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ABSTRACT

This study aimed to investigate the sedative-hypnotic effects of bioactive fractions derived from Dioscorea oppositaThunb. extract (DM) in a mouse model of insomnia. The two isolated active fractions, DM-1 and DM-2, were administered to mice for two weeks following the establishment of an insomnia model induced by p-chlorophenylalanine (PCPA). Behavioral assessments, including the open field test (OFT), tail suspension test (TST), Morris water maze (MWM), and pentobarbital-induced sleep test (PIST), were conducted to evaluate neurological recovery. Additionally, enzyme-linked immunosorbent assay (ELISA) was used to measure changes in neurotransmitter and inflammatory cytokine levels. Results demonstrated that both DM-1 and DM-2 exerted significant sedative-hypnotic effects, as evidenced by reduced locomotor distance, decreased immobility time, shortened escape latency and sleep latency, and mitigation of PCPA-induced insomnia. Mechanistically, treatment increased the levels of 5-hydroxytryptamine (5-HT), γ-aminobutyric acid (GABA), and 5-hydroxyindoleacetic acid (5-HIAA), while lowering glutamate (Glu), dopamine (DA), and norepinephrine (NE) levels. Furthermore, DM-1 and DM-2 regulated key inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α). This study provides the first evidence supporting the sedative-hypnotic properties of bioactive fractions from Dioscorea oppositaThunb. extract DM and elucidates a potential mechanism involving modulation of both neurotransmitter systems and inflammatory pathways.

Keywards : Dioscorea Opposita Thunb; P-chlorophenylalanine; Insomnia; Inflammatory cytokines; Neurotransmitters

1. Introduction

Insomnia is a prevalent sleep disorder characterized by persistent difficulties in initiating or maintaining sleep, or a lack of restorative sleep [1]. It significantly impairs daytime executive functions and contributes to cognitive dysfunction, including deficits in attention, learning performance, memory capacity, and emotional regulation [2]. The prevalence of insomnia symptoms significantly surpasses that of most chronic medical conditions. Epidemiological studies reveal that 50-60% of the general population experiences transient insomnia symptoms, while 10-15% meet the diagnostic criteria for clinically significant insomnia disorder (ICSD-3), a condition associated with severe functional impairment [3]. Extensive research results indicated that multifactorial causes are involved in the etiology of insomnia, however, the specific pathological mechanisms remain largely unclear [4]. A growing body of research indicates that insomnia involves complex interactions between multiple biological systems, including central neurotransmitter activity, proinflammatory markers, melatonin regulation, and hypothalamic-pituitary-adrenal (HPA) axis function. Concurrently, psychological factors such as chronic stress, anxiety disorders, and mood dysregulation have been shown to significantly contribute to both the development and persistence of insomnia symptoms [5].

Over the past decade, the increasing global incidence of insomnia, along with greater societal focus on mental well-being and self-care, has driven exponential growth in demand for sedative-hypnotic medications. In modern medicine, the clinical treatment of insomnia primarily involves pharmacological therapies. These include benzodiazepines,

antihistamines, tricyclic antidepressants, and melatonin receptor agonists. These medications mainly target the γ-aminobutyric acid (GABA) receptor, melatonin receptor, histamine receptor, orexin system, and serotonin receptor. Additionally, interventions for insomnia primarily encompass psychotherapy, cognitive-behavioral therapy, and transcranial magnetic stimulation [6,7]. Although pharmacotherapy remains the cornerstone of insomnia treatment due to its proven efficacy in reducing sleep onset latency and prolonging total sleep time, it is accompanied by notable safety concerns. These include the potential for drug dependence, therapeutic tolerance, substance use disorder, and paradoxical rebound insomnia—all of which may engender substantial patient apprehension and compromise long-term treatment adherence [8]. Emerging evidence highlights that herbal bioactive compounds, medicinal plant extracts, and Traditional Chinese Medicine (TCM) formulations exhibit significant sedative-hypnotic effects, distinguished bytheir favorable safety profiles and mitigated adverse effects compared to synthetic alternatives. Owing to their substantial potential for development and utilization, herbal medicines have increasingly become a favored option in the search for natural sleep-aid agents [9].

Dioscorea opposita Thunb. (commonly known as Chinese yam) is a perennial plant of the Dioscoreaceae family, prized primarily for its edible underground tuber. It is widely cultivated and consumed as a tuber vegetable throughout Asia, Africa, and the Americas. China, in particular, possesses a long history of yam cultivation dating back thousands of years, where it has been traditionally used both as a dietary staple and as an

important component in traditional herbal medicine [10]. *Dioscorea opposita Thunb*. is rich in a variety of nutritional and functional components, including amino acids, batatasins, flavonoids, starch, polysaccharides, protein, allantoin, polyphenols, and other bioactive compounds. These constituents contribute to its significant pharmacological activities, such as antioxidant, anti-inflammatory, gut microbiota modulation, spleen and stomach fortification, antitumor effects, and gastrointestinal protection[11]. Furthermore, *Dioscorea opposita Thunb.* contains a variety of bioactive secondary metabolites, which are thought to be integral to its applications in both traditional Chinese medicine and modern phytopharmaceutical formulations [11]. However, the effects of *Dioscorea opposita Thunb.* and its bioactive constituents on nervous system disorders remain poorly documented and have rarely been systematically investigated. DM is an active compound derived from the extraction and isolation of *Dioscorea opposita Thunb.* It serves as one of the key components in a patented novel formulation for the treatment of Alzheimer's disease (AD), developed by Dr. Duolikun Mamut at Urumqi Shalemei Biotechnology Co., Ltd. in Xinjiang, China.Previous clinical observations have demonstrated that DM not only improves learning and memory abilities, mental state, and sleep quality in patients with AD [12], but also enhances cognitive function in a D-galactosecombined with AlCl3-induced AD model mice by ameliorating energy metabolism dysregulation and strengthening central cholinergic neurotransmission [13]. In this work,we identified the active pharmacological fraction of DM and evaluated its sedative-hypnotic effects in a p-chlorophenylalanine-induced insomnia mouse model. Furthermore, we explored the

potential mechanisms underlying these effects by assessing changes in relevant monoamine neurotransmitters and inflammatory cytokine levels.

2. MATERIALS AND METHODS

2.1 Drugs and reagents

p-Chlorophenylalanine (PCPA) (HY-B1368; MCE, USA) was procured from Shanghai Haoyuan Biomedical Technology Co., Ltd and suspended in 0.5% gum acacia/saline solution at a concentration of 30 mg/mL. Diazepam (DZP) was provided by the Third Affiliated Hospital of Xinjiang Medical University (Urumqi, China). Pentobarbital sodium was sourced from Merck KGaA (Darmstadt, Germany). The following ELISA kits, all purchased from ELK Biotechnology Co., Ltd. (Wuhan, China), were used: tumor necrosis factor-α (TNF-α) (ELK1387MS), interleukin (IL)-1β (ELK1271MS), IL-6 (ELK1157MS), 5-hydroxytryptamine (5-HT) (ELK7830), 5-hydroxyindoleacetic acid (5-HIAA) (ELK7838), noradrenaline (NE) (ELK7885), dopamine (DA) (ELK8262), glutamic acid (Glu) (ELK8234), and gamma-aminobutyric acid (GABA) (ELK1513).

2.2 Preparation of DM Extractions

DM-1 and DM-2 extracts were provided by Xinjiang Institute of Physicochemical Technology, Chinese Academy of Sciences. The detailed preparation procedure was as follows: 500 g of the aqueous extract powder of *Dioscorea opposita Thunb.* (DM) was mixed with 1.5 L of ultrapure water and stirred at room temperature until complete dissolution. Anhydrous ethanol was then added gradually to the solution until a final

ethanol concentration of 80% (v/v) was achieved. The mixture was allowed to stand at 4 °C for 24 hours to facilitate precipitation. The precipitate (obtained from the aqueous extract via 80% ethanol precipitation) was collected and subjected to freeze-drying at –80 °C under reduced pressure to produce a fine spray-dried powder, designated as DM-1, with a yield of 56.9% (w/w). The resulting supernatant was concentrated and similarly freeze-dried under the same conditions to obtain a fine powder, designated as DM-2, with a yield of 38.32% (w/w). Both extracts were stored at 4 °C for subsequent use.

2.3 Animals

A total of 84 specific pathogen-free (SPF) male Kunming mice, aged 10–12 weeks and weighing 20 ± 2 g, were supplied by the Laboratory Animal Center of Xinjiang Medical University (license number: 2016–0003). The mice were housed under standard laboratory conditions with ad libitum access to a standard chow diet and sterilized drinking water. The housing environment was maintained on a 12 h/12 h light/dark cycle at a temperature of 22 ± 0.5 °C and relative humidity of 50–60%. After a 3-day acclimatization period, the experiments were initiated. All experimental procedures were conducted in compliance with the guidelines of the China Council on Animal Care and were approved by the Animal Ethics Committee of Xinjiang Medical University.

2.4 P-chlorophenylalanine pretreated insomnia model and drug administration

The mice were randomly assigned to seven groups (n = 12 per group): normal control, model, Zaoren Anshen granules treatment, DM-1 low-dose, DM-1 high-dose, DM-2 low-dose, and DM-2 high-dose treatment groups. With the exception of the normal

control group, all mice received intraperitoneal injections of PCPA (300 mg/kg) once daily between 10:00 and 11:00 a.m. for two consecutive days to establish the insomnia model [14]. By 48 hours post-PCPA injection, the mice exhibited loss of circadian rhythm and complete sleep deprivation, indicating successful induction of the insomnia syndrome [15].Starting from the third day, the normal control and model groups were administered normal saline (0.2 mL/10 g body weight) once daily via intragastric gavage for 15 days. The Zaoren Anshen granules treatment group received Zaoren Anshen granule solution at 1.0 g/kg, while the DM-1 and DM-2 treatment groups were given their respective extracts at low (1.0 g/kg) and high (4.0 g/kg) doses, also once daily by intragastric administration for 15 days.

2.5 Behavioral Investigation

2.5.1 Open Field Test

The open-field test (OFT) is a widely used behavioral paradigm designed to assess locomotor activity and exploratory behavior in rodents when exposed to a novel environment [16]. The apparatus consisted of a black wooden chamber measuring 60 × 60 cm with 40 cm-high walls. The floor was subdivided into 9 equal squares to facilitate behavioral scoring.Each mouse was placed individually in the center of the arena and allowed to explore freely for 5 minutes. Following the final administration, all behavioral parameters-including total distance traveled, time spent in the central zone, total freezing time, and number of rears-were automatically recorded using the VisuTrack software system (Shanghai Xinruan Information Technology Co., Ltd). To eliminate

196 olfactory cues, the apparatus was thoroughly cleaned with 75% ethanol and then water
197 after each trial.

198 **2.5.2 Tail Suspension Test**

199 Following drug administration, the distal quarter of each mouse's tail was affixed
200 with medical tape and suspended from the tail suspension tester. After a 1-minute
201 acclimatization period, the behavior of the mice was observed, and immobility time was
202 automatically recorded using the tail suspension test system (TST-100, Taimeng,
203 Chengdu, China). The total immobility time during the final 4 minutes of the 5-minute
204 suspension period was measured for quantitative analysis.

205 **2.5.3 Morris Water Maze Test**

206 The Morris water maze (MWM) test was conducted to evaluate spatial learning and
207 memory in mice, with minor modifications based on previously described methods [17].
208 The apparatus consisted of a white circular pool (120 cm in diameter, 50 cm in height)
209 with a smooth, featureless inner surface. The pool was filled with water to a height of
210 approximately 26 cm (23 ± 2℃), which was made opaque by the addition of 500 mL of
211 milk. The pool was conceptually divided into four quadrants of equal area (designated A,
212 B, C, and D). A white platform (10 cm in diameter, 25 cm in height) was positioned in the
213 center of quadrant C (the target quadrant), submerged approximately 1 cm beneath the
214 water surface to ensure it remained invisible. No visual cues were provided either inside
215 or outside the pool to directly indicate the platform's location.Therefore, the mice were
216 required to memorize and navigate to the platform's position based solely on distal

environmental cues. Training sessions commenced 1 hour after drug administration and were conducted over a period of 5 consecutive days. Each mouse was allotted a maximum of 60 seconds per trial to locate the hidden platform. If a mouse failed to find the platform within the allotted time, it was gently guided to the platform and allowed to remain there for 10 seconds. Mice that successfully located the platform were permitted to stay on it for 5 seconds. Throughout the training process, the swimming paths and latency times to reach the escape platform were meticulously documented using image tracking and analysis software (MWM-100, Taimeng, Chengdu, China).

The spatial exploration test was conducted on day 6, following the completion of the orientation navigation trials. Each mouse was introduced into the pool from a consistent entry point and allowed to swim freely for 60 seconds. During this period, the following parameters were recorded: the number of crossings over the former platform location, the total distance swum in the target quadrant, the number of entries into the target quadrant, and the total time spent within the target quadrant.

2.5.4 Potentiation of pentobarbital-induced sleep

One hour after drug administration, mice received an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Sleep onset was defined as the loss of righting reflex for more than one minute [18]. Sleep latency was measured as the duration from pentobarbital injection to the onset of sleep, while total sleep time was recorded from the loss of righting reflex until its recovery.

2.6 Serum cytokine measurements

Serum concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 were quantified using commercial mouse-specific ELISA kits (ELK Biotechnology, Wuhan, China) according to the manufacturer's instructions, as previously described. Absorbance was measured at 450 nm using a microplate reader (Tecan Spark, Tecan, Switzerland), and cytokine levels were expressed as pg/mL of serum.

2.7 Determination of 5-HT、5-HIAA、NE、DA 、Glu、GABA neurotransmitter levels in the brain

Samples intended for monoamine neurotransmitter analysis were prepared following previously established protocols [19]. Following three freeze-thaw cycles, the samples were sonicated and centrifuged at 10,000 × g for 15 minutes. The resulting supernatants were then analyzed for concentrations of glutamic acid (Glu), γ-aminobutyric acid (GABA), serotonin (5-hydroxytryptamine, 5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), and norepinephrine (NE) using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits (ELK Biotechnology, Wuhan, China), in accordance with the manufacturer's instructions. Absorbance was measured at 450 nm with a microplate reader (Tecan Spark, Tecan, Switzerland), and the results were expressed in pg/mL.

2.8 Statistical analysis

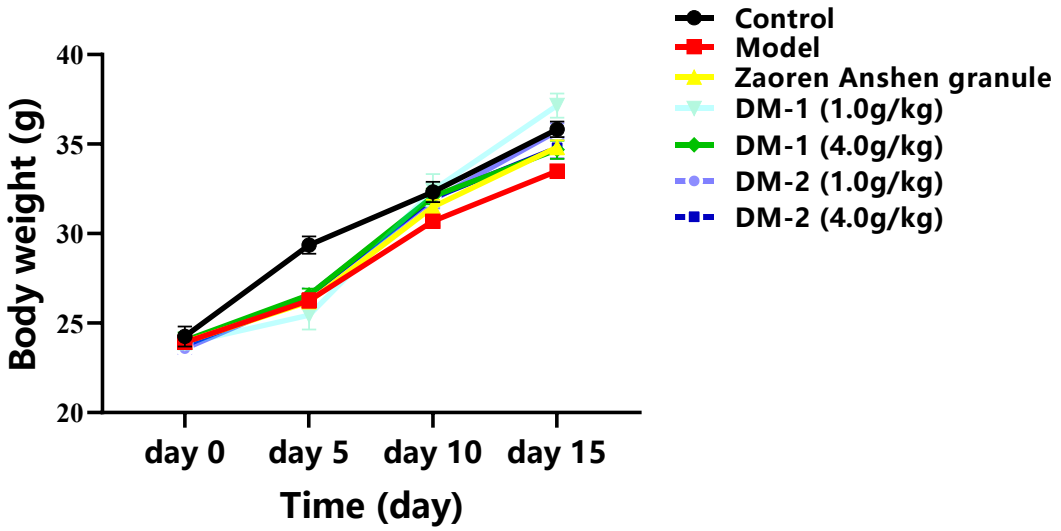
Statistical analyses were conducted using SPSS software (version 17.0). Unless otherwise stated, all data are expressed as mean ± standard error of the mean (SEM). Data of spatial learning ability in MWM task were analyzed with two-way ANOVA

repeated measure followed by a Dunnett`s post hoc-test for multiple comparisons. Other data were analyzed by one-way ANOVA analysis of variance followed by Tukey's test for multiple comparisons. Any differences were considered significantly different when $p < 0.05$.

3. Results

3.1 Measurement results of body weight changes in mice

Throughout the experimental period, body weight was measured at five-day intervals. As shown in Fig. 1, significant alterations in body weight were observed following the induction of insomnia via PCPA administration.At the onset of the study, no significant differences in baseline body weight were detected among the various groups ($p > 0.05$). However, following intraperitoneal administration of PCPA during the initial two-day period, mice in the model group displayed a reduced rate of body weight gain compared to those in the control group. Subsequently, the body weight of the model group was significantly lower than that of the control group at all three subsequent measurement timepoints ($p < 0.05 \sim p < 0.01$).On day 10, the body weight of mice treated with the lower dose of DM-1 (1.0 g/kg) was significantly higher than that of the model group ($p < 0.05$). By day 15, both the low-dose DM-1 and DM-2 treatment groups (1.0 g/kg) demonstrated a significant increase in body weight relative to the model group ($p < 0.01$).



279 **Figure 1.** Effect of DM on body weights in PCPA-induced insomnia mice (n=8 in each group).

280

281 **3.2Open field test results**

282 During the 5-minute open field test (OFT), mice in the model group demonstrated a
283 significant increase in both total movement distance and average speed compared to the
284 control group ($p< 0.05$). Conversely, administration of lower-dose DM-1 (1.0 g/kg),
285 high-dose DM-1 (4.0 g/kg), and lower-dose DM-2 (1.0 g/kg) significantly attenuated both
286 movement distance and average speed in comparison to the model group ($p< 0.05 \sim p<$
287 0.01). Although a decreasing trend in locomotion was observed in the high-dose DM-2
288 (4.0 g/kg) and Zaoren Anshen granule (1.0 g/kg) treatment groups compared to the model
289 group, the differences were not statistically significant ($p> 0.05$).These results are
290 presented in Figures 2A and 2B. Representative trajectories of all groups of mice during
291 the open - field test (OFT) are shown in Figure 2C.

292

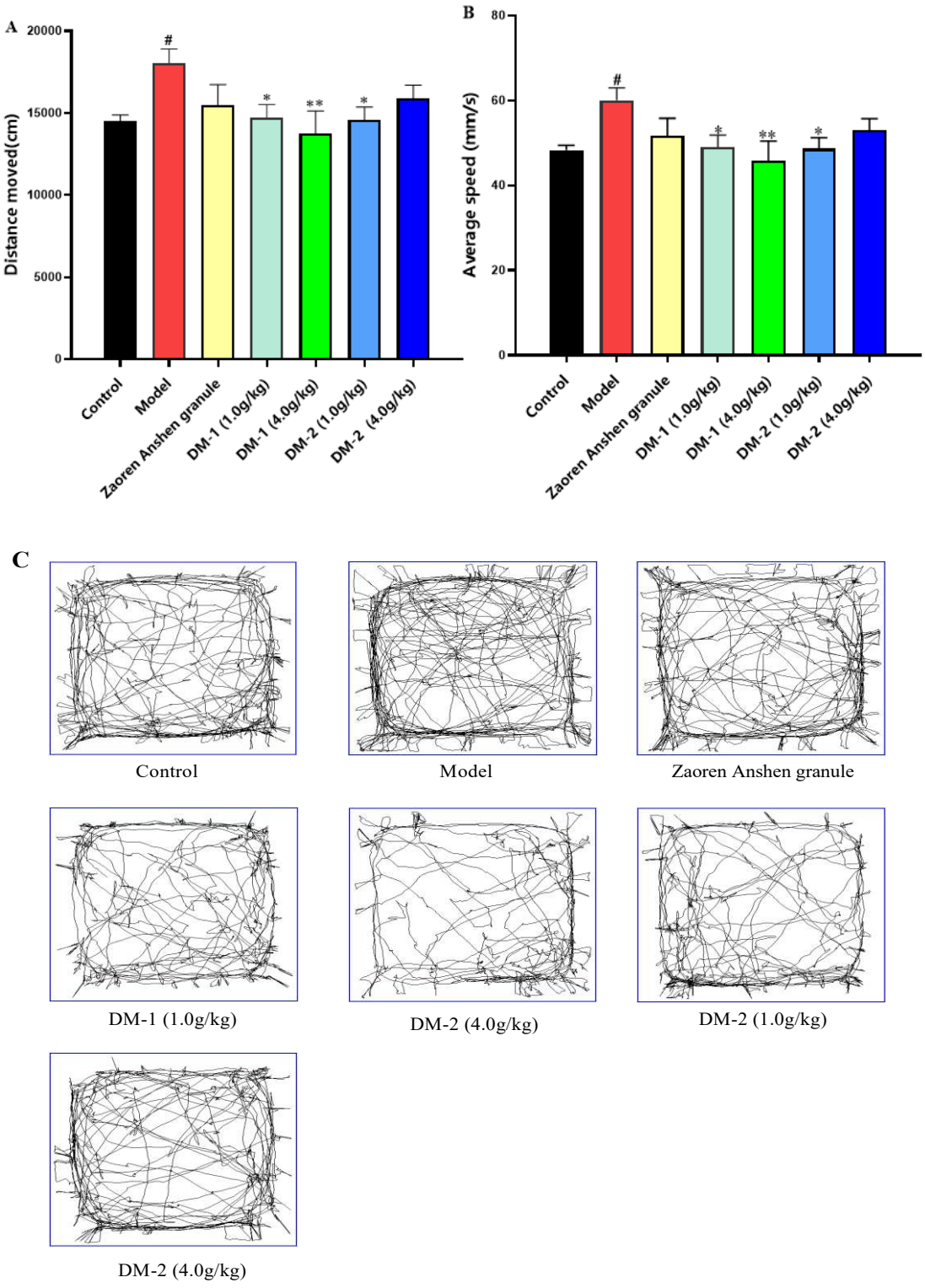


Figure 2. Effect of DM on PCPA-induced insomnia mice in open field test (n=8 in each group). A: distance moved; B: average speed; C: representative tracks of all groups of rats during the open field test. All data are expressed as means±SEM. $^{\#}p<0.05$ *vs*. the control; $^{*}p<0.05$,$^{**}p<0.01$ *vs*. the model group.

297

298 **3.3 Tail suspension test results**

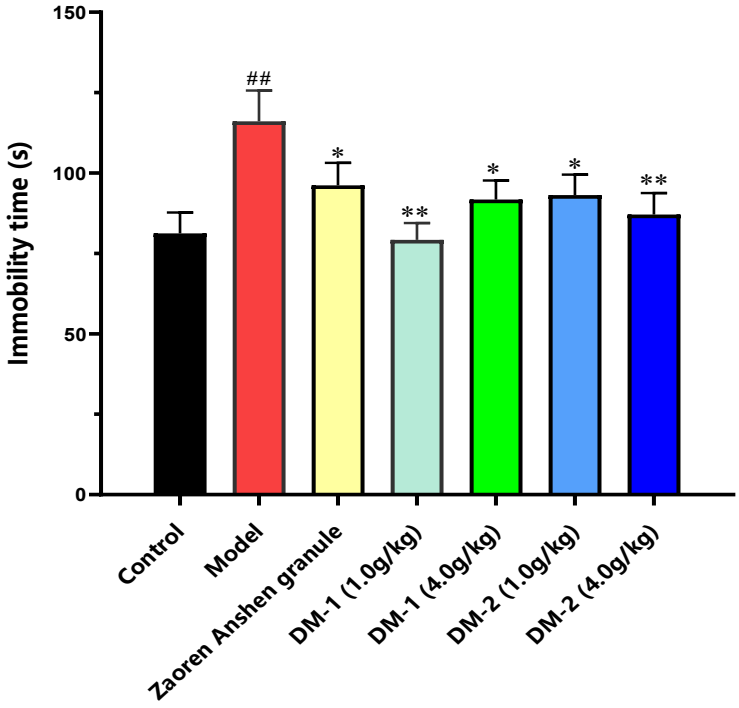
299 As depicted in Figure 3, the tail suspension test (TST) results indicated that,

300 following PCPA administration, the immobility time in the model group was significantly

301 increased compared to the control group (p< 0.01). Conversely, treatment with Zaoren

302 Anshen granule (1.0 g/kg) and both low- and high-dose DM-1 and DM-2 significantly

303 decreased immobility time relative to the model group(p < 0.05 ~0.01).



304 **Figure 3.** Effects of DM on PCPA-induced insomnia mice in tail suspension test (n=8 in each group).

305 $^{\#\#}p$<0.05 *vs*. the control; $^{*}p$<0.05,$^{**}p$<0.01 *vs*. the model group.

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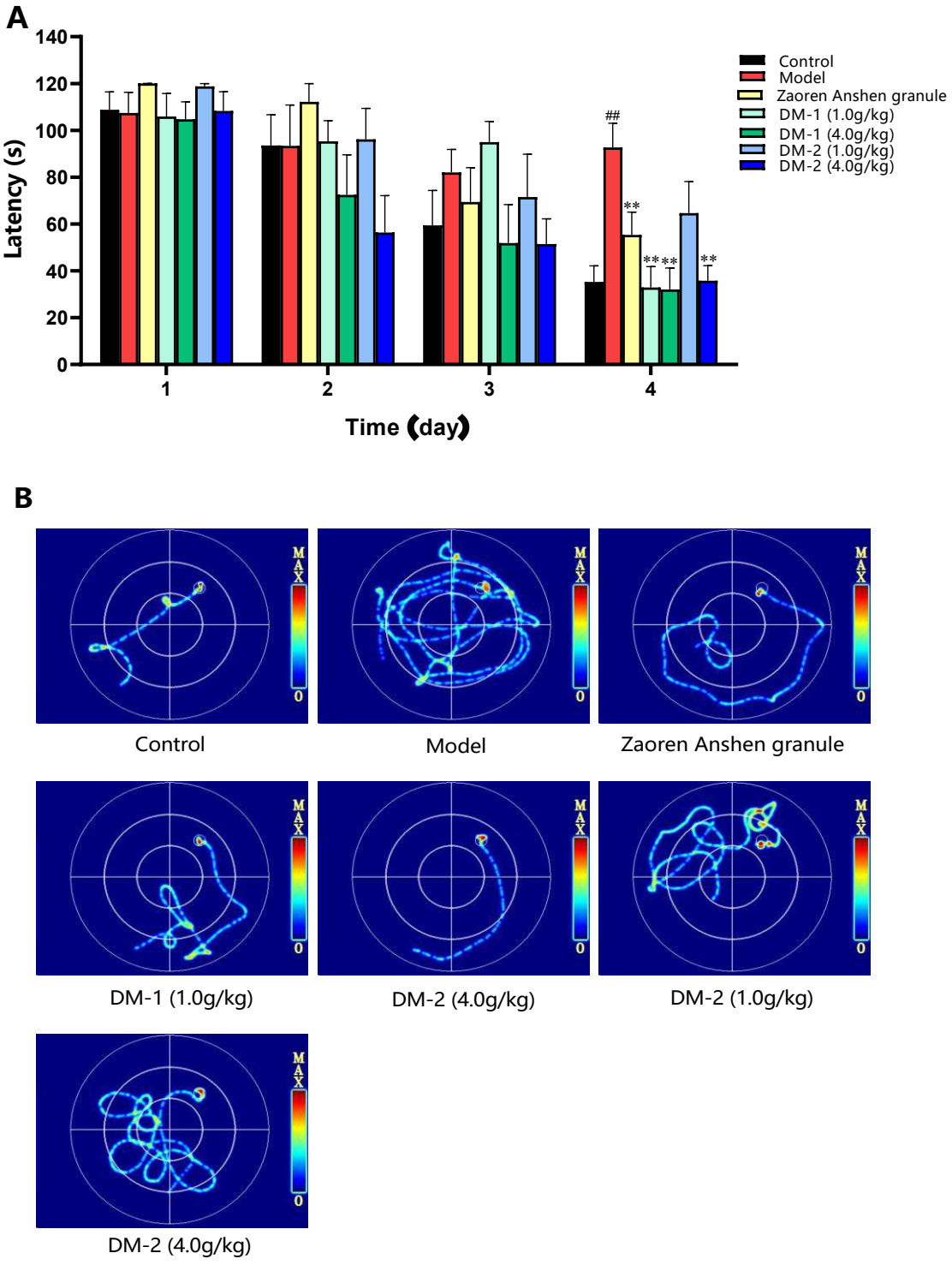
307 **3.4 Morris Water Maze Test results**

308 The Morris water maze (MWM) was employed to assess the impact of DM

309 extractions on the cognitive functions of mice with PCPA-induced insomnia. On the

310 fourth day of training trials, the insomnia model group exhibited a significantly longer

311 escape latency than the control group ($p< 0.01$; Fig. 4A). Conversely, administration of
312 both high- and low-dose DM-1 and DM-2, as well as the Zaoren Anshen granule (1.0
313 g/kg), significantly reduced the escape latency compared to the model group ($p< 0.05 \sim$
314 $p< 0.01$). Representative swimming trajectories of mice recorded on day 4 of the Morris
315 water maze (MWM) test are presented in Figure 4B.



316 **Figure 4.** Effects of DM on spatial learning in PCPA-induced insomnia mice. A: Escape latency; B:

317 Representative trajectories of all groups of rats during Morris water maze test (n=8 in each group).

318 $^{\#\#}p<0.01$ *vs*. the control; $^{*}p<0.05$,$^{**}p<0.01$ *vs*. the model group.

A spatial probe trial was performed on the 5th day to assess spatial memory capacity. The results indicated that there were no significant differences among all groups in terms of platform crossing time, swimming distance in the target quadrant, entrance times to the target quadrant, and swimming time in the target quadrant ($p > 0.05$, Fig. 5).

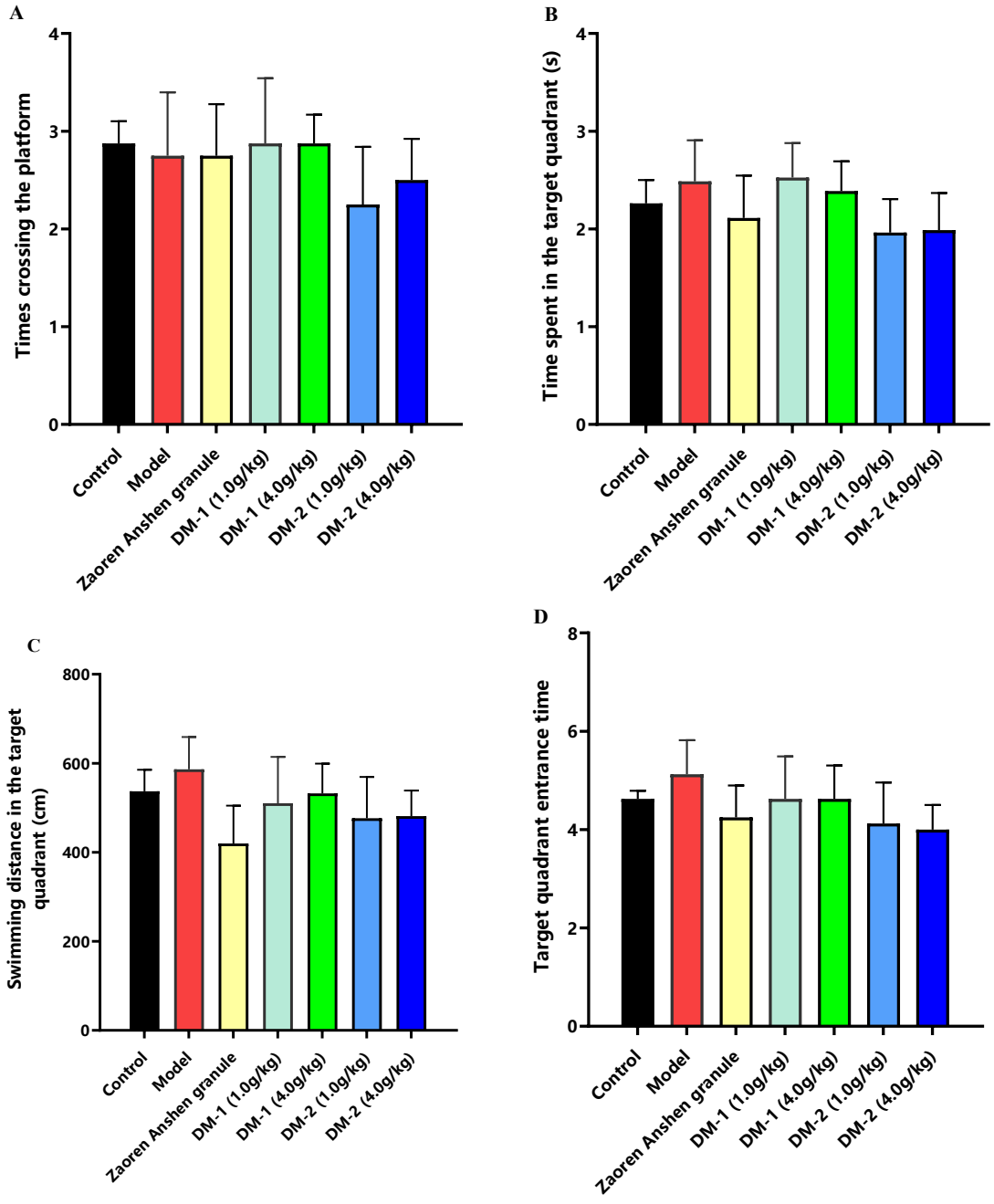


Figure 5. Effects of DM on spatial memory in PCPA-induced insomnia mice. A: Time crossing the platform; B: Time spent in the target quadrant; C: Swimming distance in the target quadrant. D:Target quadrant entrance time(n=8 in each group).

3.5 Observation of sedative and hypnotic in pentobarbital-induced mice

Compared with the control group, the model group displayed a significant increase in sleep latency and a significant decrease in sleep duration ($p< 0.001$ and $p< 0.05$, respectively; Fig. 6A, B). Treatment with high- or low-dose DM-1, DM-2, or Zaoren Anshen granule (1.0 g/kg) significantly reversed these effects, as evidenced by shortened sleep latency ($p < 0.05 \sim 0.01$). Furthermore, both DM-1 and DM-2 administration, at low and high doses, led to a prolongation of sleep duration, with the effect in the low-dose DM-2 group reaching statistical significance ($p< 0.05$, Fig. 6B). Collectively, these results demonstrate that DM extracts possess therapeutic potential against insomnia.

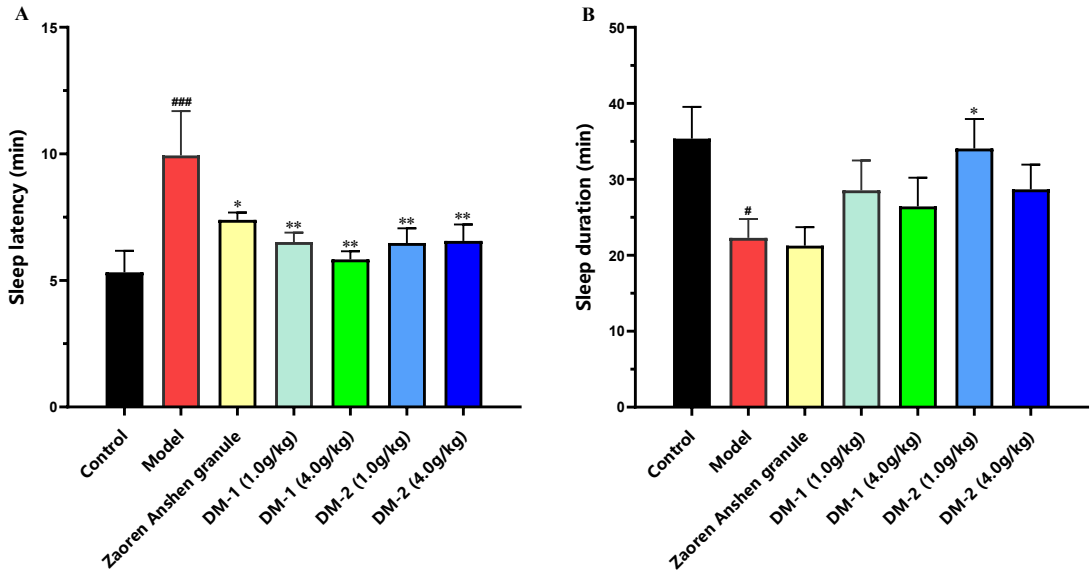


Figure 6. Hypnotic effects of DM in PCPA-induced insomnia mice.A: Sleep latency; B: Sleep duration. (n=10 in each group). All data are expressed as means±SEM. $^{\#}p<0.05$, $^{\#\#\#}p<0.001$ *vs*. the control; $^{*}p<0.05$,$^{**}p<0.01$ *vs*. the model group.

3.6 The mechanism of sedative and hypnotic effects of DM on PCPA-induced insomnia mice

3.6.1 Effects of DM on inflammatory cytokine levels in the PCPA-treated mice

Emerging evidence from PCPA-induced insomnia models implicates sleep deprivation in the dysregulation of immunological responses, driving elevated levels of inflammatory markers such as IL-1β, IL-6, and TNF-α [20, 21]. As shown in our study, serum levels of IL-1β, IL-6, and TNF-α were significantly elevated in PCPA-induced model mice compared to the normal control group(p<0.05~ 0.01). Following a two-week treatment with DM-1, DM-2, or Zaoren Anshen granule, serum levels of pro-inflammatory cytokines were significantly ameliorated compared to the PCPA model group.Specifically, TNF-α was reduced in all treatment groups (both high and low doses of DM-1, DM-2, and Zaoren Anshen granule at 1.0 g/kg), IL-1β was substantially decreased in the low- and high-dose DM-1, low-dose DM-2, and Zaoren Anshen granule groups, and IL-6 was significantly lowered in the low- and high-dose DM-2 and high-dose DM-1 groups(p<0.05~ 0.01for all; Fig. 7).

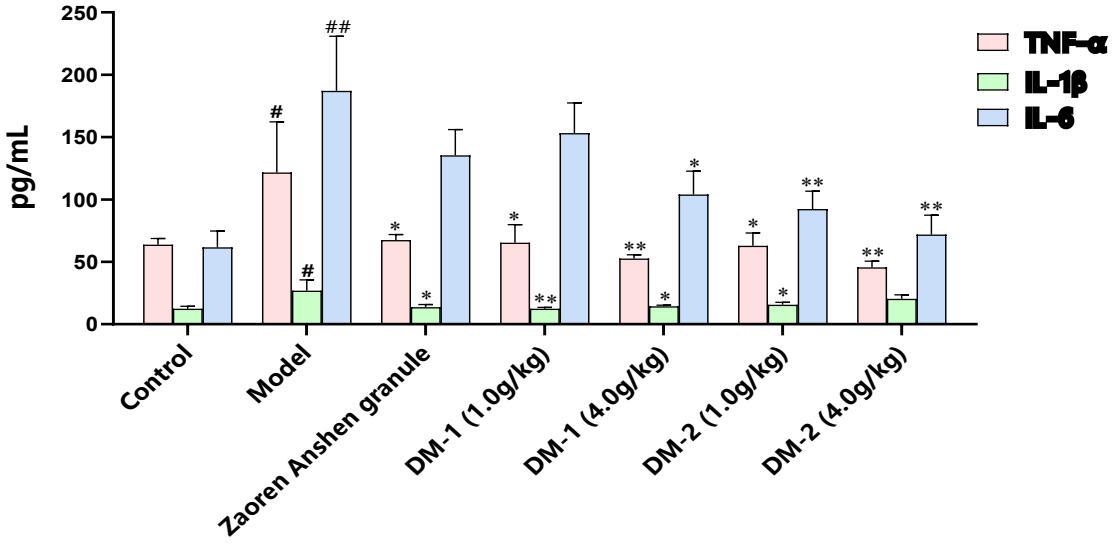
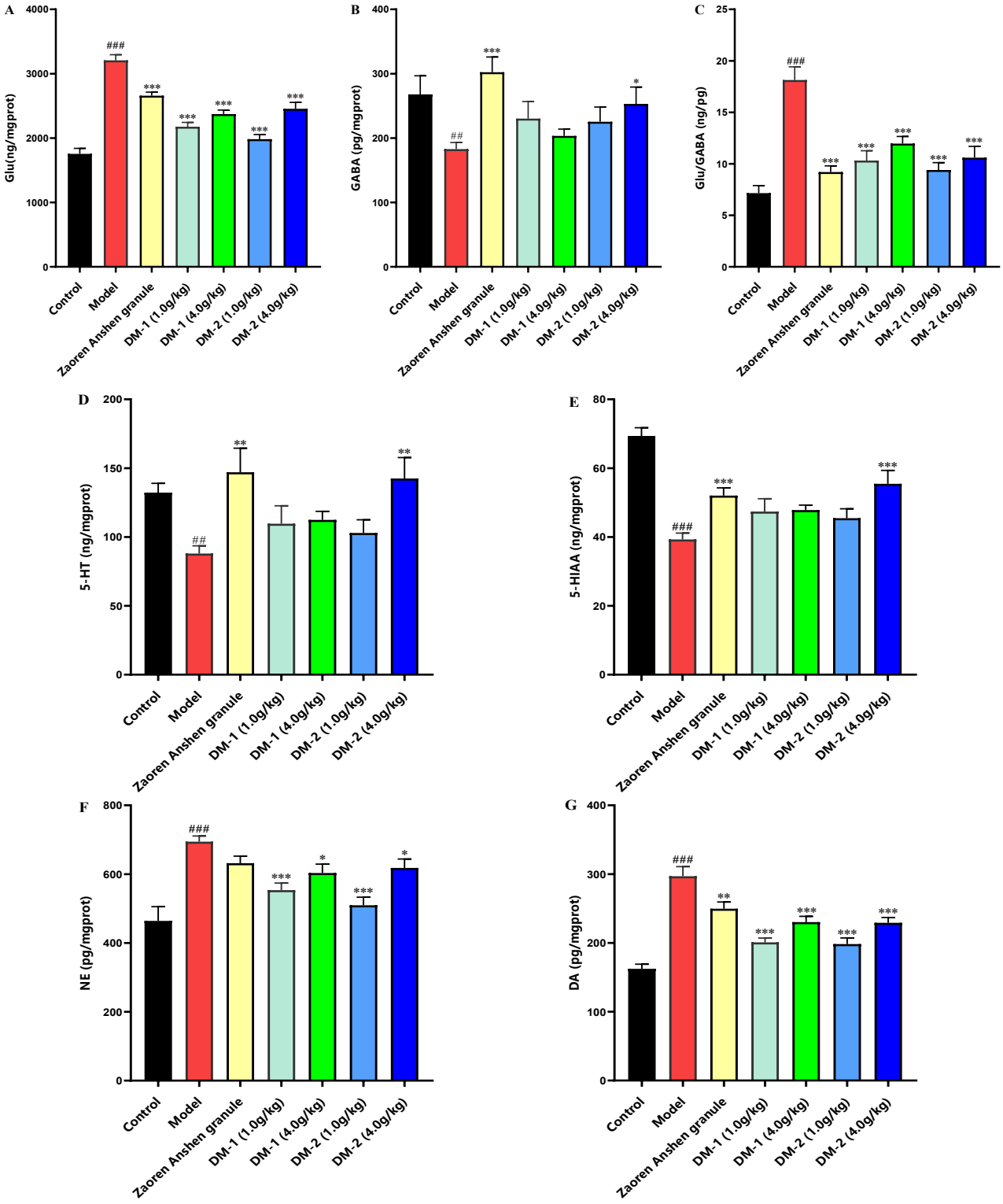


Figure 7. Effects of DM on serum level of inflammatory cytokines in PCPA-induced insomnia mice(n=10 in each group). All data are expressed as means±SEM.$^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs*. the control; $^{*}p<0.05$,$^{**}p<0.01$ *vs*. the model group.

3.6.2 Effect of DM on neurotransmitter in the PCPA-treated mice

Our results revealed significant neurochemical alterations in the brains of PCPA-induced insomnia mice compared to controls (Figure 8). In the model group, we observed a pronounced elevation in the levels ofGlu, NA, DA, and the Glu/GABA ratio ($p<0.001$).Conversely, there was a substantial reduction in5-HT,5-HIAAand GABA($p<0.01$~0.001). These alterations in neurotransmitter levels underscore the intricate neurochemical mechanisms associated with insomnia. Compared to the model group, treatment with high- and low-dose DM-1, DM-2, and Zaoren Anshen granule (1.0 g/kg) significantly reduced the levels of Glu, DA, and the Glu/GABA ratio($p<0.01$~ 0.001，Fig. 8A,C,G). Meanwhile, the levels of NA were decreased in both the DM-1 and

DM-2 treatment groups (p<0.05~ 0.001，Fig. 8F). Conversely, the levels of 5 - HT, 5 - HIAA, and GABA were significantly elevated in the high-dose DM-2 and Zaoren Anshen granule groups(p<0.05~ 0.001, Fig. 8B,D,E). These findingsindicate that DM-1 and DM-2 can effectively restore the balance of monoaminergic neurotransmitters, which are crucial for sleep-wake regulation.



376 **Figure 8.** Effects of DM on neurotransmitter levels in the brains of PCPA-induced insomnia mice
377 (n=10 in each group). All data are expressed as means±SEM. $^{\#\#}p<0.01$,$^{\#\#\#}p<0.001$ *vs*. the control;
378 $^{*}p<0.05$,$^{**}p<0.01$,$^{***}p<0.01$ *vs*. the model group.
379

4. Discussion

Sleep is important for eliminating fatigue and restoring energy. The lack of sleep can lead to physical and/or mental problems. Insomnia is highly prevalent in the general population, with a major occurrence in the elderly [22]. Although the precise function of sleep remains elusive, our understanding of the brain systems that govern sleep and wakefulness has progressed significantly. Medications utilized for the treatment of insomnia encompass benzodiazepine receptor agonists, non-benzodiazepine receptor agonists, selective melatonin receptor agonists, and sedative antidepressants [23].However, the use of these medications is often associated with side effects, including daytime "hangover" symptoms, psychomotor disturbances, drug dependence, addiction, tolerance, amnesia, and rebound insomnia. Furthermore, their clinical efficacy remains a subject of debate [24]. Consequently, research efforts aimed at discovering new sedative and hypnotic agents that exhibit fewer side effects and enhanced efficacy are ongoing. In China, due to concerns about undesirable effects such as drug resistance and withdrawal symptoms associated with contemporary sleep pharmaceuticals, there is an increasing preference for employing Chinese remedies to treat insomnia [25].

PCPA-induced insomnia is a well-established animal model in the research community for studying sleep disorders. Compared to alternative methods, this model exhibits a shorter induction period, clearly defined mechanisms of action, and pronounced insomnia characteristics, making it particularly suitable for the preliminary screening of insomnia medications. Moreover, the insomnia features induced by PCPA

401 are reliably repeatable, making it a suitable model for properly viewing and evaluating
402 the impact of sleep-inducing therapies. These traits include extended awake, disturbance
403 of sleep cycles, and alterations in sleep architecture, all of which closely match various
404 elements of human insomnia. Therefore, we utilized the PCPA-induced insomnia mice
405 model to evaluate the sedative and hypnotic effects of DM [25].

406 The result of research shows that there was no remarkable difference in basal body
407 weight among all groups before intraperitoneally injection of PCPA, after PCPA injection
408 the body weight of the mice in the PCPA model group was significantly reduced. Body
409 weight of the mice in the DM-1and DM-2 low dose treatment group increased
410 significantly. This result indicated that treatment with DM-1and DM-2 have a tendency to
411 restore normal weight to insomniac mice. The behavioral observation method refers to
412 utilizing instruments or human visual observation to systematically investigate changes in
413 an animal's spontaneous activity, specifically the increase or decrease, resulting from
414 drug-induced central nervous system inhibition or excitation [26]. In general, a decrease in
415 spontaneous activity in mice in the OFT indicates that the sedative effect of the drug, and
416 this change in behavior is thought to reflect the decrease in central nervous system
417 excitability. Inhibition of spontaneous activity indicates that DM-1 and DM-2 has
418 sedative and anti-excitatory effects. Moreover, TST results showed that immobility time
419 of the model group was prolonged after PCPA injection; the DM-1、DM-2 and Zaoren
420 Anshen granule treatment group showed a significantly reduced immobility time,
421 indicating that DM could alleviate the depression symptom of insomnia mice.

Morris water maze (MWM) was applied to investigate the influence of DM-2 and DM-2on the neurological recovery of insomnia mice. The MWM test demonstrated that DM-1、DM-2 and Zaoren Anshen granule considerably shortening the escape latency, suggesting an improvement in spatial learning and cognitive skills. This is particularly significant in the context of sleep, since cognitive capacities are generally diminished in conditions of sleep deprivation [25]. Shortening of escape latency implies a potential restorative impact of DM-1 and DM-2 on cognitive abilities impaired by inadequate sleep.

Most sleep-related experiments are based on the synergistic effect of sedative drugs and barbiturates. Pentobarbital sodium-induced sleep test is a commonly used pharmacological method to detect sedative and hypnotic effects of drugs[27]. Pentobarbital sodium is a central nervous depressant, which has sedative and hypnotic effects and can shorten the sleep latency and prolong sleep duration in laboratory animals. Experimental investigation shows that DM-1 and DM-2 has synergistic effect with pentobarbital sodium, the shortened latency of sleeping time and prolonged duration of sleeping time show the hypnotic effect of *Dioscorea Opposita Thunb* extractions.

Cytokines have an important role in immune activation, but are also transported into the central nervous system, where they influence noradrenergic, dopaminergic and serotonergic neurotransmission [28]. Abnormally elevated inflammatory responses may contribute to the central mechanism of insomnia [29]. There is a significant correlation between insomnia and inflammation levels. Numerous studies on depression and anxiety

have demonstrated that sleep duration, insomnia, and levels of inflammatory markers are interrelated [30]. It has been reported that insomnia can lead to increased levels of inflammatory factors [26]. Elevations in pro-inflammatory cytokines, particularly interleukin-6(IL-6),interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), have been implicated in the development and treatment of insomnia[31]. IL-1β and TNF-α are generally thought to enhance sleep; however, extreme elevations in IL-1β and TNF-α can impair sleep[31]. Numerous research results also have shown that an increase in the concentration of inflammatory markers such as interleukin IL-6, IL-1β , TNF-α, in blood samples has been associated with poor sleep quality [32,33]. As observed in the present study serum concentration of IL-6, IL-1β , TNF-α were increased following the injection of PCPA in insomnia model mice. To some extent, DM-1、DM-2 and Zaoren Anshen granule treatment can reduce the elevated level of IL-6, IL-1β , TNF-α in PCPA induced insomnia model mice, especially the DM-1 high dose and DM-2 low dose treatment significantly reduced all three type of inflammatory markers in the serum of PCPA induced insomnia model mice. These results indicated that the curative mechanism underlying theeffects of DM-1 and DM-2 on PCPA-induced insomnia mice are achieved by regulating cytokines level.

In the brain, endogenous neurotransmitters such as norepinephrine, serotonin, γ-aminobutyric acid (GABA) and neuropeptides have been suggested to have important roles in sleep mechanisms [28]. GABA/glutamate (Glu), a pair of inhibitory/excitatory neurotransmitters that act on neurons and are present extensively in the central nervous

system, play a role in sleep regulation.Patients with sleep disorders often display decreased levels of GABA and increased levels of Glu in the brain [34]. In our study, compared with the model group, DM-1、DM-2 and Zaoren Anshen granule treatment for 2 weeks significantly downregulated the ratio of Glu content to Glu/GABA in PCPA induced insomnia model mice and upregulated the brain GABA content. These findings suggested that DM-1and DM-2 have anticonvulsant properties through some of bio-active constituents adjusts both GABA-ergic and Glutamatergic neurotransmission.

Monoamine neurotransmitters, including dopamine (DA), norepinephrine (NE), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA), play crucial roles in various physiological processes within the human body. Previous studies have demonstrated that 5-HT significantly modulates sleep [18], particularly in cases of insomnia where imbalances in 5-HT levels can lead to sleep disturbances. p-Chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, is commonly used in experimental models to induce 5-HT depletion, thereby mimicking symptoms of insomnia. In this context, 5-HIAA, the primary metabolite of 5-HT, plays a pivotal role [25]. The ratio of 5-HIAA to 5-HT in brain tissue serves as an important metric reflecting the utilization rate of 5-HT and is a critical indicator for assessing the severity of insomnia [35]. Our study found that high-dose DM-2 and Zao Ren An Shen granule treatments led to significant increases in both 5-HT and 5-HIAA levels in the brains of PCPA-induced insomnia model mice. By promoting the utilization of 5-HT, DM-2 may help maintain the sleep state, thereby alleviating insomnia symptoms, especially in conditions where 5-HT dysregulation

contributes to sleep disturbances. NE and DA are neurotransmitters closely associated with arousal and are known to be in dynamic balance with 5-HT within brain tissue [18]. The equilibrium among these neurotransmitters is essential for regulating sleep and wakefulness. Elevated ratios of NE and DA typically indicate heightened central nervous system (CNS) excitation, which can disrupt normal sleep patterns. Our findings from the study on PCPA-induced insomniac mice revealed significantly increased ratios of NE and DA compared to the normal state, indicative of an overactive CNS aligning with the insomnia symptoms induced by PCPA. However, following the treatment with DM-1 and DM-2, there was a substantial decrease in the ratios of NE and DA, suggesting the potential of DM-1 and DM-2 to effectively inhibit CNS excitability. By regulating the levels of these arousal-related neurotransmitters, DM-1 and DM-2 appear to restore a more balanced neurochemical state conducive to sleep.

5. Conclusions

In summary, this study demonstrates that extracts of *Dioscorea opposita Thunb.* (DM) contain bioactive components with hypnotic, sedative, and anticonvulsant properties. The results further indicate that the hypnotic-sedative effects of DM are likely mediated through interactions with the GABAergic, glutamatergic, serotonergic, and immune systems. These findings not only provide a scientific foundation for the use of Dioscorea opposita Thunb. in insomnia treatment but also suggest new directions for agricultural innovation aimed at optimizing its cultivation and processing for enhanced medicinal and nutritional applications.Future work will focus on the isolation and

identification of the active constituents in DM-1 and DM-2, along with a systematic evaluation of their respective pharmacological activities. This refined strategy will establish a more robust experimental basis for developing and applying these extracts in the treatment of insomnia. Investigations along this line will be critical for elucidating the mechanisms responsible for their hypnotic-sedative effects and facilitating their translation into potential therapeutic applications.

Data Availability

The data will be available on request.

Declaration of Competing Interest

The authors declare no potential conflicts of interest related to the authorship or publication of this article.

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CRediT authorship contribution statement:

Maiwulanijiang Yizibula: conceptualization, methodology, funding acquisition, investigation, supervision, writing -reviewing and editing. All authors have read,

reviewed, and approved the final submitted manuscript. **Mayire Abudukeremu**: validation, data curation, visualization and formal analysis. **Yikelamu Yilihamu**: investigation, data curation, project administration. **Zhang jun**: investigation, data curation, project administration.**Abudureyimujiang Ruze**: visualization, formal analysis, writing-reviewing and editing. **Duolikun Mamute**: resources, validation and writing of the original draft.

Ethical approval

All experimental procedures were conducted in strict compliance with the guidelines established by the China Council on Animal Care and were approved by the Animal Ethics Committee of Xinjiang Medical University (Ethical Approval Number: IACUC-20211016-53). Clinical trial number: not applicable.

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References

[1] Huang F，et al. Sedative and hypnotic activities of the ethanol fraction from Fructus Schisandrae in mice and rats. J Ethnopharmacol. 2007;110(3):471-5.

[2] Brownlow JA, Miller KE, Gehrman PR. Insomnia and Cognitive Performance. Sleep Med Clin. 2020;15(1):71-76.

[3] Wang H, et al. The effect of Bailemian on neurotransmitters and gut microbiota in p-chlorophenylalanine induced insomnia mice. Microb Pathog. 2020;148:104474.

[4] Bollu PC, Kaur H. Sleep Medicine: Insomnia and Sleep. Mo Med. 2019;116(1):68-75.

[5] Morin CM, et al. Insomnia disorder. Nat Rev Dis Primers. 2015;1:15026.

[6] Madari S, et al. Pharmacological Management of Insomnia. Neurotherapeutics. 2021;18(1):44-52.

[7] Chan NY, et al. Non-pharmacological Approaches for Management of Insomnia. Neurotherapeutics. 2021;18(1):32-43.

[8] Sun Y, et al. Shuangxia decoction alleviates p-chlorophenylalanine induced insomnia through the modification of serotonergic and immune system. Metab Brain Dis. 2020;35(2):315-325.

[9] Yang Y, et al. Nyctinastic herbs decoction improves para-chlorophenylalanine-induced insomnia by regulating the expression level of neurotransmitters. Ann Transl Med. 2021;9(20):1524.

[10] An L, et al. Comprehensive widely targeted metabolomics to decipher the molecular mechanisms of Dioscorea opposita thunb. cv. Tiegun quality formation during harvest. Food Chem X. 2024;21:101159.

[11] Li Y, et al. Chinese Yam (Dioscorea): Nutritional Value, Beneficial Effects, and Food and Pharmaceutical Applications. Trends in Food Science & Technology. 2023;134, 29-40.

[12] Abudusalamu Awuti，et al. Effect of Chinese herb extract DM －1 on energy metabolism and central cholinergic nerve function in Alzheimer`s model mice. Chin J Clin Pharmacol. 2020;36(17):2668-2671 (Chinese).

[13] MaiwulanijiangYizibula, PalidanWushuer, DuolikunMamuti. Study on the Short Term Therapeutic Effectof Chinese Medicine Compound PreparationDM-1 on Alzheimer's Disease. Pharmacy Information, 2022; 11(3): 189-194 (Chinese).

[14] Lina Q, et al. Efficacy of electroacupuncture stimulating Shenmen (HT7), Baihui (GV20), Sanyinjiao (SP6) on spatial learning and memory deficits in rats with insomnia induced by para-chlorophenylalanine: a single acupoint combined acupoints. J Tradit Chin Med. 2023;43(4):704-714.

[15] Du C, et al. Comparative pharmacokinetics of six major compounds in normal and insomnia rats after oral administration of Ziziphi Spinosae Semen aqueous extract. J Pharm Anal. 2020;10(4):385-395.

[16] Peng G, et al. Whole body vibration training improves depression-like behaviors in a rat chronic restraint stress model. Neurochem Int. 2021;142:104926.

[17] Yizibula M, et al. Cognitive enhancement and neuroprotective effects of a traditional Chinese herbal compound medicine on Aβ1-42 induced Alzheimer's disease in rats. Folia Neuropathol. 2020;58(4):365-376.

[18] Airong R, et al. GSZ Formula Enhances Sleep Quality: Exploring Its Active Ingredients and Mechanism Using a Network Medicine Approach. Clinical Traditional Medicine and Pharmacology. 2024;4(1):36-43.

[19] Wang H, et al.. The effect of Bailemian on neurotransmitters and gut microbiota in p-chlorophenylalanine induced insomnia mice. Microb Pathog. 2020;148:104474.

[20] Sheethal S, et al. Anti-insomnia Effect of a Polyherbal Formulation on P-chlorophenyalanine Induced Experimental Animal Model. Neurochem Res. 2024;49(2):327-337.

589 [21] He F, et al. Therapeutic potential of Rosa roxburghii folium extract in insomnia treatment: a
590 comprehensive evaluation of behavioral and neurochemical effects in a PCPA-induced mouse model. J
591 Sci Food Agric. 2025;105(2):1044-1056.
592 [22] Si Y, et al. A comprehensive study on the relieving effect of Lilium brownii on the intestinal flora and
593 metabolic disorder in p-chlorphenylalanine induced insomnia rats. Pharm Biol. 2022;60(1):131-143.
594 [23] Zhong Y, et al. Sedative and hypnotic effects of Perilla frutescens essential oil through GABAergic
595 system pathway. J Ethnopharmacol. 2021;279:113627.
596 [24] Yang Y, et al. Nyctinastic herbs decoction improves para-chlorophenylalanine-induced insomnia by
597 regulating the expression level of neurotransmitters. Ann Transl Med. 2021;9(20):1524.
598 [25] Bao Y, et al. Zhumian Granules improves PCPA-induced insomnia by regulating the expression level of
599 neurotransmitters and reducing neuronal apoptosis. J Ethnopharmacol. 2024;327:118048.
600 [26] Liu Y, et al. Improvement of neurological recovery in the insomnia rats by Warming Yang Strategy
601 through targeting SIRT4 by inhibiting inflammation and apoptosis. Immun Inflamm Dis.
602 2023;11(8):e964.
603 [27] Lai Y, et al. The Effect of Chinese Agarwood Essential Oil with Cyclodextrin Inclusion against
604 PCPA-Induced Insomnia Rats. Molecules. 2023;28(2):635.
605 [28] Hu Y, et al. Gan-Dan-Liang-Yi-Tang alleviates p-chlorophenylalanine-induced insomnia through
606 modification of the serotonergic and immune system. Exp Ther Med. 2016;12(5):3087-3092.
607 [29] Wang J, et al. Objective Short Sleep Duration is Related to the Peripheral Inflammasome Dysregulation
608 in Patients with Chronic Insomnia. Nat Sci Sleep. 2020;12:759-766.

[30] Kacar S, et al. Cyproheptadine causes apoptosis and decreases inflammation by disrupting thiol/disulfide balance and enhancing the levels of SIRT1 in C6 glioblastoma cells. Toxicol In Vitro. 2021;73:105135.

[31] Rethorst CD, et al. IL-1β and BDNF are associated with improvement in hypersomnia but not insomnia following exercise in major depressive disorder. Transl Psychiatry. 2015;5(8):e611.

[32] Clinton JM, et al. Biochemical regulation of sleep and sleep biomarkers. J Clin Sleep Med. 2011;7(5 Suppl):S38-42.

[33] Mullington JM, et al. Sleep loss and inflammation. Best Pract Res Clin Endocrinol Metab. 2010;24(5):775-84.

[34] Rong M, et al. The effect of modified Qiyuan paste on mice with low immunity and sleep deprivation by regulating GABA nerve and immune system. Chin Med. 2024;19(1):84.

[35] Shi R, et al. Loganin Exerts Sedative and Hypnotic Effects via Modulation of the Serotonergic System and GABAergic Neurons. Front Pharmacol. 2019;10:409.

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