, (B) the percentage of prepulse inhibition. Data represent Means ± S.E.M (n=7-8 / Group). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the MK-801-treated group. EEEP, ethanolic extract of *Eclipta prostrata* L.; CLZ, clozapine.

3.3. EEEP ameliorated MK-801-induced hyperactivity in mice

In animal models of schizophrenia, hyperactivity represents the positive symptoms of schizophrenia [21]. Therefore, we aimed to investigate the effects of EEEP in an animal model of MK-801-induced hyperactivity. One-way ANOVA revealed significant group effects on total distance moved (treatment, $F_{3,25} = 18.65$, $p < 0.001$, Fig. 3A). The MK-801-treated group increased the total distance moved compared to the control group. Hyperactivity induced by MK-801 was significantly ameliorated by the administration of EEEP (100 mg/kg) and clozapine (1 mg/kg) compared to the MK-801-treated group (Fig. 3A). However, in the analysis of the 5 min interval, there were no significant differences between groups (Fig. 3B).

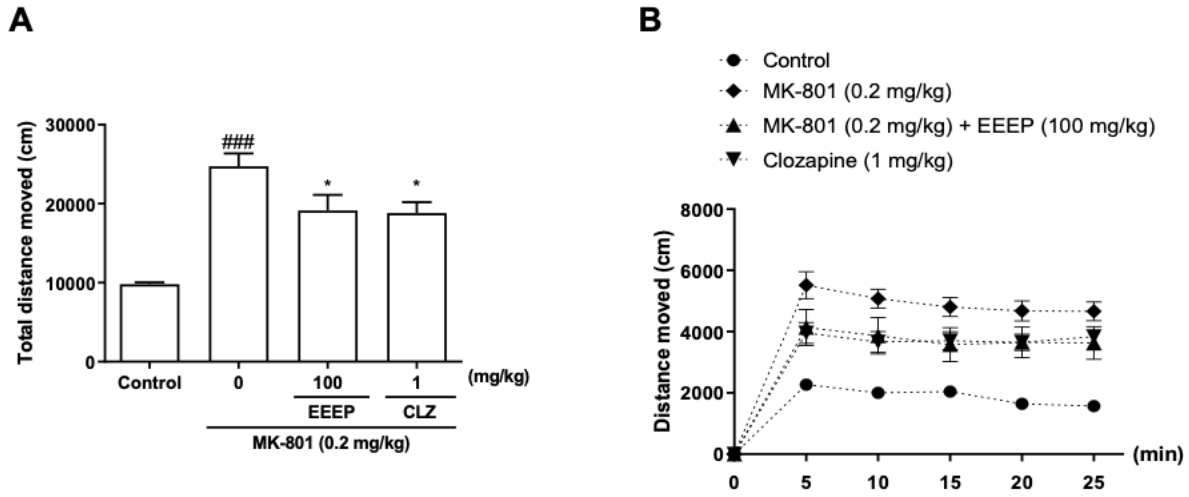

Figure 3. Effects of EEEP on MK-801-induced hyperlocomotion in the open field test. (A) total distance moved, (B) distance moved with 5 min interval. Data represent Means ± S.E.M (n=7-8 / Group) $^{###}P < 0.001$ versus the control group; $^{*}P < 0.05$ versus the MK-801-treated group. EEEP, ethanolic extract of *Eclipta prostrata* L.; CLZ, clozapine.

3.4. EEEP ameliorated MK-801-induced hyperactivity in mice

It has been reported that the increased levels of phosphorylated ERK in the prefrontal cortex are associated with the pathogenesis of schizophrenia [24]. Therefore, in this study, we examined the phosphorylated ERK protein level in the prefrontal cortex after administering MK-801 by Western blot. The MK-801-treated group showed an increase in the phosphorylation level ERK compared to the control group, while the administration of EEEP (100 mg/kg) resulted in a decrease in the phosphorylation level (Fig. 4A). Also, there were significant differences between each group (one way ANOVA, $F_{2,3} = 28.60$, $p < 0.05$) (Fig. 4B). These data suggest the normalized of ERK in prefrontal cortex by EEEP may be related with the behavioral ameliorations.

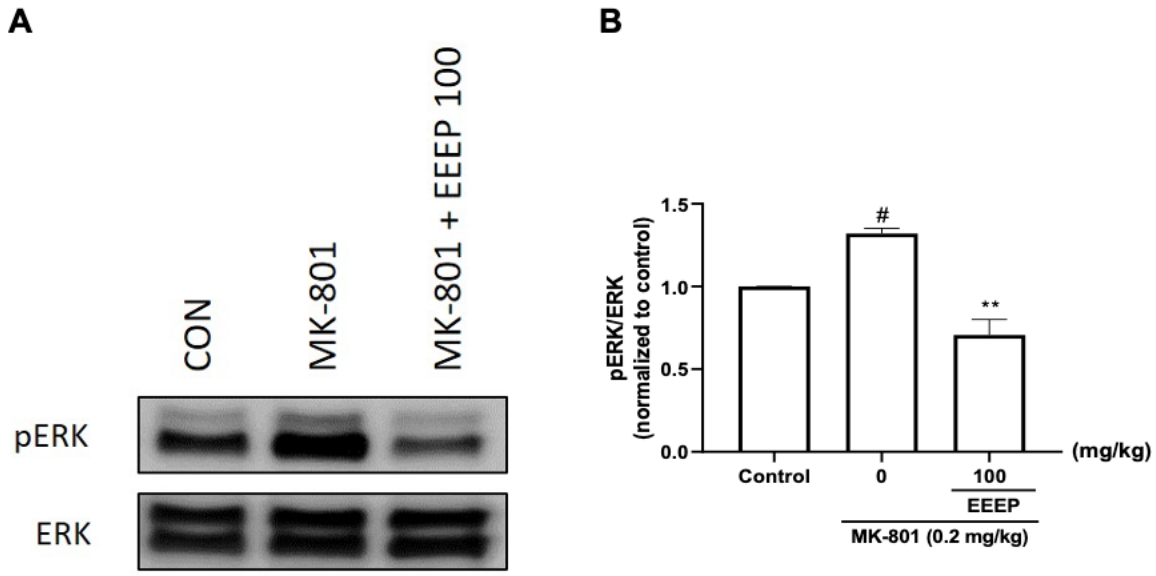

Figure 4. Effects of EEEP on ERK activation in the prefrontal cortex of MK-801-induced mice. (A) immunoblots of pERK and ERK after EEEP 100 mg/kg treatment in MK-801-induced mice, (B) ratio of phosphorylated ERK (pERK) and ERK. Data represent Means ± S.E.M (n=2 / Group) $^{#}P < 0.05$ VS versus the control group; $^{**}P < 0.01$ versus the MK-801-treated group. EEEP, ethanolic extract of *Eclipta prostrata* L.

4. Discussion

In this study, we investigated the effects of EEEP (50 or 100 mg/kg) on schizophrenia-like behavior in the MK-801-induced animal model of schizophrenia. We found that EEEP (50 or 100 mg/kg) significantly ameliorated the discrimination ratio in the novel object recognition test. In addition, EEEP (100 mg/kg) ameliorated the PPI deficit in the acoustic startle response test and the hyperactivity in the open field test. Furthermore, we analyzed the Western blot to investigate EEEP normalized the increase ERK phosphorylation induced by MK-801. Our results suggested the potential effect of EEEP as an anti-psychotics.

Due to increased incidents and accidents related to schizophrenia patients, there has been increasing social interest in curing schizophrenia. Currently, drugs used to treat schizophrenia are effective in improving positive and negative symptoms; however, their effects on cognitive dysfunction are limited, and they can also lead to side effects such as extrapyramidal symptoms and metabolic syndrome [11, 25]. Therefore, the needs for the development of schizophrenia treatments that not only exhibit effective therapeutic effects but also have minimal side effects. *E. prostrata,* which was used in this study, is known to contain various plant bioactive compounds such as flavonoid, phenolic acid, steroid, saponin, and triterpenoid saponin [26, 27]. Of these, bioactive compounds such as saponins have been reported to exhibit various pharmacological anti-Alzheimer's disease, anti-cancer, anti-inflammatory, antioxidant, etc. [28-31]. Furthermore, eclalbasaponin II, which belongs to the group of oleanane-type triterpenoid saponins and is isolated from *E. prostrata*. Eclalbasaponin II has been reported to improve cholinergic blockade-induced cognitive impairments through various behavioral tests, including the passive avoidance test, Y-maze test, and Morris water maze test [27]. However, there have been no studies investigating the effects of *E. prostrata* in the MK-801-induced schizophrenia animal model. Therefore, this study was conducted to evaluate the effects of *E. prostrata*.

In schizophrenia patients, cognitive dysfunction manifests as decreased concentration, impaired spatial, and cognitive abilities [21]. Although the etiology underlying pathogenetic mechanism of schizophrenia have not been clearly elucidated, it is known to be associated with imbalances of various neurotransmitters such as glutamate, dopamine, serotonin, and acetylcholine [6]. Recent research on the etiology of schizophrenia has focused on the hypothesis that impaired function of NMDA receptors may contribute to the development of schizophrenia [32]. Thus, NMDA receptor antagonists such as MK-801, ketamine, and phencyclidine have been used to establish animal models of schizophrenia [3]. In the present study, we found that EEEP improved the cognitive dysfunction induced by MK-801 with dose-dependent manner. These results suggest that EEEP may be considered to have the potential to exhibit therapeutic effects on cognitive and memory impairments.

PPI exhibits inappropriate and acute responses to environmental stimuli, which manifest as sensorimotor impairment and deficits in presynaptic inhibition as observed in schizophrenia [3, 33]. Since these symptoms can be improved through typical and atypical antipsychotics, the acoustic startle response test can be used as a representative for verifying new treatments for schizophrenia [33]. PPI deficits have been reported not only in patients with schizophrenia but also in patients with dementia [34]. Specifically, PPI deficits occur when the production of dopamine, a neurotransmitter involved in sensorimotor function, was suppressed. The balance of neurotransmitter secretion within the brain was closely related to cognitive function [34, 35]. In this study, we observed an increase in startle amplitude with the administration of MK-801, and we observed that the increased startle amplitude induced by MK-801 was reduced with the administration of clozapine. Although the administration of EEEP did not directly reduce startle amplitude, we found that the PPI deficits were significantly ameliorated by EEEP and clozapine. These results suggest the potential effect of EEEP to attenuate sensorimotor gating impairment.

It is well known that hyperactivity in schizophrenia animal models mimic the positive symptoms of schizophrenia patients [36]. We observed a significant increase in total distance moved in the group administered with MK-801 compared to the control group. Furthermore, clozapine, which is used for the treatment of positive symptoms of schizophrenia [37], significantly reduced the increased total distance moved induced by MK-801. Similarly, the administration of EEEP reduced the increased total distance moved. These results suggest that EEEP may contribute to the improvement of positive symptoms in schizophrenia.

In patients with schizophrenia, it has been reported an imbalance of neurotransmitters and an abnormal signal transduction system in neurons [38]. Among them, the expression changes of kinase have been shown to exhibit significant differences between healthy individuals and patients. In mice, MK-801 treatment increased extracellular glutamate levels in the prefrontal cortex and suppressed NMDA receptors, leading to the activation of the ERK signaling pathway [24, 39, 40]. On the other hand, clozapine is known to improve schizophrenia symptoms by inhibiting ERK that

activated by NMDA receptor antagonists in the prefrontal cortex [24, 41]. In this study, EEEP reduced the phosphorylation of ERK, which was increased by MK-801 treatment, suggesting that MK-801-induced behavioral improvement in a schizophrenia-like animal model is associated with changes in ERK activity. The results of this study suggest the potential effects of EEEP to improve cognitive and memory impairments as well as deficits in prepulse inhibition observed in schizophrenia patients. These effects are likely attributed to the inhibitory effect of EEEP on the activation of ERK induced by MK-801, thereby ameliorating schizophrenia-related symptoms.

Through the results of this study, we observed that EEEP improved cognitive dysfunction, sensorimotor gating deficits, and hyperactivity, which were driven to normalize the hyper phosphorylation of ERK induced by MK-801 through the ERK pathway. Additionally, the efficacy of EEEP in this study may be due to the presence of oleanane-type triterpenoid saponins, eclalbasaponin II. Therefore, further research on the components and precise mechanisms of EEEP should be pursued, it might be an effective treatment for schizophrenia with minimal side effects.

Acknowledgments: This research was supported by the Korean Ministry of Environment (2018002270002).

Conflicts of Interest: There is no conflict of interest

References

[1] Morera-Fumero, A.L. and P. Abreu-Gonzalez, Role of melatonin in schizophrenia. Int J Mol Sci, 2013. 14(5): p. 9037-50.

[2] Winship, I.R., et al., An overview of animal models related to schizophrenia. The Canadian Journal of Psychiatry, 2019. 64(1): p. 5-17.

[3] Oh, H.K., et al., Kami-ondam-tang, a traditional herbal prescription, attenuates the prepulse inhibition deficits and cognitive impairments induced by MK-801 in mice. J Ethnopharmacol, 2013. 146(2): p. 600-7.

[4] Kéri, S. and Z. Janka, Critical evaluation of cognitive dysfunctions as endophenotypes of schizophrenia. Acta Psychiatrica Scandinavica, 2004. 110(2): p. 83-91.

[5] Green, M.F., et al., Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"? Schizophr Bull, 2000. 26(1): p. 119-36.

[6] Jo, J.-H., et al., Effects of olanzapine on gene expression changes in MK-801-induced neurotoxicity using a high-density DNA microarray. Molecular & Cellular Toxicology, 2007. 3(4): p. 282-291.

[7] Plichta, P., et al., Cognitive Dysfunctions Measured with the MCCB in Deficit and Non-Deficit Schizophrenia. J Clin Med, 2023. 12(6).

[8] Adityanjee, et al., Dementia praecox to schizophrenia: the first 100 years. Psychiatry Clin Neurosci, 1999. 53(4): p. 437-48.

[9] Onitsuka, T., et al., Toward recovery in schizophrenia: Current concepts, findings, and future research directions. Psychiatry Clin Neurosci, 2022. 76(7): p. 282-291.

[10] Marder, S.R. and W. Fenton, Measurement and Treatment Research to Improve Cognition in Schizophrenia: NIMH MATRICS initiative to support the development of agents for improving cognition in schizophrenia. Schizophr Res, 2004. 72(1): p. 5-9.

[11] Miyamoto, S., et al., Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry, 2012. 17(12): p. 1206-27.

[12] Townsend, G. and D. Curtis, Case report: rapidly fatal bowel ischaemia on clozapine treatment. BMC Psychiatry, 2006. 6: p. 43.

[13] Devinsky, O., G. Honigfeld, and J. Patin, Clozapine-related seizures. Neurology, 1991. 41(3): p. 369-71.

[14] Kruk-Slomka, M. and G. Biala, Cannabidiol Attenuates MK-801-Induced Cognitive Symptoms of Schizophrenia in the Passive Avoidance Test in Mice. Molecules, 2021. 26(19).

[15] Kang, Y.M., et al., Anti-inflammatory effects of Eclipta prostrata Linne on house dust mite-induced atopic dermatitis in vivo and in vitro. J Ethnopharmacol, 2022. 292: p. 115233.

[16] Jung, W.Y., et al., The ethanolic extract of the Eclipta prostrata L. ameliorates the cognitive impairment in mice induced by scopolamine. J Ethnopharmacol, 2016. 190: p. 165-73.

[17] Torrisi, S.A., et al., Buspirone counteracts MK-801-induced schizophrenia-like phenotypes through dopamine D3 receptor blockade. Frontiers in pharmacology, 2017. 8: p. 306310.

[18] Fijał, K., P. Popik, and A. Nikiforuk, Co-administration of 5-HT6 receptor antagonists with clozapine, risperidone, and a 5-HT2A receptor antagonist: effects on prepulse inhibition in rats. Psychopharmacology, 2014. 231: p. 269-281.

[19] Huang, W., et al., Effects of the co‐administration of MK‐801 and clozapine on MiRNA expression profiles in rats. Basic & Clinical Pharmacology & Toxicology, 2021. 128(6): p. 758-772.

[20] Miki, R., et al., Effects of tipepidine on MK-801-induced cognitive impairment in mice. Brain Research, 2019. 1710: p. 230-236.

[21] Hong, S.I., et al., Danshensu isolated from Prunella vulgaris var. Lilacina attenuates MK-801-induced prepulse inhibition deficits in mice. Korean Journal of Pharmacognosy, 2013. 44(2): p. 97-103.

[22] Hill, X.L., A. Richeri, and C. Scorza, Measure of anxiety-related behaviors and hippocampal BDNF levels associated to the amnesic effect induced by MK-801 evaluated in the modified elevated plus-maze in rats. Physiol Behav, 2015. 147: p. 359-63.

[23] Geyer, M.A., et al., Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl), 2001. 156(2-3): p. 117-54.

[24] Yu, W., et al., Reversible changes in BDNF expression in MK-801-induced hippocampal astrocytes through NMDAR/PI3K/ERK signaling. Frontiers in Cellular Neuroscience, 2021. 15: p. 672136.

[25] Divac, N., et al., Second-generation antipsychotics and extrapyramidal adverse effects. Biomed Res Int, 2014. 2014: p. 656370.

[26] Timalsina, D. and H.P. Devkota, Eclipta prostrata (L.) L. (Asteraceae): Ethnomedicinal Uses, Chemical Constituents, and Biological Activities. Biomolecules, 2021. 11(11).

[27] Jung, W.Y., et al., Eclalbasaponin II Ameliorates the Cognitive Impairment Induced by Cholinergic Blockade in Mice. Neurochem Res, 2018. 43(2): p. 351-362.

[28] Juang, Y.P. and P.H. Liang, Biological and Pharmacological Effects of Synthetic Saponins. Molecules, 2020. 25(21).

[29] Rao, A. and M.-K. Sung, Saponins as anticarcinogens. The Journal of nutrition, 1995. 125: p. 717S-724S.

[30] Oyeleke, M.B. and B.V. Owoyele, Saponins and flavonoids from Bacopa floribunda plant extract exhibit antioxidant and anti-inflammatory effects on amyloid beta 1-42-induced Alzheimer's disease in BALB/c mice. Journal of Ethnopharmacology, 2022. 288: p. 114997.

[31] Zhou, Q., et al., Anti-inflammation effects of the total saponin fraction from Dioscorea nipponica Makino on rats with gouty arthritis by influencing MAPK signalling pathway. BMC complementary medicine and therapies, 2020. 20: p. 1-13.

[32] Olney, J.W. and N.B. Farber, Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry, 1995. 52(12): p. 998-1007.

[33] Powell, S.B., et al., Atypical antipsychotics clozapine and quetiapine attenuate prepulse inhibition deficits in dopamine transporter knockout mice. Behav Pharmacol, 2008. 19(5-6): p. 562-5.

[34] Nakaya, K., et al., Pharmacological characterizations of memantine-induced disruption of prepulse inhibition of the acoustic startle response in mice: involvement of dopamine D2 and 5-HT2A receptors. Behav Brain Res, 2011. 218(1): p. 165-73.

[35] Weber, M., et al., The effects of the dopamine D2 agonist sumanirole on prepulse inhibition in rats. European Neuropsychopharmacology, 2010. 20(6): p. 421-425.

[36] Xi, D., et al., Dizocilpine (MK-801) induces distinct changes of N-methyl-D-aspartic acid receptor subunits in parvalbumin-containing interneurons in young adult rat prefrontal cortex. Int J Neuropsychopharmacol, 2009. 12(10): p. 1395-408.

[37] Breier, A., et al., Effects of clozapine on positive and negative symptoms in outpatients with schizophrenia. Am J Psychiatry, 1994. 151(1): p. 20-6.

[38] Yang, A.C. and S.-J. Tsai, New targets for schizophrenia treatment beyond the dopamine hypothesis. International journal of molecular sciences, 2017. 18(8): p. 1689.

[39] Jeong, Y., et al., 4-Methoxycinnamic acid attenuates schizophrenia-like behaviors induced by MK-801 in mice. J Ethnopharmacol, 2022. 285: p. 114864.

[40] Lopez-Gil, X., et al., Clozapine and haloperidol differently suppress the MK-801-increased glutamatergic and serotonergic transmission in the medial prefrontal cortex of the rat. Neuropsychopharmacology, 2007. 32(10): p. 2087-97.

[41] Ishii, D., et al., Effects of aripiprazole on MK-801-induced prepulse inhibition deficits and mitogen-activated protein kinase signal transduction pathway. Neurosci Lett, 2010. 471(1): p. 53-7.

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