SEDATIVE EFFECTS OF THE NATURALLY DERIVED PRODUCT NSDVT IN EXPERIMENTAL ANIMALS

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Sleep disturbance is a prominent and debilitating feature of chronic fatigue syndrome, underscoring the need for safe sedative agents. The present study investigated the sedative effects of NSDVT using standardized experimental models in mice. NSDVT was administered orally at 2.5 and 5 g/kg, with diazepam serving as a positive control. Spontaneous locomotor activity, Rotarod test, and hexobarbital-induced sleeping time were assessed. NSDVT significantly reduced both horizontal and vertical locomotor activity compared with baseline and control values. Diazepam produced a more pronounced reduction at 1 hour post-administration, whereas NSDVT, particularly at 5 g/kg, exhibited a stronger effect at 3 hours, indicating a delayed but sustained sedative action. In the rotarod test, both doses of NSDVT and diazepam significantly decreased the latency to fall. Similarly, diazepam exerted a greater effect at 1 hour, while NSDVT at 5 g/kg showed a more marked reduction at 3 hours. In the hexobarbital-induced sleep test, NSDVT did not affect sleep latency but significantly prolonged sleeping time to 113.45% and 150.83% of control values, with statistical significance observed at the higher dose. These findings demonstrate that NSDVT produces a clear sedative effect characterized by central nervous system depression with a delayed onset.

Keywords: NSDVT, sedative, experimental animals, mice.

I. INTRODUCTION

Chronic fatigue syndrome (CFS), also known as myalgic encephalomyelitis, is a debilitating condition characterized by persistent, unexplained fatigue lasting at least six months, accompanied by symptoms such as post-exertional malaise, sleep disturbance, cognitive impairment, and musculoskeletal pain, which significantly affect patient functioning and quality of life.[1] Chronic fatigue syndrome is observed globally across diverse populations, with prevalence estimates varying according to diagnostic criteria but generally ranging from approximately 0.2 % to 0.7 % in community-based studies and up to approximately 0.68 % in meta-analytic estimates. The condition affects both genders and a wide age range, although many studies report a higher prevalence among females than males.[2]

Despite decades of research, the etiology and pathophysiology of CFS remain incompletely understood. This is reflected in systematic reviews that highlight the complex, multifactorial nature of the syndrome. Current evidence suggests that CFS is a multifactorial disorder involving complex interactions among immunological dysfunction, neuroendocrine imbalance, and heightened oxidative stress, rather than a single identifiable cause.[3]

Among the diverse clinical manifestations

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of CFS, sleep disturbances represent one of the most prevalent and debilitating symptoms, commonly presenting as non-restorative sleep, difficulty initiating or maintaining sleep, and altered sleep-wake rhythms. Accumulating evidence indicates that disordered sleep not only exacerbates fatigue severity but also contributes to cognitive dysfunction, mood disturbances, and reduced stress tolerance in patients with CFS.[4] Consequently, improving sleep quality and restoring normal sleep architecture have been increasingly recognized as important therapeutic targets in the comprehensive management of the syndrome.

Current medical management of CFS largely focuses on symptom-oriented and supportive strategies rather than curative interventions. Systematic evidence indicates that non-pharmacologic approaches such as graded exercise and cognitive behavioral therapy are widely used, but their efficacy for sleep outcomes specifically has been inconsistent across studies.[4] Pharmacological treatments targeting sleep disorders, such as hypnotics, sedatives, and antidepressants, may provide short-term symptomatic relief; however, their long-term efficacy is limited, and they are often associated with potential adverse effects, particularly with prolonged use.[3] These limitations underscore the need for safer and more effective interventions targeting sleep dysregulation in CFS.

Alongside conventional medical approaches, traditional herbal medicines have long been used as complementary or alternative therapies for fatigue-related disorders and sleep disturbances. Meta-analyses of traditional Chinese herbal medicines in CFS suggest potential benefits on symptom scores, although quality remains variable.[5]

NSDVT is a natural-based multi-herbal product originating from the Song Dynasty, China, consisting of fourteen medicinal ingredients: *Radix Ginseng, Poria cocos, Rhizoma Atractylodis macrocephalae, Radix Glycyrrhizae, Radix Angelicae sinensis, Radix Rehmanniae glutinosae praeparata, Radix Paeoniae lactiflorae, Radix Astragalis membranacei, Cortex Cinnamomi, Radix Polygalae, Pericarpium Citri reticulatae perenne, Fructus Schisandrae, Fructus Ziziphi jujubae,* and *Rhizoma Zingiberis*. This formula has long been used to tonify qi and blood, nourish the heart, and exert sedative effects[6]-therapeutic actions that correspond closely with symptoms such as insomnia, mental fatigue, emotional instability, and poor sleep quality observed in patients with CFS.

Although several individual constituents of NSDVT, such as *Radix Polygalae*, *Fructus Ziziphi jujubae*, and *Fructus Schisandrae*, have been reported to exhibit sedative, hypnotic, or neuroregulatory effects in experimental studies, suggesting potential mechanisms worthy of further investigation, the sedative and sleep-modulating effects of the combined NSDVT formula have not yet been systematically evaluated.[7,8] Moreover, because multi-herbal formulations may produce synergistic effects or interactions that differ from those of individual components, inferring the pharmacological properties of the entire formula solely from studies on single herbs is insufficient. Therefore, the present study was designed to evaluate the sedative effects of NSDVT using standardized experimental models, with the aim of providing scientific evidence to support its traditional use and to clarify its potential role in the management of sleep disturbances associated with chronic fatigue-related conditions.

II. MATERIALS AND METHODS

1. Subject

The preparation of NSDVT

For experimental purposes, NSDVT was prepared in the form of a liquid extract with an extraction ratio of 2:1, equivalent to 2 g of raw herbal materials per 1 mL of extract, comprising the following ingredients: 10,3mg *Radix Ginseng*, 77,3mg *Poria cocos*, 103mg *Rhizoma Atractylodis Macrocephalae,* 103mg *Radix Glycyrrhizae,* 103mg *Radix Angelicae Sinensis,* 77,3mg *Radix Rehmanniae Praeparata,* 103mg *Radix Paeoniae Lactiflorae,* 103mg *Radix Astragali Membranacei, 103mg Cortex Cinnamomi, 51,5mg Radix Polygalae, 103mg Pericarpium Citri Reticulatae, 77,3mg Fructus Schisandrae,* 154,6mg *Fructus Ziziphi Jujubae,* 51,5mg *Rhizoma Zingiberis.*

Experimental animals

Albino *Swiss* mice of both genders, with an average body weight 20 ± 2 g, were supplied by the National Institute of Hygiene and Epidemiology. The animals were acclimatized for 3–7 days before the experiment and maintained throughout the study under standard laboratory conditions, with free access to food and water, at the Department of Pharmacology, Hanoi Medical University. All animal experiments were conducted in accordance with the Vietnamese regulatory guideline issued under Decision No. 141/QĐ-K2ĐT (2015) by the Administration of Science Technology and Training.[9]

Drugs and chemicals

Diazepam (Seduxen®, Gedeon Richter, 5 mg/tablet) was used as the positive control drug. Hexobarbital (Sigma-Aldrich, 1mg/mL) was used as a sleep-inducing agent in the hexobarbital-induced sleep model. Sedative effects were evaluated using an activity cage (Model 1431, Ugo Basile, Italy) and a rotarod apparatus (Ugo Basile, Italy).

2. Methods

Spontaneous locomotor activity

The activity cage 1431 system (Ugo Basile, Italy) measures horizontal and vertical activity in mice using infrared beams. Movements are detected unobtrusively and automatically logged by the stand-alone electronic controller. This apparatus was used to evaluate the effects of NSDVT on spontaneous locomotor activity in mice.[10] Healthy albino *Swiss* mice of both sexes were randomly divided into four groups (n = 10 per group) and administered orally once daily for five consecutive days at 8:00 a.m. Group 1 received 0.9% physiological saline (control), group 2 received diazepam at 2.4 mg/kg (positive control, equivalent to the human therapeutic dose); and groups 3 and 4 received NSDVT at 2.5 g/kg and 5 g/kg, respectively.

Locomotor activity was recorded at two experimental time points: before treatment initiation (T_0) and after five days of administration (T_1). On day 5, locomotor activity was further recorded at 1 hour and 3 hours after oral dosing. The sedative effect of the test substance was evaluated by comparing the mean locomotor activity counts among the experimental groups.

Rotarod test

The rotarod test was conducted with minor modifications based on a previously described method.[10] Albino Swiss mice of both sexes were allocated into experimental groups and orally administered treatments in the same manner as described in the spontaneous locomotor activity model.

The assessments were performed at two main time points: before treatment initiation (T_0) and after five consecutive days of treatment (T_1). On the fifth day, measurements were taken at two post-administration intervals, namely 1 hour and 3 hours after dosing. At each time point, motor coordination and balance were evaluated by measuring the latency to fall from the rotating rod. Mice were first placed individually on the compartments of the rotarod apparatus and allowed to acclimate at a low

rotational speed of 3-5 revolutions per minute for 2 minutes. The apparatus was then set to an accelerating mode until a constant speed of 35 revolutions per minute was reached, after which the speed was maintained at this fixed level.

The latency to fall was recorded as the time elapsed from the moment the mouse was placed on the rod rotating at the constant speed of 35 revolutions per minute until it fell off the apparatus. The sedative effect of the test substance was assessed by comparing the mean latency to fall among the experimental groups.

Hexobarbital sleep models in mice

The effect of NSDVT on hexobarbital-induced sleep was evaluated according to a previously described method with minor modifications.[11] Albino *Swiss* mice of both sexes were randomly assigned to three groups (n = 10 per group). The control group received 0.9% sodium chloride solution, while the treatment groups received NSDVT at 2.5 g/kg and 5 g/kg, respectively. All animals were administered the test substances orally once daily at 8:00 a.m. for five consecutive days. On the fifth day, 60 minutes after the final oral administration, hexobarbital was administered intraperitoneally at 100 mg/kg (0.1 mL/10 g body weight). Following hexobarbital injection, each mouse was placed individually in a thermostatically controlled chamber maintained at 37°C. Sleep latency and sleep duration were recorded for each animal. Sleep latency was defined as the time interval from hexobarbital administration to the loss of the righting reflex, whereas sleep duration was defined as the time from the loss of the righting reflex until the spontaneous recovery of the righting reflex.[12]

Statistical analysis

The data were expressed as the mean ± standard deviation (SD), and statistical analysis was carried out employing Student's T-test. Both analyses were performed using SPSS 25.0. The p-value < 0.05 indicates statistical significance.

III. RESULTS

1. Effect of NSDVT on spontaneous locomotor activity

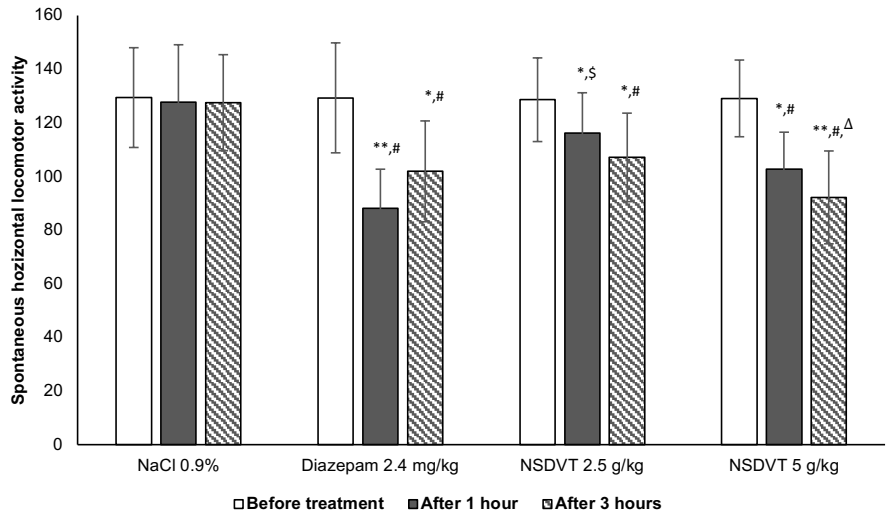

Figure 1. Effect of NSDVT on spontaneous horizontal locomotor activity

**p<0.05; **p<0.01 compared to before treatment; #p<0.05 compared to NaCl 0.9% group; $p<0.05 compared to diazepam group; Δp<0.05 compared to NSDVT 2.5 g/kg group.*

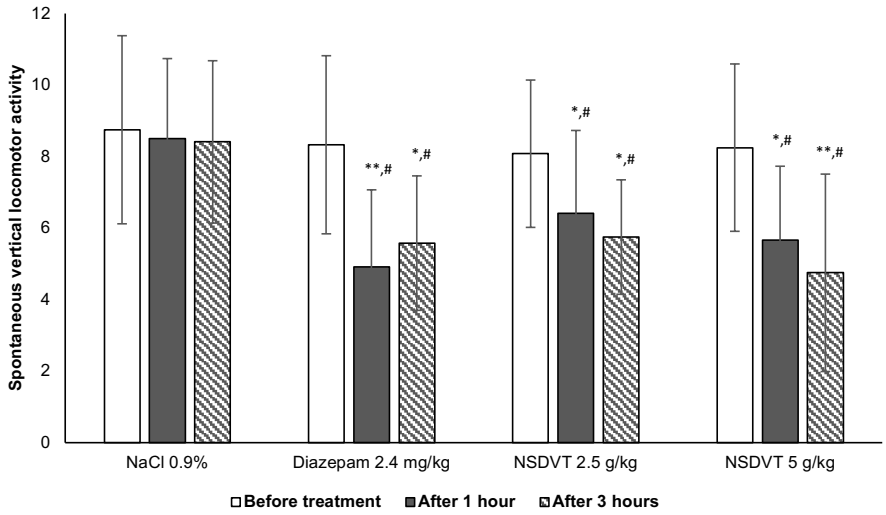

Figure 2. Effect of NSDVT on spontaneous vertical locomotor activity

**p<0.05; **p<0.01 compared to before treatment; #p<0.05 compared to NaCl 0.9% group.*

As shown in Figures 1 and 2, both doses of NSDVT (2.5 and 5 g/kg) and diazepam significantly reduced horizontal and vertical locomotor activity compared with baseline values and the control group ($p < 0.05$ or $p < 0.01$). At 1 hour after oral administration, diazepam produced a more pronounced reduction in locomotor activity, particularly horizontal movement, than both NSDVT-treated groups, with a statistically significant difference compared with the NSDVT 2.5 g/kg group ($p < 0.05$). In contrast, at 3 hours, the greatest reduction in horizontal activity was observed in the NSDVT 5 g/kg group, which was significantly greater than that in the NSDVT 2.5 g/kg group ($p < 0.05$) and comparable to diazepam. For vertical activity, diazepam at 1 hour and NSDVT at 5 g/kg at 3 hours showed only nonsignificant decreasing trends compared with the other treatment groups.

2. Effect of NSDVT on latency to fall in the Rotarod test

Table 1. Effect of NSDVT on latency to fall in the Rotarod test

Group	n	Latency to fall (s)		
		Before treatment	After treatment	
			1 hour	3 hours
NaCl 0.9%	10	130.64 ± 22.31	131.18 ± 19.05 **-0.41%**(1)	130.18 ± 18.51 **0.35%**(1)
Diazepam 2.4 mg/kg	10	131.15 ± 18.51	83.39 ± 20.86 **36.41%****#(1)	92.85 ± 16.83 **29.20%***#(1)
NSDVT 2.5 g/kg	10	131.46 ± 16.56	122.15 ± 18.53 **7.08%***$(1)	106.08 ± 21.75 **19.31%***#$+(1)

Group	n	Latency to fall (s)		
		Before treatment	After treatment	
			1 hour	3 hours
NSDVT 5 g/kg	10	131.09 ± 19.85	110.82 ± 18.84 **15.46%**$^{*\#(1)}$	89.64 ± 12.07 **31.62%**$^{**\#+(1)}$

**p<0.05; **p<0.01 compared to before treatment; $^{\#}$p<0.05 compared to NaCl 0.9% group; $^{\$}$p<0.05 compared to diazepam group; $^{\Delta}$p<0.05 compared to NSDVT 2.5 g/kg group; $^{+}$p<0.05 compared to after 1 hour; (1) percentage decrease in latency to fall compared with before treatment.*

As shown in Table 1, both doses of NSDVT (2.5 and 5 g/kg) and diazepam significantly reduced the latency to fall on the rotarod compared with baseline and the control group ($p < 0.05$). Significant differences were also observed between the two NSDVT-treated groups at the assessed time points ($p < 0.05$). At 1 hour after oral administration, diazepam produced a greater reduction in latency to fall than either NSDVT dose ($p < 0.05$). In contrast, at 3 hours, the NSDVT 5 g/kg group exhibited a more pronounced decrease in latency to fall than the NSDVT 2.5 g/kg group ($p < 0.05$), while showing only a non-significant decreasing trend compared with the diazepam group.

3. Effects of NSDVT on hexobarbital sleep models in mice

Table 2. Effects of NSDVT on hexobarbital sleep models in mice

Group	n	Sleep latency (mins)	Sleep duration (mins)	% of control (mean value)
NaCl 0.9%	10	2.58 ± 1.28	36.65 ± 13.44	
NSDVT 2.5 g/kg	10	2.46 ± 0.88	41.58 ± 6.94	113.45
NSDVT 5 g/kg	10	2.27 ± 0.58	55.28 ± 3.72$^{\#}$	150.83

$^{\#}$p<0.05 compared to NaCl 0.9% group.

As shown in Table 2, NSDVT at 2.5 g/kg and 5 g/kg did not significantly affect sleep latency ($p > 0.05$), but significantly prolonged hexobarbital-induced sleeping time to 113.45% and 150.83% of the control value, respectively. The increase observed in the NSDVT 5 g/kg group was statistically significant compared with the control group ($p < 0.05$).

IV. DISCUSSION

Sleep disturbances are a core and highly prevalent feature of chronic fatigue syndrome (CFS) and are considered an important contributor to symptom persistence and disease burden.[13] Although not all studies have consistently identified specific sleep architecture abnormalities, the preponderance of evidence indicates that dysregulated sleep physiology is implicated in the pathophysiology of CFS rather than merely a secondary complaint.[14] In this context, experimental evaluation of sedative and sleep-modulating interventions is of particular relevance for identifying agents.

The present study provides experimental

evidence supporting the sedative effects of NSDVT through a combination of behavioral and pharmacological models commonly used to evaluate central nervous system (CNS) depressant activity. By integrating spontaneous locomotor activity assessment, rotarod performance, and hexobarbital-induced sleeping time, the study offers a multidimensional evaluation of the sedative profile of NSDVT.

Effects on spontaneous locomotor activity

Normal mice exhibit by spontaneous walking and jumping behaviors, which primarily reflect horizontal locomotion and vertical rearing; therefore, assessment of both parameters is essential for accurately evaluating changes in central nervous system (CNS) activity. Spontaneous locomotor activity is a widely accepted preliminary screening model for assessing CNS stimulation or inhibition.[15] In this study, an automated activity cage was employed to quantify spontaneous locomotor activity. In modern preclinical pharmacological research, automated activity cage systems using infrared beam breaks or similar sensors allow objective, high-throughput, and continuous measurement of both ambulatory (horizontal) and rearing (vertical) activity in rodents without manual scoring, thereby reducing observer bias and error.[10] Automated systems can collect detailed locomotor data at frequent sampling intervals and for extended recording periods that are impractical for manual observation, enhancing the sensitivity and reproducibility of activity measurements. Importantly, automated activity monitoring reduces environmental stress on animals by minimizing human intervention and allows detection of subtle changes across different movement parameters that may not be captured by traditional open-field observations alone.

In the present study, oral administration of NSDVT at 2.5 g/kg and 5 g/kg produced significant reductions in both ambulatory and rearing activities at 1 hour and 3 hours post-dosing compared with baseline and the control group ($p < 0.05$). Reductions in spontaneous locomotor activity have been widely used as an indicator of central nervous system (CNS) depressant effects in rodent models, where known sedative agents reliably decrease locomotion and exploratory behavior. Diazepam served as a standard benzodiazepine positive control for sedative effects. Benzodiazepines such as diazepam are established positive controls in locomotor assays because they produce quantifiable decreases in rodent activity due to CNS depressant action.[16] These findings indicate that NSDVT exerts a pronounced inhibitory effect on spontaneous motor behavior consistent with central sedation. The use of spontaneous locomotor activity as a primary behavioral endpoint allows for sensitive detection of CNS inhibitory effects comparable to those produced by known sedative drugs.

Notably, a distinct time course of effect was observed between NSDVT and diazepam. *Diazepam induced a more marked reduction in locomotor activity within 1 hour after administration.* Diazepam has an oral dosing onset of 15-60 minutes, and peak plasma levels occur within 1-1.5 hours, consistent with locomotor effects emerging within 1 hour post-dose.[17] As a benzodiazepine, diazepam enhances GABA-A receptor-mediated inhibitory neurotransmission, which mediates its sedative and locomotor-suppressive actions.[18]

In contrast, NSDVT exhibited a more pronounced suppressive effect on locomotor activity at 3 hours post-administration, especially at the higher dose, although this difference did not reach statistical significance

compared with the diazepam group. This delayed onset suggests that NSDVT may exert its sedative action via slower pharmacokinetic processes, modulation of neuromodulators, or indirect regulatory mechanisms rather than direct receptor potentiation-a pattern consistent with the complex composition and multi-target nature of traditional herbal formulations.

Effects on the Rotarod test

The mouse rotarod test of motor coordination and sedation is widely used to predict clinically relevant sedation and motor impairment induced by central nervous system depressant drugs. Drugs with sedative and/or muscle-relaxant properties, such as benzodiazepines, reduce the latency to fall by impairing motor coordination and reflexes.[19] In this study, NSDVT significantly decreased the rotarod retention time at both tested doses, indicating an effect on motor performance consistent with CNS depression. Similar to the locomotor activity findings, diazepam produced a stronger effect at the early time point, whereas NSDVT demonstrated a more pronounced effect at later time points, particularly at the higher dose. This pattern further supports the notion that NSDVT exerts a gradual but sustained CNS inhibitory effect rather than an acute sedative action. Importantly, the reduction in rotarod performance occurred in parallel with decreased spontaneous activity, suggesting that the observed effects are attributable to central sedation rather than nonspecific motor toxicity.

Effects on hexobarbital sleep models in mice

To further confirm the CNS inhibitory effects of NSDVT, we investigated its influence on hexobarbital-induced sleep. Hexobarbital and other barbiturates exert central nervous system depressant effects primarily by enhancing inhibitory neurotransmission at GABA-A receptors.[20] In addition, hexobarbital is predominantly metabolized in the liver via cytochrome P450-mediated pathways, and it has been well documented that enhancement of its sedative effect is closely associated with reduced metabolic clearance.[21] Consequently, inhibition of hepatic metabolic enzymes can lead to a prolongation of hexobarbital-induced sleeping time.

In this study, NSDVT significantly prolonged hexobarbital-induced sleep duration without markedly altering sleep latency. This suggests that rather than acting as a primary hypnotic to initiate sleep, NSDVT may sustain CNS inhibitory activity once sedation is established. Such effects support its classification as a sedative-type CNS depressant rather than a direct hypnotic agent. This prolongation likely stems from a synergistic interaction within the central nervous system and/or the modulation of hepatic metabolic enzymes. Nevertheless, further pharmacokinetic and mechanistic investigations are required to determine the precise contribution of direct CNS interactions versus hepatic enzyme inhibition.

The sedative effects of NSDVT may be attributed, at least in part, to the combined actions of its constituent herbs, several of which have been reported to modulate CNS activity in previous experimental studies. For example, *Radix Polygalae*, a classic herbal pair within the formula, have demonstrated sedative-hypnotic effects in animal models, significantly shortening sleep latency and prolonging pentobarbital-induced sleeping time via modulation of neurotransmitter systems.[7] *Fructus Schisandrae* has also been reported to potentiate barbiturate-induced sleep and improve sleep architecture in rodents, supporting its traditional use for insomnia.[8]

Importantly, multi-herbal formulations, such as NSDVT, often exert synergistic or complementary effects that are not predictable solely from individual components. Interactions among diverse bioactive compounds may enhance CNS inhibitory effects, extend the duration of action, or mitigate adverse effects typically associated with single-agent sedatives. This complex interaction may also explain why certain individual herbs have been reported to reduce sleep latency, whereas the combined NSDVT formula in this study did not. It is possible that the co-presence of multiple constituents leads to competitive binding or metabolic adjustments that attenuate the initial hypnotic onset while significantly reinforcing sleep maintenance.

Taken together, the findings demonstrate that NSDVT exerts a sedative effect characterized by reduced spontaneous activity, impaired motor coordination, and potentiation of hexobarbital-induced sleep, with a delayed but sustained onset compared to diazepam. These properties may be particularly relevant for conditions such as chronic fatigue syndrome. Nevertheless, the study has limitations. The precise molecular mechanisms underlying the sedative effects of NSDVT remain to be elucidated, and future studies incorporating neurotransmitter assays, receptor binding analyses, and pharmacokinetic evaluations are warranted. Additionally, translating these findings into clinical settings requires further investigation.

V. CONCLUSIONS

NSDVT at 2.5 g/kg and 5 g/kg exhibited sedative effects, as evidenced by decreasing spontaneous locomotor activity in the Activity Cage test and reducing latency to fall in the Rotarod test. Furthermore, NSDVT showed a synergistic interaction with hexobarbital; while it did not significantly affect sleep latency, it prolonged the sleep duration of mice.

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