

Neuropharmacological Potential of a Combined Herbal Preparation of *Valeriana officinalis* L. and *Leonurus sibiricus* L.

Yeruult Chultemsuren¹, Bilegdemberel Khurelbaatar¹, Chimgee Tserendash¹, Maral-Erdene Gerelt-Od¹, Batdavaa Batbayar¹, Mend-Amar Batbaatar¹, Perleidlum Bunddulam², Enerel Yeruult³, Narankhajid Myadagsuren⁴, Li ChangSheng⁵, Purevjal Otgonbayar^{6*}, Enkhmaa Gonchig^{1*}

ABSTRACT

Introduction: Neuropsychiatric disorders are a global health burden. The World Health Organization estimates that 264 million individuals experience anxiety, 50 million experience epilepsy, and up to 30% of adults experience insomnia. Despite the high reliance on conventional medicine in developing nations, a critical shortage of scientifically validated herbal formulations for these conditions exists. We aimed to evaluate the neuropharmacological potential of a novel combined herbal preparation (CHP) derived from *Valeriana officinalis* L. (roots) and *Leonurus sibiricus* L. (aerial parts). **Methods:** Acute oral toxicity was assessed in C57BL/6 mice. Subchronic toxicity was evaluated in Wistar rats following the Organization for Economic Co-operation and Development guideline 407, with daily oral administration of CHP at doses of 1,000 and 1,500 mg/kg for 28 days. Sedative and locomotor effects were quantified. Hypnotic efficacy was assessed, and anticonvulsant activity was measured against pentylenetetrazole-induced seizures. **Results:** CHP demonstrated an excellent safety profile with an LD₅₀ of 7,483 mg/kg, classifying it as “practically nontoxic.” No signs of subchronic toxicity were observed. In the open-field test, doses of 750 and 1,000 mg/kg significantly suppressed locomotor activity. The preparation significantly enhanced hypnotic effects by shortening sleep latency and prolonging total sleep duration. Furthermore, CHP exerted notable anticonvulsant activity compared with the control group. **Conclusions:** Combining *V. officinalis* and *L. sibiricus* provides a safe and effective botanical synergy with potent sedative, hypnotic, and anticonvulsant properties. Therefore, CHP is a promising therapeutic candidate for managing anxiety, insomnia, and epilepsy, warranting further clinical investigation into its underlying GABAergic or serotonergic mechanisms. **Keywords:** acute and subchronic toxicity; sedative, mice; open-field test; pentylenetetrazole; thiopental sodium

¹Department of Pharmacology, School of Biomedicine, Mongolian National University of Medical Sciences, Ulaanbaatar 14210, MONGOLIA.

²Department of Clinical Pharmacy and Management, School of Pharmacy, Mongolian National University of Medical Sciences, Zorig street, post office 48/111, 14210 Ulaanbaatar, MONGOLIA.

³School of Pharmacy, Mongolian National University of Medical Sciences, Zorig street, post office 48/111, 14210 Ulaanbaatar, MONGOLIA.

⁴Department of Biology, School of Biomedicine, Mongolian National University of Medical Sciences, Zorig street, post office 48/111, 14210 Ulaanbaatar, MONGOLIA.

⁵Department of Mongolian Medicine Surgery, College of Mongolian Medicine, Inner Mongolia Medical University, China; No 5 Xinhua Street, Hohhot, Inner Mongolia Autonomous region, CHINA.

⁶Department of Hematology, School of Medicine, Mongolian National University of Medical Sciences, Zorig street, post office 48/111, 14210 Ulaanbaatar, MONGOLIA.

Correspondence

Enkhmaa Gonchig

Department of Pharmacology, School of Biomedicine, Mongolian National University of Medical Sciences, Zorig street, post office 48/111, Ulaanbaatar 14210, MONGOLIA.

E-mail: enkhmaa.g@mnums.edu.mn

Purevjal Otgonbayar

Department of Hematology, School of Medicine, Mongolian National University of Medical Sciences, Zorig street, post office 48/111, 14210 Ulaanbaatar, MONGOLIA.

E-mail: purevjal@mnums.edu.mn

History

- Submission Date: 14-04-2026;
- Review completed: 20-05-2026;
- Accepted Date: 14-06-2026.

DOI : 10.5530/pj.2026.18.142

Article Available online

http://www.phcogj.com/v18/i3

Copyright

© 2026 Phcogj.Com. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

INTRODUCTION

Neuropsychiatric and neurological disorders, including anxiety, insomnia, and epilepsy, represent significant global public health challenges that diminish quality of life and impede socioeconomic development. In 2015, the World Health Organization (WHO) estimated that anxiety disorders affected approximately 264 million people, which accounts for 3.6% of the global population¹. Insomnia affects 10–30% of adults worldwide, with a higher prevalence among women, older adults, and those with comorbid psychiatric conditions than their counterparts². These disorders frequently manifest as a clinical triad that increases the risk of depression, occupational impairment, and suicidal ideation^{3,4}.

Among the chronic neurological conditions, epilepsy remains one of the most prevalent, affecting nearly 50 million people globally, with 80% of patients with epilepsy residing in low- and middle-income countries⁵. In Mongolia, the prevalence is approximately 57 cases per 10,000 people, accounting for 7.7% of all neurological diseases, a statistic that underscores the significant localized clinical burden⁶.

Although conventional pharmacotherapies, such as benzodiazepines and barbiturates, are effective,

their long-term utility is frequently compromised by a narrow therapeutic window and adverse effects, including cognitive impairment, tolerance, physical dependence, and withdrawal syndromes. Furthermore, the high rate of treatment-resistant cases necessitates the exploration of safer, multi-target, and sustainable therapeutic alternatives.

Phytotherapy offers a vast but underutilized reservoir for drug discovery. Although 70–95% of the population in developing countries relies on plant-based medicines for primary health care, only approximately 15% of the world's 300,000 plant species have been systematically evaluated for pharmacological efficacy^{7,8}. Mongolia, characterized by its unique biodiversity and robust heritage of traditional medicine, is well-positioned to contribute to the scientific validation of neuroactive phytotherapeutics.

Among the candidates for neuropsychiatric intervention, *Valeriana officinalis* L. and *Leonurus sibiricus* L. are particularly interesting. Historically used as a sedative and anticonvulsant since antiquity, *V. officinalis* contains bioactive constituents, such as valerenic acid, valepotriates, and sesquiterpenes, that modulate the γ -aminobutyric acid (GABA) system by enhancing GABA receptor function and increasing synaptic GABA levels^{9–11}. Additionally, its neuroprotective effects are supported by the

Cite this article: Yeruult Ch, Bilegdemberel Kh, Chimgee Ts, Maral-Erdene G, Batdavaa B, Mend-Amar B, Perleidlum B, Enerel Ye, Narankhajid M, Li C, Purevjal O, Enkhmaa G. Neuropharmacological Potential of a Combined Herbal Preparation of *Valeriana officinalis* L. and *Leonurus sibiricus* L.. Pharmacogn J. 2026;18 (3): 252-264.

upregulation of brain-derived neurotrophic factor^{11–21}. Similarly, *L. sibiricus* L. is traditionally used across Eurasia because of its calming and cardioprotective properties²². Its phytochemical profile, which is rich in leonurine, stachydrine, and labdane-type diterpenes, has revealed sedative, analgesic, and anti-inflammatory activities through interactions with the GABAergic pathways^{23–35}.

Given that GABA is the primary inhibitory neurotransmitter in the central nervous system (CNS), restoring the inhibitory–excitatory balance is a central goal in the treatment of neuronal hyperexcitability^{36–38}. As *V. officinalis* and *L. sibiricus* target complementary components of the GABAergic system, their combination may yield combined neuropharmacological effects, potentially enhancing efficacy while minimizing doses.

Therefore, in this study, we aimed to evaluate the sedative, hypnotic, and anticonvulsant activities of a combined herbal preparation (CHP) of *V. officinalis* and *L. sibiricus*. By integrating ethnomedical knowledge with rigorous experimental protocols, we sought to establish a scientific basis for a safe, plant-based, and multi-target therapeutic strategy for the management of anxiety-related symptoms, insomnia, and epilepsy.

MATERIALS AND METHODS

Experimental animals

C57BL/6 mice were obtained from the Central Laboratory of Mongolian National University of Medical Sciences (MNUMS). Wistar rats were obtained from the Drug Research Institute of Monos Group, LLC. The animals were housed in the vivarium of the Department of Pharmacology, MNUMS, under standard laboratory conditions (controlled room temperature, natural light–dark cycle) with ad libitum access to food and water.

Ethical statement

The study protocol was approved by the Ethical Review Committee of the Mongolian National University of Medical Sciences (approval no. 2020/3-05, protocol no. 2020/D-02). This study was conducted in accordance with the Animal Research: Reporting of In Vivo Experiments guidelines.

Chemicals and reagents

Distilled water and 0.9% sodium chloride solution (Aiviko LLC, Mongolia), diazepam (5 mg/mL, KRKA, Slovenia), pentylenetetrazole (PTZ) (5 g, Shanghai Macklin Biochemical Co., Ltd., China), thiopental sodium (1 g, Kurgan Pharmaceuticals, Russia), and ketamine hydrochloride (50 mg/10 mL, Tritttau Pharma, Germany) were obtained and used throughout the experiment.

Preparation of plant extracts and CHP

The roots of *Valeriana officinalis* L. were imported from the Russian Federation, whereas the aerial parts of *Leonurus sibiricus* L. were collected from the Terelj region of Mongolia on August 25, 2018. Botanical identification of *L. sibiricus* was performed by Prof. E. Ganbold, PhD, Department of Biology, Ulaanbaatar University. The dried roots of *V. officinalis* and the aerial parts of *L. sibiricus* were separately extracted with 70% ethanol. The solvent was removed under reduced pressure, and the remaining aqueous fraction was lyophilized using a freeze dryer (Biobase, China) to obtain dry extracts. The two extracts were combined at a 30:70 ratio (*V. officinalis*: *L. sibiricus*) to prepare the CHP for subsequent experiments.

Acute toxicity study (LD₅₀)

Acute oral toxicity (LD₅₀) of the CHP was evaluated in C57BL/6 mice (body weight, 19–27 g; age, 6–8 weeks) using the method described by

Lorke (1983),³⁹ which was conducted in two phases.

In phase I, healthy male mice were randomly assigned to the control and experimental groups. The control group received distilled water (10 mL/kg), whereas the experimental groups (n = 3 per group) received the CHP via gavage at doses of 500, 1,000, 2,000, and 3,000 mg/kg. The physical conditions of the mice and signs of toxicity were monitored for 2 h, and mortality was recorded at 24, 48, and 72 h.

In phase II, based on the phase I results, three additional groups (n = 3 per group) received the CHP at doses of 4,000, 5,000, 6,000, 7,000, and 8,000 mg/kg.

Subchronic toxicity

Subchronic toxicity was assessed according to the Organization for Economic Co-operation and Development (OECD) guideline 407.⁴⁰ Wistar rats were randomly allocated into three groups using a computer-generated randomization sequence: an untreated healthy control group (n = 6), which received distilled water daily; a group that received CHP at 1,000 mg/kg (n = 6); and a group that received CHP at 1,500 mg/kg (n = 6), administered via oral gavage. All treatments were administered for 28 consecutive days.

The sample size (n = 6 per group) was selected based on previous pilot toxicological studies and commonly used group sizes in subchronic screening studies, in accordance with OECD guideline 407. This approach also followed the ethical principles of the 3Rs (Replacement, Reduction, and Refinement), thereby reducing animal use while maintaining an adequate preliminary safety assessment.

Body weight was recorded, and blood samples were collected for hematological evaluation to determine the safety profile of the CHP. The analyses focused on key hematological indices, including red blood cell count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular Hb (MCH), MCH concentration, red cell distribution width–standard deviation, RDW–coefficient of variation, platelet (PLT) count, mean PLT volume, PLT distribution width, PLT-to-large cell ratio, plateletcrit, and white blood cell count. All measurements were performed using a Sysmex PochH-100i hematology autoanalyzer (Sysmex Corporation, Japan)⁴¹.

The levels of biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, and creatinine, were measured using an automated biochemical analyzer (ICUBIO iChem-520, Shenzhen, China), and histopathological analyses were performed, as previously described⁴¹. All outcome assessments were performed by investigators blinded to group allocation to minimize potential observer bias.

Evaluation of sedative activity (open-field test)

Sedative activity was assessed using the open-field test according to Wang et al. (2003).⁴² C57BL/6 mice (24–27 g) were randomly assigned to four groups (n = 6 per group), with the control group (negative control) receiving distilled water (10 mL/kg, per oreo [p.o.]). The standard group (positive control) received diazepam (1 mg/kg, intraperitoneally), whereas the treatment groups received the CHP at doses of 750 or 1,000 mg/kg via gavage. One hour after oral administration (or 30 min after diazepam injection), each mouse was placed in the center of an open-field apparatus (40 × 40 cm, divided into 16 equal squares). Locomotor activity (number of squares crossed) and rearing behavior were recorded for 5 min.

Thiopental-induced sleeping test

The effect of the CHP on thiopental-induced sleep was evaluated following the method of Turner et al. (1965).⁴³ The mice (20–26 g) were divided into four groups (n = 6 per group). The control group (negative

control) received distilled water (10 mL/kg, p.o.). The standard group (positive control) received diazepam (1 mg/kg, intraperitoneally [i.p.]), whereas the treatment groups received the CHP at doses of 750 or 1,000 mg/kg via gavage. After 1 h (or 30 min for diazepam), the mice were injected with thiopental sodium (40 mg/kg, i.p.). Sleep onset and duration were recorded based on the loss and recovery of the righting reflex over 6 h.

Pentylenetetrazole (PTZ)-induced seizure model

Anticonvulsant activity was assessed using a PTZ-induced seizure model.⁴⁴ C57BL/6 mice (20–25 g) were assigned to four groups (n = 6 per group): the control group (negative control) received distilled water (10 mL/kg, p.o.). The standard group (positive control) received diazepam (2 mg/kg, i.p.), whereas the treatment groups received the CHP at doses of 750 or 1,000 mg/kg via gavage. One hour after oral dosing (or 30 min after diazepam administration), PTZ (70 mg/kg, i.p.) was administered. The latency to myoclonic and tonic-clonic seizures, seizure duration, and mortality were recorded for 30 min.

Statistical analyses

Data were analyzed using STATA version 13. The normality of data distribution was assessed using the Shapiro–Wilk test. Results are expressed as means ± standard deviations. Groups were compared using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for normally distributed data, or the Kruskal–Wallis test for non-normally distributed data, as appropriate. Statistical significance was set at $p < 0.05$.

RESULTS

Acute toxicity study

The mice were monitored for 24 and 72 h after treatment. In phase I, the control group received distilled water (10 mL/kg, p.o.), whereas the treatment groups were administered a single oral dose of the CHP at 500, 1,000, 2,000 and 3,000 mg/kg via gavage. The animals were observed continuously for the first 2 h to identify clinical signs or behavioral changes (Table 1). Dose-dependent behavioral variations were noted. At a dose of 500 mg/kg, the mice exhibited increased locomotor activity, characterized by frequent grooming, rearing, and exploratory stretching. At 1,000 mg/kg, an initial reduction in locomotion was observed, along with prolonged rearing and excessive grooming; however, activity returned to baseline within 1 h. Mice treated with 2,000 mg/kg displayed decreased activity and drooping of the ears (ptosis), with recovery beginning after 1 h. At the highest dose (3,000 mg/kg), the mice exhibited reduced movement, mild sedation,

and slightly diminished responsiveness to tactile stimuli, with normal alertness restored within 2 h. No mortality occurred in any treatment group within the first 24 h; consequently, the study proceeded to phase II.

In phase II, the mice received higher oral doses of the CHP (4,000–8,000 mg/kg) and were observed for 2 h (Table I). At 4,000 mg/kg, the clinical signs included tachypnea, ear ptosis, hunched posture (kyphosis), eye closure, and pronounced sedation with a loss of response to tactile stimuli. Recovery occurred within 2 h. At 5,000 mg/kg, the animals exhibited transient tail stiffness, tachypnea, and intermittent tremors that resolved within 1 h, followed by decreased locomotion and sedative behavior. At 6,000 mg/kg, increased respiration and circling behavior were observed alongside tremors, followed by marked sedation and eye closure. At 7,000 mg/kg, the mice displayed kyphosis, tachypnea, and body tremors, leading to prolonged sedation. The highest dose (8,000 mg/kg) induced severe toxicity, including dyspnea, chest retraction, ataxia, and tonic-clonic seizures, lasting 1–2 min. Mortality was recorded at 8,000 mg/kg: one mouse died within 2 h, and a second mouse died within 24 h after failing to recover from post-seizure sedation. Overall, oral administration (1,000–7,000 mg/kg) induced a dose-dependent reduction in locomotor activity. At a dose of 3,000 mg/kg, diminished tactile responsiveness and somnolence were evident, suggesting that the CHP possesses CNS depressant and sedative properties. Although transient tachypnea was noted at doses between 4,000 and 7,000 mg/kg, these symptoms typically resolved spontaneously within the first hour.

Administration of the CHP at doses of 5,000–7,000 mg/kg induced transient tremors, indicative of dose-dependent toxicity. Similar to the observed respiratory effects, these tremors subsided spontaneously within approximately 1 h. Mortality was monitored 24, 48, and 72 h after administration (Table 2).

All mice treated with doses ranging from 500 to 7,000 mg/kg survived the entire 72-h observation period. In contrast, the 8,000 mg/kg group exhibited severe toxic manifestations, including tachypnea and tonic-clonic seizures, resulting in mortality within the first 2 h, as well as at 24- and 48-h intervals. The median lethal dose (LD_{50}) was calculated using the following formula:

$$LD_{50} = \sqrt{Dn \times D100} = \sqrt{7000 \text{ mg/kg} \times 8000 \text{ mg/kg}} = 7483 \text{ mg/kg}$$

Dn presents the highest dose resulting in 0% mortality (7,000 mg/kg).

$D100$ presents the lowest dose resulting in 100% mortality (or the specific lethal threshold observed, 8,000 mg/kg).

Table 1. Behavioral and physiological changes in mice during acute oral toxicity

Parameter	Dose									
	Phase I					Phase II				
	Distilled water 10 mL/kg	500 mg/kg	1,000	2,000	3,000	400	5,000	600	7,000	8,000
Response to touch	+	+	+	+	↓	↓	↓	↓	↓	↓
Respiration	+	+	+	↑	↑	↑	↑	↑	↑	↑
Locomotor activity	+	+	↓	↓	↓	↓	↓	↓	↓	↓
Salivation	+	+	+	+	+	+	+	+	+	+
Urination	+	+	+	+	+	+	+	+	+	+
Defecation	+	+	+	+	+	+	+	+	+	+
Convulsions	-	-	-	-	-	-	-	-	-	a
Tremors	-	-	-	-	-	-	a	a	a	a
Edema	-	-	-	-	-	-	-	-	-	-
Sedation	-	-	-	-	a	a	a	a	a	a

Symbols: + = normal, – = not observed, a = observed, ↓ = decreased, ↑ = increased.

Table 2. Mortality of mice within 72 h after acute oral administration

Phase	Group	Dose (mg/kg)	Mortality		
			24 h	48 h	72 h
Phase I	I	500	0/3	0/3	0/3
	II	1,000	0/3	0/3	0/3
	III	2,000	0/3	0/3	0/3
	IV	3,000	0/3	0/3	0/3
	V	4,000	0/3	0/3	0/3
	VI	5,000	0/3	0/3	0/3
Phase II	VII	6,000	0/3	0/3	0/3
	VIII	7,000	0/3	0/3	0/3
	IX	8,000	2/3	1/1	-

Values represent the number of dead animals (0, 1, or 2), relative to the total number of animals tested ($n = 3$).

Consequently, the LD₅₀ of the CHP was 7,483 mg/kg. According to the OECD toxicity classification guidelines, this value categorizes the CHP as practically nontoxic⁴⁵.

Body weight changes in mice

Changes in body weight serve as a sensitive indicator of systemic toxicity; a reduction > 10% of the baseline weight is generally considered a significant toxic effect.⁴⁶ In this study, the body weights of mice treated with the CHP (500–8,000 mg/kg) were recorded on days 1, 7, and 14 and compared with those of the control group (Figure 1).

Oral administration of the CHP at doses up to 7,000 mg/kg did not induce significant changes in body weight at any time point compared with the control ($p > 0.05$). These findings suggest that the tested preparation does not exert growth-suppressive effects or systemic toxicity at these doses over the 14-day observation period.

Organ weight changes in mice

Absolute and relative organ weights are critical indices of physiological and pathological changes during toxicological assessments. The liver, spleen, heart, lungs, and kidneys, primary to metabolic and systemic homeostatic responses, were specifically evaluated for potential treatment-related toxicities⁴⁷.

On day 14, the mice were euthanized, and the absolute weights of these target organs were recorded. Relative organ weights (organ-to-body weight ratios) were subsequently calculated (Figure 2 and Figure 3).

Statistical analysis revealed that oral administration of the CHP (500–7,000 mg/kg) induced no significant changes in either the absolute or relative weights of the liver, spleen, kidneys, heart, or lungs compared with the control group ($p > 0.05$). These results indicate that the preparation does not induce organ-specific hypertrophy, atrophy, or inflammation, further supporting the absence of systemic toxicity in the major internal organs.

Subchronic toxicity study

Body weight changes in rats

Rats treated with the CHP at doses of 1,000 and 1,500 mg/kg exhibited a statistically significant increase in body weight on days 7, 14, 21, and 28 compared with baseline (day 1, $p > 0.05$). This weight gain was consistent with normal physiological growth patterns (Figure 4).

After 28 days of oral administration, comparative analysis of absolute and relative organ weights revealed no statistically significant differences between the control and treatment groups ($p > 0.05$, Figure 5). These results indicate the absence of treatment-related organ weight alterations, suggesting that the CHP is well-tolerated during subchronic exposure.

Hematological and biochemical analysis

Hematological analysis of rats treated with 1,000 and 1,500 mg/kg of the CHP revealed no statistically significant differences in key blood parameters, such as red and white blood cell counts, PLT counts, Hb levels, and HCT count, compared with the control group (Table 3).

Similarly, biochemical assessments revealed no significant differences in the liver (AST, ALT, and ALP) or renal function markers (creatinine and urea) between the experimental and control groups after 28 days (Table 4).

Histopathological study

Histopathological examination of the liver, kidneys, and heart revealed no pathological alterations in rats administered the CHP at doses of 1,000 and 1,500 mg/kg compared with the control group (Figure 6). Microscopic examination of liver sections from the experimental group and the control group showed no evidence of necrosis, fatty degeneration, inflammation around the portal areas, or fibrosis. Kidney tissue analysis revealed normal glomerular structure without focal or diffuse necrosis in the proximal or distal tubules. Moreover, the histological assessment of the cardiac muscle revealed normal cardiomyocyte morphology, with no pathological changes observed in either the control or treatment groups.

Sedative activity assessment

The open-field test was used to evaluate the sedative potential of the CHP, as it is a highly sensitive and reliable method for assessing spontaneous locomotor activity⁴⁸. The mice were orally administered the preparation at doses of 750 and 1,000 mg/kg. Behavioral parameters, including the total number of squares crossed and rearing frequency, were recorded over a 5-min observation period.

In the open-field test, spontaneous locomotor activity measured by the total number of squares crossed was significantly lower in all treatment groups compared with the control group (114.5 ± 22.8). Specifically, locomotor activity decreased by 72.1% in the standard group (32.0 ± 23.8), 39.2% in the 750 mg/kg group (69.66 ± 29.07), and 63.0% in the 1,000 mg/kg group (42.33 ± 33.67). These reductions were statistically significant ($p < 0.05$, Figure 7), supporting the dose-dependent sedative effects of the preparation.

In the open-field test, the frequency of vertical rearing, an indicator of exploratory behavior, was significantly lower in all treatment groups compared with the control group (25.5 ± 3.01). The standard group exhibited a pronounced reduction to 6.33 ± 5.71 (75.2% decrease). Similarly, rearing frequency decreased to 15.33 ± 7.96 in the 750 mg/kg group (39.9% reduction) and to 9.16 ± 6.88 in the 1,000 mg/kg group (64.1% reduction). These differences were statistically significant ($p < 0.05$, Figure 8).

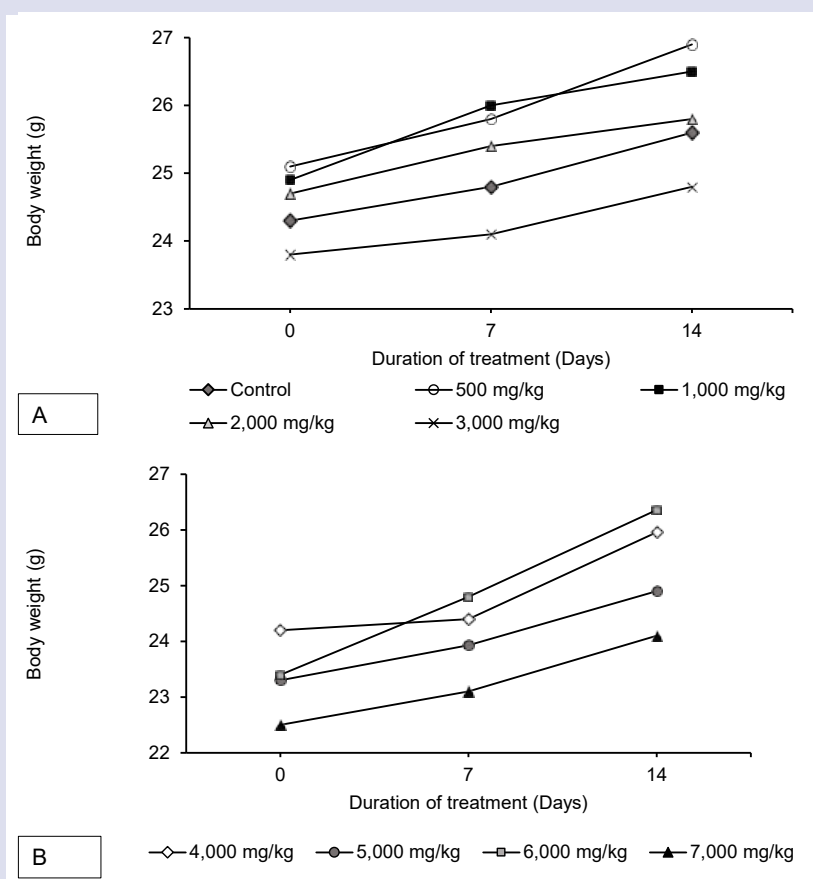


Figure 1. Body weight changes in mice during subchronic administration

(A) Control group and groups administered the combined herbal preparation at doses of 500–3,000 mg/kg.

(B) Groups administered the combined herbal preparation at doses of 4,000–7,000 mg/kg.

No statistically significant differences were observed compared with the control group ($p > 0.05$).

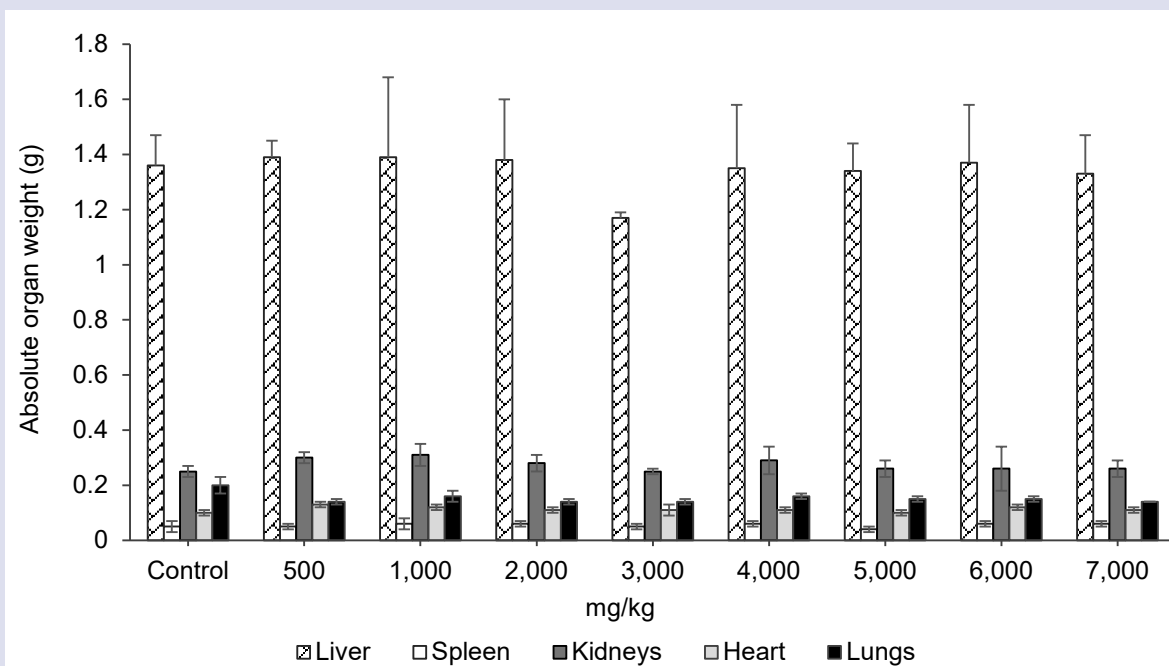


Figure 2. Absolute organ weights of mice after oral administration

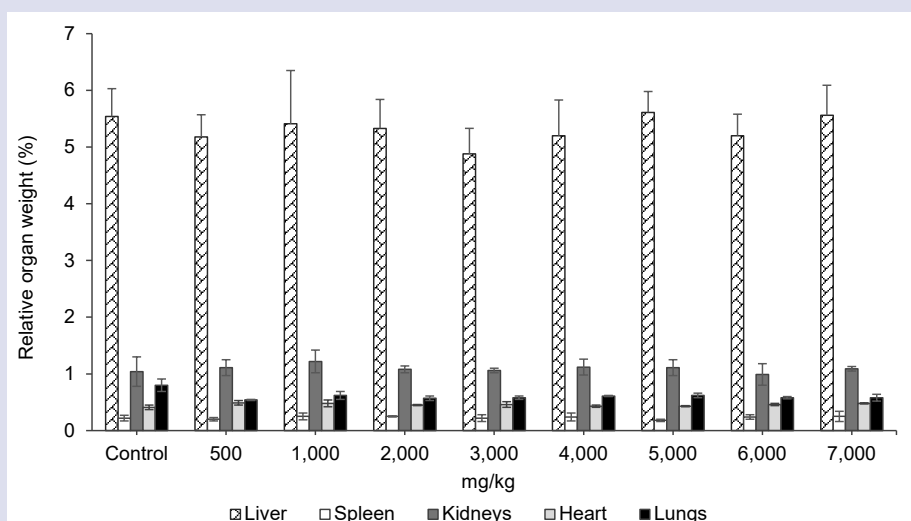


Figure 3. Relative organ weights of mice treated with the combined herbal preparation. No statistically significant differences were observed compared with the control group ($p > 0.05$).

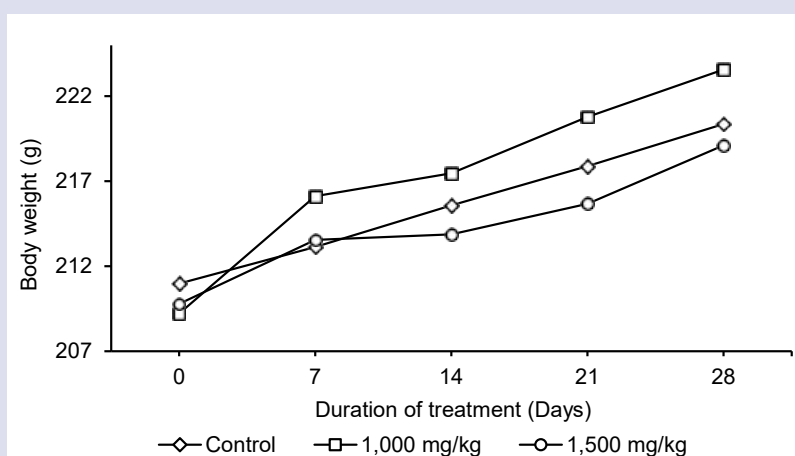


Figure 4. Changes in body weight of rats following the oral administration of the combined herbal preparation

Table 3. Hematological parameters of rats during subchronic toxicity study

Parameter	Control	1,000 mg/kg	1,500 mg/kg
WBC $10^3 \mu\text{l}$	8.3 ± 0.38	8.8 ± 2.2	8.28 ± 1.08
RBC $10^6 \mu\text{l}$	6.39 ± 1.27	7.47 ± 0.45	6.83 ± 0.52
HGB g/dl	12.08 ± 1.76	13.93 ± 0.76	13.26 ± 0.9
HCT %	38.35 ± 4.27	43.33 ± 2.11	40.65 ± 2.79
MCV fl	61.43 ± 9.44	58.08 ± 2.63	59.65 ± 3.89
MCH pg	19.16 ± 1.94	18.66 ± 0.82	19.46 ± 1.13
MCHC g/dl	31.4 ± 2.06	32.15 ± 0.94	32.66 ± 1.28
PLT $10^3 \mu\text{l}$	$1,083.83 \pm 176.81$	968.5 ± 157.62	935.66 ± 120.13
RDW-SD fl	30.33 ± 1.79	29.12 ± 1.52	29.75 ± 4.0
RDW-CV fl	12.36 ± 1.7	12.13 ± 1.83	11.93 ± 1.75
PDW %	8.32 ± 0.45	8.033 ± 0.46	8.4 ± 0.44
MPV fl	7.3 ± 0.35	7.12 ± 0.19	7.4 ± 0.24
PLCR %	7.28 ± 1.94	6.13 ± 0.67	7.52 ± 1.39
PCT %	0.81 ± 0.17	0.68 ± 0.11	0.69 ± 0.08

WBC, White Blood Cells; RBC, Red Blood Cells; HGB, Hemoglobin; HCT, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; PLT, Platelet Count; RDW-SD, Red Cell Distribution Width-Standard Deviation; RDW-CV, Red Cell Distribution Width-Coefficient of Variation; PDW, Platelet Distribution Width; MPV, Mean Platelet Volume; PLCR, Platelet Large Cell Ratio; PCT, Plateletcrit.

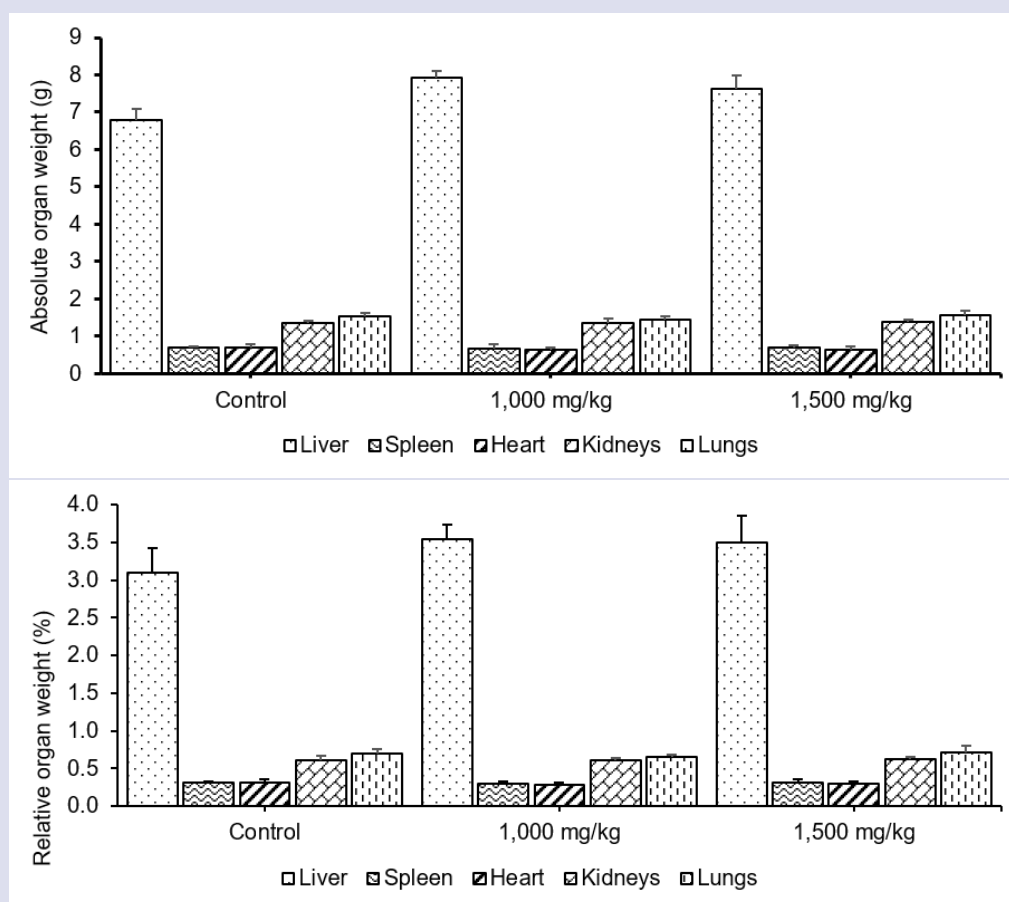


Figure 5. Absolute and relative organ weights of rats following the oral administration of the combined herbal preparation

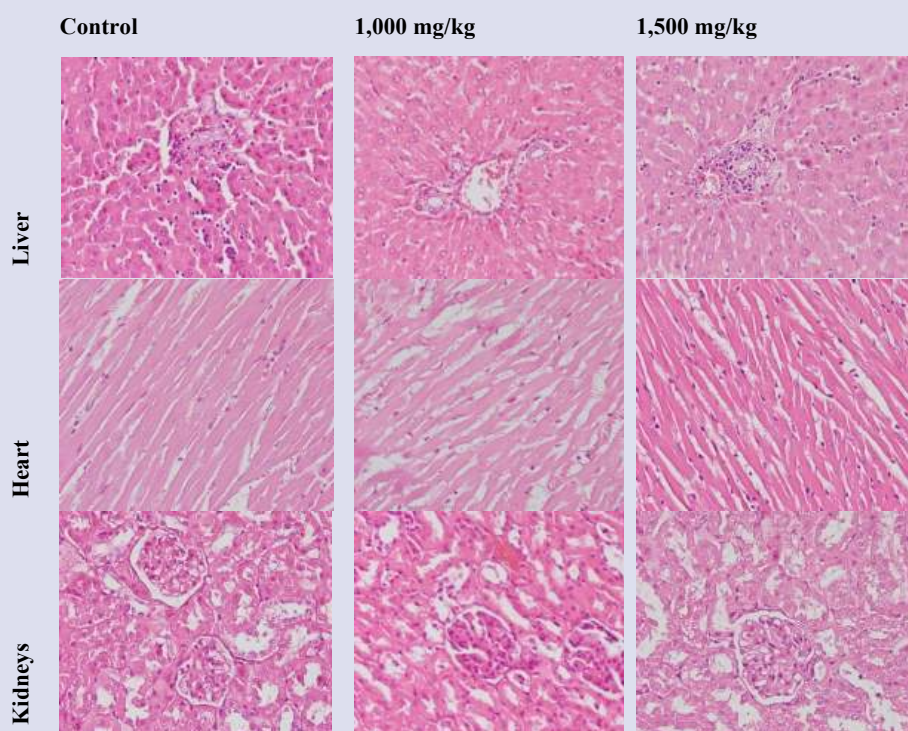


Figure 6. Histopathological features of the liver, heart, and kidneys in experimental rats

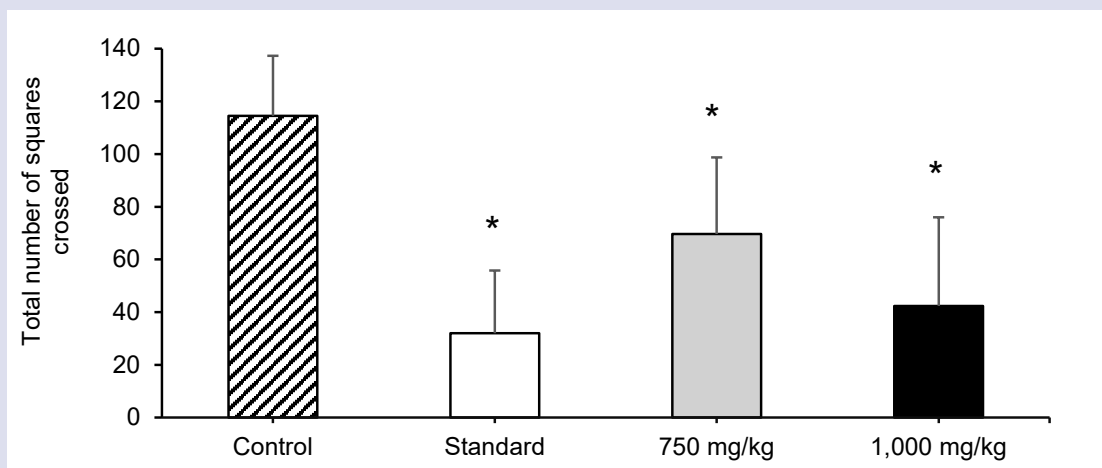


Figure 7. Total number of squares crossed by mice in each experimental group

*Significantly different from the control group ($p < 0.05$)

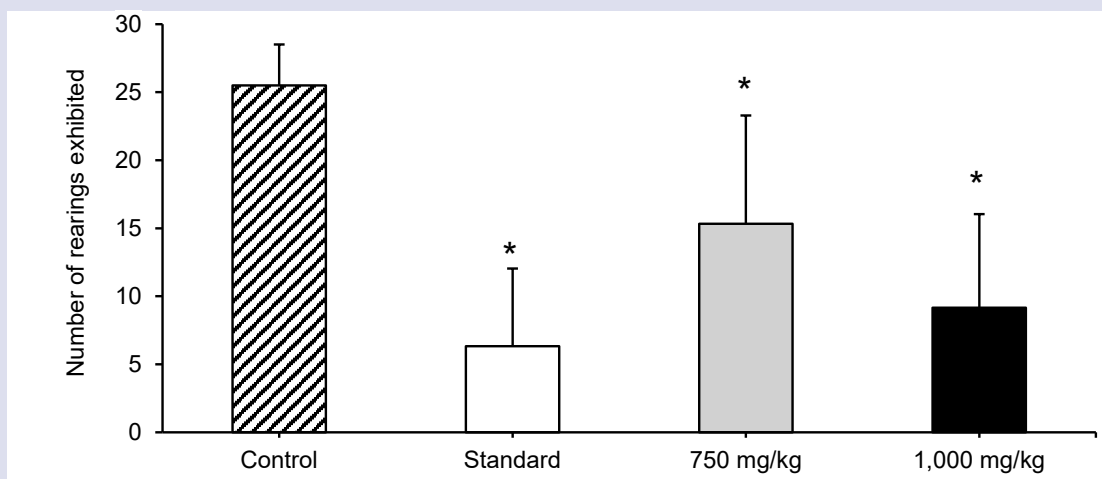


Figure 8. Number of rearings exhibited by mice in each experimental group

*Significantly different from the control group ($p < 0.05$)

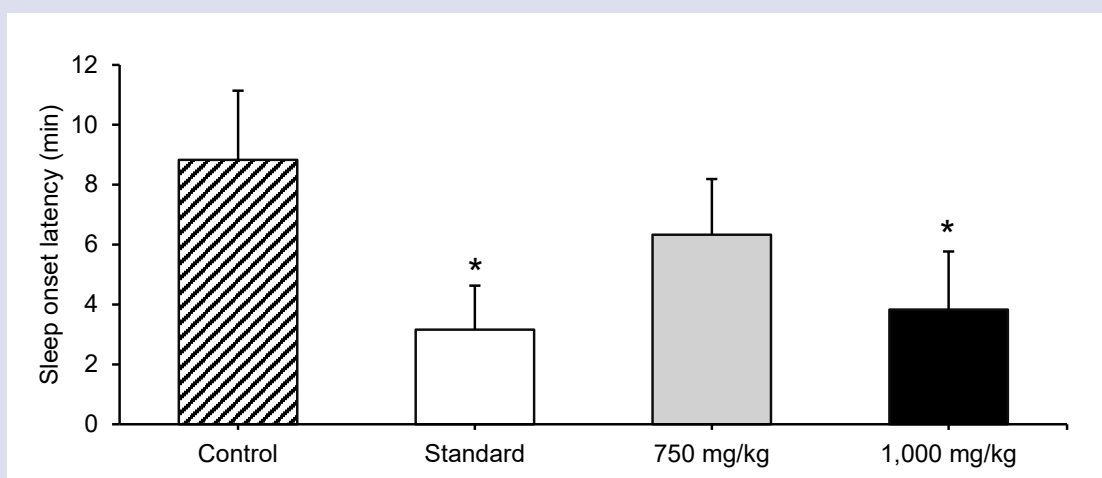


Figure 9. Sleep onset latency in mice following thiopental sodium administration

*Significantly different from the control group ($p < 0.05$)

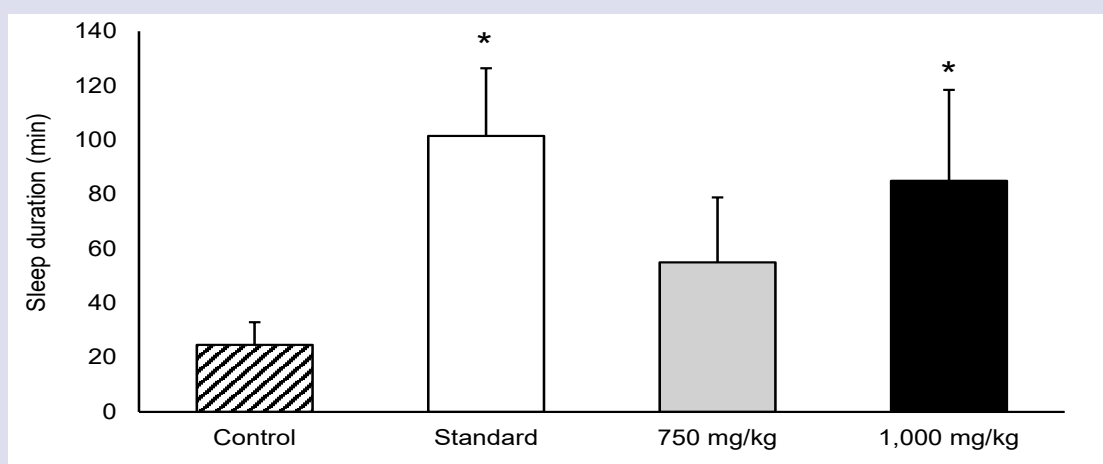


Figure 10. Duration of thiopental sodium-induced sleep in mice

*Significantly different from the control group ($p < 0.05$)

Table 4. Serum biochemical parameters of rats during subchronic toxicity study

Parameter	Control	1,000 mg/kg	1,500 mg/kg
ALT U/L	62.61 ± 3.18	66.05 ± 3.4	65.27 ± 5.69
AST U/L	115.6 ± 6.84	125.76 ± 12.66	128.75 ± 10.75
ALP U/L	239.42 ± 20.86	251.97 ± 20.2	252.885 ± 20.7
UREA mmol/L	7.17 ± 1.35	7.41 ± 0.87	7.58 ± 1.25
CREA μmol/L	45.7 ± 2.25	42.79 ± 2.91	43.86 ± 3.19

ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase; UREA, Urea; CREA, Creatinine.

Table 5. Effects of the combined herbal preparation on PTZ-induced myoclonic seizures

Group	Dose	Onset time of myoclonic seizures (s) Mean ± SD	Duration of myoclonic seizures (s)
Control (distilled water)	10 mL/kg	76.33 ± 6.59	74.33 ± 11.43
Standard (diazepam)	2 mg/kg	1,800.00 ± 00*	0.00 ± 0.00*
Experimental group I	750 mg/kg	98.83 ± 8.25*	49.16 ± 3.86*
Experimental group II	1,000 mg/kg	104.66 ± 10.74*	16 ± 7.79*

*Significantly different from the control group ($p < 0.05$)

Table 6. Effects of the combined herbal preparation on PTZ-induced tonic-clonic seizures

Group	Dose	Onset time of tonic-clonic seizures (s) Mean ± SD	Duration of tonic-clonic seizures (s)	Seizure protection (%)	Mortality protection (%)
Control (distilled water)	10 mL/kg	88.83 ± 8.95	99 ± 10.48	6/6 (0)	6/6 (0)
Standard (diazepam)	2 mg/kg	1,800.00 ± 00*	0.00 ± 0.00*	0/6 (100)	0/6 (100)
Experimental group I	750 mg/kg	163.5 ± 54.76*	42.33 ± 8.01*	6/6 (0)	0/6 (100)
Experimental group II	1,000 mg/kg	206 ± 95.06*	24.5 ± 8.5*	6/6 (0)	0/6 (100)

*Significantly different from the control group ($p < 0.05$)

Collectively, oral administration of the CHP at 750 and 1,000 mg/kg significantly suppressed both spontaneous locomotor activity and exploratory behavior in a dose-dependent manner. These results suggest a potent sedative effect, which may be mediated by bioactive constituents, such as alkaloids, terpenoids, and flavonoids, which are known for their CNS depressant properties.

Thiopental-induced sleep modulation

Thiopental sodium was used to evaluate the sleep-modulating potential of CHP⁴⁹. The mice were orally administered the preparation at doses of 750 and 1,000 mg/kg. After 1 h, thiopental sodium (40 mg/kg, i.p.) was administered, and sleep behavior was monitored for 6 h. Sleep onset

(latency) and duration were determined by the loss and recovery of the righting reflex, defined as the inability of the animal to return to an upright position within 5 s when placed in a supine position⁵⁰.

In the thiopental sodium-induced sleep test, sleep onset latency was significantly shortened in all treatment groups compared with the control group (8.83 ± 2.31 min). The standard group exhibited the most pronounced effect, with latency reduced by 64.2% (3.16 ± 1.47 min). Administration of the CHP resulted in a dose-dependent reduction in latency: 28.3% at 750 mg/kg (6.33 ± 1.86 min) and 56.6% at 1,000 mg/kg (3.83 ± 1.94 min). These reductions were statistically significant ($p < 0.05$, Figure 9).

Sleep duration was significantly prolonged in all treatment groups compared with the control group (24.66 ± 8.33 min). The standard group exhibited the greatest increase in sleep duration (101.5 ± 24.9 min). Consistent with latency results, the CHP induced a dose-dependent extension of sleep time, reaching at 55.0 ± 23.89 min at 750 mg/kg and 85.0 ± 33.44 min at 1,000 mg/kg. These increases were statistically significant across all treatment groups ($p < 0.05$, Figure 10).

Collectively, the significant reduction in sleep latency and prolongation of total sleep duration demonstrate that the CHP markedly potentiates thiopental-induced hypnosis. These findings suggest that the bioactive constituents of the preparation act synergistically with thiopental sodium to enhance its hypnotic effects. This potentiation likely occurs through the modulation of central inhibitory neurotransmission, potentially involving the GABA receptor complex, the primary site of action for barbiturates, such as thiopental.

PTZ-induced seizure protection

PTZ was used to evaluate the anticonvulsant potential of the CHP.⁵¹ The mice received oral doses of 750 or 1,000 mg/kg, followed by a PTZ challenge (70 mg/kg, i.p.) after 1 h. Latency to seizure onset, duration of myoclonic and tonic-clonic seizures, and mortality were monitored over a 30-min period.

The CHP significantly prolonged the latency to myoclonic seizure onset compared with the control group (76.33 ± 6.59 s). Latency increased by 22.7% at 750 mg/kg (98.83 ± 8.25 s) and 27.0% at 1,000 mg/kg (104.66 ± 10.74 s). Correspondingly, the duration of myoclonic seizures was significantly reduced from 74.33 ± 11.43 s (control) to 49.16 ± 3.86 s (750 mg/kg) and 16.0 ± 7.79 s (1,000 mg/kg). The standard group (diazepam) exhibited complete protection, with no myoclonic activity observed ($1,800 \pm 0.00$ s, $p < 0.005$) (Table 5).

Similarly, the latency to tonic-clonic seizures was significantly extended in a dose-dependent manner. Compared with the control group (88.83 ± 8.95 s), latency increased by 45.7% at 750 mg/kg (163.5 ± 54.76) and 56.9% at 1,000 mg/kg (206.0 ± 95.06 s). Seizure duration was also markedly reduced, with reductions of 57.2% and 75.3% at 750 and 1,000 mg/kg doses, respectively ($p < 0.05$, Table 6).

Although the control group experienced complete seizure progression, the standard group (diazepam, 2 mg/kg) showed 100% seizure protection. Notably, both the standard treatment and the CHP at all tested doses provided 100% protection against PTZ-induced mortality. Collectively, these findings demonstrate that the CHP possesses significant anticonvulsant activity, characterized by delayed seizure onset and reduced seizure severity.

DISCUSSION

Nature provides a vast diversity of molecular structures that serve as primary sources for the development of novel drugs. Advancements in isolation and structural elucidation have enabled rigorous evaluation of the pharmacological and toxicological profiles of bioactive plant compounds⁵². According to the WHO, traditional medicine remains globally relevant; notably, nearly 25% of modern medicines and 60% of anticancer agents are plant-derived^{53,54}. Although approximately 300,000 plant species exist, only 15% have been pharmacologically validated. Despite the efficacy of synthetic drugs, challenges, such as high costs and adverse systemic effects, have driven the global herbal medicine market to an estimated \$83 billion⁸⁻⁹. In Mongolia, where 90% of pharmaceuticals are imported, investigating indigenous flora is a critical strategy for enhancing national pharmacopeia and economic sustainability⁵⁵.

Building on Mongolian and Tibetan traditional medicine, which utilizes *Valeriana officinalis* L. and *Leonurus sibiricus* L. for their sedative,

hypnotic, and anticonvulsant properties, we evaluated the CHP of these species. We aimed to characterize its pharmacological potential as a monotherapy or adjunct treatment for epilepsy, anxiety, and insomnia.

Valeriana officinalis (Valerian), found in Europe, Asia, and several Mongolian provinces, has been used as a sedative.⁵⁶ Similarly, *Leonurus sibiricus* (Lamiaceae), widely distributed across Mongolia, is traditionally used to treat uterine spasms, cardiovascular conditions, and anxiety⁵⁷.

Safety is a prerequisite for new medicinal products. In this study, acute oral toxicity testing yielded an LD₅₀ of 7,483 mg/kg, classifying the CHP as “practically nontoxic” per OECD guidelines. No mortality or severe clinical signs were observed at doses of up to 7,000 mg/kg. Furthermore, subchronic toxicity assessments revealed no significant alterations in body weight, organ weight, hematology, or serum biochemical parameters. Histopathological analysis confirmed the absence of necrosis, inflammation, and fibrosis in the liver, kidneys, and heart, indicating that CHP does not exert systemic toxicity upon repeated exposure.

These safety findings are consistent with those reported in the existing literature. Previous studies on *V. officinalis* ethanol extracts reported an LD₅₀ of 3.3 g/kg (i.p.) in mice, whereas essential oils showed an oral LD₅₀ of 15,000 mg/kg^{58,59}. Clinical reports have also suggested that *V. officinalis* remains non-life-threatening even at 20 times the therapeutic dose⁶⁰. Regarding *L. sibiricus*, researchers have noted low toxicity for its dry extracts and alkaloids, with oral LD₅₀ values $> 5,000$ mg/kg^{61,62}.

The sedative efficacy of CHP was validated using the open-field test, where doses of 750 and 1,000 mg/kg significantly reduced motor activity. These effects are likely mediated via GABAergic pathways, enhancing inhibitory neurotransmission; however, this mechanism is hypothesized and was not experimentally confirmed in the present study. These results mirror the findings of Neamati et al. and de Almeida et al., who documented significant reductions in the rearing and exploration of mice treated with *V. officinalis*^{62,63}. Similarly, Koshovyi et al. reported synergistic sedative effects when combining *Leonurus* species with amino acids⁶⁴.

Furthermore, 750 and 1,000 mg/kg CHP significantly potentiated thiopental-induced sleep, shortening the onset latency and extending the duration of sleep. Thiopental induces sleep by prolonging the chloride channel opening at the GABA-A receptor; CHP appears to enhance barbiturate activity⁶⁵. Our results corroborate those of Chow et al. and Hendricks et al., who demonstrated that *V. officinalis* extracts (250–1,000 mg/kg) significantly enhanced phenobarbital-induced sleep^{66,67}.

Hiller et al. confirmed that *V. officinalis* at 100 mg/kg significantly prolonged thiopental-induced sleep duration⁶⁸, whereas Ahmed et al. demonstrated that methanolic extracts of *L. sibiricus* enhanced phenobarbital-induced sleep by reducing latency and increasing duration⁶⁹. Similar hypnotic properties have been reported by Chultemsuren et al. using diverse *Leonurus* species^{25,70}.

Epilepsy affects approximately 50 million people worldwide. In low- and middle-income countries, nearly 75% of patients lack access to adequate treatment.⁵ Given the chronic nature of this disorder and the potential adverse effects of long-term conventional antiepileptic drug use, an urgent need for safer plant-based alternatives exists. In this study, the anticonvulsant activity of the CHP was evaluated using the PTZ-induced seizure model. PTZ induces seizures by antagonizing the GABA receptor at the picrotoxin binding site, leading to neuronal depolarization via sodium and calcium ion influx⁷¹.

Oral administration of CHP (750 and 1,000 mg/kg) significantly prolonged seizure onset latency and reduced seizure duration. Notably,

CHP provided 100% protection against mortality in both the myoclonic and tonic-clonic seizure models. These anticonvulsant effects are likely mediated by sesquiterpenes and alkaloids, which enhance GABAergic neurotransmission. Our results are consistent with those of previous studies; Nouri et al. demonstrated the protective effects of *V. officinalis* (250–1,000 mg/kg) against PTZ-induced seizures⁷², and Ebrahim et al. reported its efficacy in temporal lobe epilepsy models⁷³. Similarly, significant anticonvulsant activity has been documented for *Leonurus deminutus*²⁵ and *Leonotis leonurus*, with the latter's effects attributed to its high alkaloid content⁷⁴.

Maintenance of the homeostatic balance between excitatory and inhibitory neurotransmitters is essential for CNS function. Disruption of this balance is fundamental to the pathogenesis of anxiety, insomnia, and epilepsy. As the primary inhibitory neurotransmitter in the CNS, GABA is a molecular target for many anxiolytic and hypnotic drugs⁷⁵. Extensive research has confirmed that the bioactive compounds in *V. officinalis* and *L. sibiricus* enhance GABAergic activity. Trauner et al. showed that valerian-derived sesquiterpenes exhibited a strong binding affinity for GABA receptors⁷⁶, specifically at the β_3 subunit^{77,78}. Furthermore, Mulyawan et al. observed increased *GABRB3* expression following *V. officinalis* administration⁷⁹.

Regarding the *Leonurus* component, Rauwald et al. identified leonurine as a compound with a high affinity for GABA binding sites²⁶. In addition to GABAergic modulation, these plants may influence the serotonin, adenosine, and prolyl oligopeptidase pathways^{80–87}. Collectively, the terpenes, iridoids, alkaloids, and flavonoids in CHP likely exert their sedative, hypnotic, and anticonvulsant effects through synergistic mechanisms, including the enhancement of inhibitory transmission and inhibition of enzymatic degradation pathways. These findings highlight the potential use of *V. officinalis* and *L. sibiricus* as novel herbal therapeutics and functional supplements.

A limitation of the present study is the absence of biochemical, receptor-binding, and phytochemical analyses to confirm the underlying mechanisms of action or to standardize the active constituents of CHP. Future studies are therefore warranted to characterize the chemical profile of CHP using advanced and specific analytical techniques. In addition, detailed mechanistic investigations are needed to elucidate its effects on the GABAergic system, particularly at the receptor level. Furthermore, chronic toxicity studies are required to establish the long-term safety of the preparation.

CONCLUSIONS

The CHP demonstrated a favorable safety profile, with an LD₅₀ of 7,483 mg/kg, classifying it as “practically nontoxic” according to the OECD guidelines. Repeated oral administration in subchronic studies revealed no significant alterations in body weight or hematological or serum biochemical parameters. Histopathological examination confirmed the absence of structural damage to the major organs. Pharmacological assays have established that CHP possesses significant sedative and hypnotic activities and provides potent protection against PTZ-induced seizures. These results suggest that this CHP is a safe and effective candidate for further development as a primary or adjunct therapy for anxiety, insomnia, and epilepsy.

ACKNOWLEDGMENTS

The authors are grateful to the Department for Science, Technology and Innovation, and the Science and Technology Support Fund of MNUMS. We would like to thank Editage (www.editage.com) for English language editing.

REFERENCES

1. World Health Organization. Depression and other common mental disorders: global health estimates; 2017. p. 10-1.

2. Zhang Y, Ren R, Lei F, Zhou J, Zhang J, Wing YK, et al. Worldwide and regional prevalence rates of co-occurrence of insomnia and insomnia symptoms with obstructive sleep apnea: a systematic review and meta-analysis. *Sleep Med Rev*. 2019;45:1-17.
3. Blake MJ, Trinder JA, Allen NB. Mechanisms underlying the association between insomnia, anxiety, and depression in adolescence: implications for behavioral sleep interventions. *Clin Psychol Rev*. 2018;63:25-40.
4. Bjorøy I, Jørgensen VA, Pallesen S, Bjorvatn B. The prevalence of insomnia subtypes in relation to demographic characteristics, anxiety, depression, alcohol consumption and use of hypnotics. *Front Psychol*. 2020;11:527.
5. World Health Organization. Epilepsy; June 20, 2019. [cited Nov 23, 2019]. Available from: <https://www.who.int/news-room/fact-sheets/detail/epilepsy>.
6. Center for Health Development. *Health indicators*. 2019:154-8.
7. World Health Organization. The world traditional medicines situation. In: Traditional medicines: global situation, issues and challenges. Geneva; 2011. p. 1-14.
8. De Luca V, Salim V, Atsumi SM, Yu F. Mining the biodiversity of plants: a revolution in the making. *Science*. 2012;336(6089):1658-61.
9. Volodya Ts. Methods of using medicinal plants in clinical practice. Ulaanbaatar: Bitpress; 2021. p. 340, 371.
10. Santos MS, Ferreira F, Cunha AP, Carvalho AP, Macedo T. An aqueous extract of valerian influences the transport of GABA in synaptosomes. *Planta Med*. 1994;60(3):278-9.
11. Patočka J, Jakl J. Biomedically relevant chemical constituents of *Valeriana officinalis*. *J Appl Biomed*. 2010;8(1):11-8.
12. Malva JO, Santos S, Macedo T. Neuroprotective properties of *Valeriana officinalis* extracts. *Neurotox Res*. 2004;6(2):131-40.
13. Gonulalan EM, Bayazeid O, Yalcin FN, Demirezer LO. The roles of valerenic acid on BDNF expression in the SH-SY5Y cell. *Saudi Pharm J*. 2018;26(7):960-4.
14. Torres-Hernández BA, Del Valle-Mojica LM, Ortiz JG. Valerenic acid and *Valeriana officinalis* extracts delay onset of Pentylenetetrazole (PTZ)-Induced seizures in adult Danio rerio (Zebrafish). *BMC Complement Altern Med*. 2015;15:228.
15. Jung HY, Yoo DY, Nam SM, Kim JW, Choi JH, Yoo M, et al. Valerenic acid protects against physical and psychological stress by reducing the turnover of serotonin and norepinephrine in mouse hippocampus-amygdala region. *J Med Food*. 2015;18(12):1333-9.
16. Chen HW, He XH, Yuan R, Wei BJ, Chen Z, Dong JX, et al. Sesquiterpenes and a monoterpenoid with acetylcholinesterase (AChE) inhibitory activity from *Valeriana officinalis* var. *latifolia* in vitro and in vivo. *Fitoterapia*. 2016;110:142-9.
17. Rodríguez-Cruz A, Romo-Mancillas A, Mendiola-Precoma J, Escobar-Cabrera JE, García-Alcocer G, Berumen LC. Effect of valerenic acid on neuroinflammation in a MPTP-induced mouse model of Parkinson's disease. *IBRO Rep*. 2019;8:28-35.
18. Zare A, Khaksar Z, Sobhani Z, Amini M. Analgesic effect of valerian root and turnip extracts. *World J Plast Surg*. 2018;7(3):345-50.
19. Taherianfard M, Karamifard M. Evaluation of the GABAA receptor on pain sensitivity in male rat pretreated with *Valeriana officinalis* extract using formalin test. *Physiol Pharmacol* 2018;22:118-23.
20. Caudal D, Guinobert I, Lafoux A, Bardot V, Cotte C, Ripoché I, et al. Skeletal muscle relaxant effect of a standardized extract of *Valeriana officinalis* L. after acute administration in mice. *J Tradit Complement Med*. 2017;8(2):335-40.
21. Li Y, Liu YR, Xiao BK, Yang JY, Huang RQ. Dynamic comparison of free radical scavenging abilities of *Hypericum perforatum* L., *Herba Verbenae Officinalis*, and *Valeriana Officinalis* L. Extracts. *Pharmacogn J*. 2015;7:198-204.

22. Chua HP, Aminah A. Determination of antioxidant activities of dried kacangma (*Leonurus sibiricus*) extract in three bioassay systems. *J Trop Agric Food Sci.* 2013;41(2):221-9.
23. Zhang RH, Liu ZK, Yang DS, Zhang XJ, Sun HD, Xiao WL. Phytochemistry and pharmacology of the genus *Leonurus*: the herb to benefit the mothers and more. *Phytochemistry* 2018;147:167-83.
24. World Health Organization. *Medicinal plants in Mongolia.* 2013:107-9.
25. Chultemsuren M. Phytochemical and pharmacological study of some species of *Leonurus* (*Leonurus* L.) growing in the Mongolian People's Republic. Doctoral dissertation of Medical Sciences. Ulaanbaatar; 1973.
26. Rauwald HW, Savtschenko A, Merten A, Rusch C, Appel K, Kuchta K. GABA-A Receptor binding assays of standardized *Leonurus cardiaca* and *Leonurus japonicus* extracts as well as their isolated constituents. *Planta Med.* 2015;81(12-13):1103-10.
27. Shin HY, Kim SH, Kang SM, Chang IJ, Kim SY, Jeon H, et al. Anti-inflammatory activity of motherwort (*Leonurus sibiricus* L.). *Immunopharmacol Immunotoxicol.* 2009;31(2):209-13.
28. Song X, Wang T, Zhang Z, Jiang H, Wang W, Cao Y, et al. Leonurine exerts anti-inflammatory effect by regulating inflammatory signaling pathways and cytokines in LPS-induced mouse mastitis. *Inflammation.* 2015;38(1):79-88.
29. Sitarek P, Skala E, Wysokińska H, Wielanek M, Szemraj J, Toma M, et al. The effect of *Leonurus sibiricus* Plant extracts on stimulating repair and protective activity against oxidative DNA damage in CHO cells and content of phenolic compounds. *Oxid Med Cell Longev.* 2016;2016:5738193.
30. Schmidt S, Jakab M, Jav S, Streif D, Pitschmann A, Zehl M, et al. Extracts from *Leonurus sibiricus* L. increase insulin secretion and proliferation of rat INS-1E insulinoma cells. *J Ethnopharmacol.* 2013;150(1):85-94.
31. Li X, Liu R, Zhang W, Wang B, Ma R, Chi X, et al. Up-regulation of Fas/FASL activation contribute to the apoptosis enhancement of RU486 by Gong-Qing Decoction, a traditional Chinese prescription. *J Ethnopharmacol.* 2011;134(2):386-92.
32. Hang-Ching L. Antiplatelet effect of leonurine from *Leonurus sibiricus*. *Chin Pharm J.* 2007;59:149-52.
33. Lee MJ, Lee HS, Park SD, Moon HI, Park WH. *Leonurus sibiricus* herb extract suppresses oxidative stress and ameliorates hypercholesterolemia in C57BL/6 mice and TNF-alpha induced expression of adhesion molecules and lectin-like oxidized LDL receptor-1 in human umbilical vein endothelial cells. *Biosci Biotechnol Biochem.* 2010;74(2):279-84.
34. Wu H, Fronczek FR, Ferreira D, Burandt CL, Zjawiony JK. Labdane diterpenoids from *Leonurus sibiricus*. *J Nat Prod.* 2011;74(4):831-6.
35. Sayed MA, Alam MA, Islam MS, Ali MT, Ullah ME, Shibly AZ, et al. *Leonurus sibiricus* L. (honeysuckle): a review of its phytochemistry and pharmacology. *Asian Pac J Trop Biomed.* 2016;6(12):1076-80.
36. Treiman DM. GABAergic mechanisms in epilepsy. *Epilepsia* 2001;42(3)(Suppl 3):8-12.
37. Nuss P. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. *Neuropsychiatr Dis Treat* 2015;11:165-75.
38. Plante DT, Jensen JE, Winkelman JW. The role of GABA in primary insomnia. *Sleep.* 2012;35(6):741-2.
39. Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol.* 1983;54(4):275-87.
40. Organization for Economic Co-operation and Development. Guideline for testing of chemicals Test No. 407: repeated dose 28-day oral toxicity study in rodents. Paris: OECD Publishing; 2008.
41. ChangSheng L, Tserendash C, Gerelt-Od ME, Batbayar B, Altankhuyag U, Batbaatar MA, et al. Acute and subchronic toxicity study of Sampilnorov Wwure. *Pharmacogn J.* 2025;17(2):203-10.
42. Wang YQ, Zhu CY, Wei EQ. A new model for evaluating locomotor activity and central depressive drugs in different strain mice. *Bull Sci Technol.* 2003;19:292-6.
43. Turner RA. Screening methods in pharmacology. New York: Academic Press; 1965. p. 64-9.
44. Swinyard EA, Kupferberg HJ. Antiepileptic drugs: detection, quantification, and evaluation. *Fed Proc.* 1985;44(10):2629-33.
45. OECD Test No. 423: acute Oral toxicity—Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing; 2002.
46. Vaghasiya YK, Shukla VJ, Chanda SV. Acute oral toxicity study of *Pluchea arguta boiss* extract in mice. *J Pharmacol Toxicol.* 2011;6:113-23.
47. Dybing E, Doe J, Groten J, Kleiner J, Brien JO, Renwick AG. Hazard characterization of chemicals in food and diet: dose response, mechanism and extrapolation issues. *Food Chem Toxicol* 2002;42:237-82.
48. Belovicova K, Bogi E, Csatosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. *Interdiscip Toxicol.* 2017;10(1):40-3.
49. McIntyre TD, Alpern HP. Thiopental, phenobarbital, and chlordiazepoxide induce the same differences in narcotic reaction as ethanol in long-sleep and short-sleep selectively-bred mice. *Pharmacol Biochem Behav.* 1986;24(4):895-8.
50. Feather-Schussler DN, Ferguson TS. A battery of motor tests in a neonatal mouse model of cerebral palsy. *J Vis Exp.* 2016;117:53569.
51. Kandravicius L, Balista PA, Lopes-Aguiar C, Ruggiero RN, Umeoka EH, Garcia-Cairasco N, et al. Animal models of epilepsy: use and limitations. *Neuropsychiatr Dis Treat* 2014;10:1693-705.
52. Odontuya G. Secondary metabolites of some medicinal plants of Mongolia: structure and scientific basis for application [dissertation]. Ulaanbaatar; 2021.
53. Brower V. Back to nature: extinction of medicinal plants threatens drug discovery. *J Natl Cancer Inst.* 2008;100(12):838-9.
54. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod.* 2012;75(3):311-35.
55. Mongolia drug product registration. Ulaanbaatar; 2018.
56. Al-Attaqchi OHA, Deb PK, Al-Attaqchi NHA. Review of the phytochemistry and pharmacological properties of *Valeriana officinalis*. *Curr Tradit Med.* 2020;6(4):260-77.
57. Lamjav Ts, Dorjantsan D, Tserenbaljir D. Medicinal plants of Mongolia. Ulaanbaatar; 1971. p. 209-10.
58. Rosecrans JA, Defeo JJ, Youngken HW. Pharmacological investigation of certain *Valeriana officinalis* L. extracts. *J Pharm Sci.* 1961;50:240-4.
59. Von Skramlik EV. On the toxicity and tolerance of ethereal oils. *Pharmazie.* 1959;14:435-45.
60. Willey LB, Mady SP, Cobaugh DJ, Wax PM. Valerian overdose: a case report. *Vet Hum Toxicol.* 1995;37(4):364-5.
61. Chua HP, Murugaiyah M, Aminah A. Effects of kacangma herb (*Leonurus sibiricus*) intake on blood chemistry, clinical pathology, body and organ weight changes in rabbit. *J Trop Agric Food Sci.* 2007;35(2):221-30.
62. De Almeida ER, Xavier HS, da Silva AR, Chaves TM, Aragão-Neto AC. Evaluation potential of valerian roots extract and valepotriates on behavioral tests in mice. *Journal Pharm.* 2011;2(5):164-70.

63. Neamati A, Chaman F, Hosseini M, Boskabady MH. The effects of *Valeriana officinalis* L. hydro-alcoholic extract on depression like behavior in ovalbumin sensitized rats. J Pharm Bioallied Sci. 2014;6(2):97-103.
64. Koshovyi O, Raal A, Kireyev I, Tryshchuk N, Ilina T, Romanenko Y, et al. Phytochemical and psychotropic research of motherwort (*Leonurus cardiaca* L.) modified dry extracts. Plants (Basel). 2021;10(2):230.
65. Moniruzzaman M, Atikur Rahman M, Ferdous A. Evaluation of sedative and hypnotic activity of ethanolic extract of *Scoparia dulcis* linn. Evid Based Complement Alternat Med. 2015;2015:873954.
66. Chow NK, Fretz M, Hamburger M, Butterweck V. Telemetry as a tool to measure sedative effects of a valerian root extract and its single constituents in mice. Planta Med. 2011;77(8):795-803.
67. Hendriks H, Bos R, Allersma DP, Malingré TM, Koster AS. Pharmacological screening of valeranal and some other components of essential oil of *Valeriana officinalis*. Planta Med. 1981;42(1):62-8.
68. Hiller KO, Zetler G. Neuropharmacological studies on ethanol extracts of *Valeriana officinalis* L.: behavioural and anticonvulsant properties. Phytother Res. 1996;10(2):145-51.
69. Ahmed F, Tslam MA, Choudhuri MK. Central nervous system depressant activity of *Leonurus sibiricus*. Niger J Nat Prod Med. 2005;9:35-7.
70. Chultemsuren M, Jamyandagva S. Effect of a combined preparation of *Leonurus* and *Valeriana* on chloral hydrate-induced sleep. Sci Rep Inst Nat Comp.d 1985;7:57.
71. Watanabe M, Miyai A, Danjo S, Nakamura Y, Itoh K. The threshold of pentylenetetrazole-induced convulsive seizures, but not that of nonconvulsive seizures, is controlled by the nitric oxide levels in murine brains. Exp Neurol. 2013;247:645-52.
72. Nouri MH, Abad AN. Gabaergic system role in aqueous extract of *Valeriana officinalis* L. root on PTZ-induced clonic seizure threshold in mice. Afr J Pharm Pharmacol. 2011;5(9):1212-7.
73. Rezvani ME, Roohbakhsh A, Allahtavakoli M, Shamsizadeh A. Anticonvulsant effect of aqueous extract of *Valeriana officinalis* in amygdala-kindled rats: possible involvement of adenosine. J Ethnopharmacol. 2010;127(2):313-8.
74. Bienvenu E, Amabeoku GJ, Eagles PK, Scott G, Springfield EP. Anticonvulsant activity of aqueous extract of *Leonotis Leonurus*. Phytomedicine. 2002;9(3):217-23.
75. Sieghart W. Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. Pharmacol Rev. 1995;47(2):181-234.
76. Trauner G, Khom S, Baburin I, Benedek B, Hering S, Kopp B. Modulation of GABA-A receptors by valerian extracts is related to the content of valerenic acid. Planta Med. 2008;74(1):19-24.
77. Khom S, Baburin I, Timin E, Hohaus A, Trauner G, Kopp B, et al. Valerenic acid potentiates and inhibits GABA(A) receptors: molecular mechanism and subunit specificity. Neuropharmacology. 2007;53(1):178-87.
78. Benke D, Barberis A, Kopp S, Altmann KH, Schubiger M, Vogt KE, et al. GABA A receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. Neuropharmacology. 2009;56(1):174-81.
79. Mulyawan E, Ahmad MR, Islam AA, Massi MN, Hatta M, Arif SK. Analysis of GABRB3 Protein Level after Administration of valerian Extract (*Valeriana officinalis*) in BALB/c mice. Pharmacogn J. 2020;12(4):821-7.
80. Wu A, Ye X, Huang Q, Dai WM, Zhang JM. Anti-epileptic Effects of Valepotriate Isolated from *Valeriana jatamansi Jones* and Its Possible Mechanisms. Pharmacogn Mag. 2017;13(51):512-6.
81. Park JY, Lee Y, Lee HJ, Kwon YS, Chun W. In silico screening of GABA aminotransferase inhibitors from the constituents of *Valeriana officinalis* by molecular docking and molecular dynamics simulation study. J Mol Model. 2020;26(9):228.
82. Müller CE, Schumacher B, Brattström A, Abourashed EA, Koetter U. Interactions of valerian extracts and a fixed valerian-hop extract combination with adenosine receptors. Life Sci. 2002;71(16):1939-49.
83. Dietz BM, Mahady GB, Pauli GF, Farnsworth NR. Valerian extract and valerenic acid are partial agonists of the 5-HT5a receptor in vitro. Brain Res Mol Brain Res. 2005;138(2):191-7.
84. Fernández S, Wasowski C, Paladini AC, Marder M. Sedative and sleep enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*. Pharmacol Biochem Behav. 2004;77:399-404.
85. Marder M, Viola H, Wasowski C, Fernández S, Medina JH, Paladini AC. 6-methylapigenin and hesperidin: new valeriana flavonoids with activity on the CNS. Pharmacol Biochem Behav. 2003;75:537-45.
86. Wasowski C, Marder M, Viola H, Medina JH, Paladini AC. Isolation and identification of 6-methylapigenin, a competitive ligand for the brain GABA(A) receptors from *Valeriana wallichii*. Planta Med. 2002;68:934-6.
87. Zachow LL, Ávila JM, Saldanha GA, Mostardeiro MA, da Silva UF, Morel AF, et al. Chemical composition and evaluation of prolyl oligopeptidase and acetylcholinesterase inhibitory activities of *Leonurus sibiricus* L. from Brazil. Nat Prod Res. 2017;31(12):1459-63.

Cite this article: Yeruult Ch, Bilegdemberel Kh, Chimgee Ts, Maral-Erdene G, Batdavaa B, Mend-Amar B, Perleidulam B, Enerel Ye, Narankhajid M, Li C, Purevjal O, Enkhmaa G. Neuropharmacological Potential of a Combined Herbal Preparation of *Valeriana officinalis* L. and *Leonurus sibiricus* L.. Pharmacogn J. 2026;18 (3): 252-264.