Available online at http://www.iorajournal.org/index.php/ijgor/index

International Journal of Global Operations Research

e-ISSN: 2722-1016
p-ISSN: 2723-1739

Vol. 5, No. 1, pp. 10-14, 2024

Citronella (Cymbopogon nardus (L.) Rendle) Leaves Infusion: Sedative Activity in Swiss-Webster Strain Male Mice

Cszahreyloren Vitamia[1*], Wahyuni Mega Handayani[2]

[1,2] *Departement of Pharmacy, Akademi Farmasi Bumi Siliwangi, Jl. Rancabolang No.104, Bandung 40286, Indonesia*

**Corresponding author email: cszahreyloren@akfarbumisiliwangi.ac.id*

Abstract

Insomnia is one of the sleep disorders and more severe symptoms can culminate in the onset of stress disorder. The high threat of being affected by COVID-19 causes insomnia, so drugs such as sedatives are required to treat this disorder. Research has been carried out on the sedative activity of infusion of citronella leaves (Cymbopogon nardus (L.) Rendle) in Swiss webster strains of male mice by the traction test method. The study used twenty-five mice randomly divided into five groups. Each group was given different preparations such as negative control given Na CMC 0.5%, the positive control group was given suspension of diazepam and the infusion group of citronella leaves with concentrations of 10% w/v, 30% w/v and 50% w/v as the test groups sequentially. The results showed that citronella leaves infusion had increased sedative activity along with in conjunction with an increment in test concentration. The infusion concentration of citronella leaves that have maximum sedative activity was found at a concentration of 50% w/v in the variations tested and have significantly different results compared to the control group.

Keywords: Cymbopogon nardus, citronella, sedative activity, sedative activity, mice.

1. Introduction

The Covid-19 pandemic that's right now underway in Indonesia has made gigantic changes which can be seen from changes in way of life and social relations in daily life, adjusting to these unusual changes was not a simple thing (Sharifi and Khavarian-Garmsir 2020). The impacts that arise are as varied as the case in terms of health which produces psychological pressure and symptoms of anxiety which have a negative impact on sleep quality such as insomnia and more severe symptoms which can culminate in the emergence of stress disorders (Cohen, et al., 2017). Sleep seems to be important in restoring our daily functions, but sleep deprivation makes us more emotionally agitated and more susceptible to stressful stimuli and events (Vandekerckhove and Wang 2018). According to research by Yasmin et al (2021) there were 20.05% of a total of 5641 respondents who experienced insomnia based on the threat of being affected by Covid-19.

The condition of the Covid-19 pandemic tends to have a negative impact on the quality of one's sleep because of the many changes that occur in daily routines, fear of health, worry about the situation and the prolonged duration of the pandemic, resulting in loss of entertainment venues and reduced social interaction (Altena et al., 2020). The high threat of stress due to the impact of Covid-19 has caused insomnia so that drugs are needed to overcome this, including sedative-hypnotic drugs. This class of drugs belongs to the class of depressants that suppress the central nervous system (CNS), the sedative class can cause loss of physical or mental response but does not affect consciousness while those that because drowsiness are hypnotics (National Institute on Drug Abuse, 2022).

Person with insomnia tend to require sedative-hypnotic drugs and this is considered reasonable because the drugs utilized too incorporates a reasonably quick pharmacological effect. Excessive utilize is additionally not great since the side effect can worsen health, this is considering the number of cases of drug abuse on this class of sedative and hypnotic agent. This problem can be solved by traditional medicine which developed to overcome by provide sedative activity which is taken from natural ingredients, namely citronella (Cymbopogon nardus (L.) Rendle) (Yulianita et al., 2019).

Citronella leaves have several uses, including as an anti-mosquito can also relieve pain, stiff muscles, reduce insomnia, anxiety and provide a sedative effect (Shah et al., 2011). However, studies that have been carried out using extracts from all parts of the citronella grass, so that the sedative action within the form of leaf infusion has of citronella has not been carried out. Subsequently, this research is exceptionally interesting since the infusion of citronella leaves can be utilized effectively by individuals who require it for traditional medication. One of the

extraction methods used in a simple, easy and economical way is the infusion method. Infusion is also more applicable in the manufacture of traditional medicines where it can be made directly by the community without using special tools and the solvent used is sufficient with water so that it can be used in serving (Stéphane et al., 2021).

2. Material and Methods

2.1. Materials and Instrumentation

The materials used in this study: simplisia citronella leaves (Cymbopogon nardus (L.) Rendle) is obtained directly from the Manoko Lembang plantation, West Bandung Disctrict, West Java, Indonesia; 0.5% Na CMC, distilled water and Diazepam suspension (Stesolid®). The instrumentation used in this research is Test animal scales (triple beam balance), analytical balance (Fujitsu®), oral sonde, stopwatch (casio®), gloves (remedi®), measuring flask (Pyrex®), beaker glass (Pyrex®), mortar, stamper, stir bar, thermometer (GEA®) and traction test tool.

2.2. Plant Collection and Extraction

The whole plant of citronella (Cymbopogon nardus (L.) Rendle) was collected from the Manoko Lembang plantation in January 2022. The samples were then identified and determined by the Herbarium Laboratory of Plant Taxonomy, Department of Biology FMIPA Padjadjaran University. The concentration of citronella leaves infusion used was 10% w/v, 30% w/v and 50% w/v. This infusion is made by weighing 10 grams, 30 grams, 50 grams of simplicia citronella leaves and then putting it into an infusion pot, adding 100 ml of water and then heating it for 15 minutes after the temperature in the pot reaches 90°C, while stirring occasionally, then after chilling filtered using filter paper (Svenson and Paigen 2019).

2.3. Animals

The animals test with 20–30 g weight were collected from Local Cultivation Animal Lab Cimahi, West Java, Indonesia. The mice used in this study were male mice of the swiss webster strain, male mice were used because their biological conditions were relatively stable compared to female mice which were affected by the estrous cycle period and also used the Swiss-webster strain because the price was more economical. Mice were first acclimatized for 7 days with the aim of adapting in the lab so that mice would not experience stress that might affect the research (McKinney et al., 2022). Mice were also fasted for 18 hours (ad libitum) to reduce the effect of food on research results (Jensen et al., 2013).

2.4. Drugs and Treatment

Twenty-five animals were divided into 5 treatment groups with 5 mice in each group for negative control, standard (positive control), and test sample for every experiment, then weighed one by one and recorded the weight and marked on the tail. Each group was given different preparations such as the negative control group was given 0.5% Na CMC, the positive control group was given diazepam (1 mg/kg) (Moniruzzaman, et al., 2015) and the citronella leaf infusion group with concentrations of 10% w/v, 30% w/v and 50% w/v as the test groups sequentially.

All tests were administered the preparation was given to the mice orally by previously preparing the preparations that had been placed in the oral sonde syringe then positioning the mice as if they were going to be given the preparations, positioning the mice vertically leaning slightly forward. The oral probe was inserted into the mouse's mouth by walking along the roof of the mouse's mouth until it touched the throat. Be careful not to get the preparation into the throat. Press the syringe to remove the preparation from the oral tube (Ichorbio, 2021).

2.5. Traction Test Method

Determination of sedative activity in mice was conducted by traction test method. This method aims to measure the decrease or muscle power of the test animal after it is hung on a tool in the form of a wire. The mice were hanging on the traction test equipment then was observed from the 5th to the 120th minute by observing of the body turning reflex, and measuring the falling time after hung using the Traction Test (Sudewi, et al., 2021).

2.6. Statistical Analysis

Distribution (distribution) of normal data was analyzed using the Shapiro Wilk test. The normality test is a test to measure whether the data has a normal distribution or not, so that if the data is normally distributed, parametric statistics can be used. The Shapiro Wilk test was chosen because the number of samples was less than 50 samples.

To analyzed normality of the data test, with this formula below

$$T_3 = \frac{1}{D}\left[\sum_{i=1}^{k} a_i\left(X_{n-i+1} - X_i\right)\right]^2 \quad (1)$$

Data variance was analyzed using the Levene Statistic homogeneity test. Levene's test is used to test the similarity of the variances of several populations. The Levene's Test statistical formula is given by:

$$W = \frac{(N-k)\sum_{i=1}^{k} n_i(\overline{Z}_{i.} - \overline{Z}_{..})^2}{(k-1)\sum_{i=1}^{k}\sum_{j=1}^{n_i}(Z_{ij} - \overline{Z}_{i.})^2} \quad (2)$$

Data that were normally distributed were then analyzed using an analysis of variant (ANOVA) test to see if the average difference was significant or not and continued with the Post Hoc Test with the LSD (Least Significant Different) test which aims to determine differences between treatment groups. Statistical analysis was processed using the Statistical Product and Service Solution (SPSS) 26.0 for Windows program (Pringgandini et al., 2018).

3. Result and Discussion

3.1. Turning Reflex

The results of the turning reflex test in Table 1 showed a decrease in activity in the four groups of mice that were given positive control preparations, citronella leaves infusion (CLI) concentrations of 10%, concentrations of 30% (30% CLI)), and concentrations of 50% (50% CLI) could provide sedative activity. However, this was not the case with the group of mice that provided a negative control preparation because the administration of Na CMC did not affect the mice in terms of its characteristics which functioned as a suspending agent, so it had no effect and caused a decrease in activity in the mice (Alnamer et al., 2012).

Table 1: Turning reflex on mice

Groups	Results on turning reflex				
	1	2	3	4	5
Negative control	√	√	√	√	√
Positive control	×	×	×	×	×
10% CLI	×	×	×	×	×
30% CLI	×	×	×	×	×
50% CLI	×	×	×	×	×

3.2. Fall Time on Traction test

The results obtained from the fall time test can be seen in Table 2 showing that the negative control group did not have sedative activity because it had a longer fall time than the other treatment groups. However, in the treatment of the positive control group and citronella leaf infusion at concentrations of 10%, 30% and 50%, they showed sedative activity because they would immediately fall and take a long time to condition their bodies back to remain balanced (Hanrahan et al., 2011).

Table 2: Fall Time on mice in Traction test

Sample	Fall time (second) on mice number-					Mean ±SD
	1	2	3	4	5	
Negative control	99	100	93	112	110	102.80 ± 7.981
Positive control	23	16	7	38	31	23.00 ± 12.186
10% CLI	61	61	83	72	71	69.60 ± 9.154
30% CLI	52	49	45	45	49	48.00 ± 3.000
50% CLI	40	20	42	30	34	33.20 ± 8.786

The fall time obtained is getting faster as the infusion concentration increments. It can be seen at the 50% concentration citronella leaves infusion where the data shows a fast fall time. This is due to the presence of chemical compounds such as flavonoids which have the potential to provide sedative activity. These flavonoid compounds are thought to have an influence on Gamma-aminobutyric acid (GABA) receptors. GABA is a mediator that can activate the inhibition of neurons so that nerve activity to deliver stimuli is disrupted. If disturbed, this can result in changes in

muscle work causing a decrease in muscle tone and a decrease in sensitivity to the surrounding environment which is marked by a decrease in activity in mice.18

3.3. Statistical Result

The results of the data were tested for normality using the Shapiro-Wilk test because the sample used was less than 50. The results of each treatment group showed a value of more than 0.05 can be ($p > 0.05$) so that it can be seen that all the data is normally distributed. The data was then tested for homogeneity using the Levene's Test. The test results showed $p > 0.05$. This indicated that there were similarities between the treatment groups, which meant that the data was homogeneous. so that the research data fulfills the parametric test requirements for using the One Way Anova test. The results of the ANOVA statistical test obtained significant results ($p < 0.05$) which showed that the average fall time for each treatment group was significantly different.

4. Conclusion

Infusion of citronella leaves (Cymbopogon nardus (L.) Rendle) in swiss webster strain male mice using the traction test method had sedative activity which increased with increasing test concentrations. The concentration of citronella leaf infusion (Cymbopogon nardus (L.) Rendle) which has maximum sedative activity at a concentration of 50% w/v in the variation tested. The ANOVA statistical test of sedative activity of infusion of citronella leaves in vary concentrations had significantly different ($p < 0.05$). By this significantly different result in ANOVA test, LSD (Least Significant Different) test followed to determine which significant difference of each group. the negative control and positive control treatment groups and the three concentrations (10%,30%,50%) showed significantly different results ($p < 0.05$). The significant value of the positive control to the concentration of 10% and 30% was significantly different ($p < 0.05$). The 50% concentration differed significantly from the negative control, the 10% and 30% concentrations ($p < 0.05$), while the 50% concentration compare with the positive control was not significantly different ($p > 0.05$). This can be interpreted that the results of sedative activity at 50% concentration are almost similar with the positive control group.

References

Alnamer, R., Alaoui, K., Bouidida, E. H., Benjouad, A., & Cherrah, Y. (2012). Sedative and hypnotic activities of the methanolic and aqueous extracts of Lavandula officinalis from Morocco. *Advances in Pharmacological and Pharmaceutical Sciences, 2012*.

Altena, E., Baglioni, C., Espie, C. A., Ellis, J., Gavriloff, D., Holzinger, B., ... & Riemann, D. (2020). Dealing with sleep problems during home confinement due to the COVID-19 outbreak: Practical recommendations from a task force of the European CBT-I Academy. *Journal of sleep research, 29*(4), e13052.

Cohen, S., Kaplan, Z., Zohar, J., & Cohen, H. (2017). Preventing sleep on the first resting phase following a traumatic event attenuates anxiety-related responses. *Behavioural Brain Research, 320*, 450-456.

Hanrahan, J. R., Chebib, M., & Johnston, G. A. (2011). Flavonoid modulation of GABAA receptors. *British journal of pharmacology, 163*(2), 234-245.

Ichorbio. (2021). *A Beginner's Guide on How to Inject Mice*. https://ichor.bio/beginners-guide-of-how-to-inject-mice/ Accessed: 2021-06-10

Jensen, T. L., Kiersgaard, M. K., Sørensen, D. B., & Mikkelsen, L. F. (2013). Fasting of mice: a review. *Laboratory animals, 47*(4), 225-240.

McKinney, M. M., Dupont, W. D., Corson, K. J., Wallace, J. M., & Jones, C. P. (2022). Physiologic and Behavioral Effects in Mice Anesthetized with Isoflurane in a Red-tinted or a Traditional Translucent Chamber. *Journal of the American Association for Laboratory Animal Science, 61*(4), 322-332.

Moniruzzaman, M., Rahman, A., & Ferdous, A. (2015). Evaluation of sedative and hypnotic activity of ethanolic extract of Scoparia dulcis Linn. *Evidence-Based Complementary and Alternative Medicine, 2015*.

NIDA. (2022). *Prescription CNS Depressants DrugFacts National Institute on Drug Abuse (NIDA).* https://nida.nih.gov/publications/drugfacts/prescription-cns-depressants. Accessed: 2022-04-16

Pringgandini, L. A., Indarti, G. Y., Melinda, M., & Sari, M. (2018). Efektivitas spray nanokolagen limbah sisik ikan mas (Cyprinus carpio) untuk mempercepat proses penyembuhan luka insisi Effectiveness of nano-collagen spray of goldfish

(Cyprinus carpio) scales waste in accelerating the incision wound healing process. *Jurnal Kedokteran Gigi Universitas Padjadjaran, 30*(2), 113-119.

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. (2011). Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). *Journal of advanced pharmaceutical technology & research, 2*(1), 3-8.

Sharifi, A., & Khavarian-Garmsir, A. R. (2020). The COVID-19 pandemic: Impacts on cities and major lessons for urban planning, design, and management. *Science of the total environment, 749*, 142391.

Stéphane, F. F. Y., Jules, B. K. J., Batiha, G. E., Ali, I., & Bruno, L. N. (2021). *Extraction of bioactive compounds from medicinal plants and herbs*. Nat Med Plants.

Sudewi, S., Karimah, N., & Nadia, S. (2021). Uji Efektivitas Sedatif Ekstrak Etanol Daun Rambusa (Passiflora Foetida L.) Terhadap Mencit Jantan (Mus musculus). *Journal of Pharmaceutical And Sciences, 4*(2), 93-100.

Svenson, K. L., & Paigen, B. (2019). Recommended housing densities for research mice: filling the gap in data-driven alternatives. *The FASEB Journal, 33*(3), 3097.

Vandekerckhove, M., & Wang, Y. L. (2018). Emotion, emotion regulation and sleep: An intimate relationship. *AIMS neuroscience, 5*(1), 1.

Yasmin, F., Najeeb, H., Moeed, A., Naeem, U., Asghar, M. S., Chughtai, N. U., ... & Pakpour, A. H. (2021). COVID-19 vaccine hesitancy in the United States: a systematic review. *Frontiers in public health, 9*, 770985.

Yulianita, Y., Effendi, E. M., & Firdayani, E. M. (2019). Sedative Effect of Citronella (Cymbopogon nardus (L.) Rendle) Towards Male Mice (Mus musculus*). Indonesian Journal of Pharmaceutical Science and Technology, 1*(1), 16-23.