Analysis of GABRB3 gene mRNA expression and motor coordination after administration of valerian extracts (Valeriana officinalis) in BALB/c mice [version 1; peer review: awaiting peer review]

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Abstract

**Background:** Valeriana officinalis has often been used to treat sleep disorders as a traditional medicine for 2000 years. The sedative effect of valerian extract is facilitated by the GABAA receptor β3 subunit. The aim of this study is to determine the effect of valerian extract on GABRB3 gene mRNA expression and sedative effect in BALB/c mice.

**Methods:** This is an experimental preclinical study using a posttest-only control group design. A total of 20 BALB/c mice were randomly allocated into four groups consisting of five mice each. Group I was given 5 ml of Aqua Dest (distilled water), group II was given 0.025 mg/10 g of diazepam, group III was given 2.5 mg/10 g of valerian extract, and group IV was given 5 mg/10 g of valerian extract. The drugs were administrated for seven days through a gastric gavage. The rotarod test was performed on the seventh day. A blood sample was taken on the first day before drug administration and after the rotarod test on the seventh day to be analyzed using quantitative real-time PCR.

**Results:** GABRB3 gene mRNA expression showed a significant increase in groups II, III, and IV (p <0.0001). There was significant difference between group III and IV. The examination of motor coordination (rotarod test) showed a significant difference (p <0.05) between group I and group II, between group I and group III, and between group I and group IV. There was no significant difference between group II and both groups III and IV.
Conclusions: GABRB3 gene mRNA expression was significantly increased after the administration of valerian extract. Based on the rotarod test, valerian extract and diazepam had a clinically similar sedation effect. A higher dose of valerian extract does not yield a higher level of GABRB3 gene mRNA expression nor sedative effects.

Keywords
Valeriana officinalis, GABRB3 genes, sedative, real-time polymerase chain reaction, rotarod, BALB/c
Introduction

GABRB3 is the gene that encodes the GABA_4 receptor β subunit, which is one of the chloride sub-channels of the gamma aminobutyric acid (GABA) receptor in humans. The protein encoded by the GABRB3 gene is one of 13 sub-units of the multiple chloride ion channel ligand channels. GABA is a very important inhibitory neurotransmitter of the central nervous system. GABA has an important role in reducing the excitation of neurons by inhibiting the transmission of nerve impulses in the brain and also plays a role in the regulation of muscle tone. GABA is classified as an amino acid because it has an amine group. GABA receptors are the target workplace of GABA. GABA_4 is one of the GABA receptors that works in 40% of the mammalian central nervous system as a major inhibitory neurotransmitter. GABA_4 receptors are the site of action of a variety pharmacological drugs including barbiturates, benzodiazepines and ethanol. The majority of gene coding in the GABA_4 receptor sub-units is arranged into four clusters on chromosomes 4, 5, 15 and X in the human genome. The cluster of genes is assumed to contribute to the expression of genes. There are two clusters that have three genes, namely α5, β3 and γ3 on chromosome 15. The similarity of all clusters is that there is a β subunit that shows the orientation of the reverse transcription process.

Sleep is a vital physiological phenomenon, which maintains the balance of human body regulation. Insomnia is a temporary or persistent difficulty in starting or maintaining sleep. Insomnia cannot be underestimated, because it can lead to more serious conditions and endanger health and safety. Therefore, every insomniac needs to find the right solution. Diazepam is one of the oldest and most commonly prescribed drugs for treating sleep disorders and is a class of benzodiazepines. Benzodiazepines exert their sedative-hypnotic properties by increasing or potentiating the binding of major inhibitory neurotransmitter GABA at the GABA_4 receptor. These receptors are found in the central nervous system and consist of five subunits of proteins: two alpha sub-units, two beta sub-units, and one gamma sub-unit. Under normal conditions, GABA binds to alpha sub-units at the GABA_4 receptor and facilitates the entry of negatively charged chloride ions into neurons. Benzodiazepines bind allosterically to the gamma subunit of the GABA_4 receptor and increase GABA binding to alpha sub-units. This results in more chloride ions being diffused into the neurons and hyperpolarization ensues, thus making neurons less responsive to excitatory postsynaptic potential stimulation. In other words, benzodiazepines suppress the central nervous system, hence the sedative effects. The use of benzodiazepines is countered by several adverse effects, such as daytime drowsiness and dizziness or lightheadedness.

According to the World Health Organization (WHO), up to 80% of the world’s population uses natural herbal medicines. The valerian plant (Valeriana officinalis) is often used to treat sleep disorders. The use of valerian to reduce tremors, depression, treat headaches, and as antispasmodics has also been reported. The three main chemical ingredients of this plant include essential oils (valeranic acid), valepotriates, and alkaloids. Valerian extract contains GABA, which works by depressing the central nervous system. Valerian’s mechanism of action is similar to benzodiazepines, which increases the number of chloride ions that enter the neurons. One of the ingredients of valerian is glutamine, which has the ability to cross the blood-brain barrier and will then be converted into GABA. Valerenic acid inhibits the breakdown of enzymatic GABA, causing the amount of GABA in the synaptic space to remain high. Valerian works by inhibiting GABA reuptake, so that the concentration of GABA in the synaptic cleft remains high, which will induce sedation.

However, unlike benzodiazepines, valerian binds to the beta sub-unit of the GABA receptor. Valerian also inhibits GABA metabolism, which increases the abundance of GABA and prolongs the effects of hyperpolarization. The sedative effect of valerian extract is facilitated by GABA receptor sub-unit β3. A previous study has found that consumption of 400–450 mg valerian extract before bedtime can improve sleep latency and sleep quality. Administration of 2000 mg/kg valerian liquid suspension daily for seven days in mice resulted in not only sedation, but also hyperthermia, defecation, and reflex disorder. The available evidence suggests that valerian might improve sleep quality without producing side effects.

To the best of the author’s knowledge, there have been no published studies at the level of gene expression yet. We aimed to evaluate the level of GABRB3 gene mRNA expression and sedative effect after administration of valerian extract. We hypothesized that the administration of valerian extract results in increased GABRB3 gene mRNA expression and sedation in BALB/c mice. BALB/c mice have the characteristics of easy breeding and minimal weight variations between males and females. Therefore, it is one of the most extensively used strains in experimental studies, particularly in neurobiology and neuroscience research. BALB/c mice were also used in our reference study regarding the use of valerian extract.

Methods

Ethical statement

Animals were treated according to the Ethical Principles and Guidelines for Scientific Experiments on Animals (2005) by the Swiss Academy of Medical Sciences and ARRIVE guidelines for animal studies. All experimental protocols employed in this study were approved by the Medical Research Ethics Committee of Hasanuddin University Makassar, Indonesia (903/H4.8.4.5.31/PP36-KOMTEK/2017). Principles for the ethical treatment of animals in research environments are described (Government Regulation of Republic of Indonesia No. 95 2012). Specific and detailed directions for the care and use of animals in biomedical research are available in the guidelines, developed in 2011 by Indonesia Health Research Ethics Committee of the Ministry of Health (Indonesia National Guidelines on Health Research Ethics 2011).
Study setting and design
This study was conducted at the Laboratory of Molecular Biology, Faculty of Medicine, Hasanuddin University, Makassar. This study was an experimental laboratory study with BALB/c mice as an animal model, using a posttest-only control group design.

Animals
This is an experimental study using 28 healthy adult male BALB/c mice that were eight weeks old, weighed approximately 25–35 grams, with no anatomical abnormality, that had never been used in experimental studies before. Mice were obtained from the maintenance and development unit of the experimental animal laboratory of Molecular Biology Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Out of 28 mice, 20 mice that could last ≥180 seconds in at least two of the three trials were included. All mice were housed in standard polycarbonate cages (60 cm long × 30 cm wide × 30 cm high) with rice husks, with each cage consisting of five mice. The animal housing kept was at 25±2°C under a 12-hour light/dark cycle. Mice were fed on standard diet in the form of mouse pellets (food made by Japfa Comfeed Indonesia Inc) and given tap water ad libitum for a period of seven days during this study. We assessed the clinical status of the mice every day during routine checks to prevent any clinical changes in the laboratory mice. No mice were found dead or having decreased appetite/growth during the study period. The mice were handled in accordance with the regulations set by the Animal Care and Use Committee at Indonesian National Guidelines on Health Research Ethics.28

Drugs and treatments
Valerian extracts used in this study were Blackmores Valerian Forte, a standardized herbal pill equivalent to 2 g (2000 mg) of Valeriana officinalis dry root or rhizome. The extract was diluted with Aqua Dest (distilled water) to 2 mg/0.1 mL. The drugs were administered via gastric gavage with 1 ml syringe. The dose was determined with the dose used in humans as a reference; the maximum tolerated dose in humans is 81.2 mg/kg as determined by Kakeshi (2014)29, or 51 mg/kg by Al-Majed (2006)30. The Food and Drugs Administration (FDA) recommends converting the doses from human to mice by using 12.3 as a conversion factor, which led to the final dose of 2.5 mg/10 g and 5 mg/10 g as the dose for mice based on the average weight of 20 grams. Meanwhile, the dose of diazepam was adjusted from the human daily dose of 0.2 mg/kg, leading to the final dose of 2.5 mg/kg or 0.025 mg/10 g31,32.

Motor coordination/sedation (rotarod test)
The rotarod test is one of the most commonly used methods to assess motor coordination and balance in mice. It has also been used to assess sedation level effectively in previous studies. The latency of falling from the rod at a constant speed is sensitive to the presence of a biological change (i.e. sedation) that affects motor coordination and balance33,34. A rotarod consists of rods with multiple pointed edges along the seam, with a rod diameter of 3–7 cm, speed control, and a lever that halts the timer after the mice fell from the rod35. The mice underwent a “trial” using the rotarod machine for 300 seconds, at a speed of 20 rotations per minute (rpm), repeated three times with intervals of 10–15 minutes. The trial was conducted 24 hours before the study began. All mice underwent rotarod machine “training” for 60 seconds, at a speed of 20 rpm, repeated three times with five minute intervals, for seven days, 30 minutes before drug administration. On the seventh day, 30 minutes after drug administration, mice were placed on the rotarod machine for 300 seconds, speed of 20 rpm, repeated five times with 10–15 minute intervals. The timer starts when the rotary engine is running. The timer stops when the mouse falls from the rod or until 300 seconds is reached. The time taken to fall from the rod was recorded.

Study protocol
After two-weeks acclimatization the mice were randomly divided into four groups (group I Aqua Dest, group II diazepam, group III valerian 2.5 mg and group IV valerian 5 mg) consisting of five animals each using the GraphPad calculator tool to prevent any bias. The sample size in this study refers to the minimum sample size according to the WHO for research on traditional short-term treatments using animal models, which is five animals per group and Federer’s formula.36 The intervention group, group I, was given 5 ml of Aqua Dest; group II was given 0.025 mg/10 g of diazepam diluted with distilled water to 0.5 mg/0.1 mL; group III was given 2.5 mg/10 g valerian extract diluted with distilled water to 2 mg/0.1 mL; and group IV was given 5 mg/10 g valerian extract diluted with distilled water to 2 mg/0.1 mL. All interventions were administered once daily every morning at 08:00 am for seven days through a gastric gavage in the animal laboratory. All mice were fasted for three hours before drug administration. The body weight of mice was also recorded before drug administration. On the seventh day, 30 minutes after drug administration, mice were placed on the rotarod machine. The time taken to fall from the rod was recorded. Mice were returned to the cage to rest during the intervals. A blood sample of 0.2 ml was taken on the first day before drug administration and after the rotarod test on the seventh day to be analyzed using quantitative real-time PCR (Bio-Rad CFX Connect, USA). The blood was taken from the tail vein without anesthesia. Efforts were made to minimize suffering, such as limiting the frequency of blood sampling, providing adequate space for living and performing no other experimentation or pain inducing procedures.

RNA nucleic acid extraction
Three important steps in this method are lysing, binding, and washing according to the Boom methods.37 A 100 µg/µl of blood volume sample was dissolved into 900 µl of L6 solution, which consists of 120 g of guanidium thycocyanate (GuSCN) (Fluka Chemie AG, Buchs, Switzerland, cat no. 50990) in 100 ml of 0.1 M Tris HCl, pH 6.4. 22 ml 0.2 M ethylenediaminetetraacetate (EDTA) pH 8.0 and 2.6 g Triton X-100 (Packard, Instrument) with a final concentration of 50 mM Tris HCl, 5 M GuSCN, 20 mM EDTA, and 0.1% Triton X-100. The solution was centrifuged at 12,000 rpm. After the lysing process, 20 µl of diatom suspension consisting of 50 ml H2O and 500 µl of 32% (w/v) Celite (diatom) (Jansen Chimica,
manufacturer's instructions. All measurements were conducted using CFX Manager software (Bio-Rad) for calculating gene expression in the analysis mode. Analysis was performed by using iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad, Hercules, CA, USA, Cat No 172503) according to the manufacturer’s instructions. The synthesized cDNA was diluted 10 times and used to determine GABAAR subunit mRNA levels. Following reverse transcription, quantitative RT-PCR (qRT-PCR) was performed in triplicate using the iQ SYBR Green Supermix (Bio-Rad, Cat No 170-888) PCR mix containing 100 mM KCl, 40 mM Tris-HCl pH 8.4, 0.4 mM of each dNTP, 50 U/mL DNA Polymerase (iTaq), 5 µL cDNA template, 2.5 µL of each primer, and 12.5 µL RNase-<sub>H</sub> (5 µL Hercules, CA, USA) in a final volume of 25 µL. The PCR conditions: initial heating at 95°C for three minutes to denature the cDNA and activate the Taq DNA Polymerase, followed by 40 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 60°C for 60 seconds, and extension at 72°C for two minutes. The reaction was then stopped with a final step at 72°C for 15 minutes. Qiagen QuantiTect Primer Assays with the following 10X primers were used: GABRB3 (product number 249900, NM_021912, final concentration 1X), forward (5’ GGTTCTTGGCCTCTGGT -3’) and reverse (5’- AACGAAGTGGACCCATTCACCTCC -3’). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (product number 249900, NM_002046, final conc.1X) was used as an internal control for normalization, GAPDH primer, forward (5’ TCCAGGGTGTTGCTTATGG -3’) and reverse (5’- TTCCACCAGGCTTCTGCTC -3’). Analysis was performed using CFX Manager software (Bio-Rad) for calculating gene expression in the analysis mode ΔΔC(t) according to the manufacturer’s instructions. All measurements were conducted as per manufacturer’s instructions and repeated three times to ensure the validity. Target mRNA expression was read as fold change<sub>40,41</sub>.

### Statistical analysis
Statistical analysis was performed using the IBM<sup>®</sup> SPSS<sup>®</sup> Statistics version 26.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). Data normality tests were performed using the Shapiro-Wilk statistical test. ANOVA and Kruskal Wallis tests were used to analyze numerical differences between the four groups. The paired t-test was used to analyze mean values before and after treatment between each group, while the independent t-test was used to analyze mean values before and after treatment between two different groups. The analyzed variables were mouse weight, GABRB3 gene mRNA expression, and rotarod test results. A p-value of <0.05 was considered significant.

### Results

#### Normality test
**GABRB3** mRNA expression before and after treatment showed normal data distribution, with a p-value of >0.05 within each group (Table 1)<sup>42</sup>. The Shapiro-Wilk normality test was also performed on motor coordination functions (Table 2). In this study, motor coordination functions were tested with the rotarod test, which showed a mean of 183.71 seconds with a median of 215.9 seconds<sup>40</sup>. The normality test showed normal data distribution with p-value of >0.05 in each group.

#### Mice body weight
The Kruskal Wallis test (Table 3) showed that there was no significant difference between the weight of each mouse (p>0.05)<sup>42</sup>.

#### GABRB3 gene mRNA expression
Table 4 shows the mean value of GABRB3 mRNA expression before and after treatment. In group I, there was no significant difference (p = 0.653). In group II, there was a significant increase (p <0.0001) from 5.88 to 8.064. The increase was most significant in group III (p <0.0001), from 6.321 to 12.796. In group IV, there was an increase from 6.028 to 9.778 (p <0.0001). The ANOVA test (Table 5) did not show any significant difference between each group before treatment (p = 0.562). However, there was a significant difference after treatment (p <0.0001). The independent t-test (Table 6) did not show any significant difference (p >0.05) between two groups before treatment. However, there were significant differences between two groups after treatment (p <0.0001) (Table 7).

#### Rotarod test
Table 8 shows the rotarod test results after treatment between two groups using the independent t-test. There were significant differences (p<0.05) between group I (Aqua Dest) and group II (diazepam), between group I (Aqua Dest) and the first treatment group (valerian 2.5 mg/10 g), and between group I (Aqua Dest) and the second treatment group (valerian 5 mg/10 g). There were no significant differences between the positive control group and either treatment group (p >0.05).

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**Table 1**

| Group | Treatment | p-value |
|-------|-----------|---------|
| I     | Aqua Dest | >0.05   |
| II    | Diazepam  | >0.05   |

**Table 2**

| Group | Treatment | p-value |
|-------|-----------|---------|
| I     | Aqua Dest | >0.05   |
| II    | Diazepam  | >0.05   |

**Table 3**

| Group | Treatment | p-value |
|-------|-----------|---------|
| I     | Aqua Dest | >0.05   |
| II    | Diazepam  | >0.05   |

**Table 4**

| Group | Treatment | p-value |
|-------|-----------|---------|
| I     | Aqua Dest | >0.05   |
| II    | Diazepam  | >0.05   |

**Table 5**

| Group | Treatment | p-value |
|-------|-----------|---------|
| I     | Aqua Dest | >0.05   |
| II    | Diazepam  | >0.05   |

**Table 6**

| Group | Treatment | p-value |
|-------|-----------|---------|
| I     | Aqua Dest | >0.05   |
| II    | Diazepam  | >0.05   |

**Table 7**

| Group | Treatment | p-value |
|-------|-----------|---------|
| I     | Aqua Dest | >0.05   |
| II    | Diazepam  | >0.05   |

**Table 8**

| Group | Treatment | p-value |
|-------|-----------|---------|
| I     | Aqua Dest | >0.05   |
| II    | Diazepam  | >0.05   |
Table 1. Shapiro-Wilks normality test on GABRB3 mRNA expression.

| No. | Variable                      | N  | Mean  | SD    | Min  | Max  | Median | p-value |
|-----|-------------------------------|----|-------|-------|------|------|--------|---------|
| 1   | GABRB3 mRNA before treatment  | 20 | 6.027 | 0.539 | 5.059| 6.889| 6.151  |         |
|     | Aqua Dest 5 ml                | 5  | 5.88  | 0.469 | 5.448| 6.409| 5.617  | 0.069   |
|     | Diazepam 0.025 mg/10 g        | 5  | 5.88  | 0.693 | 5.059| 6.654| 5.913  | 0.577   |
|     | Valerian 2.5 mg/10 g          | 5  | 6.321 | 0.548 | 5.509| 6.889| 6.256  | 0.598   |
|     | Valerian 5 mg/10 g            | 5  | 6.028 | 0.467 | 5.379| 6.585| 6.115  | 0.951   |
| 2   | GABRB3 mRNA after treatment   | 20 | 9.171 | 2.57  | 5.622| 13.193| 8.897  |         |
|     | Aqua Dest 5 ml                | 5  | 6.046 | 0.377 | 5.622| 6.603| 5.916  | 0.769   |
|     | Diazepam 0.025 mg/10 g        | 5  | 8.064 | 0.451 | 7.529| 8.746| 8.019  | 0.885   |
|     | Valerian 2.5 mg/10 g          | 5  | 12.796| 0.416 | 12.158|13.193|12.892  |0.563    |
|     | Valerian 5 mg/10 g            | 5  | 9.778 | 0.486 | 9.048|10.347| 9.809  | 0.898   |

Data were analyzed with the Shapiro-Wilks normality test; a p-value of >0.05 was considered as normal data distribution. The unit used to measure the expression was fold change.

Table 2. Shapiro-Wilks normality test on rotarod test.

| Variable        | N  | Mean  | SD    | Min  | Max  | Median | p-value |
|-----------------|----|-------|-------|------|------|--------|---------|
| Rotarod test    | 20 | 183.71| 95.946| 7.4  | 300  | 215.9  |         |
| Aqua Dest 5 ml  | 5  | 274.88| 29.332| 243  | 300  | 288    | 0.05    |
| Diazepam 0.025 mg/10 g | 5 | 181.68| 86.214| 71.2 | 282.2| 212    | 0.678   |
| Valerian 2.5 mg/10 g | 5 | 121.44| 109.921|7.4 | 251 | 99     | 0.371   |
| Valerian 5 mg/10 g | 5 | 156.84| 84.893|15.2 |221.8|200.4  | 0.1     |

Data were analyzed with the Shapiro-Wilks normality test; a p-value of >0.05 was considered as normal data distribution. The values indicate seconds before falling.

Table 3. Comparison of BALB/C mouse weight within seven days of observation.

| Observation | Aqua Dest 5 ml | Diazepam 0.025 mg/10 g | Valerian 2.5 mg/10 g | Valerian 5 mg/10 g | p-value |
|-------------|----------------|------------------------|----------------------|-------------------|---------|
| Day 1       | 31.96          | 31.86                  | 32.38                | 31.66             | 0.963   |
| Day 2       | 32.22          | 31.66                  | 32.08                | 31.62             | 0.955   |
| Day 3       | 31.98          | 31.62                  | 32.04                | 30.92             | 0.841   |
| Day 4       | 32.22          | 31.92                  | 32.2                 | 30.98             | 0.943   |
| Day 5       | 32.3           | 32.18                  | 32.04                | 30.94             | 0.757   |
| Day 6       | 32.8           | 32.16                  | 32                   | 30.8              | 0.635   |
| Day 7       | 32.8           | 32.02                  | 32.3                 | 30.44             | 0.28    |

Data were analyzed by the Kruskal-Wallis test; a p-value >0.05 was considered significant.
Discussion

Sedation is defined as a condition of decreased awareness of the surrounding environment and reaction to external stimuli. The sedative effect of several active substances in valerian extract work synergistically. Valerenic acid inhibits the breakdown of GABA, which results in an abundance of GABA in the synaptic space. Valerian extract also contains GABA, which suppresses the central nervous system. Furthermore, glutamine, one of the valerian extract substances, can cross the blood-brain barrier and will be converted into GABA. Lastly, valerian also acts by blocking GABA reuptake.\(^{28,43}\)

\(\text{GABRB3}\) is a heteromeric gene that encodes the \(\text{GABA}_A\) receptor \(\beta_3\)-subunit, a member of the ligand-gated ion channel family in humans. \(\text{GABA}_A\) receptors are the major inhibitory transmitter receptors in the brain and the site of action of a variety of pharmacologically and clinically important drugs.\(^{1-4}\) Meguro et al. (1997) have identified \(\text{GABRB3}\) expression in mouse A9 hybrids that contained either a paternal or maternal human chromosome.\(^{44}\)

Administration of diazepam and valerian extract in this study resulted in a significant increase in \(\text{GABRB3}\) gene mRNA expression.

Table 4. Comparison of \(\text{GABRB3}\) gene mRNA expression before and after treatment.

| No | Group                  | N  | Mean  | SD   | Min   | Max   | Median | p-value |
|----|------------------------|----|-------|------|-------|-------|--------|---------|
| 1  | Aqua Dest 5 ml         | 5  | 5.88  | 0.469| 5.448 | 6.409 | 5.617  | 0.653   |
|    | Before treatment       | 5  | 5.88  | 0.469| 5.448 | 6.409 | 5.617  |         |
|    | After treatment        | 5  | 6.046 | 0.377| 5.622 | 6.603 | 5.916  |         |
| 2  | Diazepam 0.025 mg/10 g | 5  | 5.88  | 0.693| 5.059 | 6.654 | 5.913  | <0.0001 |
|    | Before treatment       | 5  | 5.88  | 0.693| 5.059 | 6.654 | 5.913  |         |
|    | After treatment        | 5  | 8.064 | 0.451| 7.529 | 8.746 | 8.019  |         |
| 3  | Valerian 2.5 mg/10 g   | 5  | 6.321 | 0.548| 5.509 | 6.889 | 6.256  | <0.0001 |
|    | Before treatment       | 5  | 6.321 | 0.548| 5.509 | 6.889 | 6.256  |         |
|    | After treatment        | 5  | 12.796| 0.416| 12.158| 13.193| 12.892 |         |
| 4  | Valerian 5 mg/10 g     | 5  | 6.028 | 0.467| 5.379 | 6.585 | 6.115  | <0.0001 |
|    | Before treatment       | 5  | 6.028 | 0.467| 5.379 | 6.585 | 6.115  |         |
|    | After treatment        | 5  | 9.778 | 0.486| 9.048 | 10.347| 9.809  |         |

Data were analyzed by the paired t-test; a p-value of <0.05 was considered significant. The unit used to measure the expression was fold change.

Table 5. Comparison of \(\text{GABRB3}\) gene mRNA expression before and after treatment between four groups.

| No | Variable         | N  | Mean  | SD   | Median | p-value | F-value |
|----|------------------|----|-------|------|--------|---------|---------|
| 1  | \(\text{GABRB3}\) mRNA before |    |       |      |        |         |         |
|    | Aqua Dest 5 ml   | 5  | 5.88  | 0.469| 5.617  | 0.562   | 0.71    |
|    | Diazepam 0.025 mg/10 g | 5  | 5.88  | 0.693| 5.913  |         |         |
|    | Valerian 2.5 mg/10 g | 5  | 6.321 | 0.548| 6.256  |         |         |
|    | Valerian 5 mg/10 g | 5  | 6.028 | 0.467| 6.115  |         |         |
| 2  | \(\text{GABRB3}\) mRNA after |    |       |      |        | <0.0001 | 216.61  |
|    | Aqua Dest 5 ml   | 5  | 6.046 | 0.377| 5.916  |         |         |
|    | Diazepam 0.025 mg/10 g | 5  | 8.064 | 0.451| 8.019  |         |         |
|    | Valerian 2.5 mg/10 g | 5  | 12.796| 0.416| 12.892 |         |         |
|    | Valerian 5 mg/10 g | 5  | 9.778 | 0.486| 9.809  |         |         |

Data were analyzed with the ANOVA test; a p-value of <0.05 was considered significant. The unit used to measure the expression was fold change.
expression. The first treatment group and second treatment group had the highest motor coordination function impairment compared to other groups, although not significant. Thus, it can be interpreted that valerian extract exhibited a sedation effect on both the clinical and molecular levels. Based on the result of the rotarod test, there were significant differences between group I and the rest of the groups, while there were no significant differences between groups II, III, and IV. This result indicated that valerian extract and diazepam have clinically comparable sedation effects, as shown in reports by Khom et al. and Budzinski et al. Valerian has been shown to have a sedative effect and prolong sleep time in mice, as reported by Hendrik et al. and Fernandez et al. Regarding motoric coordination function, Khom et al. also reported that the administration of ≥10 mg/kg of valerian extract caused a significant reduction in locomotion in mice. The mice in this study have

| No | Variable          | Mean | SD   | Min  | Max  | Median | p-value |
|----|-------------------|------|------|------|------|--------|---------|
| 1. | Aqua Dest 5 ml    | 5.88 | 0.47 | 5.448| 6.409| 5.616  | 0.999   |
|    | Diazepam 0.025 mg/10 g | 5.88 | 0.693| 5.058| 6.653| 5.913  |         |
| 2. | Aqua Dest 5 ml    | 5.88 | 0.470| 5.448| 6.409| 5.616  | 0.209   |
|    | Valerian 2.5 mg/10 g | 6.321| 0.548| 5.509| 6.889| 6.256  |         |
| 3. | Aqua Dest 5 ml    | 5.88 | 0.470| 5.448| 6.409| 5.616  | 0.631   |
|    | Valerian 5 mg/10 g | 6.028| 0.467| 5.379| 6.585| 6.115  |         |
| 4. | Diazepam 0.025 mg/10 g | 5.88 | 0.693| 5.058| 6.653| 5.913  | 0.298   |
|    | Valerian 2.5 mg/10 g | 6.321| 0.548| 5.509| 6.889| 6.256  |         |
| 5. | Diazepam 0.025 mg/10 g | 5.88 | 0.693| 5.058| 6.653| 5.913  | 0.703   |
|    | Valerian 5 mg/10 g | 6.028| 0.467| 5.379| 6.585| 6.115  |         |
| 6. | Valerian 2.5 mg/10 g | 6.321| 0.548| 5.509| 6.889| 6.256  | 0.39    |
|    | Valerian 5 mg/10 g | 6.028| 0.467| 5.379| 6.585| 6.115  |         |

Data were analyzed with the independent t-test; a p-value of<0.05 was considered significant.

| No | Variable          | Mean | SD   | Min  | Max  | Median | p-value |
|----|-------------------|------|------|------|------|--------|---------|
| 1. | Aqua Dest 5 ml    | 6.046| 0.377| 5.622| 6.603| 5.915  | <0.0001 |
|    | Diazepam 0.025 mg/10 g | 8.064| 0.451| 7.529| 8.745| 8.019  |         |
| 2. | Aqua Dest 5 ml    | 6.046| 0.377| 5.622| 6.603| 5.915  | <0.0001 |
|    | Valerian 2.5 mg/10 g | 12.796| 0.416| 12.158| 13.193| 12.892 |         |
| 3. | Aqua Dest 5 ml    | 6.046| 0.377| 5.622| 6.603| 5.915  | <0.0001 |
|    | Valerian 5 mg/10 g | 9.778| 0.486| 9.048| 10.347| 9.809  |         |
| 4. | Diazepam 0.025 mg/10 g | 8.064| 0.451| 7.529| 8.745| 8.019  | <0.0001 |
|    | Valerian 2.5 mg/10 g | 12.796| 0.416| 12.158| 13.193| 12.892 |         |
| 5. | Diazepam 0.025 mg/10 g | 8.064| 0.451| 7.529| 8.745| 8.019  | <0.0001 |
|    | Valerian 5 mg/10 g | 9.778| 0.486| 9.048| 10.347| 9.809  |         |
| 6. | Valerian 2.5 mg/10 g | 12.796| 0.416| 12.158| 13.193| 12.892 | <0.0001 |
|    | Valerian 5 mg/10 g | 9.778| 0.486| 9.048| 10.347| 9.81   |         |

Data were analyzed with the independent t-test; a p-value of<0.05 was considered significant. The unit used to measure the expression was fold change.
homogeneous characteristics (no difference in mean weight), thus not presenting as a confounding factor or bias that might affect the study’s results.

Interestingly, the mice in first treatment group (valerian 2.5 mg/10 g) had the most significant increase in the mean value of \textit{GABRB3} gene mRNA expression after treatment (up to two-fold), exceeding that of second group treatment (valerian 5 mg/10 g), which had a higher dose of valerian extract. Furthermore, the mice in first treatment group also demonstrated the shortest mean duration in the rotarod test. These results indicate that increasing the dose of valerian extract (2.5 mg to 5 mg) was not directly proportional to the increase in both \textit{GABRB3} gene mRNA expression and sedation.

This study has several limitations. First, there are various available formulations of valerian extract without a clear standard of processing method. Second, there are no previous studies capable of proving that valerian extract exerts its sedative effects by increasing the level of \textit{GABRB3} gene mRNA expression. Finally, this study does not perform a brain biopsy for determining the level of \textit{GABRB3} gene mRNA expression through immunohistochemistry tests. Further research is needed to establish the standardized dosage of valerian extract. More definitive studies to determine the correlation between the level of \textit{GABRB3} gene mRNA expression and sedation effect are also warranted.

**Conclusion**

Valerian extract exerts its sedative properties by binding to the GABA\textsubscript{A} receptor \(\beta3\) subunit. As the gene that encodes the GABA\textsubscript{A} receptor \(\beta3\) sub-unit, \textit{GABRB3} gene mRNA expression was increased after the administration of valerian extract. Valerian extract had a clinically similar sedation effect to diazepam. However, a higher dose of valerian extract did not yield a higher level of \textit{GABRB3} gene mRNA expression or sedative effects.

**Data availability**

**Underlying data**

Open Science Framework: Effect of Valerian Extracts on \textit{GABRB3} Gene mRNA Expression and Motor Coordination in BALB/c mice. https://doi.org/10.17605/OSF.IO/CT52W<sup>4</sup>.

This project contains the following underlying data:

- Mice_Body_Weight.xlsx
- mRNA_expression_of_GABRB3.xlsx
- Rotarod.xlsx

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

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