

# Echinacoside Exhibits Antidepressant-like Effects Through AMPAR–Akt/ERK–mTOR Pathway Stimulation and BDNF Expression in Mice

Han-Wen Chuang

China Medical University

Tse-Yen Wang

China Medical University

Chih-Chia Huang (✉ [chihchiahuang@yahoo.com.tw](mailto:chihchiahuang@yahoo.com.tw))

Tsaotun Psychiatric Center Ministry of Health and Welfare <https://orcid.org/0000-0001-8703-1878>

I-Hua Wei

China Medical University

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## Research Article

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# Abstract

**Background:** Several natural products have been demonstrated to be effective in the treatment of depressive disorders. Echinacoside, a naturally occurring phenol extracted from *Cistanche tubulosa*, *Echinacea angustifolia*, and *Cistanche* spp, has a wide range of physiological effects, such as antioxidation, neuroprotection, anti-inflammatory, and immunoregulation, which are closely related to depression. In addition, echinacoside can activate protein kinase B (Akt), extracellular signal-regulated kinase (ERK), and brain-derived neurotrophic factor (BDNF) in the brain. A key downstream event of the Akt, ERK, and BDNF signaling pathways, namely mechanistic target of rapamycin (mTOR) signaling, plays a crucial role in generating an antidepressant effect. Thus, echinacoside is a promising therapeutic agent for depression. However, research regarding the role of echinacoside in brain mTOR activation and antidepressant effect remains lacking.

**Materials and methods:** The forced swimming test in C57BL/6 mice was used to investigate the antidepressant-like activities of echinacoside and the underlying mechanism involved in glutamatergic signaling.

**Results:** We confirmed the suggestions by previous reports that echinacoside activates Akt/ERK signaling and further demonstrated that echinacoside triggers mTOR signaling and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor activation in the Akt/ERK signaling pathway downstream and upstream, respectively, and upregulates BDNF in the hippocampus of mice to exhibit antidepressant-like activities.

**Conclusion:** To the best of our knowledge, our study is the first to reveal that echinacoside is a potential treatment for depressive disorders. Moreover, the present study suggests a mechanism for the neuroprotective effect of echinacoside.

## Introduction

Major depressive disorder is a common and serious psychiatric disorder that is characterized by a depressed mood or loss of interest. Although various antidepressants are available, only 50% of patients who receive current treatments recover within the first 6 months, and their recovery rate declines sharply over time; thus, many patients continue to have depressive episodes even with regular antidepressant treatment [1]. Several recent studies have indicated that antidepressants, in addition to targeting monoamine receptors, target glutamate receptors, which play a pivotal role in both the pathophysiology and treatment of depression [2, 3]. These findings expand on the unexpected findings of the rapid-acting antidepressant properties of ketamine, an N-methyl-D-aspartate receptor (NMDAR) antagonist [4]. Ketamine induces glutamate release. When the NMDAR is blocked, ketamine stimulates another glutamate receptor— $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)—and enhances the accumulation of brain-derived neurotrophic factor (BDNF); it then activates protein kinase B (Akt)/extracellular signal-regulated kinase (ERK) signal transduction cascades followed by the mechanistic target of rapamycin (mTOR). These processes cause rapid-onset antidepressant-like effects

[4, 5]. Thus, other agents that can trigger the biochemical cascades responsible for the antidepressant effect of ketamine may also have antidepressant potential.

Echinacoside, a naturally occurring phenol isolated from *Cistanche tubulosa*, *Echinacea angustifolia*, and *Cistanche* spp, has been used as a traditional herbal medicine in Chinese and Western countries for its antioxidation, neuroprotection, anti-inflammatory, antitumor, antiaging, hepatoprotection, immunoregulation, and learning memory improvement effects [6]. Moreover, preclinical studies conducted elsewhere have indicated that echinacoside enhances memory through the activation of the phosphoinositide 3-kinase-Akt pathway in the hippocampus of rats with Alzheimer-like disease [7]. Furthermore, it prevents injuries induced by rotenone through Trk–ERK pathway activation and BDNF accumulation [8]. These mechanisms are closely related to depression treatment.

On the basis of the known roles of echinacoside in the increased activation of glutamate system signaling, including Akt, ERK, and BDNF, in the brain and the involvement of the Akt/ERK/BDNF downstream target mTOR signaling in treating depression, we reasoned that echinacoside may exhibit antidepressant properties. However, the effects of echinacoside on brain mTOR signaling remain unclear, and the antidepressant effects of echinacoside are also unexplored. Therefore, we investigated the antidepressant-like responses of echinacoside by using the forced swimming test (FST) in mice. Furthermore, Western blot analysis was conducted to examine the role of Akt/ERK/BDNF signaling downstream and upstream in mTOR signaling and AMPAR signaling, respectively, in the brain, underlying the possible antidepressant effect of echinacoside.

## Materials And Methods

### Animals

Male C57BL/6 mice aged 6–8 weeks and weighing 23–25 g were adapted to our laboratory animal center for at least 7 days before the experiment. The mice were maintained in a controlled environment with 23°C ± 1°C temperature, 55% ± 5% humidity, 12-h light/dark cycle, and ad libitum food and water. The mice were transferred to the experimental room for subsequent studies. All experiments were approved by the Institutional Animal Care and Use Committee of China Medical University, Taiwan (permit No. CMUIACUC-2016-072-1).

### Drug administration

Mice were treated with echinacoside (Sigma Aldrich), desipramine (Sigma Aldrich), MK2206 (MedChemExpress), NBQX (Sigma Aldrich) dissolved in 0.9% saline, SL327 (Sigma Aldrich), and rapamycin (Toku-E) dissolved in 0.5% ethanol through an intraperitoneal injection of 0.01 mL/g body weight. The mice in the dose-dependent group were given normal saline, desipramine (20 mg/kg, a tricyclic antidepressant as a positive control) [9], and echinacoside (20, 30, or 40 mg/kg) 30 min before the FST. The other group was treated with the mTOR inhibitor rapamycin (20 mg/kg) [10] or AMPAR inhibitor NBQX (30 mg/kg) [11] 30 min before echinacoside (30 mg/kg, ideal dosage derived from the

dose-dependent group) and the Akt inhibitor MK2206 (60 mg/kg) [12] or ERK inhibitor SL327 (40 mg/kg) [13] 1 h before echinacoside administration.

## **Forced swim test**

The forced swim test is a rodent behavioral test adopted to evaluate the efficacy of antidepressant drugs, and the index is immobility time [14, 15]. First, we conducted a 15-min swim procedure 24 h before the 5-min formal FST in a transparent cylindrical tank with water, in accordance with previous reports. The formal FST was executed 30 min after the injections. We deposited the experimental mice in a cylinder (height: 40 cm; diameter: 20 cm) filled with water (height: 10 cm) at 25°C for 5 min and recorded their behavioral performance by using a digital camera. The immobility periods were quantified automatically by using EthoVision.

## **Open field test**

We used an open field test (OFT) to analyze the locomotor ability of mice for excluding the possibilities of false-positive results in the FST [15]. The mice were placed into a plastic cage with a 60 cm × 60 cm board and surrounded by 50-cm-high walls; subsequently, the mice were allowed to explore the cage for 5 min after drug administration. Mice movements were recorded using a digital camera, and the total movement in the 5-min period was computed using EthoVision.

## **Animal tissue preparation for Western blot analysis**

After the OFT was conducted, four mice were sacrificed and their hippocampus removed and dried with nitrogen for further Western blot analysis. The collected tissues were stored at – 80°C until use. Subsequently, 250 µL of lysis buffer was added per tube for the grinding of the collected tissues. The mixture was centrifuged at 15,000 rpm for 15 min at 4°C after grinding. The supernatant was transferred to new centrifugal tubes and heated for 7 min with sample buffer. After cooling down the mixture by using a cooling machine set at 4°C, we preserved all samples at – 80°C. Subsequently, we conducted Western blot analysis to measure the immunoreactions of proteins in the hippocampus. Previously described procedures for Western blotting were followed [10]. The details are provided in the supplemental material.

## **Data analysis**

SPSS 12.0 was used for statistical analysis. The data of behavioral experiments were measured using one-way analysis of variance followed by Tukey post hoc tests. Variations in Western blot analysis were analyzed using the nonparametric Mann–Whitney test to compare the two groups. All results were considered significant at  $P < 0.05$ , and the tests were two-tailed.

## **Results**

# **Antidepressant-like effects and activation of mTOR signaling pathway following echinacoside treatments**

The FST, the most popular animal test for predicting antidepressant effects [14, 15], was used to measure the antidepressant-like effects of echinacoside in mice (Fig. 1A). Furthermore, the OFT was used to evaluate the possibilities of false-positive results in the FST [15] (Fig. 1B). Echinacoside at the doses of 20, 30, and 40 mg/kg significantly reduced the immobility of mice by 17.5%, 50.0%, and 21.5% compared with control mice (Fig. 1C). Furthermore, a single injection of the reference drug, desipramine (20 mg/kg), significantly reduced immobility. Echinacoside at any of the tested doses and desipramine did not influence the locomotor activity of mice (Fig. 1D). Thus, echinacoside generates antidepressant effects. In addition, an inverted U-shaped dose–response relationship was observed in the decreased immobility in the FST following echinacoside treatment.

To determine whether the antidepressant-like effects of echinacoside are associated with increases in activated Akt, ERK, and Akt/ERK downstream mTOR signaling, the intensities of the activated Akt, ERK, and mTOR were evaluated using Western blot analysis (Fig. 2A). Here, mTOR activation was dose dependent, occurring at relatively low doses (20 and 30 mg/kg) but not at a higher dose (40 mg/kg; Fig. 2B). The trend was consistent with the decreased immobility in the FST. Moreover, we found that all doses (20, 30, and 40 mg/kg) of echinacoside significantly increased pAkt activation in mice (Fig. 2C). In addition, echinacoside at 30 mg/kg resulted in significant increases in pERK levels, but the lower (20 mg/kg) and higher doses (40 mg/kg) did not have a significant influence (Fig. 2D). Desipramine did not significantly influence pmTOR, pAkt, and pERK.

## **Glutamatergic system is involved in echinacoside-induced antidepressant effects**

We found that echinacoside at 30 mg/kg could provide antidepressant effects with significant increases in the intensities of pmTOR, pAkt, and pERK. Therefore, we used echinacoside at 30 mg/kg for all further experiments.

To confirm the involvement of AMPAR and mTOR in antidepressant-like responses induced by echinacoside, we applied NBQX (an AMPAR inhibitor) and rapamycin (an mTOR inhibitor) before echinacoside administration during the FST. The timeline of inhibitor pretreatment is presented in Fig. 3A. The reduction in the immobility duration induced by echinacoside was completely reversed by NBQX and rapamycin (Fig. 3B). This indicates that echinacoside-induced antidepressant-like action required the activation of mTOR and AMPAR. Subsequently, we analyzed whether the AMPAR–mTOR signaling pathway could be altered through the administration of NBQX or rapamycin in echinacoside-treated mice. A single injection of echinacoside resulted in significant increases in the immunoreactions of pmTOR, pAkt, and pERK (Fig. 3C). The levels of total mTOR, Akt, and ERK remained unchanged. NBQX treatment before echinacoside blocked the echinacoside-induced increase in the activations of pmTOR (Fig. 3D), pAkt (Fig. 3E), and pERK (Fig. 3F). The increased pmTOR levels induced by echinacoside was completely abolished through pretreatment with rapamycin (Fig. 3D), but echinacoside-induced increases in pAkt and pERK were not attenuated (Fig. 3E, F). These data indicate that echinacoside activates the mTOR signaling pathway, which requires AMPAR stimulation.

We applied ERK inhibitor SL327 and Akt inhibitor MK2206 before echinacoside treatment to validate the roles of ERK and Akt in echinacoside-induced antidepressant responses. The timeline of inhibitor pretreatment is presented in Fig. 4A. Echinacoside-induced antidepressant-like effects were blocked by SL327 and MK2206 (Fig. 4B). Thus, the antidepressant-like action of echinacoside also depends on the activations of ERK and Akt signaling. To elucidate the Akt and ERK upstream and downstream signaling events on the AMPAR–mTOR pathway, we also measured pmTOR, pAkt, and pERK activation through the treatment of mice with SL327 or MK2206 before echinacoside administration. Figure 4C indicates that ERK inhibitor SL327 administered before echinacoside blocked the echinacoside-induced increase in pmTOR (Fig. 4D) and pERK (Fig. 4F) but did not affect pAkt (Fig. 4E) immunoreactions. Moreover, Akt inhibitor MK2206 prevented the increase in pmTOR (Fig. 4D) and pAkt (Fig. 4E) engendered by echinacoside but did not modulate echinacoside-induced increase in pERK (Fig. 4F). These results confirm that the antidepressant actions of echinacoside depend on the activation of AMPAR–Akt/ERK–mTOR signal transduction.

## **Modulations of AMPAR membrane insertion after treatment with echinacoside**

AMPA membrane insertion is considered to be involved in the antidepressant effects of several glutamate-based antidepressants [10, 16–18]. Thus, we investigated whether echinacoside modulates the activated forms of the AMPAR subunit of GluA1 on its PKA (GluA1ser845) [19–21] and PKC (GluA1ser831) [22, 23] sites to assess the influence of echinacoside on the insertion of the AMPAR membrane. As shown in Fig. 5A, increases in the phosphorylation of GluA1ser845 and GluA1ser831 were observed in mice treated with 30 mg/kg echinacoside, which was not observed in mice treated with 20 mg/kg echinacoside and desipramine (Fig. 5B, C). Although 40 mg/kg echinacoside upregulated pGluA1ser845 and pGluA1ser831, the increase was not significant.

NBQX treatment before echinacoside reversed echinacoside-induced increases in the levels of pGluA1ser845 and pGluA1ser831 (Fig. 5E, F). However, treatment with rapamycin 30 min before echinacoside did not attenuate the echinacoside-induced increase. In addition, SL327 or MK2206 pretreatment before echinacoside did not significantly attenuate echinacoside-induced increases in both (Fig. 5H, I). The total levels of GluA1 remained unchanged following treatments (Fig. 5A, D, G). These data indicate that echinacoside could enhance AMPAR membrane insertion, which required AMPAR activation.

## **Effect of echinacoside on BDNF expression**

BDNF is a type of neurotrophin, which also plays a key role in neuronal development, differentiation, survival, and emotional regulation [24]. Moreover, BDNF released through ERK signaling results in a rapid antidepressant response [25]. We examined BDNF expression in mice treated with drugs. As presented in Fig. 6A, 20 and 30 mg/kg echinacoside treatment significantly upregulated BDNF expressions in the hippocampus; by contrast, desipramine and 40 mg/kg echinacoside did not affect the BDNF expressions. The trend was consistent with mTOR activation and antidepressant-like action in the FST. In addition, NBQX and rapamycin treatments before echinacoside blocked echinacoside-induced increase in BDNF

(Fig. 6B). Finally, SL327 and MK2206 completely inhibited echinacoside-induced increases in BDNF (Fig. 6C).

## Discussion

On the basis of the known effects of echinacoside on the increased activation of Akt and ERK signaling in the brain [7, 8], the present study was designed to investigate whether treatment with echinacoside can increase Akt/ERK downstream mTOR signaling in the hippocampus and induce antidepressant-like effects. We found that a single injection of 30 mg/kg echinacoside could exert antidepressant-like effects in the FST, accompanied by a significant increase in the activated forms of Akt, ERK, and Akt/ERK downstream mTOR signaling. Subsequently, based on pharmacological inhibitions, we observed that the antidepressant-like effects of echinacoside required the activation of the AMPAR–Akt/ERK–mTOR pathway, which is same as that of ketamine. Moreover, echinacoside facilitated AMPAR membrane insertion and BDNF expression. These findings suggest that echinacoside is a potential therapeutic agent with rapid-onset antidepressant effects and indicate a possible mechanism of the neuroprotective effect of echinacoside.

Echinacoside was investigated in this study because of evidence suggesting that it can upregulate brain Akt and ERK signaling [7, 8], and Akt/ERK downstream mTOR signaling is closely related to antidepressant effects and depression pathophysiology [4, 26–28]. Echinacoside also exhibits neuroprotective, antioxidant, anti-inflammation effects and therapeutic effects against Alzheimer and Parkinson diseases [6]. Because oxidative stress, inflammation, and these neurodegenerative diseases are highly correlated with depression, these reports imply the possibility of antidepressant-like actions of echinacoside [29, 30]. First, we used the FST to examine antidepressant-like effects of different doses of echinacoside. We found that a single injection of 20, 30, or 40 mg/kg echinacoside could induce significantly reduced immobility in the FST without alteration of the locomotor activity in the OFT, verifying that echinacoside has antidepressant-like effects. Echinacoside exhibited an inverted U-shaped dose–response relationship in decreased immobility in the FST. Reduction in immobility time was more pronounced with 30-mg/kg echinacoside treatment than with 20- or 40-mg/kg echinacoside treatment; furthermore, 30-mg/kg echinacoside treatment significantly increased all immunoreactivities at the pAkt, pERK, pmTOR, and BDNF levels. The behavioral actions of echinacoside in the FST occurred in a nonlinear dose–response manner, consistent with the induction of pmTOR and BDNF expression. However, the effect of echinacoside on mTOR upstream signaling, including pAkt and pERK, followed a linear or sigmoidal dose–response curve. The reason for the inverted U-shaped dose–response relationship with echinacoside in downstream pmTOR and BDNF and behavioral actions remains unclear. However, this U-shaped dose–response phenomenon is not uncommon in pharmacological studies [31].

We subsequently focused on the effect of 30 mg/kg echinacoside to elucidate the antidepressant-like mechanism of echinacoside. To examine whether the antidepressant-like response to echinacoside is mediated through mTOR activation directly, we administered the mTOR inhibitor rapamycin before echinacoside; then, we observed the behavior of the mice in the FST. We found that a pharmacological

block of mTOR activation by rapamycin inhibited the antidepressant effects of echinacoside, indicating that echinacoside's antidepressant-like effects depend on mTOR activation. This is relevant to the pathology of depression and antidepressant mechanism.

Duman's group were the first to discover that mTOR activation in brain is required for the rapid antidepressant responses of ketamine [4]; soon after that, mTOR became a major target in the studies of depression and antidepressant action. Subsequently, various studies confirmed the crucial role of mTOR in the treatment and pathophysiology of depression. Furthermore, many clinically used antidepressants (escitalopram, fluoxetine, and paroxetine) have mTOR activation-enhancing effects [32]. To date, data published on the effect of echinacoside on mTOR signaling are limited to two in vitro studies for bone and intestine cells, and their results are contradictory [33, 34]. The first study found that echinacoside applied at doses of  $10^{-8}$  to  $10^{-6}$  M could promote mTOR activation in high-glucose-injured osteoblastic MC3T3-E1 cells [33]. By contrast, in the other in vitro study, echinacoside administered at 10 and 20  $\mu\text{g/mL}$  inhibited mTOR activation caused by lipopolysaccharide in rat intestinal IEC-6 cells [34]. Although studies have revealed that echinacoside facilitates Akt and ERK production in the brain, neither an in vitro nor in vivo investigation has studied the effects of echinacoside on mTOR activation in the brain. This is the first in vivo study to demonstrate that echinacoside could significantly increase the activation of hippocampal mTOR signaling. In addition, the mTOR inhibitor rapamycin blocked the antidepressant responses and mTOR activation caused by echinacoside, suggesting that the activation of hippocampal mTOR is required in the antidepressant responses of echinacoside.

Several studies have suggested that AMPAR functioning has a critical role as an upstream messenger to activate the mTOR signaling pathway and provides antidepressant actions [4, 35, 36]. To understand the role of AMPAR in the echinacoside-induced antidepressant effect and mTOR signaling activation, we investigated whether the behavioral antidepressant action of echinacoside required AMPAR and whether this signaling was upstream of mTOR activation. The NBQX, an AMPA inhibitor, was administered to echinacoside-treated mice. Echinacoside had no antidepressant-like effect on NBQX-pretreated mice, demonstrating that the antidepressant-like effects of echinacoside also depend on AMPAR stimulation.

Western blot analysis indicated that the AMPAR inhibitor NBQX prevented echinacoside-induced increases in activated mTOR and both mTOR upstream regulator kinases, Akt and ERK. These results confirmed that mTOR signaling activation through AMPAR participates in the antidepressant-like responses of echinacoside and suggests that echinacoside is an antidepressant that targets the glutamatergic pathway. Finally, regarding the Akt/ERK-mediated mechanisms of echinacoside, our study also revealed that hippocampal Akt and ERK expressions were upregulated after echinacoside treatment. The present results confirm those of previous reports [7, 8]. In addition, the blocking of Akt or ERK inhibited the antidepressant-like effect. The results also demonstrated that Akt and ERK are involved in the antidepressant-like actions of echinacoside. The antidepressant-like responses of echinacoside on Akt/ERK signaling occur during AMPAR and mTOR signaling downstream and upstream, respectively, which are parallel to those of glutamate-based antidepressants [4, 28, 37]. Taken together, the results

indicate that the antidepressant actions of echinacoside depend on the activations of AMPAR–Akt/ERK–mTOR signal transduction.

AMPA, a subtype of ionotropic glutamate receptor, consists of GluA2 and GluA1, GluA3, or GluA4 subunits [38]. GluA1 is centrally involved in synaptic plasticity [39]. AMPAR trafficking is crucial for antidepressant and neuroprotective effects [10, 16–18, 40]. To further investigate the effects of echinacoside on AMPAR trafficking, we analyzed the phosphorylation of AMPAR subunits GluA1 PKA (GluA1ser845) and GluA1 PKC (GluA1ser831), both of which are indicators of GluA1 membrane insertion [19–23]. Our immunohistochemical analyses demonstrated that the *in vivo* treatment with echinacoside increased AMPAR phosphorylation at GluA1ser845 and GluA1ser831. Our data verified that echinacoside can facilitate AMPAR insertion to the synapses, which can increase postsynaptic AMPAR levels, thus leading to the increase in the AMPAR:NMDAR ratio. Subsequently, increased AMPAR throughput activated mTOR, and finally, echinacoside provided antidepressant-like effects. These results indicate the antidepressant properties of echinacoside and are also correlated with those of ketamine, which also can increase GluA1 insertion into the postsynaptic membrane [36, 41]. The increased AMPAR throughput has been found in several antidepressants and proposed as a convergent response [42, 43].

Early studies have reported that echinacoside benefits human health, such as with neuroprotective effects in Parkinson and Alzheimer diseases [6]. Mitogen-activated protein kinase, NF-kappa B, caspase 3 and 8, and reactive oxygen species/activating transcription factor 3/C/EBP-homologous protein pathways have been proposed as the underlying neuroprotective mechanisms of echinacoside [6]. However, the actual mechanism remains unclear. Our *in vivo* study is the first to show that echinacoside induces AMPAR membrane insertion. Our findings suggest an alternative potential cellular mechanism to explain how echinacoside exerts its neuroprotective effect. Following AMPAR insertion facilitation, echinacoside regulates the expression of synapse-related proteins and potentially protects against maladaptive neurosynaptic deficit and cellular damage, resulting in neuroprotective effects in some diseases, particularly those of malalterations in synaptic plasticity.

A study reported that echinacoside exerts neuroprotective effects through the activation of the Trk/ERK signaling pathway, and BDNF was involved in this reaction [8]; furthermore, another study found that echinacoside treatment can reduce dopaminergic function and increase BDNF mRNA expression and its protein [44]. In addition to neuroprotection, BDNF plays a crucial role in depression and the therapeutic mechanisms of antidepressants [45]. To understand whether the antidepressant response of echinacoside is accompanied by BDNF variation, we examined the expression of BDNF through Western blotting. We found that echinacoside could upregulate BDNF. This finding is consistent with previous studies and is also relevant to the antidepressant effect of echinacoside. The same properties of echinacoside and ketamine affect the BDNF level. However, the inhibitors of AMPAR, Akt, ERK, and mTOR could block BDNF expression induced by echinacoside, suggesting that BDNF expression is downstream of AMPAR–Akt/ERK–mTOR pathway signaling. The action is different from that of ketamine. Studies have indicated that ketamine can enhance glutamate release and block NMDAR, which activate another glutamate receptor, AMPAR; this in turn triggers BDNF release, which causes downstream mTOR

activation [45]. Moreover, we suggest that BDNF plays a role in the antidepressant-like properties and AMPAR–Akt/ERK–mTOR signaling pathway activation caused by echinacoside, although future mechanistic studies of this interaction are required.

## Conclusions

Our results verified that the natural phenol echinacoside exhibits antidepressant-like effects through the activation of the AMPAR–Akt/ERK–mTOR signaling pathway, which is similar to that of ketamine. Moreover, echinacoside enhances AMPAR membrane insertion and BDNF expression, indicating that echinacoside potentially provides neuroprotection by triggering AMPAR membrane insertion and BDNF upregulation. Our findings serve as preclinical evidence that echinacoside can target the hippocampal glutamatergic pathway and act as a rapid-acting antidepressant.

## Abbreviations

NMDAR: N-methyl-D-aspartate; AMPAR:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BDNF: brain-derived neurotrophic factor; Akt: protein kinase B; ERK: extracellular signal–regulated kinase; mTOR: mechanistic target of rapamycin; FST: forced swimming test; OFT: open field test; GluA1ser845: AMPAR subunit of GluA1 on PKA site; GluA1ser831: AMPAR subunit of GluA1 on PKC site.

## Declarations

### Acknowledgements

Not applicable.

### Authors' contributions

IHW and CCH contributed to conception and design of the study. HWC performed the experiments. HWC and TYW wrote the manuscript. IHW and CCH provided the method of target prediction. CCH analyzed the data. IHW and CCH implemented the study and modified the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

The data can be requested from the author upon reasonable request.

## Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

## Consent for publication

We declare that the Publisher has the Author's permission to publish the relevant contribution

## Competing interests

The authors declare that they have no competing interests.

## References

1. Keller MB, Lavori PW, Mueller TI, Endicott J, Coryell W, Hirschfeld RM, Shea T: **Time to recovery, chronicity, and levels of psychopathology in major depression. A 5-year prospective follow-up of 431 subjects.** *Arch Gen Psychiatry* 1992, **49**:809-816.
2. Deutschenbaur L, Beck J, Kiyhankhadiv A, Muhlhauser M, Borgwardt S, Walter M, Hasler G, Sollberger D, Lang UE: **Role of calcium, glutamate and NMDA in major depression and therapeutic application.** *Prog Neuropsychopharmacol Biol Psychiatry* 2016, **64**:325-333.
3. Huang CC, Tsai MH, Wu YC, Chen KT, Chuang HW, Chen Y, Tseng GW, Fu PI, Wei IH: **Activity Dependent Mammalian Target of Rapamycin Pathway and Brain Derived Neurotrophic Factor Release Is Required for the Rapid Antidepressant Effects of Puerarin.** *Am J Chin Med* 2018:1-16.
4. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS: **mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists.** *Science* 2010, **329**:959-964.
5. Gunduz D, Troidl C, Tanislav C, Rohrbach S, Hamm C, Aslam M: **Role of PI3K/Akt and MEK/ERK Signalling in cAMP/Epac-Mediated Endothelial Barrier Stabilisation.** *Front Physiol* 2019, **10**:1387.
6. Liu J, Yang L, Dong Y, Zhang B, Ma X: **Echinacoside, an Inestimable Natural Product in Treatment of Neurological and other Disorders.** *Molecules* 2018, **23**.
7. Wu CR, Lin HC, Su MH: **Reversal by aqueous extracts of Cistanche tubulosa from behavioral deficits in Alzheimer's disease-like rat model: relevance for amyloid deposition and central neurotransmitter function.** *BMC Complement Altern Med* 2014, **14**:202.
8. Zhu M, Lu C, Li W: **Transient exposure to echinacoside is sufficient to activate Trk signaling and protect neuronal cells from rotenone.** *J Neurochem* 2013, **124**:571-580.
9. Zhang H-T, Whisler LR, Huang Y, Xiang Y, O'Donnell JM: **Postsynaptic  $\alpha$ -2 Adrenergic Receptors are Critical for the Antidepressant-Like Effects of Desipramine on Behavior.** *Neuropsychopharmacology* 2009, **34**:1067-1077.

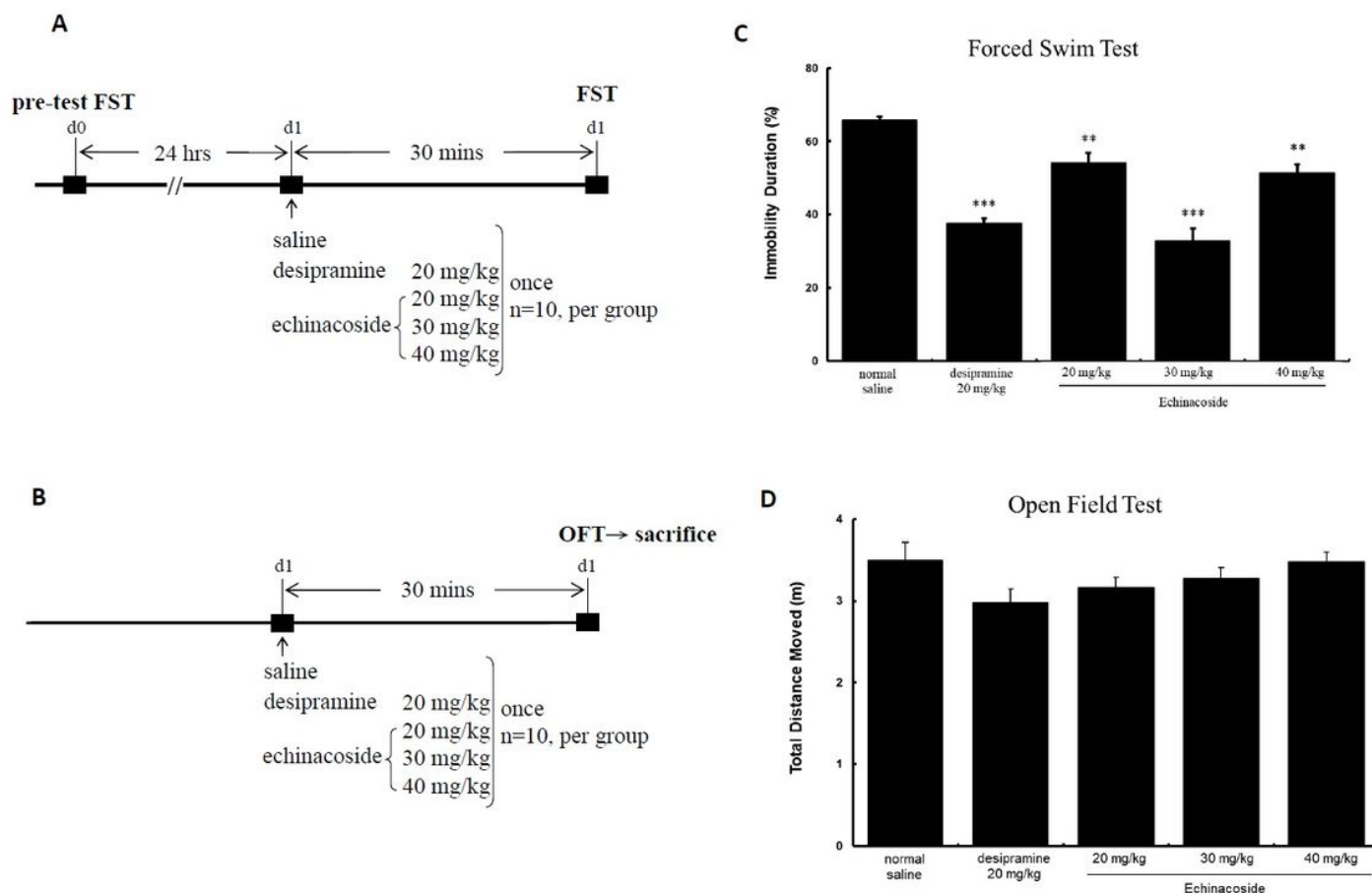
10. Chen KT, Tsai MH, Wu CH, Jou MJ, Wei IH, Huang CC: **AMPA Receptor-mTOR Activation is Required for the Antidepressant-Like Effects of Sarcosine during the Forced Swim Test in Rats: Insertion of AMPA Receptor may Play a Role.** *Front Behav Neurosci* 2015, **9**:162.
11. Bauer MR, Garcy DP, Boehm SL, 2nd: **Systemic Administration of the AMPA Receptor Antagonist, NBQX, Reduces Alcohol Drinking in Male C57BL/6J, But Not Female C57BL/6J or High-Alcohol-Preferring, Mice.** *Alcohol Clin Exp Res* 2020, **44**:2316-2325.
12. Leszczynska KB, Foskolou IP, Abraham AG, Anbalagan S, Tellier C, Haider S, Span PN, O'Neill EE, Buffa FM, Hammond EM: **Hypoxia-induced p53 modulates both apoptosis and radiosensitivity via AKT.** *The Journal of Clinical Investigation* 2015, **125**:2385-2398.
13. Lee Y-S, Ehninger D, Zhou M, Oh J-Y, Kang M, Kwak C, Ryu H-H, Butz D, Araki T, Cai Y, et al: **Mechanism and treatment for learning and memory deficits in mouse models of Noonan syndrome.** *Nature Neuroscience* 2014, **17**:1736-1743.
14. Porsolt RD, Le Pichon M, Jalfre M: **Depression: a new animal model sensitive to antidepressant treatments.** *Nature* 1977, **266**:730-732.
15. Porsolt RD, Anton G, Blavet N, Jalfre M: **Behavioural despair in rats: a new model sensitive to antidepressant treatments.** *Eur J Pharmacol* 1978, **47**:379-391.
16. Du J, Suzuki K, Wei Y, Wang Y, Blumenthal R, Chen Z, Falke C, Zarate CA, Jr., Manji HK: **The anticonvulsants lamotrigine, riluzole, and valproate differentially regulate AMPA receptor membrane localization: relationship to clinical effects in mood disorders.** *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2007, **32**:793-802.
17. Du J, Machado-Vieira R, Maeng S, Martinowich K, Manji HK, Zarate CA, Jr.: **Enhancing AMPA to NMDA throughput as a convergent mechanism for antidepressant action.** *Drug discovery today Therapeutic strategies* 2006, **3**:519-526.
18. Reus GZ, Stringari RB, Ribeiro KF, Ferraro AK, Vitto MF, Cesconetto P, Souza CT, Quevedo J: **Ketamine plus imipramine treatment induces antidepressant-like behavior and increases CREB and BDNF protein levels and PKA and PKC phosphorylation in rat brain.** *Behavioural brain research* 2011, **221**:166-171.
19. Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, Malinow R: **PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity.** *Nat Neurosci* 2003, **6**:136-143.
20. Smith KE, Gibson ES, Dell'Acqua ML: **cAMP-dependent protein kinase postsynaptic localization regulated by NMDA receptor activation through translocation of an A-kinase anchoring protein scaffold protein.** *J Neurosci* 2006, **26**:2391-2402.
21. Banke TG, Bowie D, Lee H, Huganir RL, Schousboe A, Traynelis SF: **Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase.** *J Neurosci* 2000, **20**:89-102.
22. Barria A, Muller D, Derkach V, Griffith LC, Soderling TR: **Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation.** *Science* 1997, **276**:2042-2045.
23. Tavalin SJ: **AKAP79 selectively enhances protein kinase C regulation of GluR1 at a Ca<sup>2+</sup>-calmodulin-dependent protein kinase II/protein kinase C site.** *J Biol Chem* 2008, **283**:11445-11452.

24. Duman RS, Monteggia LM: **A neurotrophic model for stress-related mood disorders.** *Biol Psychiatry* 2006, **59**:1116-1127.
25. Lepack AE, Bang E, Lee B, Dwyer JM, Duman RS: **Fast-acting antidepressants rapidly stimulate ERK signaling and BDNF release in primary neuronal cultures.** *Neuropharmacology* 2016, **111**:242-252.
26. Jernigan CS, Goswami DB, Austin MC, Iyo AH, Chandran A, Stockmeier CA, Karolewicz B: **The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder.** *Progress in neuro-psychopharmacology & biological psychiatry* 2011, **35**:1774-1779.
27. Ota KT, Liu RJ, Voleti B, Maldonado-Aviles JG, Duric V, Iwata M, Duthiel S, Duman C, Boikess S, Lewis DA, et al: **REDD1 is essential for stress-induced synaptic loss and depressive behavior.** *Nature medicine* 2014, **20**:531-535.
28. Voleti B, Navarria A, Liu RJ, Banasr M, Li N, Terwilliger R, Sanacora G, Eid T, Aghajanian G, Duman RS: **Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses.** *Biol Psychiatry* 2013, **74**:742-749.
29. Bakunina N, Pariante CM, Zunszain PA: **Immune mechanisms linked to depression via oxidative stress and neuroprogression.** *Immunology* 2015, **144**:365-373.
30. Galts CPC, Bettio LEB, Jewett DC, Yang CC, Brocardo PS, Rodrigues ALS, Thacker JS, Gil-Mohapel J: **Depression in neurodegenerative diseases: Common mechanisms and current treatment options.** *Neurosci Biobehav Rev* 2019, **102**:56-84.
31. Baldi E, Bucherelli C: **The inverted "u-shaped" dose-effect relationships in learning and memory: modulation of arousal and consolidation.** *Nonlinearity in biology, toxicology, medicine* 2005, **3**:9-21.
32. Park SW, Lee JG, Seo MK, Lee CH, Cho HY, Lee BJ, Seol W, Kim YH: **Differential effects of antidepressant drugs on mTOR signalling in rat hippocampal neurons.** *The international journal of neuropsychopharmacology* 2014, **17**:1831-1846.
33. Gong W, Zhang N, Cheng G, Zhang Q, He Y, Shen Y, Zhang Q, Zhu B, Zhang Q, Qin L: **Rehmannia glutinosa Libosch Extracts Prevent Bone Loss and Architectural Deterioration and Enhance Osteoblastic Bone Formation by Regulating the IGF-1/PI3K/mTOR Pathway in Streptozotocin-Induced Diabetic Rats.** *Int J Mol Sci* 2019, **20**.
34. Li L, Wan G, Han B, Zhang Z: **Echinacoside alleviated LPS-induced cell apoptosis and inflammation in rat intestine epithelial cells by inhibiting the mTOR/STAT3 pathway.** *Biomed Pharmacother* 2018, **104**:622-628.
35. Maeng S, Zarate CA, Jr., Du J, Schloesser RJ, McCammon J, Chen G, Manji HK: **Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors.** *Biological psychiatry* 2008, **63**:349-352.
36. Wei IH, Chen KT, Tsai MH, Wu CH, Lane HY, Huang CC: **Acute Amino Acid d-Serine Administration, Similar to Ketamine, Produces Antidepressant-like Effects through Identical Mechanisms.** *J Agric Food Chem* 2017, **65**:10792-10803.
37. Duman RS, Shinohara R, Fogaca MV, Hare B: **Neurobiology of rapid-acting antidepressants: convergent effects on GluA1-synaptic function.** *Mol Psychiatry* 2019.

38. Lu W, Shi Y, Jackson AC, Bjorgan K, During MJ, Sprengel R, Seeburg PH, Nicoll RA: **Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach.** *Neuron* 2009, **62**:254-268.
39. Diering GH, Huganir RL: **The AMPA Receptor Code of Synaptic Plasticity.** *Neuron* 2018, **100**:314-329.
40. Beattie MS, Ferguson AR, Bresnahan JC: **AMPA-receptor trafficking and injury-induced cell death.** *Eur J Neurosci* 2010, **32**:290-297.
41. Logan SM, Sarkar SN, Zhang Z, Simpkins JW: **Estrogen-induced signaling attenuates soluble Abeta peptide-mediated dysfunction of pathways in synaptic plasticity.** *Brain Res* 2011, **1383**:1-12.
42. Schloesser RJ, Huang J, Klein PS, Manji HK: **Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder.** *Neuropsychopharmacology* 2008, **33**:110-133.
43. Zarate CA, Jr., Singh J, Manji HK: **Cellular plasticity cascades: targets for the development of novel therapeutics for bipolar disorder.** *Biol Psychiatry* 2006, **59**:1006-1020.
44. Zhao Q, Gao J, Li W, Cai D: **Neurotrophic and neurorescue effects of Echinacoside in the subacute MPTP mouse model of Parkinson's disease.** *Brain Res* 2010, **1346**:224-236.
45. Castrén E, Monteggia L: **Brain-Derived Neurotrophic Factor Signaling in Depression and Antidepressant Action.** *Biol Psychiatry* 2021.

## Figures

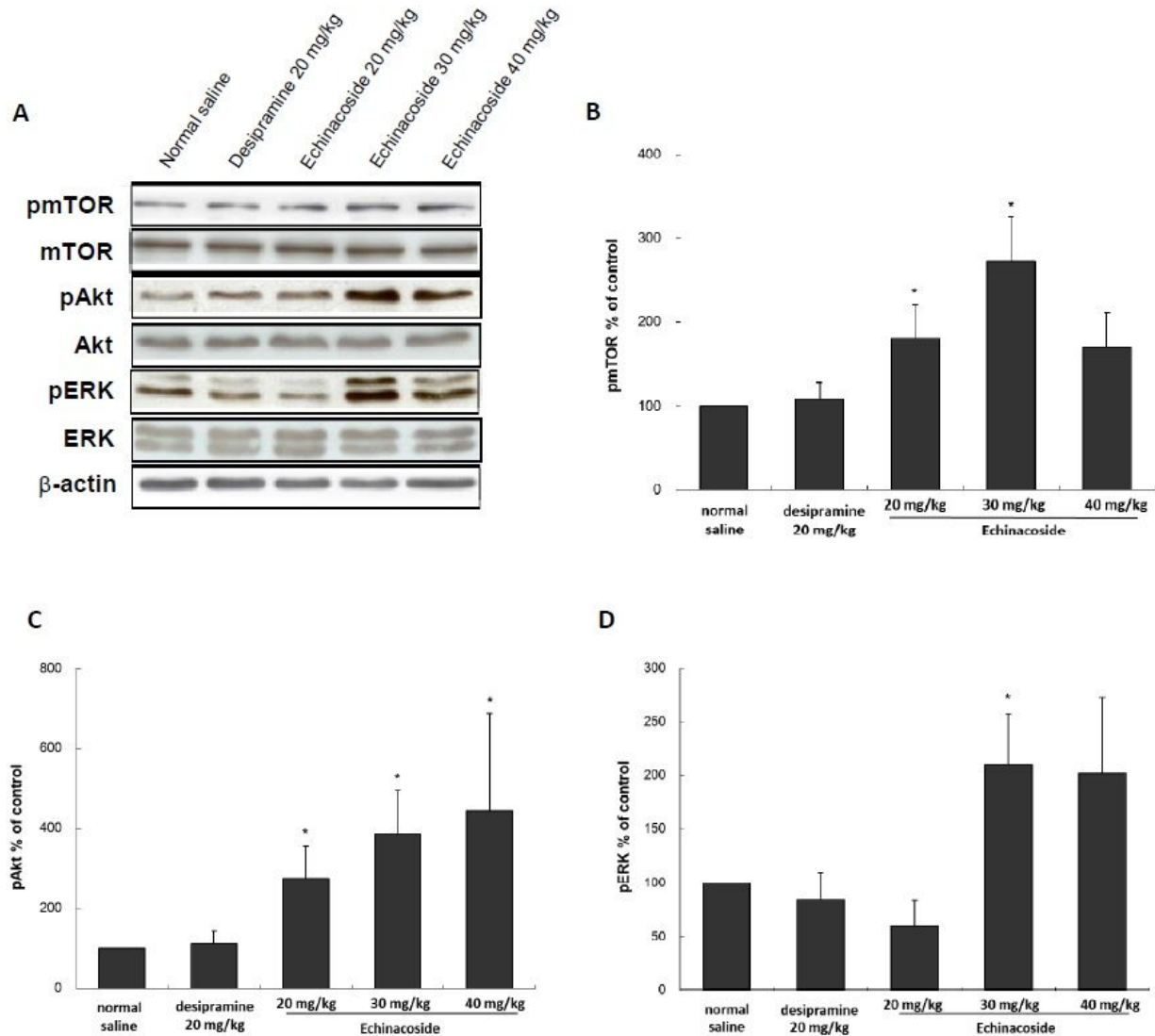
**Figure 1**



**Figure 1**

Experimental mice treated with saline, desipramine (20mg/kg, a tricyclic antidepressant as a positive control) and three different doses of echinacoside (20, 30 or 40mg/kg) by intraperitoneally injection then detected behavior by forced swimming test (FST) (A) or open field test (OFT) (B). Mice were respectively administrated by normal saline, desipramine (20mg/kg) and echinacoside (20, 30 or 40 mg/kg) 30 minutes before FST and 15-minute pre-swimming were conducted at 24 hours before the experiment (A). The percentage of immobility time significantly reduced in the groups of three different doses of echinacoside and desipramine-treated group (C) (ANOVA,  $F(4,45)=32.454$ ,  $p < 0.001$ ,  $n = 10$  per group). Mice were injected by normal saline, desipramine (20mg/kg) and echinacoside (20, 30 or 40 mg/kg) 30 minutes before OFT (B). The total distance moved linked to the animals' locomotor activity ability showed no difference between saline and all treating groups (D) (ANOVA,  $F(4,45)=1.859$ ,  $p > 0.05$ ,  $n = 10$  per group). (\*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with saline-treated group with Tukey post hoc analysis); values shown are mean  $\pm$  SEM.

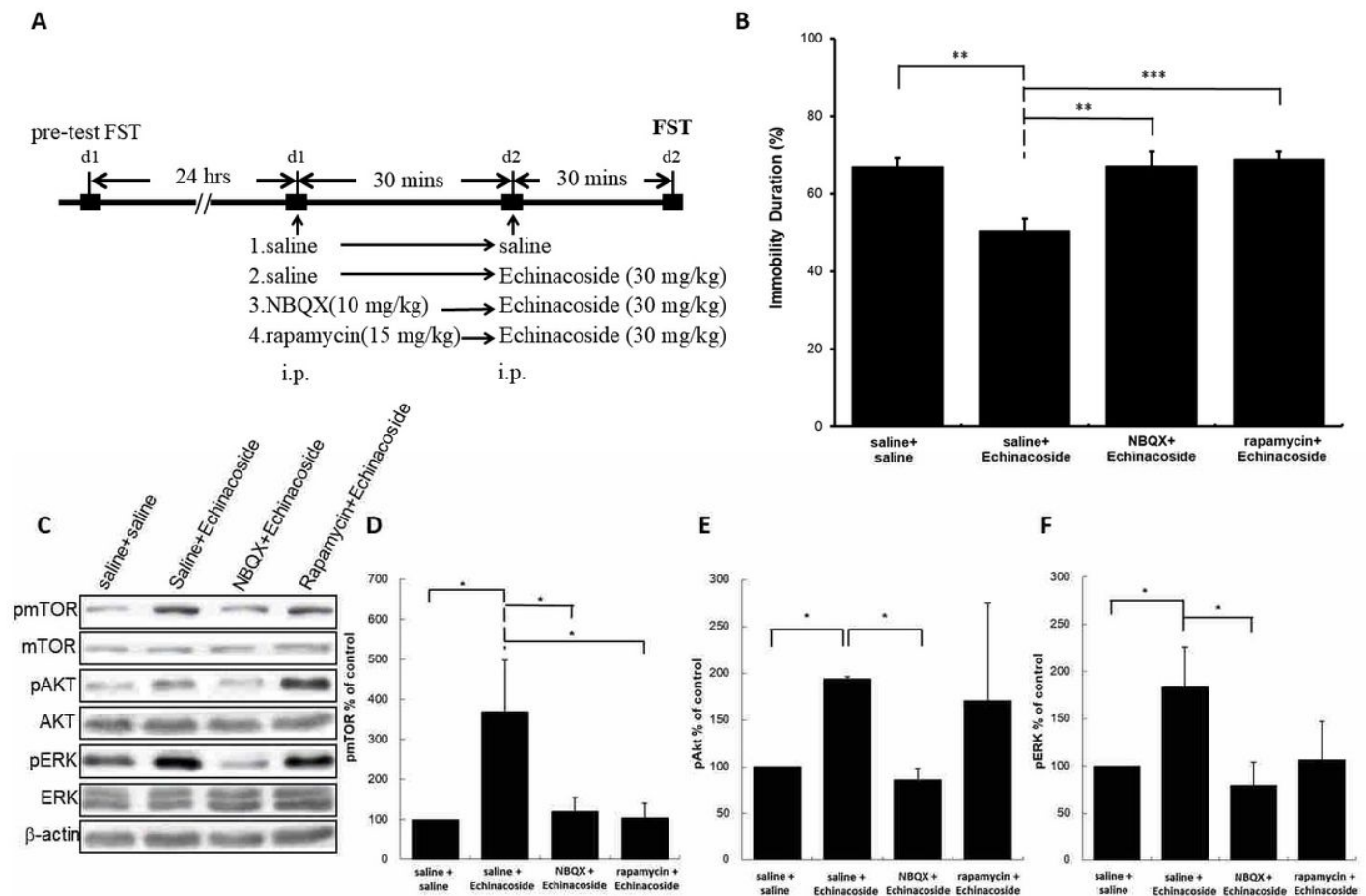
**Figure 2**



**Figure 2**

Effects of different echinacoside doses (20mg/kg, 30mg/kg or 40mg/kg) and desipramine (20mg/kg) intraperitoneally injected on the phosphorylation of mTOR, Akt, and ERK in the hippocampus of mice. Western blot analysis of phosphorylation of mTOR, Akt and ERK were conducted (A). The densitometry analyses of the blot (normalized to  $\beta$ -actin) verify the enhanced activity of pmTOR in group treated with echinacoside at 20 mg/kg and 30 mg/kg, not in groups treated with echinacoside at 40 mg/kg and desipramine (B). The increased pAkt in the mice hippocampus following treatment with echinacoside were showed in a dose-dependent manner (C). Only echinacoside at 30 mg/kg significantly increased pERK (D). Total levels of mTOR, Akt, and ERK were not different among the groups. (\* $p < 0.05$  compared with saline-treated group by Mann-Whitney U test);  $n=4$  per group, values shown are mean  $\pm$  SEM.

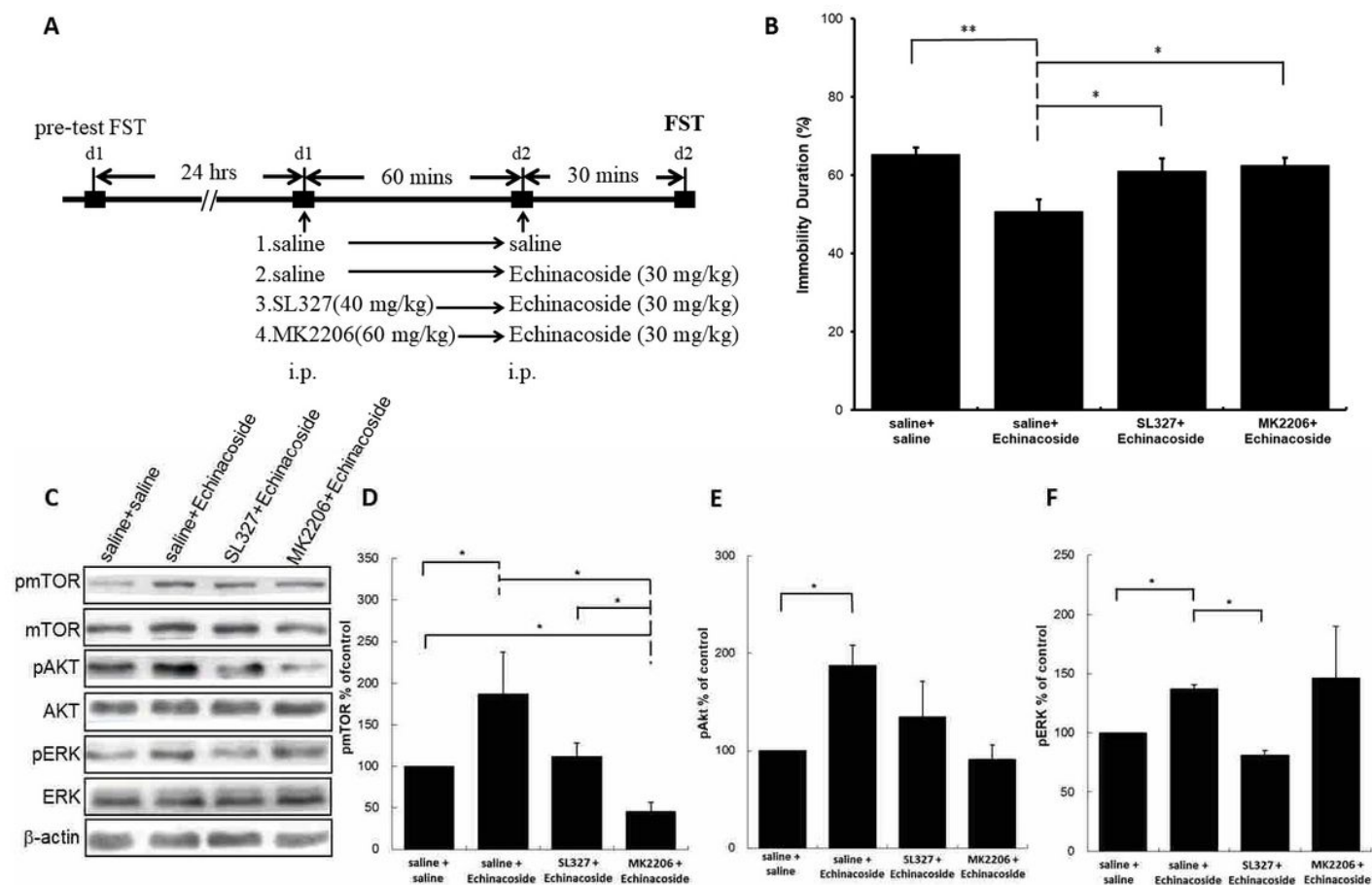
**Figure 3**



**Figure 3**

Effects of NBQX (30 mg/kg) or rapamycin (20 mg/kg) on the immobility duration in forced swim test (FST) and Western blotting of pmTOR, pAkt, and pERK from hippocampus of echinacoside (30 mg/kg)-treated mice. The timeline exhibits the experimental procedure under administration of drugs (A). Statistically analysis showed that the effect of reduction of immobility duration in FST resulted from echinacoside treatment is inhibited when the mice were pretreated with NBQX and rapamycin (B) (ANOVA,  $F(3,36)=9.083$ ,  $p < 0.001$ ;  $n = 10$  per group; \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with saline-treated group with Tukey post hoc analysis). Western blot analysis of phosphorylation of mTOR, Akt, and ERK was performed (C). The densitometry analyses of the blot (normalized to β-actin) confirmed the increased activity of pmTOR (D), pAkt (E), and pERK (E) in the echinacoside administrated group. The increased expression of pmTOR (D), pAkt (E), and pERK (F) resulted from echinacoside treatment being blocked when mice were pretreated with NBQX. The increased expression of pmTOR (D) resulted from echinacoside treatment is blocked when mice were pretreated with rapamycin and the increased expression of pAkt (E) and pERK (F) resulted from echinacoside treatment is not blocked;  $n = 4$  each group; \*  $p < 0.05$ , Mann-Whitney U test; Values shown are mean  $\pm$  SEM.

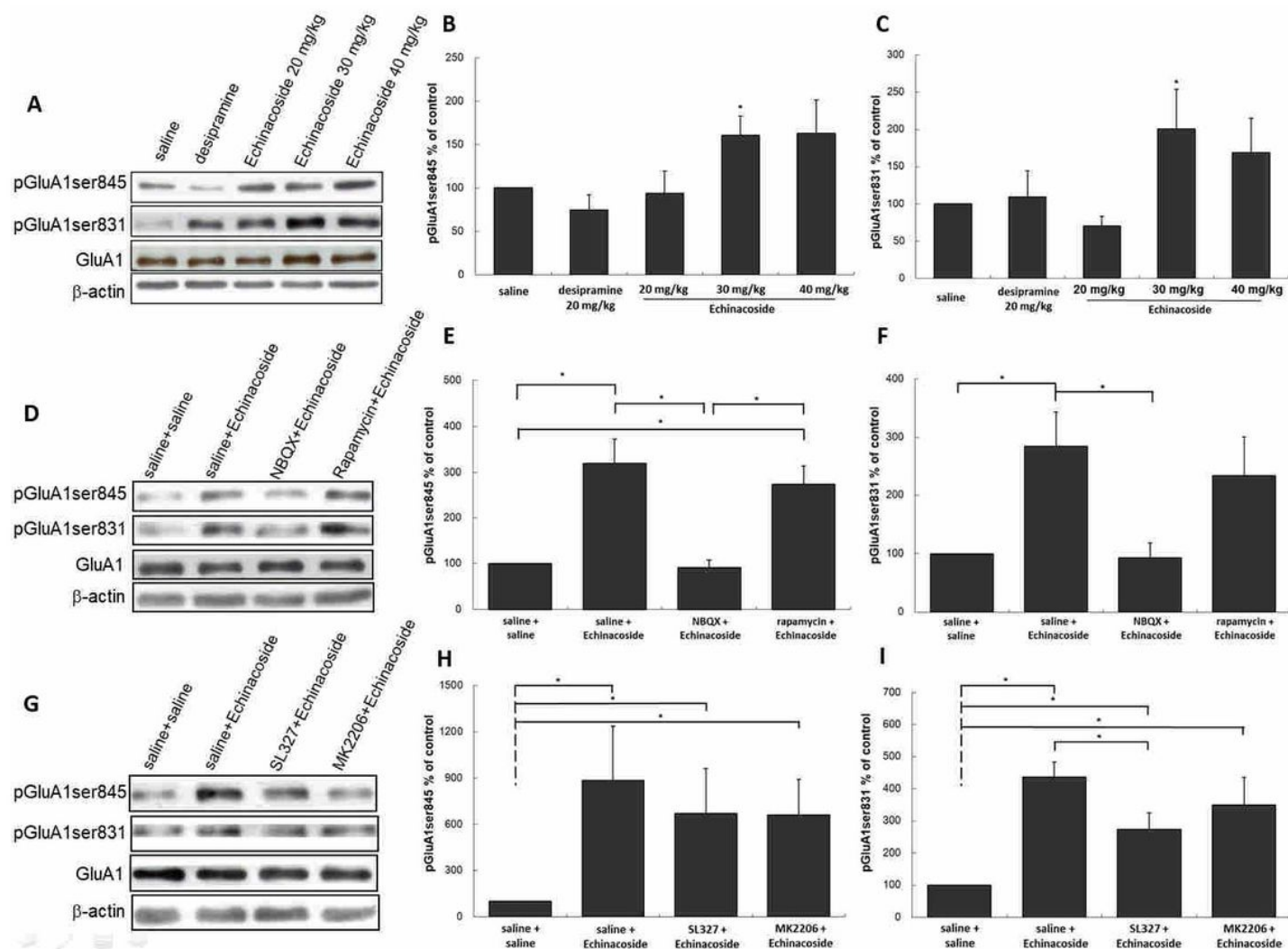
**Figure 4**



**Figure 4**

Effects of SL327 (40 mg/kg) or MK2206 (60 mg/kg) on the antidepressant-like effect and Western blotting of pmTOR, pAkt, and pERK of echinacoside (30 mg/kg)-treated mice. The timeline exhibits the experimental procedure under administration of drugs (A). Statistically analysis showed that the effect of reduction of immobility duration in FST resulted from echinacoside treatment is inhibited when the mice were pretreated with SL327 and MK2206 (B) (ANOVA,  $F(3,36)=5.867$ ,  $p < 0.01$ ;  $n=10$  per group; \* $p < 0.05$ , \*\* $p < 0.01$  compared with saline-treated group with Tukey post hoc analysis). Western blot analysis of phosphorylation of mTOR, Akt, and ERK was performed (C). The densitometry analyses of the blot (normalized to  $\beta$ -actin) confirmed the increased activity of pmTOR (D), pAkt (E), and pERK (E) in the echinacoside administrated group. Western blotting shows the increased expression of pmTOR caused by echinacoside treatment is blocked when the mice were pretreated with SL327 and MK2206 (D). The enhanced expression of pAkt (E) is blocked by pretreatment of MK2206, not SL327. The enhanced expression of pERK (F) is blocked by pretreatment of SLE 327, not MK2206. Total levels of Akt, ERK and mTOR were not different among the four groups.  $n = 4$  each group; \*  $p < 0.05$ , Mann-Whitney U test; Values shown are mean  $\pm$  SEM.

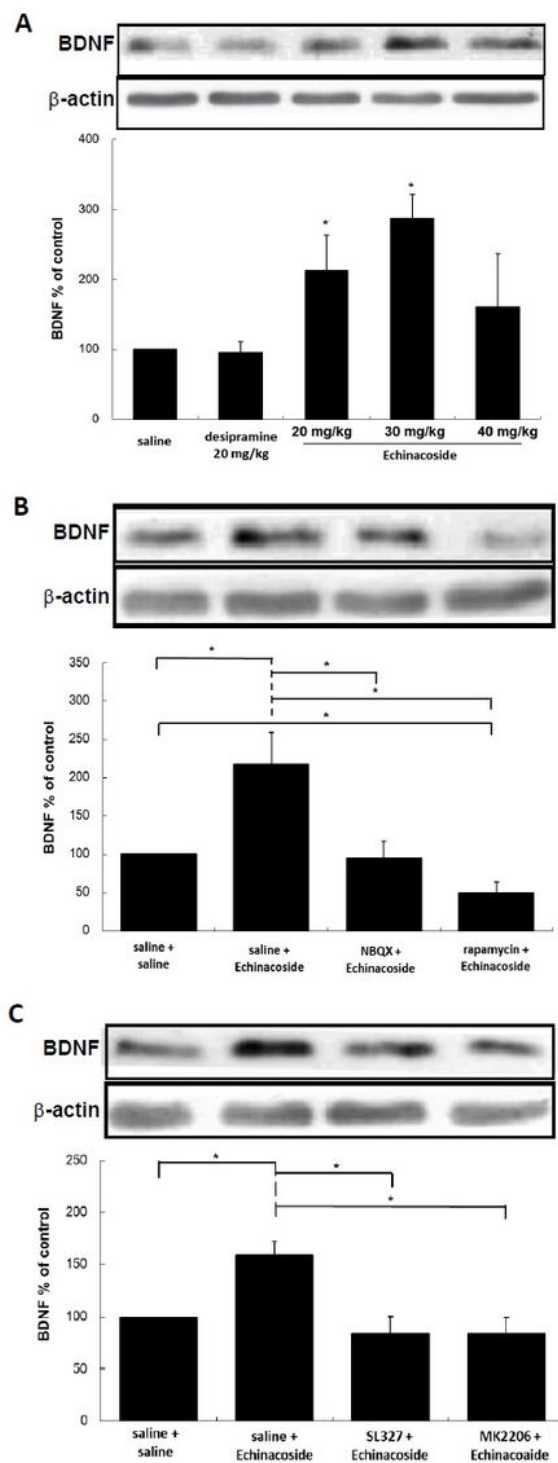
**Figure 5**



**Figure 5**

Representative Western blotting of pGluA1ser845 and pGluA1ser831 from hippocampus of mice treated with saline, desipramine (20 mg/kg) or echinacoside (20, 30 or 40 mg/kg) (A), after echinacoside (30 mg/kg) administration with pretreatment with NBQX (30 mg/kg) or rapamycin (20 mg/kg) (D) and after echinacoside (30 mg/kg) administration with pretreatment with SL327 (40 mg/kg) or MK2206 (60 mg/kg) (G). Echinacoside at 30 mg/kg treatment significantly increases the expression of pGluA1ser845 (B) and pGluA1ser831 (C), which were not observed in mice with other treatments. The densitometry analyses of the blot (normalized to β-actin) verify the enhanced activities of pGluA1ser845 (E), and pGluA1ser831 (F) in the echinacoside at 30 mg/kg administrated group and decreased activities in the NBQX, but, not in rapamycin pretreated groups (D, E, F). The up-regulations of pGluA1ser845 and pGluA1ser831 were not blocked by pretreatment of SL327 or MK2206 (G, H, I). n = 4 each group; \* p < 0.05, Mann-Whitney U test; Values shown are mean ± SEM.

**Figure 6**



**Figure 6**

Representative Western blotting of the expression of BDNF from hippocampus of mice treated with saline, desipramine (20 mg/kg) or echinacoside (20, 30 or 40 mg/kg) (A), after echinacoside (30 mg/kg) administration with pretreatment with NBQX (30 mg/kg) or rapamycin (20 mg/kg) (B) and after echinacoside (30 mg/kg) administration with pretreatment with SL327 (40 mg/kg) or MK2206 (60 mg/kg) (C). Echinacoside at 20, 30 mg/kg treatment significantly increases the expression of BDNF (B),

which was not observed in mice with other treatments. The densitometry analyses of the blot (normalized to  $\beta$ -actin) verify the enhanced expression of BDNF (B) in the echinacoside at 30 mg/kg administrated group and decreased activities in the NBQX and rapamycin pretreated groups (B). The up-regulation of BDNF was also blocked by pretreatment of SL327 or MK2206 (C). n = 4 each group; \* p < 0.05, Mann-Whitney U test; Values shown are mean  $\pm$  SEM.

## Supplementary Files

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