

Total saponins from *Oplopanax elatus* Nakai: An optimization study of the extraction process using response surface methodology and its psychopharmacological activities

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Research

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Abstract

Background: *Oplopanax elatus* Nakai is an ancient angiosperm plant member. It belongs to the Araliaceae family and has biological activities similar to those of *Panax ginseng*. However, the psychopharmacological effects of *O. elatus* Nakai have not been characterized. Thus, in the present study, we determined the optimal process for extracting total saponins and then screened the psychopharmacological activities of *O. elatus* Nakai extract (ONE).

Methods: A three-level Box-Behnken design with 3 variables was used to evaluate the treatment variables that influenced the total saponin content (TSC) extracted from *O. elatus* Nakai. ONE was orally administered to mice and drosophila to evaluate its psychomotor, sedative-hypnotic, anti-stress, and anxiolytic effects.

Results: The optimal variables for ethanol extraction were 60 min, 70% ethanol, 30 g/mL and 80 °C, and the total saponin yield reached $18.06 \pm 0.17\%$. Treatment with ONE extended cold swimming endurance time, reduced anxiety-like behaviour, decreased spontaneous locomotor activity, prolonged sleep duration, and ameliorated the sleep-wake rhythm. The ginseng extract (GE) is characterized by low-dose excitation and high-dose inhibition of the central nervous system.

Conclusion: Some of the properties of ONE and GE were not identical, but ONE has potentially psychopharmacological activities.

Introduction

Oplopanax elatus Nakai is commonly known as Zamanikha (Заманиха) in Russia and as Ci Shen in China [1]. It is used to treat different disorders in the medical systems of China, Russia, and Korea. In Russia, it is designated a classical adaptogen [2]. In Korea, people traditionally use it to treat a variety of ailments, such as asthenia, depressive state, diabetes, cough, rheumatism, gastrointestinal disorders, and wounds [3-4]. *O. elatus* Nakai is mainly distributed over a 1400 m band of the Chang-bai mountain region in China's northeast [5]. In traditional Chinese medicine, *O. elatus* Nakai possesses similar actions to those of *Panax ginseng*. In Chinese folk medicine, ginseng has been used to treat neurasthenics, hypotension, schizophrenia, cardiovascular disease, diabetes mellitus, and rheumatism. *O. elatus* Nakai also possesses antifungal, fever-relieving, pain-easing, and anti-ageing activities [6]. Chinese people take the stem of *O. elatus* Nakai as an analgesic medicine to treat arthritis [7]. The root of *O. elatus* Nakai has a well-known history of use in the treatment of diseases such as neurasthenia, cardiovascular disorders, and cancer [8]. The chemical components of this plant have been widely investigated. Earlier work has indicated that this plant contains volatile oils [9], aliphatic acids [10], saponins [11], flavonoids [12], and anthraquinones [13].

O. elatus Nakai and ginseng both belong to the Araliaceae family and have similar biological activities. Previous literature on ginseng has reported that it has comprehensive activities, including central nervous regulation, immune system modulation, protection of the cardiovascular system, and antitumour

activities [14-15]. Compared with ginseng, there is comparatively little known about the chemical composition and pharmacological effects of *O. elatus* Nakai. Particularly, the psychopharmacological effects and active ingredient of *O. elatus* Nakai have not been extensively characterized.

This study intends to optimize the ethanol extraction process of total saponins from *O. elatus* Nakai by response surface analysis. The ethanol concentration, solvent/raw material ratio, extraction temperature, and extraction time were taken as factors to optimize the best process parameters and screen the psychopharmacological activities of *O. elatus* Nakai extract (ONE).

Methods

2.1. Materials and chemicals

We obtained *O. elatus* Nakai and ginseng from a wild medicinal plant base in Ji'an, Jilin Province. In autumn, the roots and rhizome of *O. elatus* Nakai were harvested, washed, and cut into sections for later use. Professor Zhenyue Wang of the Heilongjiang University of Traditional Chinese Medicine identified the samples. Ethanol and methanol were of analytical grade. Vanillin, glacial acetic acid, and perchloric acid were supplied by Carmel (Tianjin, China). Oleanolic acid was obtained from the National Institutes for Food and Drug Control (Beijing, China).

2.2 Extraction procedure

We prepared 1 kg of dried powdered *O. elatus* Nakai. Samples were diluted using different concentrations of ethanol (10, 30, 50, 70, or 90%) at a solvent/raw material ratio of 10, 20, 30, 40, or 50 mL/g for extraction times (20, 40, 60, 80, or 100 min) and at different extraction temperatures (50, 60, 70, 80, 90, or 100 °C) for reflux extraction. After filtration, the solvent was removed in a rotary evaporator at 50 °C (N-1210BS-WB, EYELA, Japan). With the total saponins yield as the evaluation index, analysis of variance (ANOVA) was carried out near each factor's maximum yield according to the single-factor experimental results. The method of preparation of the ginseng extract (GE) was the same as that described above.

2.3 Response surface methodology

After studying the four factors, we obtained variances of the maximum total saponins of 0.6081, 1.024, 0.3558, and 0.1207, respectively. Therefore, we selected three factors with the larger conflict among the four aspects of the extraction process for investigation, namely, the concentration of ethanol (A), the solvent/raw material ratio (B) and the temperature (C), to carry out the next experimental design (Table S1). Table 1 presents the experimental design. The quality of the polynomial equation was judged by the determination coefficient (R^2). ANOVA was conducted to determine the significance of the model and regression coefficients. Fischer's exact test verified statistical significance. The response surface and contour map determined the maximum value of the predictive variable.

2.4 Total saponin content (TSC)

TSC of the extracts was measured using the oleanolic acid reagent method. A six-point standard curve (10 to 60 µg/mL) was constructed using oleanolic acid as the reference standard and the results were calculated as TSC per gram dried extract (mg/g) using the following equation

$$\text{TSC (mg/g)} = \frac{nCV}{m \times 1000} \quad (1)$$

where C(mg/g): total content of saponin compounds (mg/g), C: concentration of oleanolic acid obtained established from the calibration curve (mg), V: volume of ethanol solvent (mL), m: weight of *O. elatus* Nakai (g) and n: dilution ratio.

2.5 Bioactivity of total saponins

This study's subjects were 150 male ICR mice (20 ± 2 g), SPF grade, from the Drug Safety Evaluation Center of the Heilongjiang University of Traditional Chinese Medicine. The chambers were maintained at 22 ± 2 °C with a 12 h light/dark cycle, and food and water were available ad libitum. All procedures performed in these studies were approved by the Heilongjiang University of Traditional Chinese Medicine Animal Care and Use Committee.

We randomly separated mice into five groups: control, positive control (GE, 100 mg/mL), low-dose ONE (50 mg/mL), medium-dose ONE (100 mg/mL), and high-dose ONE (150 mg/mL). Each mouse was given the corresponding treatment at the dosage of 2 mL/10 g for 7 days.

2.5.1 Open field test

Mice were gently placed in the centre of the open field to freely explore the whole arena. Medicine was given to mice 1h before the start of each session. Mice were allowed to habituate to the open field arena for 2 min to eliminate novelty bias. An automated system (OFT-100, Chengdu Taming Technology Co., Ltd., SichuangV) recorded the number of accesses to each animal's centre and the centre detention time (s) for 10 min.

2.5.2 Elevated plus-maze test

After administration, mice were put into the elevated plus-maze (EPM). After entering the open arm's junction and the closed arm, timing began and the mice were allowed to move freely in the maze. The activity trajectory of each mouse was observed artificially, and their behaviour in EPM was recorded for 5 min. The observation index included the following: the number of entries and times spent entering into any open arm.

2.5.3 Cold swimming test

After 1h of the last oral administration, the mice were put into the cold water ($16\pm 1^{\circ}\text{C}$). If the animal drowned (stop swimming or barely float), then removed it from the water and record its incubation time. After each session, animals were dried with towels, placed under a warmer, and then returned to their home cages.

2.5.4 Drosophila test

Drosophila melanogaster were obtained from the Drosophila Technology Platform of the Shanghai Institute of Biochemistry and Cell Biology, CAS. *Drosophila* aged seven days were collected. ONE was dissolved in the essential medium at final concentrations of 4.00, 2.00, 0.50, 0.25 and 0.03% (w/v) ($n = 32$). The sleeping time and locomotor activities of the drosophila were detected by a Drosophila Activity Management System (DAMS system) for 24 h.

Statistical Analysis

All data are expressed as the mean and standard deviation. Data were analysed using a one-tailed unpaired T-test to compare each group versus the control group. All statistical analyses were conducted using Prism 7.0. The accepted level of significance was set at $P < 0.05$.

Results

4.1 Model fitting and statistical analysis

The results of 17 experimental runs using Box-Behnken Design along with the measured and predicted values for both responses (Y) for each trial in the experiment are shown in Table 1. In addition, the ANOVA results are presented in Table S2. The correlation coefficient (R^2) value of Y was 0.9833. The results revealed that the developed models for responses were significant ($P < 0.0001$). The value of R^2_{Adj} (0.9618) suggested that the yield of total saponins was attributed to the independent variables. To analyse the effects of the independent variable on the extraction yield of total saponins, the following second-order polynomial equation was generated: $Y = 19.11000 + 0.017500A - 0.15750B + 0.19250C + 0.36750AB - 0.15750AC + 0.36750BC - 0.88375A^2 - 1.23375B^2 - 0.11375C^2$

According to the variance analysis of the regression equation in Table S2, the model's P -value was less than 0.05, which suggested that the difference in the model was statistically significant. The lack of fit was 0.059, with no statistically significant difference ($P > 0.05$), indicating that the model was great for fitting in this experiment. Table S2 shows the influence of AB, A^2 , B^2 , and C^2 on the yield of total saponins, with statistically significant differences ($P < 0.01$). The P -value of B was 0.0355 and that of C was 0.0156, with statistically significant differences ($P < 0.05$).

4.2 Influence of the process variables on the total saponin extraction yield

The response surface plots on the extraction yield of total saponins (Y) are illustrated in Figure S1. When the ethanol concentration increased from 10 to 70%, the yield of total saponins increased steadily. We speculated that the water-soluble constituents of *O. elatus* Nakai, such as polysaccharides, proteins, and pigments were soluble in low concentrations of ethanol and affected the total saponin content. When the concentration was 70%, the high concentration of ethanol inhibited expansion of the plant cells. As illustrated in Figure S1, the maximum yield was achieved at 80 °C. By increasing the temperature, the molecular movement is accelerated, and increased the solubility of the saponins. But, when the temperature above 80 °C, the yield was slight reduce. May be the saponins in *O. elatus* Nakai are heat-unstable compounds. Overall, the total saponin extraction process predicted by the model was as follows: ethanol concentration of 70%, solvent/raw material ratio of 30 mg/mL, extraction temperature (80 °C), and time 60 min.

4.3 The effect of ONE in the open field test

As shown in Figure 1, compared with the control, the number of times entered the open field centre and the central stay time of the high-dose ONE group were significantly prolonged. It was confirmed that a specific dose of ONE indeed improves the anxiety of mice. These results also demonstrated that GE, like other ginseng varieties, can increase rodent locomotor activity in open field experiments.

4.4 The effects of ONE in the EPM test

As shown in Figure 2, These results suggested that ONE had an anxiety-like effect. In addition, the anti-anxiety effect was thought to be beneficial because it has not been reported as a side effect of dyskinesia. Notably, one of its characteristics was observed in this study.

4.5 The effects of ONE in the cold swimming test

Compared with the control group, the mice treated with ONE showed a significant increase in the incubation period of swimming in the experiments. The mice treated with GE also showed a considerable increase in the incubation period in the acute experiments (Figure S2). These results indicated that ONE and GE had anti-fatigue and anti-stress properties. This result supported the adaptability of ginseng products. Previous studies have shown that ginseng can enhance endurance and promote survival in the cold, during fatigue, and in other stressful conditions. Therefore, the cold swimming test results indicated that ONE, like other ginseng species, could improve physiological endurance.

4.6 Locomotor activity of drosophila

We found that after treatment with ONE (0.25% for females, 4.00% for males), they were significant decrease in all-daytime compared to the control group (Figure 3; A, B), while ONE had no effect during the daytime (Figure 3; C, D). However, at night, ONE decreased the number of locomotor activities (Figure 3; E, F). The administration of 4.00% GE shortened the locomotor counts in both male and female drosophila. It means that ONE could reduce the locomotor activities of drosophila throughout the all-daytime and especially at night, and may have different effects based on sex.

4.7 Sleep time of drosophila

Figure 5 shows that compared with the control group, GE at a concentration of 4.00% had a significant effect on the increase in the all-day time of drosophila (Figure 4; A, B), but 2.00% and 4.00% ONE groups significantly decreased during the daytime (Figure 4; C, D). After treatment with 0.50 and 2.00% GE at night, the sleeping time decreased, while when treated with 4.00%, the sleeping time increased. In contrast, the ONE groups (0.50, 2.00 and 4.00% for females, 0.5 and 4.00% for males) displayed an increased sleeping time (Figure 4; E, F). Simultaneously, we counted the number of sleep episodes, as displayed in Figure S3, the results are consistent with sleep time. In short, the above results showed that ONE could significantly prolong the sleeping time at night, while the influence on females was adverse during the day. In comparison, the effects of GE on the sleeping time were as follows: the low dose decreased and the high dose increased the sleeping time.

4.8 Sleep rhythm of drosophila

To determine whether ONE affected sleep rhythms, we measured the sleep rhythm of drosophila over the whole day (Figure 5). Although the period was slightly increased for male drosophila over the last 12 h relative to that in control animals, sleep rhythms were as clearly defined for female drosophila during the night time period as those in the controls. This phenomenon was most obvious when the concentration of ONE was 0.500%. Thus, ONE has a minor impact on sleep but strong effects on circadian rhythms.

Discussion

Saponins are a broad category of plant secondary metabolites found in *O. elatus* Nakai [16]. Wang and Xu performed systematic studies focusing on the saponins occurring in the leaves of *O. elatus* Nakai. The leaf total saponin content was 3.32% based on a developed colorimetric method [17]. Twenty-five cirenshenosides, including cirenshenosides A-V and glycosides II, III and IV, were isolated from *O. elatus* Nakai leaves [18-22]. The roots and rhizomes of *O. elatus* Nakai contained 6.9% crude steroid saponins [23]. Saponins are considered the main active ingredients of *O. elatus* Nakai that act on the central nervous system.

In order to improve the extraction rate of saponins, the response surface method was first used to optimize the extraction process. The concentrations of ethanol, solvent/raw material ratio, and extraction temperature were selected as the factors for investigation through one-way analysis of variance screening. Design Expert 8.0.6 software was used to determine the optimal process. Under different extraction conditions, we studied the yield of the total saponins of *O. elatus* Nakai. The yield of total saponins obtained by the above methods was $18.06 \pm 0.17\%$, which proved that the extraction process is good stability.

Stress has a significant impact on human health worldwide, leading to memory impairment, anxiety, and depressive-like behaviours [24]. Fatigue is a direct result of different harmful stimuli. It is a very complicated physiological state in the human body, and the most direct manifestation is the decline in

sports endurance. Time spent exercising has been regarded as an essential indicator that reflects athletic endurance in the field of anti-fatigue research. It has been reported that ginsenosides have significant anti-fatigue and anti-stress effects [25-26]. The results of the cold swimming experiment showed that GE and ONE could significantly prolong the swimming time of mice and improve the anti-cold and anti-fatigue effects. Nevertheless, the anti-fatigue effects of GE were more substantial than those of ONE because ONE enhances the adaptive functions of an organism, which is similar to but less effective than ginseng.

Psychological load will lead to psychological fatigue, and effective prevention and correction of psychological dysfunction could resist physiological fatigue. In this experiment, EPM, and OFT were selected to investigate the anti-anxiety effects of the ethanol extract of *O. elatus* Nakai. The results of EPM test show that an increase in the percentage of entries and time spent in the open arms are indicative of ONE's anxiolytic effects. In the open field test, the number of mice entering the central area increased, and the residence time in the central part also increased, showing that ONE had an anti-anxiety effect.

Sleep regulation involves complex mechanisms in all animal species. *Drosophila* have been favoured as an ideal model in sleep and circadian rhythm research due to their conserved mechanism and easily manageable operation [27-28]. In humans, sleep is a dynamic physiological process involving multiple transitions between the rapid eye movement (REM) stage and three other non-REM stages [29-30]. In this study, we chose the DAMS system, which allows the simultaneous monitoring of multiple drug sleep tests. We monitored changes in several important parameters, including total sleep time, number of sleep occurrences, locomotion counts and sleep rhythm, revealing the different influences on the *drosophila* sleep profile after treatment with *O. elatus* Nakai and ginseng. Then, we found very diverse results regarding the effects of these treatments on sleep regulation. For instance, the administration of ONE could reduce locomotor activities of *Drosophila* all day time, especially at night, which may have different effects based on sex. By comparing these differences, we found that high-dose ONE could prolong the sleeping time and reduce locomotor activities, particularly at night, while GE is characterized by low-dose excitation and high-dose inhibition of the central nervous system; thus, these treatments are not identical.

Conclusion

In summary, the response surface method was used to optimize the extraction process of total saponins from *O. elatus* Nakai. After optimization, the optimal extraction process of total saponins from *O. elatus* Nakai was 70% ethanol volume fraction, 30g/mL solvent/raw material ratio, extraction temperature 80 °C, extraction time 60 min, and total saponins yield was $18.06 \pm 0.17\%$.

The present results demonstrate that ONE has potentially psychopharmacological activities, such as psychomotor, sedative-hypnotic, anti-stress, and anxiolytic effects. Nevertheless, some of the properties of ONE and GE were not identical. Meanwhile, due to the limitations of the present study, the specific

constituents involved in the pharmacodynamic material basis of ONE are still unknown. This can be a worthwhile focus in future studies.

Declarations

Acknowledgement

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Authors' contributions

Wang Yanyan designed, organized, and supervised the study. Zhang Hui, Bian Hongsheng, Meng Yonghai, Wang Zhibin, Guan Tong, Yang Yue and Huang Lili contributed to literature review and data analyses. Li Tingli contributed to the project design and paper writing. All authors read and approved the final manuscript.

Availability of data and materials

All the data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All animal studies were approved by the Animal Experimental Ethical Committee of Heilongjiang University of Chinese Medicine.

Consent for publication

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

Conflicts of interest

The authors declare no conflicts of interest.

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Abbreviations

ICR Institute for Cancer Research

ONE	O. elatus Nakai Extract
TSC	Total Saponin Content
GE	Ginseng Extract
ANOVA	Analysis of Variance
EPM	Elevated Plus-Maze
DAMS	Drosophila Activity Management System
OFT	Open Field Test
REM	Rapid Eye Movement

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Table

Table 1 Box-Behnken design and observed responses.

Run	A	B	C	Total saponins yield Y /%	
				Measured Responses	Predicted Responses
1	-1	-1	0	17.01	17.50
2	1	-1	0	17.78	16.80
3	-1	1	0	18.27	16.45
4	1	1	0	19.11	17.22
5	-1	0	-1	19.39	17.75
6	1	0	-1	16.80	18.10
7	-1	0	1	17.57	18.45
8	1	0	1	18.90	18.17
9	0	-1	-1	16.45	18.10
10	0	1	-1	17.78	17.05
11	0	-1	1	18.34	17.75
12	0	1	1	18.20	18.17
13	0	0	0	18.97	19.11
14	0	0	0	19.18	19.11
15	0	0	0	17.99	19.11
16	0	0	0	17.15	19.11
17	0	0	0	18.13	19.11

Figures

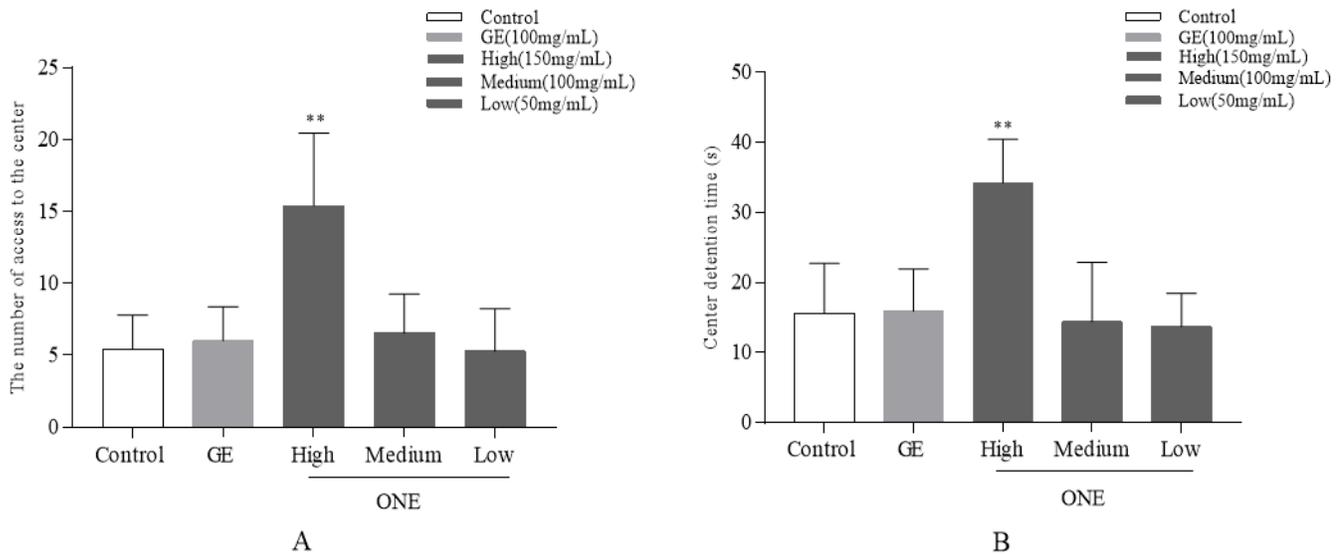


Figure 1

The effect of ONE on anxiety in mice in the OFT. A: The number of access to the center of the mice in different groups. B: The detention time in the center of the mice in different groups. The GE group had no significant difference compared with the control group. The high dose ONE group had a significant difference in the number and detention time of entering the center (n=15, *P <0.05; **P<0.01).

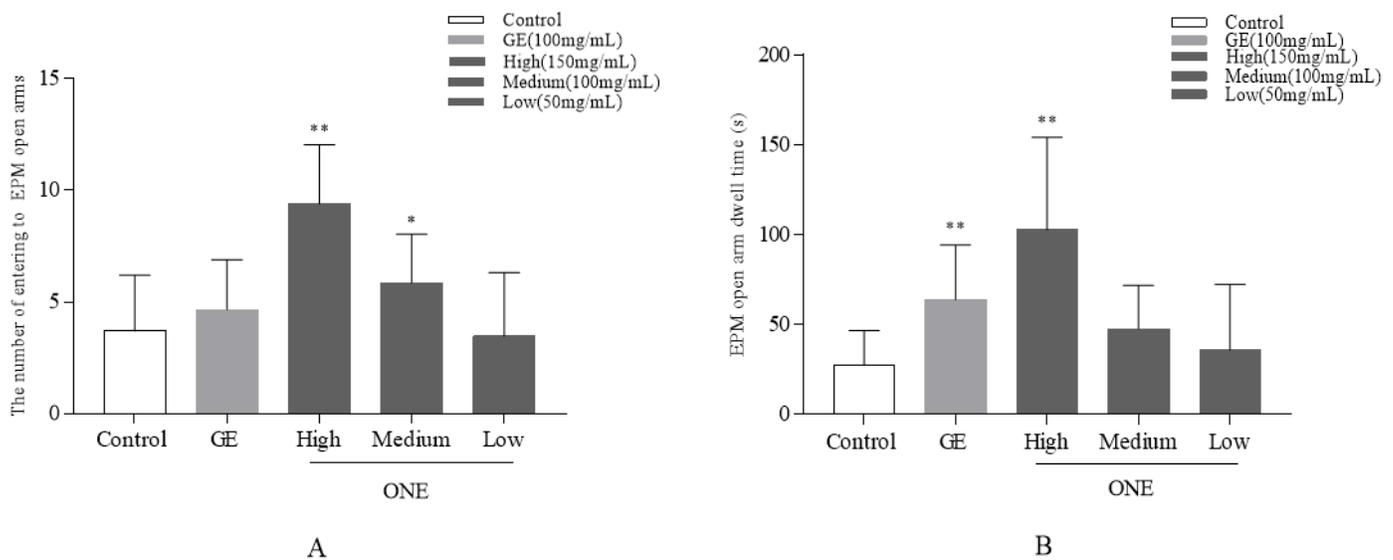


Figure 2

The anti-anxiety effect of ONE in EPM test. A: The number of entering to EPM open arms of the mice in different groups. B: The detention time in EPM open arms of the mice in different groups. The number of entering to EPM open arms of the mice in high and medium dose ONE groups had a significant difference, compared to the control group. The dwell time in EPM open arms of the mice in GE and high dose ONE groups had a significant difference, compared to the control group (n=15, *P <0.05; **P<0.01).

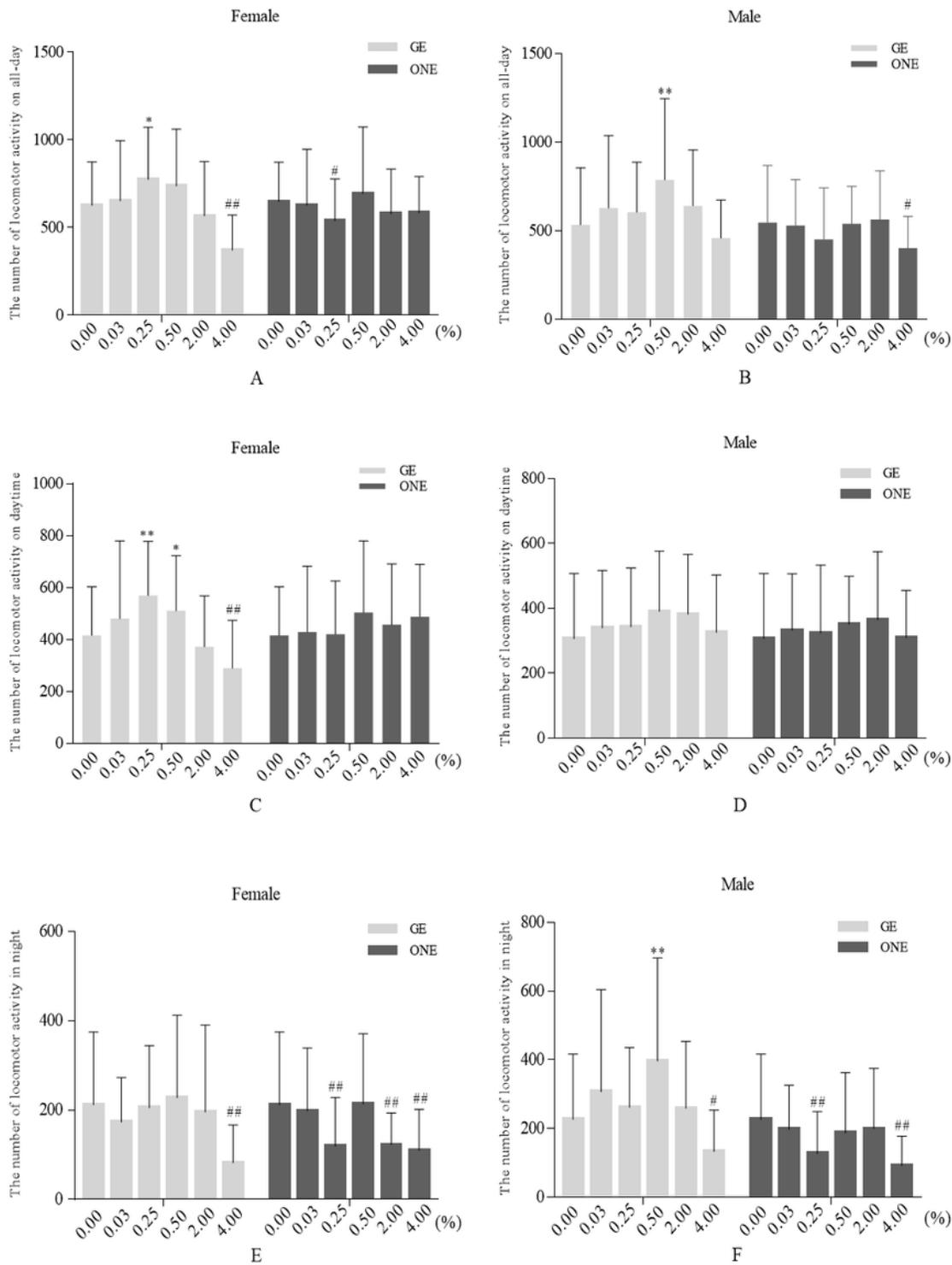


Figure 3

The effects of different concentrations of ONE on locomotor activity of *Drosophila*. A: The number of locomotor activity of the female *Drosophila* in all-day. B: The number of locomotor activity of the male *Drosophila* in all-day. C: The number of locomotor activity of the female *Drosophila* in day time. D: The number of locomotor activity of the male *Drosophila* in day time. E: The number of locomotor activity of

the female drosophila in night time. C: The number of locomotor activity of the male drosophila in night time. (n=28, *, # P <0.05; **, ## P<0.01).

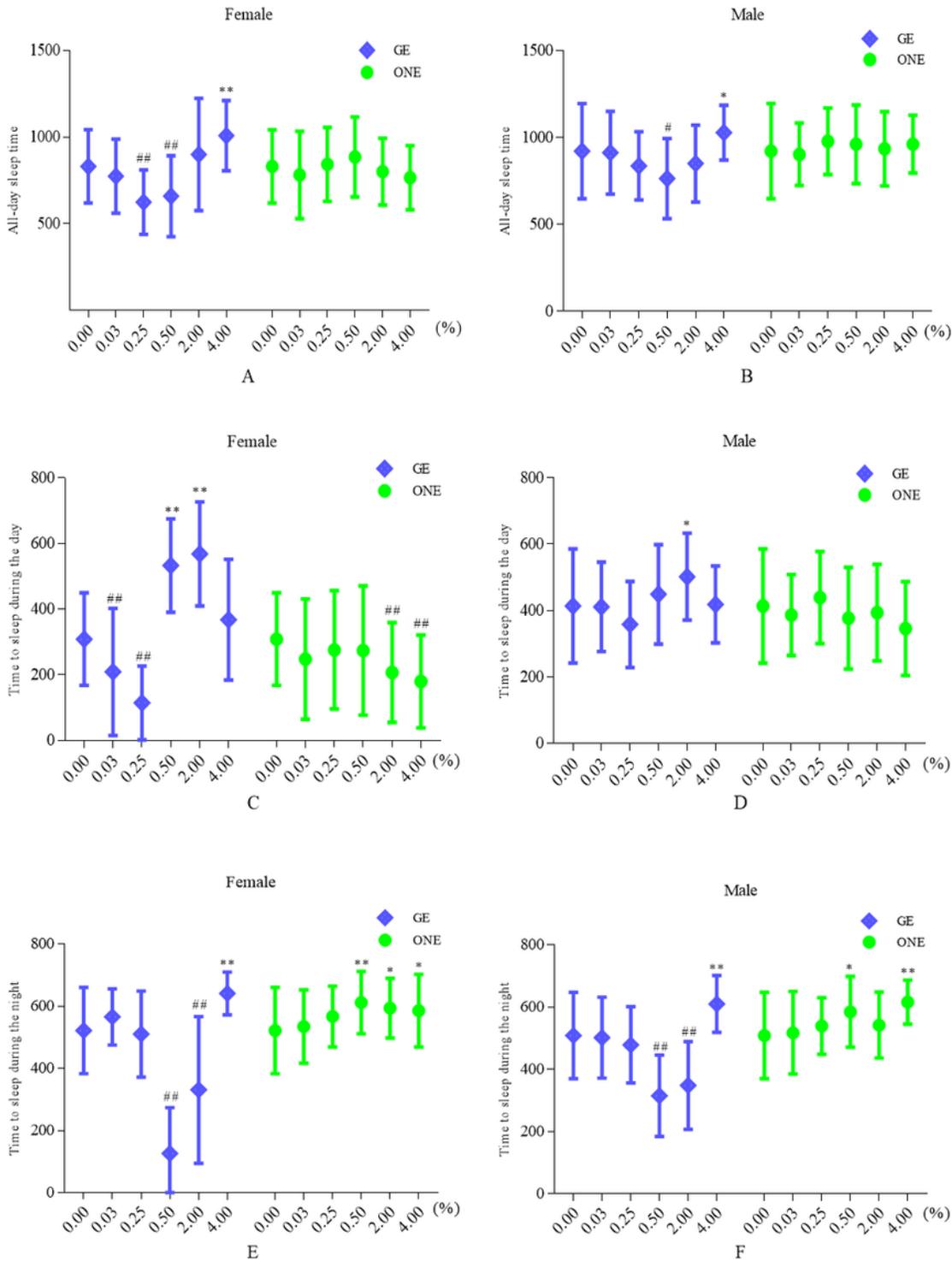


Figure 4

The effects of ONE on sleeping time of drosophila. A: The sleeping time of the female drosophila in all-daytime. B: The sleeping time of the male drosophila in all-daytime. C: The sleeping times of the female drosophila in daytime. D: The sleeping time of the male drosophila in daytime. E: The sleeping time of the

female drosophila in night. F: The sleeping time of the male drosophila in night (n=28; *, # P <0.05; **, ## P<0.01).

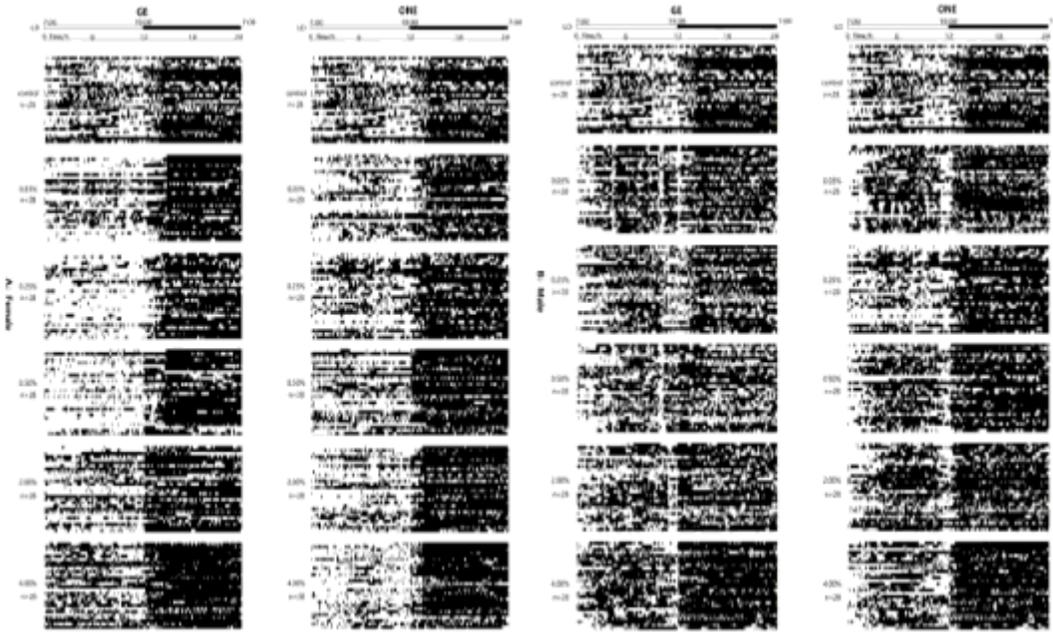


Figure 5

The effects of ONE on Sleep-Awakening of drosophila. A: The effects of ONE and GE on Sleep-Awakening of female drosophila. B: The effects of ONE and GE on Sleep-Awakening of male drosophila. The white part represents drosophila way awake, and the black represents drosophila was sleeping.

Supplementary Files

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