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ORIGINAL ARTICLE

Protective effects of jujube seed extract against oxidative stress in HT22 cells and its sleep-promoting action through GABAergic receptors

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Abstract

Jujube seed extract (JSE), rich in polyphenols and flavonoids, exhibits neuroprotective and sleep-enhancing properties. *In vitro*, JSE protected HT22 cells against oxidative stress by activating the Nrf2/HO-1 pathway and upregulating antioxidant enzymes. *In vivo*, JSE increased non-REM sleep and delta wave activity in pentobarbital- and caffeine-induced sleep models. Spinosin contributed to these effects. Long-term JSE administration upregulated $GABA_A$, $GABA_B$, and 5-HT_{1A} receptors in brain tissue. These findings suggest that JSE offers dual benefits in mitigating oxidative stress and promoting sleep through antioxidant defense and neurotransmitter modulation, supporting its potential as a natural therapeutic agent.

Keywords: Antioxidant, GABA receptors, Neuroprotection, Oxidative stress, Sleep

1. Introduction

Sleep is an essential physiological process for nearly all animals and plays a critical role in maintaining life. Sleep deprivation can cause immediate effects such as fatigue, attention deficits, and emotional dysregulation. In the long term, it can lead to severe mental and physical abnormalities, including disorientation, paranoia, hallucinations, and, in extreme cases, can be fatal [1]. Prolonged sleep deprivation has been shown to elevate reactive oxygen species (ROS) levels in the brain, leading to oxidative stress and damage to neurons. Increased ROS levels in hippocampal neurons negatively impact synaptic plasticity and cognitive function [2].

Sleep has a protective function against oxidative damage by helping to dissipate ROS in the brain. During sleep, the brain's metabolic activity decreases, and the activity of antioxidant enzymes increases, maintaining a balance between ROS production and removal. However, insomnia disrupts this balance, causing an increase in oxidative damage and impairing neuronal function [3]. Natural products with antioxidant activity have been found to reduce oxidative stress and prevent cell damage, offering potential benefits for those experiencing sleep deprivation or insomnia [4]. As sleep deprivation and insomnia can increase oxidative stress and cause brain cell damage, natural antioxidants may help reduce this stress and improve sleep quality.

Jujube seed extract (JSE), a traditional herbal medicine derived from the seeds of the Chinese jujube (*Ziziphus jujuba* Miller), is rich in antioxidants and has been used in oriental medicine to treat sleep disorders [5]. It has been shown to reduce

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oxidative stress and prevent cell damage. Studies have reported the effects of JSE on psychosocial stress, its ability to enhance pentobarbital-induced sleep by modulating the GABAergic system, and its anxiolytic effects in mice. The sleep-inducing effects of JSE are linked to its affinity for the $GABA_A$ receptor [6], which is targeted by GABAergic neurons regulating sleep and wakefulness [7]. $GABA_A$ receptors, predominantly located in key brain regions involved in sleep regulation, are important targets for many sleep disorder treatments [8].

This study aimed to demonstrate that JSE, which interacts with GABAergic neurons, protects hippocampal neurons from damage caused by oxidative stress and improves sleep. The study tested JSE's protective effects on hippocampal HT22 cells under oxidative stress induced by H_2O_2 and assessed its sleep-promoting effects through pentobarbital-induced sleep experiments and electroencephalogram (EEG) measurements. These findings suggest that JSE can protect hippocampal neurons from oxidative damage while promoting sleep, offering new possibilities for the treatment and prevention of sleep disturbances.

2. Materials and methods

2.1. JSE preparation

The Jujube Seed Extract (JSE) was supplied by NOVAREX Co., Ltd., located in Cheongju, Korea. Initially, the dried jujube seeds underwent extraction using 30% fermented food-grade ethanol, followed by filtration. The resulting filtrate was subsequently concentrated to a 10 Brix solution and then subjected to spray drying to produce JSE powder. The final product of JSE contain 4.0 mg/g of spinosin.

2.2. Analysis of radical scavenging activity and ferric reducing antioxidant power (FRAP)

The scavenging activity of JSE against 1,1-diphenyl-picrylhydrazyl (DPPH, Sigma-Aldrich, Louis, MO, USA) radicals was assessed using a slightly adapted Blois et al. method [9]. The scavenging activity against 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich) radicals was measured using the method described by Re et al. [10]. The FRAP assay was performed following the protocol outlined by Benzie & Strain [11]. Iron sulfate hexahydrate (Sigma-Aldrich) was used as the standard, and the results are expressed as the micromolar (μM) content of iron sulfate hexahydrate present in 1 mg of extract.

2.3. Analysis of polyphenols, flavonoids, and total sugars

Total polyphenol and flavonoid contents were measured by the Folin-Ciocalteu method [12] and the p-dimethylaminocinnamaldehyde method [31], respectively, with gallic acid (Sigma-Aldrich) and catechin (Sigma-Aldrich) as standards at wavelengths of 750 nm and 640 nm, respectively. Total sugars were measured using the phenol-sulfuric acid method at a wavelength of 490 nm, using glucose as the standard.

2.4. HT22 cell culture and cell viability

The cells used in this experiment were mouse hippocampus-derived neuronal cells (HT22). They were cultured in an incubator at 5% CO_2 and 37 °C using Dulbecco's Modified Eagle's Medium (Welgene, Gyeonsan, Korea) containing 10% fetal bovine serum (Welgene), 1% penicillin-streptomycin (Welgene), and 1% L-glutamate.

To investigate the effect of JSE on cell viability and H_2O_2-induced cytotoxicity, cells were seeded at a concentration of 1×10^4 cells/mL and treated with various concentrations of the JSE. Cells were exposed to 400 μM H_2O_2 for oxidative stress, and viability was assessed 24 h later by the WST-1 assay.

2.5. Analysis of ROS and malondialdehyde (MDA) in HT22 cell line treated with H_2O_2

The ROS scavenging activity of JSE was measured using a 2,7-dichlorofluorescin diacetate (DCF-DA) assay with HT22 cells. Cells (1×10^5 cells/mL) were cultured in 6-well plates for 24 h at 37 °C with 5% CO_2, treated with JSE (100 and 200 μg/mL) for 2 h, followed by 400 μM H_2O_2 for 24 h. After incubation with 10 μM DCF-DA for 30 min, the supernatant was collected, and fluorescence intensity was measured (excitation: 485 nm; emission: 535 nm).

To measure changes in MDA levels, the MDA 586 kit (Oxis Research, Portland, OR, USA) was used. Cell lysates quantified to 1 mg/mL were used to measure MDA levels by the manufacturer's instructions.

2.6. qPCR analysis

Intracellular RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, USA). Brain tissue (100 mg) was added to 1 mL of TRIzol reagent, homogenized with a tissue lyser, and processed for total RNA extraction. cDNA synthesis from RNA was performed using 1 μg of total RNA and a PrimeScript reagent kit. RT-PCR was conducted using cDNA, primer sequences (Table S1 https://doi.org/

10.38212/2224-6614.3555), and Power SYBR Green PCR Master Mix. The amount of mRNA for each expressed gene was calculated relative to GAPDH using an RT-PCR system (Applied Biosystems, CA, USA). The target genes included γ-aminobutyric acid A receptor, subunit gamma 1 (Gabrg1), γ-aminobutyric acid B receptor 1 (Gabbr1), γ-aminobutyric acid B receptor 2 (Gabbr2), and 5-hydroxytryptamine receptor 1A (Htr1a).

2.7. Western blot analysis

Intracellular proteins were separated using RIPA lysis buffer supplemented with both protease and phosphatase inhibitors. For protein homogenization, 50 mg of brain tissue was combined with 1 mL of lysis buffer. The homogenized mixture was centrifuged (12,000×*g*, 15 min, 4 °C), and the resulting supernatant was collected to obtain the protein extract. Protein concentrations were determined using the BCA protein assay, and 20 μg of the extracted protein was subjected to sodium dodecyl sulfate-polyacrylamide gel for electrophoresis. After size-based separation, proteins were transferred onto PVDF membranes. The membranes were blocked with 5% bovine serum albumin for 1 h and then incubated 18 h at 4 °C with primary antibodies [GAPDH and GABAB receptor (Cell Signaling Technology Inc., MA, USA) and GABA A receptor and 5-HT1A receptor (Abcam, Cambridge, UK)] at a dilution of 1:1000. Following this, a secondary antibody (HRP-linked anti-rabbit IgG, Cell Signaling Technology Inc.) was diluted 1:2000 and incubated for 2 h at room temperature. Protein expression levels were visualized using an ECL detection kit and captured using an image analyzer (EZ-Capture ST, Tokyo, Japan).

2.8. Experimental animals

The experimental animals used in this study included male ICR mice weighing 30 ± 2 g and male SD rats weighing 300 ± 20 g, purchased from Orient Bio Inc. (Seongnam, Korea). The study was approved by the Korea University Institutional Animal Care & Use Committee (KUIACUC-2024-0048). The animals were acclimated for over a week under conditions of 20 ± 3 °C, 50 ± 5% humidity, and a 12-h dark/light cycle.

2.9. Analysis of sleep latency and sleep duration in mice induced by pentobarbital

After 24 h of fasting, groups of six mice were given oral doses of JSE, followed 30 min later by an intraperitoneal injection of pentobarbital at 42 mg/kg. Mice that failed to fall asleep within 15 min were excluded. Sleep duration was recorded as the time between the loss and recovery of the righting reflex. To examine the effects of antagonists, picrotoxin (PIX, 4 mg/kg), bicuculline (BIC, 6 mg/kg), and flumazenil (FMZ, 10 mg/kg) were injected intraperitoneally 20 min prior to JSE. Pentobarbital was then administered 40 min after JSE, and both sleep latency and duration were measured.

2.10. EEG analysis

EEG electrodes were implanted following the method described by Hong et al. (2018), and SD rats were allowed one week to recover before an EEG transmitter was attached. EEG recordings were conducted at 15 mm/s for 7 h (10:00–17:00) after oral sample administration. Sleep structure was analyzed using the Fast Fourier Transform (FFT) algorithm and the ecgAUTO3 program (v3.3, emka Technologies), with parameters including wakefulness, REM sleep, NREM sleep, theta waves, and delta waves. In the insomnia model, caffeine (40 mg/kg) was administered to all groups except the normal group (NOR) prior to EEG analysis.

2.11. Statistical analysis

Data were analyzed using a one-way ANOVA with the Statistical Package for Social Sciences (SPSS, version 27.0, IBM, Chicago, IL, USA). Group comparisons were made at the $p < 0.05$ level using Tukey's multiple comparison test.

3. Results

3.1. Radical scavenging activity and components of JSE

JSE showed the highest total sugar content at 500.20 mg/g, with polyphenol and flavonoid contents-estimated to contribute to its radical scavenging activity-measured at 35.87 mg/g and 0.12 mg/g, respectively. The IC_{50} values for scavenging activity against ABTS and DPPH radicals were 4.68 and 6.60 mg/mL, respectively. Additionally, the reducing power, analyzed using the FRAP assay, was 51.66 mM/g.

3.2. Cell viability

A WST assay showed increased HT22 cell viability with JSE treatment (50–400 μg/mL) after 24 h (Fig. 1). Fig. 2 shows the protective effect of JSE against H_2O_2-induced oxidative stress, with cells exposed to H_2O_2 alone exhibiting 64.65% viability.

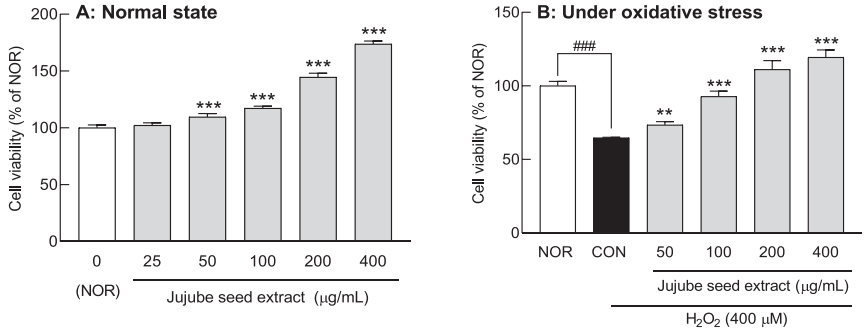

Fig. 1. ***Effect of jujube seed extract (JSE) on normal HT22 cells and H_2O_2 oxidative stress-induced HT22 cell viability.*** *NOR represents untreated control cells, and CON represents H_2O_2-treated control cells. Cells treated with JSE at concentrations of 50–400 μg/mL are also shown. Values mean ± standard deviation. $^{###}p < 0.001$ compared to NOR, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to CON (ANOVA, Tukey's test).*

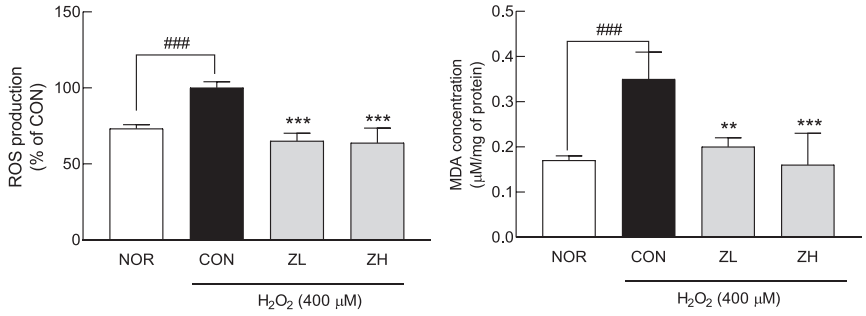

Fig. 2. ***Effect of jujube seed extract on ROS and MDA levels in HT22 cells exposed to H_2O_2-oxidative stress.*** *NOR represents untreated control cells, and CON represents H_2O_2-treated control cells. ZL indicates cells treated with jujube seed extract at a concentration of 100 μg/mL, while ZH indicates cells treated with 200 μg/mL. Values are mean ± standard deviation. $^{###}p < 0.001$ vs. NOR (T-test), $^{**}p < 0.01$ and $^{***}p < 0.001$ vs CON (ANOVA, Tukey's test).*

However, co-treatment with H_2O_2 and JSE resulted in an increase in viability with higher doses of JSE, showing 73.42% at 50 μg/mL, 92.82% at 100 μg/mL, and 111.16% and 119.39% at 200 and 400 μg/mL, respectively. These results indicate that JSE effectively inhibits H_2O_2-induced stress in nerve cells.

3.3. Changes of ROS and MDA in HT22 cells treated with H_2O_2 by JSE

The inhibitory effects of JSE on ROS and MDA were evaluated in HT22 cells subjected to H_2O_2-induced oxidative stress (Fig. 2). Treatment with JSE at concentrations of 100 μg/mL (ZL) and 200 μg/mL (ZH) effectively suppressed the increased levels of ROS and MDA caused by H_2O_2. Specifically, ROS levels decreased by 34.89% and 36.24% with 100 μg/mL and 200 μg/mL of JSE, respectively, while MDA levels were reduced by 42.86% and 54.29%. JSE has a significant inhibitory effect on oxidative stress in HT22 cells.

3.4. Changes in the expression levels of antioxidant enzymes in HT22 cells treated with H_2O_2 by JSE

To assess whether JSE protects cells from H_2O_2-induced toxicity via cellular antioxidant enzymes, their expression levels were measured (Fig. 3). H_2O_2 treatment significantly reduced the expression levels of SOD1, GSH-pX, and CAT relative to normal cells. However, in H_2O_2-treated cells, JSE treatment at both low (100 μg/mL) and high (200 μg/mL) doses restored these enzyme levels to those of normal cells or higher. Notably, the high dose of JSE resulted in significantly higher antioxidant enzyme expression relative to the control (CON). This increase in antioxidant enzyme expression due to JSE treatment appears to mitigate H_2O_2-induced oxidative stress, thereby demonstrating its protective effect on HT22 cells.

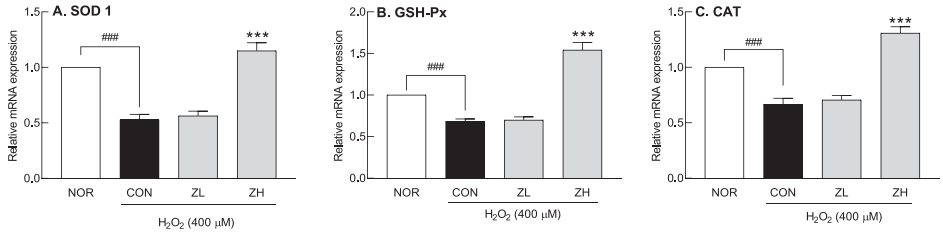

*Fig. 3. **Effect of jujube seed extract on SOD1, GSH-Px and CAT expression levels in H_2O_2 oxidative stress-induced HT22.** NOR represents untreated control cells, and CON represents H_2O_2-treated control cells. ZL indicates cells treated with jujube seed extract at a concentration of 100 μg/mL, while ZH indicates cells treated with 200 μg/mL. Values are mean ± standard deviation. $^{###}p < 0.001$ vs. NOR (T-test), $***p < 0.001$ vs CON (ANOVA, Tukey's test).*

3.5. Changes of HO-1/Nrf2 signaling protein levels in HT22 cells treated with H_2O_2 by JSE

Western blot analysis was conducted to examine whether JSE-induced antioxidant enzyme expression involves Nrf2/HO-1 signaling. As illustrated in Fig. 4, Nrf2 and HO-1 protein levels were significantly lower in the control group (CON) compared to the normal group (NOR) ($p < 0.01$), while Keap1, a negative regulator of Nrf2, was significantly higher in CON ($p < 0.05$) (Fig. 4A and B). JSE treatment increased the protein levels of Nrf2 and HO-1, while the expression of Keap1 decreased in proportion to the dosage. This suggests that JSE accelerates ROS scavenging by activating Nrf2 and promoting the production of oxidative stress-detoxifying enzymes.

3.6. Changes in sleep latency and duration by JSE

Oral administration of JSE (100 and 200 mg/kg) did not affect sleep latency, but sleep duration increased at higher doses, showing a significant difference from the NOR (Fig. 5). GABA (100 mg/kg), used as a positive control (PC), similarly showed no

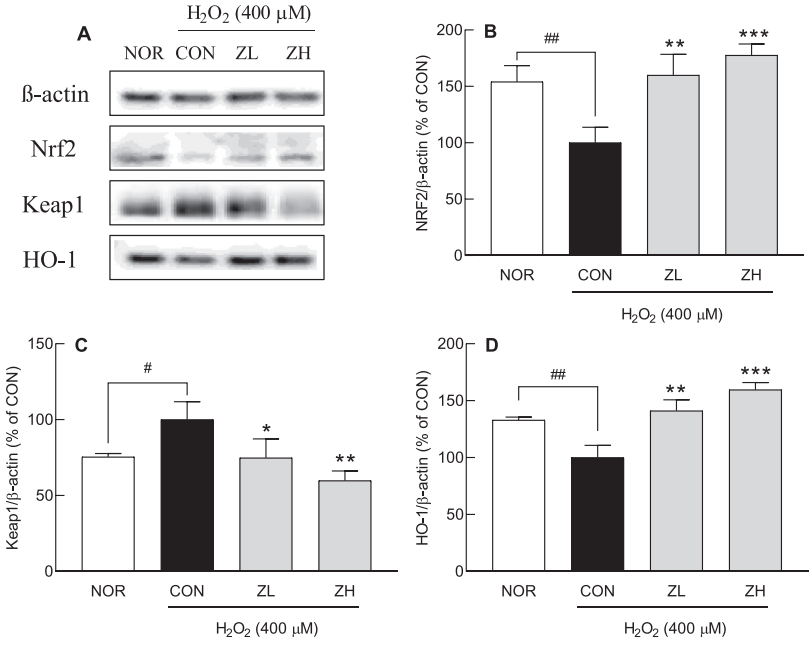

*Fig. 4. **Effect of jujube seed extract on Nrf2, Keap1 and HO-1 protein levels in H_2O_2 oxidative stress-induced HT22.** NOR represents untreated control cells, and CON represents H_2O_2-treated control cells. ZL indicates cells treated with jujube seed extract at a concentration of 100 μg/mL, while ZH indicates cells treated with 200 μg/mL. Values are mean ± standard deviation. $^{#}p < 0.05$ and $^{##}p < 0.01$ vs. NOR (T-test), $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs CON (ANOVA, Tukey's test).*

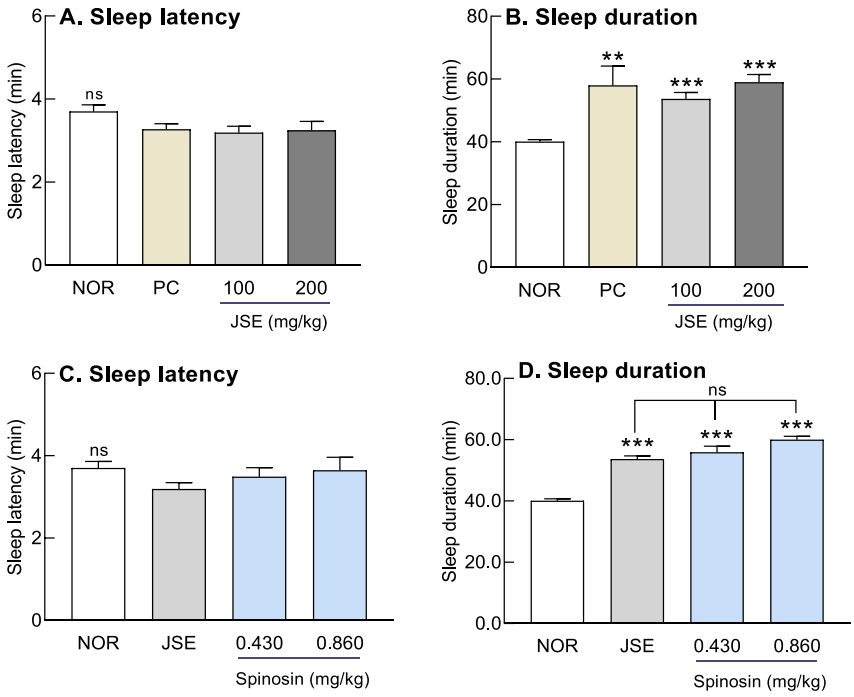

*Fig. 5. Effect of jujube seed extract (JSE) on sleep latency (A, C) and duration (B, D) in mice administered pentobarbital (42 mg/kg, i.p.). NOR: normal control, PC: positive control; γ-aminobutyric acid (100 mg/kg). Values are presented as means ± standard error of the mean (n = 7 for each group). **p < 0.01, ***p < 0.001 vs. NOR (ANOVA, Tukey's test). ns, not significant.*

significant effect on sleep latency, but increased sleep duration by 44.6% compared to NOR. JSE administration at 200 mg/kg produced a comparable increase in sleep duration to that observed with GABA.

Since spinosin, the presumed active component, is present in JSE at 4.31 mg/g, 0.43 mg of spinosin (Fig. S1 https://doi.org/10.38212/2224-6614.3555), equivalent to 100 mg of JSE, was orally administered, and sleep latency and duration were measured (Fig. 5C and D). No significant difference in sleep latency was observed with JSE at 100 mg/kg or spinosin at 0.43 and 0.86 mg/kg. Similarly, sleep durations of 53.65 and 55.62 min were recorded for JSE 100 mg/kg and spinosin 0.43 mg/kg, respectively. The effect of JSE in prolonging sleep duration may be related to its spinosin content.

3.7. Changes in NREM sleep by JSE

The changes in REM sleep and NREM sleep duration following oral administration of JSE were assessed through EEG analysis (Fig. 6). Both JSE and GABA administrations resulted in a greater increase in total sleep time compared to NOR. Sleep duration increased with JSE at 100 mg/kg and 200 mg/kg, although there was no significant difference between the two doses. While both GABA and JSE administration led to an increase in sleep time, no significant difference was observed between the two treatments. JSE administration led to a decrease in REM sleep duration, which was not significantly different from NOR, while NREM sleep duration increased significantly. With higher JSE doses, delta wave sleep also increased more compared to NOR. Therefore, the enhanced sleep effect of JSE is linked to the prolonged NREM sleep duration and the rise in delta wave sleep.

3.8. EEG changes in a caffeine-induced insomnia model by JSE

EEG changes following JSE administration were measured after inducing insomnia in SD rats with 40 mg/kg caffeine (Fig. 7). Caffeine administration resulted in increased awake time and decreased sleep time, confirming the induction of insomnia. GABA and JSE administration effectively restored the sleep time reduced by caffeine treatment. JSE administration elevated NREM sleep to a level slightly higher than NOR and also increased delta wave sleep beyond normal levels. The reduction in sleep time caused by caffeine was counteracted by JSE administration, and the normalization of sleep

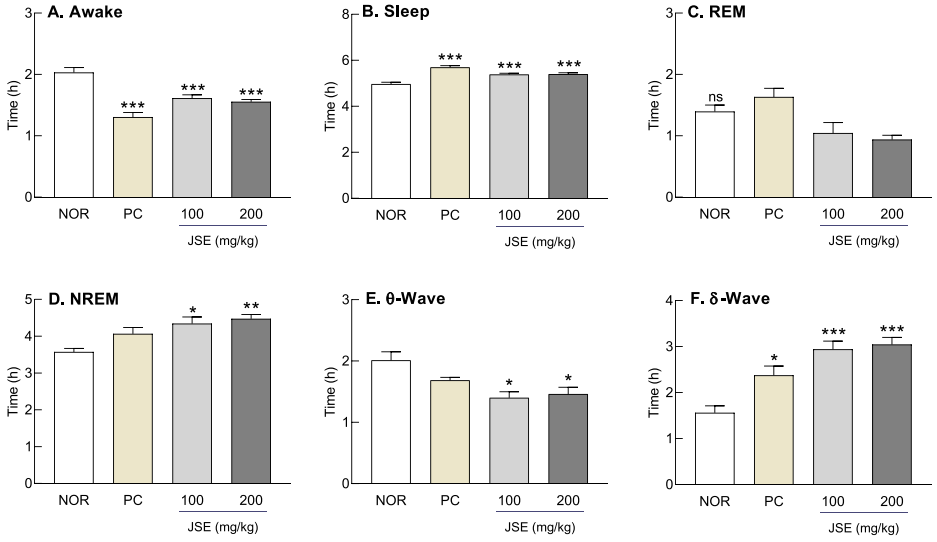

Fig. 6. ***Effect of jujube seed extract (JSE) on electrophysiological activity in rats.*** *Electroencephalography recordings were conducted over a 6-day period. (A) Time spent awake, (B) total sleep duration, (C) duration of rapid eye movement (REM) sleep, (D) duration of non-REM (NREM), (E) δ wave activity, and (F) θ wave activity during NREM. NOR: normal control, PC: positive control; γ-aminobutyric acid (100 mg/kg). Values are presented as means ± standard error of the mean (n = 6 for each group). *p < 0.05, **p < 0.01, ***p < 0.001 vs. NOR (ANOVA, Tukey's test). ns, not significant.*

time appears to be due to the increase in NREM sleep linked to elevated delta wave sleep.

3.9. Changes in sleep duration by GABA receptor antagonists

Sleep duration changes were measured with $GABA_A$ receptor antagonists alongside JSE to confirm its receptor binding (Fig. 8). Co-administration of $GABA_A$ receptor antagonists PIX, BIC, and FMZ with JSE did not show a significant change in sleep latency relative to NOR. When PIX and BIC were administered with JSE, sleep duration was comparable to that observed with JSE alone. However, the combination of FMZ and JSE led to a decrease in sleep duration in contrast to JSE alone. This decrease in sleep duration suggests that FMZ inhibits the binding of JSE to $GABA_A$ receptors.

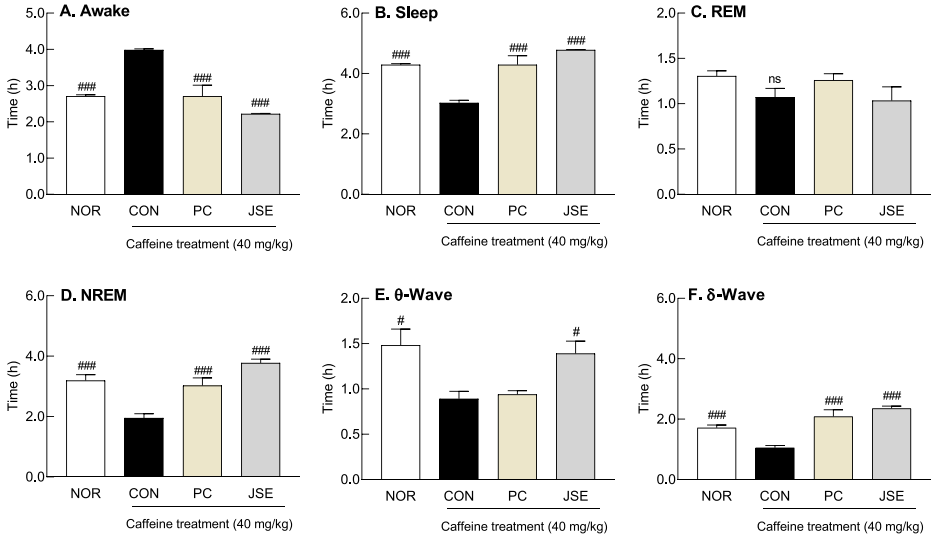

Fig. 7. ***Effect of jujube seed extract (JSE) on electrophysiologic pattern in rats.*** *Electroencephalography recordings were conducted over a 4-day period. (A) Time spent awake, (B) total sleep duration, (C) duration of rapid eye movement (REM) sleep, (D) duration of non-REM (NREM), (E) δ wave activity, and (F) θ wave activity during NREM. NOR: normal control; CON: caffeine control (40 mg/kg) group; PC: γ-aminobutyric acid (100 mg/kg), JSE: jujube seed extract (100 mg/kg). Values are presented as means ± standard error of the mean (n = 6 for each group). #p < 0.05, ###p < 0.001 vs. CON (ANOVA, Tukey's test). ns, not significant.*

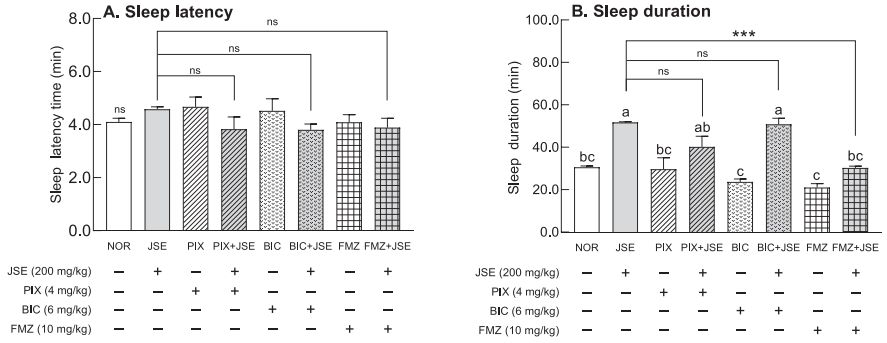

*Fig. 8. Effects of jujube seed extract (JSE) on the $GABA_A$ receptor antagonist sleep latency (A) and duration (B) in mice administered pentobarbital (42 mg/kg, i.p.). NOR: normal control, PIX: picrotoxin, BIC: bicuculline, FMZ: flumazenil, JSE: 100 mg/kg. Values are presented as means ± standard error of the mean (n = 7 for each group). ***p < 0.001 vs. NOR (ANOVA, Tukey's test). ns, not significant.*

These findings indicate that JSE may bind to the flumazenil binding site on $GABA_A$ receptors to exert its sleep-promoting effects.

3.10. Changes in brain receptors and neurotransmitters by oral administration of JSE for 4 weeks

To measure changes in brain receptors following oral administration of JSE for 4 weeks, brain tissues were extracted, and receptor expression levels were quantified using qPCR, while changes in receptor proteins were assessed through Western blot analysis (Fig. 9). The expression levels of Gabrg1, Gabbr1, and Gabbr2 receptors, which correspond to $GABA_A$ and $GABA_B$ receptors, increased with higher doses of JSE treatment. Similarly, the expression levels increased with GABA treatment, which served as a positive control (PC) (Fig. 9A–C). The expression level of Htr1a, corresponding to 5-HT_{1A} receptors, also increased in proportion to the JSE treatment dose (Fig. 9D). Western blot analysis revealed that $GABA_A$ receptor protein levels increased with JSE administration at 200 mg/kg, showing a significant difference compared to the NOR group (Fig. 9E). Additionally, treatment with GABA and JSE at 200 mg/kg contributed to increased levels of Htr1a protein (Fig. 9H).

4. Discussion

Sleep is essential for maintaining the body's equilibrium and regulating the nervous system, while sleep deprivation can lead to stress and adversely affect both bodily and brain functions.

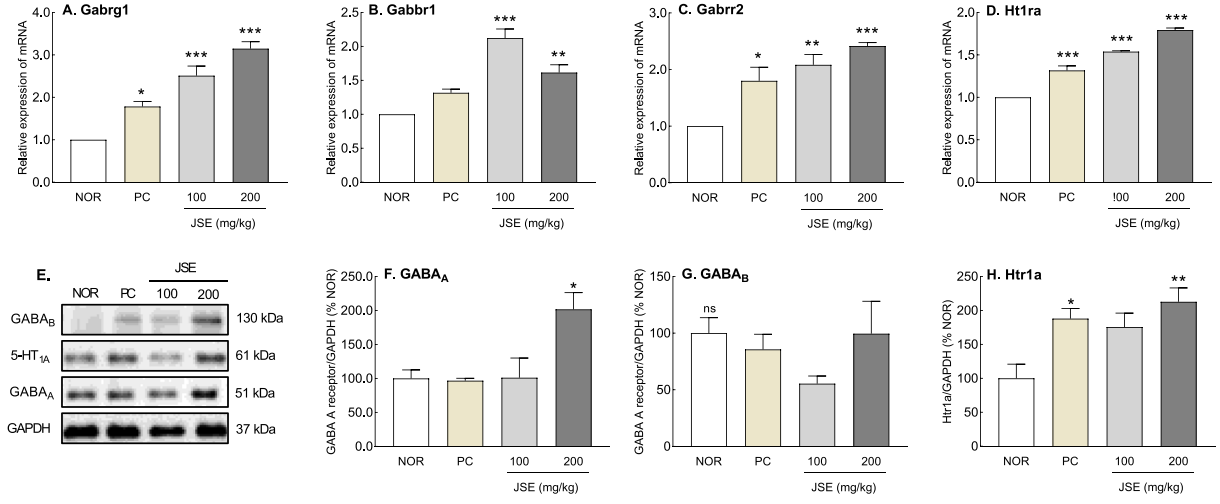

*Fig. 9. Effects of jujube extract (JSE) on gene and protein expressions of GABAergic and serotonin receptors in mice brain. NOR: normal control, PC: positive control; γ-aminobutyric acid (100 mg/kg). Values are presented as means ± standard error of the mean (n = 6 for each group). *p < 0.05, **p < 0.01 and ***p < 0.001 vs. NOR (ANOVA, Tukey's test). ns, not significant.*

Additionally, sleep is essential for the removal of ROS generated during wakefulness [13]. An increase in ROS is closely linked to sleep deprivation, which leads to the accumulation of ROS in the hippocampus—a region vital for regulating the circadian rhythm of sleep and wakefulness. This accumulation results in intracellular oxidative stress and can ultimately lead to neuronal death [14]. Flavonoids found in medicinal plant extracts have been shown to effectively eliminate ROS associated with sleep disorders [4].

Jujube contains flavonoids, saponins, and alkaloids, such as spinosin, swertisin, and acylspinosin, which exhibit sedative effects and radical scavenging activity. Table 1 shows that the IC_{50} value for DPPH radical scavenging was 0.3 mg/mL, demonstrating radical scavenging activity comparable to the IC_{50} values of 0.11–0.34 mg/mL reported by Choi et al. [15]. The radical scavenging activity of the flavonoids in JSE appears to help protect hippocampal cells from damage by inhibiting radical induction caused by H_2O_2 in HT22 cells (Fig. 1). This activity resulted in a decrease in intracellular ROS and MDA levels, which is an oxidative product (Fig. 2). The phenolic compounds in jujube seed extract have been identified as an effective source of antioxidants that enhance the viability of neuronal cells exposed to oxidative stress by reducing H_2O_2-induced oxidative damage and increasing the viability of PC-12 cells [16]. In particular, the expression of antioxidant enzymes plays a critical role in the removal of ROS in the body [17]. SOD catalyzes the conversion of superoxide into hydrogen peroxide and oxygen, which is then broken down into water and oxygen by catalase (CAT) and glutathione peroxidase (GPx), aiding in ROS elimination [18]. It seems that the increased expression levels of these antioxidant enzymes following JSE administration (Fig. 3) contributed to the removal of ROS in hippocampal cells.

Phase II enzymes like heme oxygenase-1 (HO-1) protect cells from ROS, and their expression is regulated by Nrf2, which boosts phase II enzyme levels to prevent cell damage from oxidative stress [19,20]. JSE administration contributed to the normalization of Nrf2 and HO-1 protein levels that were reduced by H_2O_2 exposure (Fig. 4). Nrf2 is a key regulator of antioxidant enzymes, and HO-1 is a target of cytosolic Nrf2 [21]. Under normal conditions, cytosolic Nrf2 binds to Keap1, but during oxidative stress, Nrf2 is released, increasing its protein levels. It then translocates to the nucleus, binds to antioxidant response elements, and promotes the expression of antioxidant proteins like HO-1 [22,23]. HO-1, influenced by the expression of cytosolic Nrf2, plays a critical role in ROS removal and is involved in neuronal protection.

Sleep plays a crucial role in maintaining the body's balance and regulating the nervous system. Since nerve cells are sensitive to environmental changes, it is essential to quickly remove waste products generated by nerve metabolism from the brain's intercellular space [24]. The oxidative stress removal effect of JSE not only helps eliminate ROS induced by insomnia but also contributes to maintaining homeostasis by increasing sleep duration.

Ziziphus spinosa, also known as *Z. jujuba*, is believed to possess sedative properties. *In vitro* studies have demonstrated the anxiolytic and sedative effects of *Z. jujuba* extracts due to their affinity for serotonin, benzodiazepine, dopamine, and GABA receptors [25]. Spinosin, a compound in *Z. jujuba*, reduces sleep latency and increases sleep duration. Among the jujuba extract compounds, saponins had stronger hypnotic effects than flavonoids and polysaccharides. Spinosin enhances pentobarbital-induced sleep through the 5-HT_{1A} receptor [26]. It decreases sleep latency while increasing total sleep time, NREM sleep time, REM sleep time, and particularly slow-wave sleep (SWS). Additionally, spinosin significantly antagonized the sleep reduction induced by 8-OH-DPAT, a 5-HT_{1A} agonist, in rats administered pentobarbital [27].

JSE, with spinosin as its active ingredient, enhanced pentobarbital-induced sleep without affecting sleep latency (Fig. 5). EEG analysis showed a decrease in REM sleep and an increase in NREM sleep, particularly in delta wave sleep (Fig. 6). After four weeks of JSE administration, the expression and protein levels of 5-HT1A and $GABA_A$ receptors increased, suggesting involvement of serotonin and GABA receptors, as previously reported. The GABA

Table 1. Radical scavenging activities and composition of jujube seed extract (JSE).

	IC_{50} value		Reducing power FRAP (mM/g)
	ABTS radical	DPPH radical	
JSE (mg/mL)	4.68 ± 0.26	6.60 ± 0.48	51.66 ± 6.08
Ascorbic acid (μg/mL)	69.75 ± 0.26	22.00 ± 0.71	-
	Polyphenols	Flavonoids	Total sugar
JSE (mg/g)	35.87 ± 0.77	0.12 ± 0.002	500.20 ± 4.75

receptor, a major target for improving insomnia, is an ion channel protein that binds to GABA, an inhibitory neurotransmitter in the central nervous system. GABA receptors are distributed throughout the brain and play an essential role in regulating the nervous system by inhibiting excessive nerve cell activity. Among the GABA receptors, the $GABA_A$ receptor is most closely associated with sleep [28]. The $GABA_A$ receptor is known to be the target of benzodiazepine hypnotics, which enhance GABA's effects to induce sleep [29]. Spinosin, found in *Z. jujuba*, is known to interact with both $GABA_A$ and 5-HT1A receptors, with its activity being modulated by antagonists of the $GABA_A$ and 5-HT_{1A} receptors, respectively [30]. Additionally, JSE has been shown to engage with the flumazenil binding site among the various binding sites of $GABA_A$ receptors (Fig. 8).

This study demonstrated that JSE mitigates oxidative stress and reduces oxidative damage in hippocampal cells by increasing the expression of HO-1, a phase II antioxidant enzyme regulated by other antioxidant enzymes such as SOD, CAT, and GPX, as well as the Nrf2 protein. Furthermore, JSE promotes sleep through the involvement of 5-HT_{1A} and $GABA_A$ receptors, particularly by binding to the benzodiazepine site associated with flumazenil among the $GABA_A$ receptors. Therefore, JSE is expected to be utilized as a functional food ingredient due to its ROS scavenging activity and sleep-promoting effects.

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Declaration of competing interest

The authors have declared that there are no conflicts of interest related to this study.

References

[1] Palmer CA, Bower JL, Cho KW, Clementi MA, Lau S, Oosterhoff B, et al. Sleep loss and emotion: a systematic review and meta-analysis of over 50 years of experimental research. Psychol Bull 2024;150:440.
[2] Davinelli S, Medoro A, Savino R, Scapagnini G. Sleep and oxidative stress: current perspectives on the role of NRF2. Cell Mol Neurobiol 2024;44:52.
[3] Terzi A, Ngo KJ, Mourrain P. Phylogenetic conservation of the interdependent homeostatic relationship of sleep regulation and redox metabolism. J Comp Physiol B 2024:1–12.
[4] David AVA, Parasuraman S, Edward EJ. Role of antioxidants in sleep disorders: a review. J Pharmacol Pharmacother 2023; 14:253–8.
[5] Chen J, Liu X, Li Z, Qi A, Yao P, Zhou Z, et al. A review of dietary *Ziziphus jujuba* fruit (Jujube): developing health food supplements for brain protection. Evid Based Complement Alternat Med 2017;2017:3019568.
[6] Ghit A, Assal D, Al-Shami AS, Hussein DEE. GABA receptors: structure, function, pharmacology, and related disorders. J Genet Eng Biotechnol 2021;19.
[7] Li L, Zhang MQ, Sun X, Liu WY, Huang ZL, Wang YQ. Role of dorsomedial hypothalamus GABAergic neurons in sleep-wake states in response to changes in ambient temperature in mice. Int J Mol Sci 2022;23.
[8] Michels G, Moss SJ. GABA receptors: properties and trafficking. Crit Rev Biochem Mol Biol 2007;42:3–14.
[9] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199–200.
[10] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 1999;26:1231–7.
[11] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70–6.
[12] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999;299:152–78.
[13] Hachul de Campos H, Brandao L, D'Almeida V, Grego B, Bittencourt L, Tufik S, et al. Sleep disturbances, oxidative stress and cardiovascular risk parameters in postmenopausal women complaining of insomnia. Climacteric 2006;9:312–9.
[14] Klein JA, Ackerman SL. Oxidative stress, cell cycle, and neurodegeneration. J Clin Investig 2003;111:785–93.
[15] Choi SH, Ahn JB, Kim HJ, Im NK, Kozukue N, Levin CE, et al. Changes in free amino acid, protein, and flavonoid content in Jujube (*Ziziphus jujube*) fruit during eight stages of growth and antioxidative and cancer cell inhibitory effects by extracts. J Agric Food Chem 2012;60:10245–55.
[16] Choi J, An X, Lee BH, Lee JS, Heo HJ, Kim T, et al. Protective effects of bioactive phenolics from jujube (*Ziziphus jujuba*) seeds against H_2O_2-induced oxidative stress in neuronal PC-12 cells. Food Sci Biotechnol 2015;24:2219–27.
[17] Chen XL, Kunsch C. Induction of cytoprotective genes through Nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. Curr Pharm Des 2004;10:879–91.
[18] Choi Y, Lee SM, Kim Y, Yoon J, Jeong HS, Lee J. A tocotrienol-rich fraction from grape seeds inhibits oxidative stress induced by tert-butyl hydroperoxide in HepG2 cells. J Med Food 2010;13:1240–6.
[19] Zhao CR, Gao ZH, Qu XJ. Nrf2-ARE signaling pathway and natural products for cancer chemoprevention. Cancer Epidemiol 2010;34:523–33.
[20] Jaiswal AK. Nrf2 signaling in coordinated activation of antioxidant gene expression. Free Radic Biol Med 2004;36:1199–207.
[21] Kwon DH, Choi EO, Hwang H-J, Kim KJ, Hong SH, Lee DH, et al. Socheongja and Socheong 2 extracts suppress lipopolysaccharide-induced inflammation and oxidative stress in RAW 264.7 macrophages through activating Nrf2/HO-1 signaling and suppressing MAPKs pathway. J Life Sci (Seoul) 2018;28:207–15.
[22] Kim WS, Kim YE, Cho EJ, Byun EB, Park WY, Song HY, et al. Neuroprotective effect of Annona muricata-derived polysaccharides in neuronal HT22 cell damage induced by hydrogen peroxide. Biosci Biotechnol Biochem 2020;84:1001–12.
[23] Yang J, Sung J, Kim Y, Jeong HS, Lee J. Inhibitory effects of butein on adipogenesis through upregulation of the Nrf2/HO-1 pathway in 3T3-L1 adipocytes. Prev Nutr Food Sci 2017;22:306–11.
[24] Eugene AR, Masiak J. The neuroprotective aspects of sleep. MEDtube Sci 2015;3:35.
[25] Hua Y, Xu XX, Guo S, Xie H, Yan H, Ma XF, et al. Wild jujube (*Ziziphus jujuba* var. spinosa): a review of its phytonutrients,

health benefits, metabolism, and applications. J Agric Food Chem 2022;70:7871–86.
[26] Wang B, Zhu H-T, Wang D, Yang C-R, Xu M, Zhang Y-J. New spinosin derivatives from the seeds of Ziziphus mauritiana. Nat Prod Bioprospect 2013;3:93–8.
[27] Belwal T, In Nabavi, Nabavi SM, editors. Naturally occurring chemicals against Alzheimer's disease. Cambridge: Academic Press; 2020. p. 1–30.
[28] Winsky-Sommerer R. Role of GABAA receptors in the physiology and pharmacology of sleep. Eur J Neurosci 2009; 29:1779–94.
[29] Goldschen-Ohm MP. Benzodiazepine modulation of GABAA receptors: a mechanistic perspective. Biomolecules 2022;12:1784.
[30] Liu J, Zhai W-M, Yang Y-X, Shi J-L, Liu Q-T, Liu G-L, et al. GABA and 5-HT systems are implicated in the anxiolytic-like effect of spinosin in mice. Pharmacol Biochem Behav 2015; 128:41–9.
[31] Vivas N, Glories Y, Lagune L, Saucier C, Augustin M. Estimation du degré de polymérisation des procyanidines du raisin et du vin par la méthode au p-dimethylaminocinnamaldéhyde. J Int Sci Vigne Vin 1994;28:319–36.