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Purwoceng (*Pimpinella pruatjan* Molk.) nanosuspension repairs spatial white albino wistar strains' spatial memory degeneration after sleep deprivation

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ABSTRACT. Paradoxical sleep deprivation (PSD) induces oxidative stress and interferes permanent memory consolidation in hippocampus. Purwoceng (*Pimpinella pruatjan* Molk.) is herbal medicine with antioxidant effect. These effects will be more effective with nanosuspension technology. The research purposes were to find effect of PSD to rats' spatial memory reduction and to find Purwoceng nanosuspension extract effect to improve rats' spatial memory after PSD stress induction. The research method is randomized controlled trial with posttest only with control group approach to 36 white male albino rats Wistar strain which is induced by 96 h PSD stress model. Experiment groups is regular extract 25 mg/300g BW/day, Purwoceng nanosuspension extract 25 mg/300 gBW/day, 50 mg/300 gBW/day, and 75 mg/300 gBW/day. Statistical analysis found that there is no significant effect of Purwoceng extract to memory traveling time (p=0.414) and memory track length (p=0.316) between pre-PSD and post-PSD group. There is significant effect of PSD stress after Purwoceng administration to memory track length and memory travelling time (p=0,005). The conclusion is that there is significant correlation between the difference of memory latency and memory traveling time of morris water maze (MWM) test between the various Purwoceng nanoemulsion dose. The 25-50 mg/300 gBW/day is effective dose of Purwoceng nanoemulsion extract to repairs spatial memory degeneration after PSD stress.

Keywords: Antanan gunung; nanoemulsion; paradoxical sleep deprivation; spatial memory; sleep deprivation

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INTRODUCTION

Sleep deprivation (SD) or lack of sleep can disrupt circadian rhythm and physiology mechanism of body (Ma *et al*., 2019). It induces oxidative stress which damages temporary and permanent memory consolidation as we find in Alzheimer, Parkinson, and another brain degenerative disease (Wu *et al*. 2019). Characteristics of these diseases are reduction of quality and quantity of post synapses neuron and hippocampus cell (Havekes, 2017). Paradoxical sleep deprivation elevates glucose metabolisme up to 30 % until there is excess of free radicals, disrupt sleep-and-wake cycle, leads to antioxidant imbalance, oxidative stress in hippocampus and the other central nervous system part (Owens *et al*., 2016). Sleep deprivation intensifies corticosterone level, induces CA3 neuron atrophy and reduces arborization (dendrite branching) in hippocampus. Those effect generates spatial memory problem and anxiety behavior to experimental animals (Konankanchi, 2022).The potential of Purwoceng as an antioxidant is found in bioflavonoid compounds, the exogenous antioxidants that can increase antioxidant levels and suppress free radical formation (Nurcahyanti *et al.*, 2018). Bioflavonoids also activate ascorbic acid which is useful as an antioxidant and neuroprotectant (Teleanu *et al*., 2019). Tests on the antioxidant activity of Purwoceng ethanol extract showed that Purwoceng was able to capture free radicals (Qodri *et al*., 2013).

Indonesian unique herbal medicine (2019), Purwoceng (*Pimpinella pruatjan* Molk.) has antioxidant effect because of phenol and bioflavonoid content). Nanosuspension technology makes colloid particle can be dispersed into submicron size less than 1 μm, with mean sizes is 200 - 600 nm

(Ansari *et al.*, 2012). The technology has been expected to increase absorption, bioavailability, onset, peak level and penetration compared with regular extract. Bioflavonoid as exogen antioxidant can escalate antioxidant body level and reduces ROS free radicals as product of oxidative stress. This research purposes were to find sleep deprivation effect to spatial memory ability and antioxidant ability of Purwoceng nanosuspension. This research uses nano preparation technology that can increase the bioavailability and absorption of active ingredients so that the selectivity, effectiveness and safety of herbs are more higher (Ansari *et al.*, 2012). The release of active compounds can be controlled to minimize side effects and with nano size can be given in high concentrations due to their small size and high loading capacity (Dewandari *et al.*, 2017). We expected that these results can eliminate the disobedience of patient because the available formulations usually use large doses and minimize the lack of drug target specificity for chronic disease.

MATERIALS AND METHODS

This is experimental, randomized controlled trial with posttest only, control group design research. The subjects are 36 white male albino rats Wistar strain, divided into 6 groups: Goup A: PSD with sleep recovery; group B: PSD without sleep recovery; group C: PSD and Purwoceng extract 25 mg/300 gBW/day, group D: PSD + Purwoceng nanosuspension extract 25 mg/300 gBW/day, group E: 50 mg/300 gBW/day; group F: 75 mg/300 gBW/day. The chosen dose is based on recent research by Pribadi, (2012) and Nuryadin dan Nabilla (2018). Their research doses are 25 mg/mL/day for 300-gram body weight or 83,25 mg/kgBW. Nanoemulsion extract dose is 25 mg/3 mL by single dose 3 mL nanoemulsion solution given by oral tube.

The Purwoceng has been obtained from Batur village, Dieng plateau, Banjarnegara. Plant determination has been done in plant taxonomy laboratory of Biology Faculty, Universitas Jenderal Soedirman, Purwokerto. Nanoemulsion process has been done in Pharmacy Laboratory of Pharmacy Faculty, Jenderal Soedirman University, Purwokerto. Purwoceng nanoemulsion extract has been made by mixing purwoceng ethanol extract with Tween 80, stirred with magnetic stirrer 500 rpm for 15 minutes, added by PEG 400 and Virgin coconut oil, and then stirred for 15 minutes. The solution is added by a few aqudest slowly, and then stirred by magnetic stirrer 1000 rpm for 15 min, and then sonicated in 25 – 40°C temperature for 30 min. The nanoemulsion phytochemicals are being screened to measure alkaloid and flavonoid components, followed by nanoemulsion characteristics test, such as organoleptic, transmittance percentage (%T), particle size, and particle size distribution and also Zeta Potential.

Paradoxical Sleep Deprivation. Experimental animals were being placed in stainless steel cage filled with water. It has 24x26x29 cm dimension, two platform with 6 cm diameter. The foothold has muscle atonia shock device which automatically turns on every 10 min to give a shock effect for 1 second that causes a wake-up effect. This treatment is carried out for 96 h with a break between 07.00 – 11.00 WIB (Arjadi *et al*., 2021).

Morris Water Maze (MWM) test. Assessment of the spatial memory ability of rats using the MWM test in a pool with a diameter of 180 cm and a height of 76 cm divided into 4 parts, one of which is given a footing (on the shallow bottom of the water, not on the surface of the water) to stand and stand on. The water filled with the pond consists of water and coconut milk/milk, which is useful for clouding the water, so the rats are judged to find a foothold based on memory. The test takes 7 days, namely the first 5 days the experimental animals are swam 4 times a day within 60 s, until they find a foothold (acquisition trial / acquisition test phase). On the 6^{th} day, the stepping platform was taken and the experimental animals were allowed to swim to find a foothold according to what was remembered (probe trial/probe phase) and recorded the results of the trial probe as initial data for the memory test of the experimental mice and on the 7^{th} day a spatial ability test/sensorineural MWM test/examination trial phase was conducted.

The treatment was documented by video camera and observed the spatial latency time ability test the spatial memory capabilities of the rats by counting the time recorded from the time it was entered

into the pond until it was on a platform. The spatial ability test was tested for the length of the test track to assess memory capabilities. We get spatial data of the rats by measuring the area and distance of the path traveled in finding a foothold, and the results of the comparison of the distance measured in the actual pond diameter with the diameter of the pond in the video, multiplied by the distance in the video. The data is presented in tabular and graphical form, where the X-axis represents the day session from the acquisition trial to the 7th day of testing and the Y-axis represents latency time (in seconds) or track length (in cm) (Buccafusco, 2009).

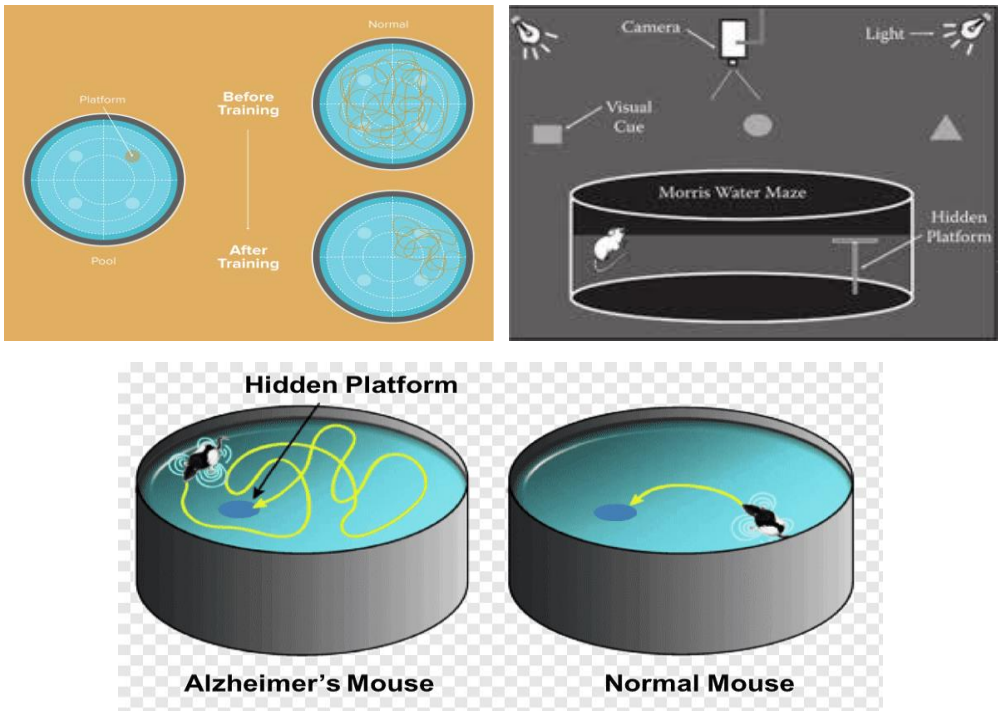

Fig. 1. Test of spatial memory ability of experimental animals: A. Morris Water Maze pool; B. Acquisition test phase; C. Test results between normal and impaired memory.

The MWM test was carried out at the end of each treatment phase as reference data for indicators of spatial memory ability and became the basis for the analysis of the data used. The degree of degeneration of spatial memory abilities is determined by the longer the path length and the longer the latency time required to reach the target. Tests a few days before the sensorineural test (acquisition phase and probe phase) were carried out as a means of practice and trials to increase the frequency of target recognition by footing. The data displayed in the pre-PSD, post-PSD, and post-Purwoceng phases are descriptive data to see the pattern of spatial memory development per experimental rat in the MWM pool and analyzed to get a conclusion. Research ethics were obtained from the Health Research Ethics Commission, Faculty of Medicine, Universitas Jenderal Soedirman, Number 097/KEPK/V/2021 dated 10 May 2021.

Data analysis. The research data was tested for the normality of the data distribution using the Shapiro-Wilk and homogeneity test and data variance using Levene's Test. If the data transformation is normal and heterogeneous, it is analyzed using One Way of Variance (ANOVA) as a parametric test and if it is not normal, it is tested with Kruskal Wallis. The test was continued with the Tukey Post-Hoc test to determine whether there was a significant difference between the control group and the treatment group with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

The transmittance test showed that the size of the clarity and purity of the nanoemulsion formed was 84.5% and although it did not reach 100%, it was sufficient as a marker of the validity of the nanoemulsion solution structurally. The particle size distribution test of the Purwoceng nanoemulsion solution was found to be 123.87±28.9 nm which indicates that the particle size distribution is ideal

because it is in the range of 20-200 nm, so that the stability and distribution of molecules in the dissolution medium is optimal. The results of the polydispersity index of the Purwoceng nanoemulsion solution obtained the number 0.37±0.11 which indicates that the intermediate disparity is between homogeneous and heterogeneous. The results of the zeta potential of the Purwoceng nanoemulsion solution were found to be -16.56±1.19 mV, which indicated good molecular stability of the nanoemulsion solution from particle attraction aggregation, because it was in the range of -30 to 30 mV. The results of the phytochemical screening test showed that the Purwoceng extraction solution was reactive in the alkaloid and flavonoid testing which indicated that the extraction solution was positive for qualitatively qualitative alkaloids and flavonoids.

Table 1. Comparison of observation results of mwm of latency time test data.

Groups	Latency time (seconds)		
	Pre-PSD	Post-PSD	After Purwoceng Treatment
A (PSD Non-Sleep)	20.25	42.25	-
B (PSD + Sleep)	5.25	9.25	-
C (PSD + 25 mg extract)	13	21.25	46
D (PSD + 25 mg nanoemulsion)	15	39.25	27.25
E (PSD + 50 mg nanoemulsion)	7.5	12.5	12.5
F (PSD + 75 mg nanoemulsion)	11	7	12.25

Table 2. Data comparison of observation results long path of mwm examination trial per phase between groups of experimental wistar rats.

Groups	Track length (cm)		
	Pre-PSD	Post-PSD	After Purwoceng Treatment
A (PSD Non-Sleep)	562.5	900.25	-
B (PSD + Sleep)	112	205	-
C (PSD + 25 mg extract)	255	511.25	1013.25
D (PSD + 25 mg nanoemulsion)	321.5	837	487.5
E (PSD + 50 mg nanoemulsion)	180	271.75	190.75
F (PSD + 75 mg nanoemulsion)	197.5	167	2715

Based on the figures in Tables 1 and 2, it can be seen that there is a decreasing trend of spatial memory ability between the average rats after PSD treatment but statistically there is no significant difference between PSD to the decrease in spatial memory ability in rats and sleep recovery does not provide a difference to spatial memory ability. The paired t-test between pre-PSD and post-PSD in group A showed travel time $p = 0.168 > 0.05$ and path length $p = 0.145 > 0.05$ and Wilcoxon test in group B obtained travel time $p = 0.180 > 0.05$ and path length $p = 0.068 > 0.05$. These results differ from the findings of several studies on memory disorders.

Recent research (Heba *et al.*, 2021) stated that 72 hours of PSD treatment through reduced catalase activity and glutathione levels, inhibition of Na/K ATPase in the hippocampus and cerebral cortex, increased acetylcholinesterase and lipid peroxidation which resulted in the induction of oxidative stress and increased brain excitability can reduce memory performance and increase anxiety levels in rats. Recent research by Kamphuis *et al*., (2017) states that the disruptive consequences of degeneration of the prefrontal cortex in experimental rats treated with sleep disturbances for 7 days caused degeneration of the prefrontal cortex with symptoms of slower motor responses and impulsive reflexes. Increased anxiety in post-PSD rats resulted in increased brain excitability and overactivity of motor behavior that made the rat's instincts move faster which increased the length of the trajectory in the sensorineural MWM test.

Atrooz *et al*. (2019) stated that prolonged sleep disturbances increase the incidence of forgetting and cause memory fragmentation such as short and medium-term memory loss due to weakened associative pathways characterized by a decrease in the number of pyramidal and dendritic cells in the CA1 hippocampus area, the main area of memory pathway transmission. According to Wu *et al*.

(2019), the pathophysiological mechanism of memory degeneration due to sleep disturbances is related to glymphatic-vascular-lymphatic clearance in brain macromolecular structures, increased oxidative stress in the brain and decreased levels of melatonin in blood circulation.

Hunter's research (2019) states that there is no evidence of a detrimental effect of REM sleep disturbances on the performance of experimental mice in the acquisition phase of the MWM test, both during retention and reversal and REM is only useful in helping improve learning performance and general memory. Research by Karabulut *et al.* (2019) states that the post-MWM sleep phase, especially during REM sleep, plays a very important role in modulating the consolidation and rapid repair of cells that play a role in memory which can improve the transcription of mRNA genes from pyramidal cells and lead to hippocampal neuroplasticity.

The explanation for the absence of a significant difference between PSD and the role of sleep recovery on spatial memory abilities in this study is still unknown but it is suspected that adequate sleep compensation after PSD and before the MWM acquisition phase to sensorineural testing. The short sleep compensation was able to provide a better consolidation effect on brain and hippocampal performance so that it could reduce the effect of decreasing spatial memory ability which is expected to exist in the rat group. Sleep compensation in PSD was more accommodating than when it was replaced with total sleep deprivation (TSD) treatment where mice were not allowed to sleep at all for several days. According to Tufik *et al*. (2009), adequate compensation from regular sleep-wake cycles can improve neurophysiological and behavioral changes significantly despite having experienced sleep disturbances, indicating that there is no significant difference in spatial memory ability between the groups of rats with sleep recovery and without sleep recovery because all groups received sleep compensation even with different duration.

Based on statistical tests, there was no significant difference between the administration of Purwoceng on spatial memory abilities in rats based on the length of the track and the latency time. The administration of purwoceng non-nanosuspension and nanosuspension extraction at a dose of 25 mg, 50 mg, or 75 mg did not give a significant difference in spatial memory ability. Data analysis using Paired t-test group C (non-nanoemulsion Purwoceng extract) showed travel time $p = 0.271 (> 0.05)$ and path length $p = 0.224 (>0.05)$, group D (Purwoceng nanoemulsion 25 mg/gBW) obtained travel time $p = 0.21 > 0.05$ and path length $p = 0.249 (>0.05)$, Wilcoxon test in group E (Purwoceng nanoemulsion 50 mg/gBW) obtained travel time $p = 0.465 (>0.05)$ and path length $p = 0.465 (>0.05)$, and group F (Purwoceng nanoemulsion 75 mg/gBW) obtained travel time $p = 0.651 (>0.05)$ and path length $p = 0.647 (>0.05)$. Bivariate analysis testing the spatial memory ability of rats after administration of Purwoceng through the One-Way ANOVA test showed a significant relationship between travel time ($p = 0.000 < 0.05$) and track length ($p = 0.001 < 0.05$) in groups C, D, E, and C. and F. The post hoc test showed that the lowest travel time was group D, followed by groups F and E, while the highest travel time was found in group C and in the section on track length, the lowest track length was group E, but not much different with groups D and F.

The explanation of the absence of significant differences between Purwoceng on post-PSD spatial memory ability is not certainly known. It is suspected that the active flavonoid substances in Purwaceng are able to reduce the level of cell aging and the level of tissue inflammation but cannot provide instantaneous improvement in the quality of spatial memory. Flavonoids can modulate neuronal signaling pathways that are important in memory processing, synaptic plasticity, and long-term potentiation mechanisms as the basis for the formation of a memory (Rendeiro *et al.*, 2009) and improve cognitive memory through activation of kinases in the MAPK and P13 kinase pathways and accelerate the process of regulation of the cAMP response element-binding protein (CREB) which is important in the memory storage process in the hippocampus and cerebral cortex (Krishnaveni, 2012), Nanoparticle preparation technology by emulsification was proven to produce significant pharmacological effects which was indicated by the difference in the level of decline in spatial memory ability at different doses of Purwoceng and non-nanoemulsion extract Purwoceng had a

higher rate of degeneration of spatial memory ability compared to the group that received Purwoceng nanoemulsion.

CONCLUSION

There was no effect between the decrease in spatial memory ability and the number of pyramidal cells and hippocampal volume in albino male wistar rats treated with PSD. There was no significant effect of giving Purwoceng nanoemulsion on improving spatial memory ability of albino male wistar rats treated with PSD. There is a significant effect on the difference in latency and path length of the MWM test between doses of Purwoceng nanoemulsion. The dose of 25 - 50 mg is the effective dose of the Purwoceng nanoemulsion which results in a shorter path length and latency time indicating an improvement in post-PSD spatial memory degeneration.

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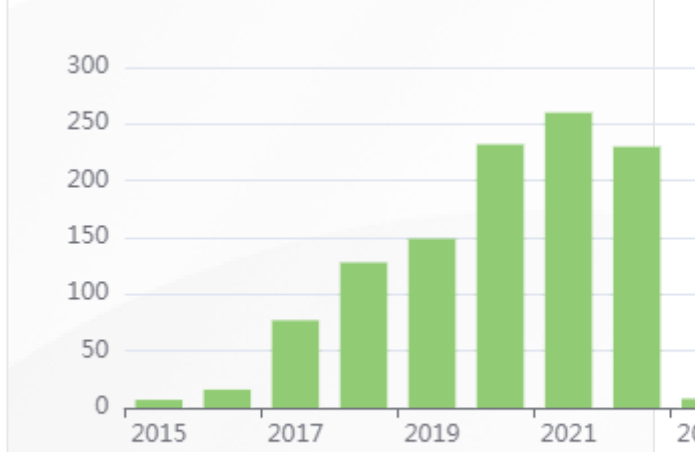

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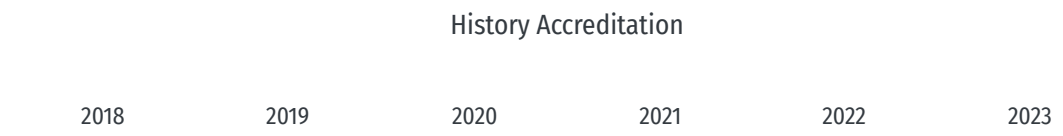

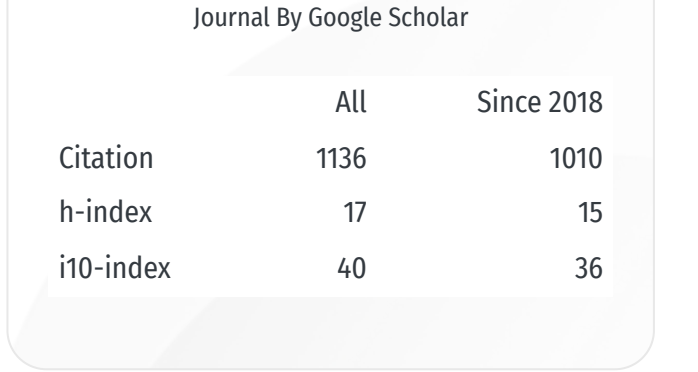

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