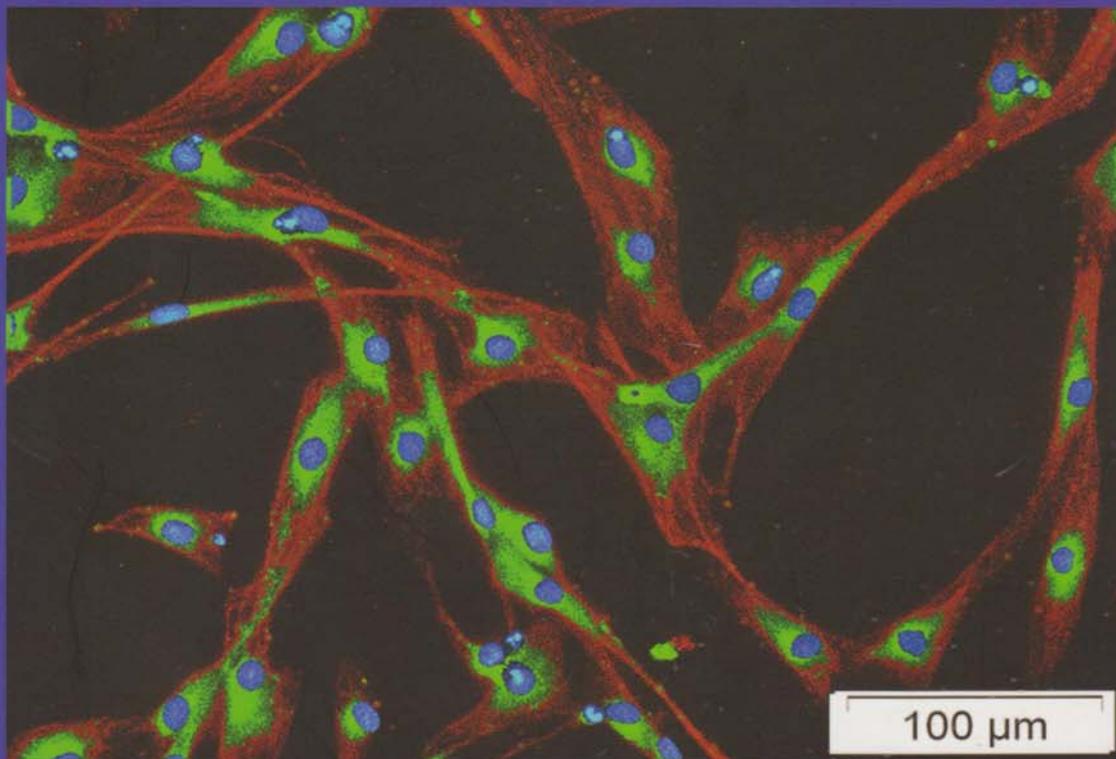


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bioactive component in this plant and the safety of this alkaloid causing adverse drug-drug or drug-herb interaction via P-gp has not been fully investigated. Therefore our main objective is to identify the effects of mitragynine on the gene and protein expression of P-gp. The effects of mitragynine on the mRNA and protein expression of P-gp were investigated in Caco-2 cell line using RT-qPCR and Western Blot analysis, respectively. Mitragynine produced a significant inhibition on the mRNA expression of P-gp at the highest concentration (10 μ M). However, the protein expression was inhibited at much lower concentration starting from 0.1 μ M and onwards (0.1 μ M, 1 μ M and 10 μ M). Mitragynine decreased the mRNA and protein expression of P-gp in a concentration-dependent manner indicating that mitragynine may cause a reduction of efflux of P-gp substrate and thereby may affect the bioavailability of the substrate or drug.

Keywords: Mitragynine, P-glycoprotein (P-gp), Caco-2 cell line.

OR-006

Effect Of Different Aerobic Physical Activities Performed 3 Times A Week And 7 Times A Week On The Concentration Of Serotonin In Brain Tissue Of Wistar Rat

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Serotonin plays an important role in the physiological functions of human body. Serotonin synthesis is determined by the availability of free tryptophan amino acid in the blood and tryptophan hydroxylase enzyme. Physical activity increases the

synthesis of tryptophan hydroxylase enzyme and free amino acid tryptophan in the blood. Physical activity affects the synthesis of serotonin in the brain, but it is not known whether there are differences effect of aerobic physical activity performed 3 times a week and 7 times a week to the concentration of serotonin in the brain tissue of wistar rats. Analyze differences levels of serotonin in the brain tissue wistar rats that treated by aerobic physical activity 3 times a week and 7 times a week. Laboratories experimental studies with post test – only control design. Statistical test using unpaired t-test. Samples were using wistar rats (*Rattus norvegicus*) 27 animals and divided into 3 groups, control group P1, treatment group P2 (physical activity 3 times a week) and treatment group P3 (physical activity 7 times a week). Treatment group P2 and P3 were treated using treadmill at a speed of 20 m/min for 30 minutes. Levels of serotonin in the brain tissue were measured using the ELISA. There was a decrease of serotonin concentration in wistar rat brain tissue between aerobic treatment group to control group. Average concentration of serotonin brain tissue in P1 group (0.66 \pm 0.26), P2 group (0.16 \pm 0.15) and P3 group (0.11 \pm 0.08). There were no significant differences ($p>0.05$) between treatment groups P2 vs P3 ($p=0.35$). Aerobic physical activity performed 3 times a week and 7 times a week had no effect to the concentration of serotonin in the wistar rats brain tissue.

Keywords : serotonin, physical activity, brain tissue.

OR-007

Optimisation of Stereotactic Implantation of Guide Cannulae in the Hippocampal CA1 region by using Motorized Stereotaxis Instrument

EFFECT OF DIFFERENT AEROBIC PHYSICAL ACTIVITIES PERFORMED 3 TIMES A WEEK AND 7 TIMES A WEEK ON THE SEROTONIN CONCENTRATION OF BRAIN TISSUE WISTAR RAT

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Abstract

Background. Serotonin plays an important role in the physiological functions of human body. Serotonin synthesis is determined by the availability of free tryptophan amino acid in the blood and tryptophan hydroxylase enzyme. Physical activity increases the synthesis of tryptophan hydroxylase enzyme and free amino acid tryptophan in the blood. Physical activity affects the synthesis of serotonin in the brain, but it is not known whether there are differences effect of aerobic physical activity performed 3 times a week and 7 times a week to the concentration of serotonin in the brain tissue of wistar rats.

Purpose. Analyze differences levels of serotonin in the brain tissue wistar rats that treated by aerobic physical activity 3 times a week and 7 times a week.

Method. Laboratories experimental studies with post test – only control design. Statistical test using unpaired t-test. Samples were using wistar rats (*Rattus norvegicus*) 27 animals and divided into 3 groups, control group P1, treatment group P2 (physical activity 3 times a week) and treatment group P3 (physical activity 7 times a week). Treatment group P2 and P3 were treated using treadmill at a speed of 20 m/min for 30 minutes. Levels of serotonin in the brain tissue were measured using the ELISA.

Results. There was a decrease of serotonin concentration in wistar rat brain tissue between aerobic treatment group to control group. Average concentration of serotonin brain tissue in P1 group (0.66 ± 0.26), P2 group (0.16 ± 0.15) and P3 group (0.11 ± 0.08). There were no significant differences ($p>0.05$) between treatment groups P2 vs P3 ($p=0.35$).

Conclusion. Aerobic physical activity performed 3 times a week and 7 times a week had no effect to the concentration of serotonin in the wistar rats brain tissue.

Keywords : Serotonin, Physical Activity, Brain

INTRODUCTION

Physical activity plays important role mainly in biosynthesis of serotonin.¹ Serotonin is a chemical substance that is essential in regulating human physiology.² The synthesis of serotonin in axon terminals of brain nerve cells is influenced by tryptophan hydroxylase, an enzyme that catalyzes tryptophan into serotonin. Physical activity increases the expression of TPH2 gene causing increased synthesis of tryptophan hydroxylase.³ It can be said that physical activity can indirectly influence the synthesis of serotonin in brain. Besides, the synthesis of serotonin is determined by the availability of tryptophan. Physical activity increases free fatty acids, so the number of tryptophan bound in albumin is decreased. As a result, the concentration of free tryptophan in blood is increased and this will increase the uptake of tryptophan through blood-brain barrier.⁴

At present, the study related to physical activity and its influence to serotonin synthesis is still limited in number. Therefore, further study is required to investigate the effect of aerobic physical activities in different frequencies to the level of serotonin of Wistar rat brain tissue. The objective of this study was to investigate the difference in serotonin level of Wistar rat brain tissue between rats given aerobic physical exercise three times a week and those given seven times a week.

METHODS

The research design was experimental study using *post test - only control design*. The experiment was conducted in animal house of Pharmacology and Therapy, Faculty of Medicine, Universitas Padjajaran Bandung. The level of serotonin was measured in Laboratory of Molecular Genetics, Universitas Padjajaran Bandung.

The research data were taken in February 2015. The size of sample was calculated using Federer's formula. Each group of study consisted of at least nine Wistar rats. So, the total number of Wistar rats used in this study was twenty seven.

The inclusion criteria for the research subject were male rat of Wistar strain, aged six to eight weeks, weight 130-250 grams, healthy, feasible to do the test, and never used as experimental animal. The exclusion criteria included the rats which were sick on the test, lazy or unable to follow the test well.

The rats were divided into three groups (P1, P2, and P3). P1 was control group, P2 was treatment group consisting of rats given aerobic physical activity three times a week, and P3 was treatment group consisting of those given aerobic physical activity seven times a week.

Before the treatments were given, all groups of study were acclimatized for 5 days by running them on a treadmill in speed of <15 m/min for 10 minutes at maximum.

Aerobic physical activity was applied to the subjects in experimental groups by running them on a treadmill in speed of 20 m/min for 30 minutes. The weight of rat was weighed before and after the treatments. After that, the rats were anaesthetized with ketamine in dose of 40 mg/kgbw, incised, and the brain tissues were taken out for serotonin analysis. The level of serotonin in the brain tissue was measured using ELISA.

All data collected in this study were analyzed statistically using SPSS for windows version 19. Independent *t* test was used to find out whether or not there was a significant difference in serotonin levels between the two groups (significance level $p < 0.05$).

RESULTS

The mean levels of serotonin in Wistar rat brain tissue can be seen below:

Table 1. The Mean Levels of Serotonin of Wistar Rat Brain Tissue in Control Group and Treatment Groups

Group	N	Mean \pm SD (ng/ml)
Control	9	0.66 \pm 0.26
Three times a week	9	0.16 \pm 0,15
Seven times a week	9	0.11 \pm 0.08
Total :	27	

Table 1 showed that there was a decrease in the level of serotonin in brain tissue of rats treated three times a week and seven times a week if compared with control group. The lowest level of serotonin occurred in group given aerobic physical activity seven times a week.

Independent *t* test was used to find out the difference in serotonin level between control group and treatments group. The results of the independent *t* test are shown in the following table:

Table 2. The Serotonin Level of Wistar Rat Brain Tissue in Control Group and Treatment Groups

Group	Mean \pm SD (ng/ml)	p*
Control	0.66 \pm 0.26	
Three times a week	0.16 \pm 0.15	0.00
Seven times a week	0.11 \pm 0.08	0.00

p* Independent *t* test compared with control group

From Table 2, it can be seen that there was a significant difference ($p < 0.05$) in serotonin level between control group and treatment groups.

Independent *t* test was also applied to find out whether or not there was a significant difference in serotonin level between rats treated with three times a week and those with seven times a week. The results of independent *t* test are shown in the following table:

Table 3. The Level of Serotonin of Rat Brain Tissue in Control Group and Treatment Groups

Group	Mean \pm SD (ng/ml)	p*
Three times a week	0.66 \pm 0.26	0.35
Seven times a week	0.11 \pm 0.08	

p* Independent *t* test among treatment groups

It can be seen from Table 3 that there was no significant difference ($p > 0.05$) between serotonin levels of rat brain tissue between group given aerobic physical exercise three times a week and that given seven times a week.

DISCUSSION

The results of this study showed that there was a significant difference ($p < 0.5$) in serotonin level of brain tissue between treatment groups and control group. The mean levels of serotonin in treatment groups both in three times a week (0.16 \pm 0.15 ng/ml) and seven times a week (0.11 \pm 0.08 ng/ml) decreased if compared with control group (0.66 \pm 0.26 ng/ml). The decrease occurring in the treatment groups of three times a week was lower than that of seven times a week (0.16 \pm 0.15 ng/ml vs 0.11 \pm 0.08 ng/ml).

This could be explained that there was a recovery phase in aerobic physical activity conducted three times a week but not in that of seven times a week. This suggests that physical activities conducted three to five times a week is recommended. The period of recovery enables our body to recover energy loss and repairs damaged cells during physical activities.⁵

Moreover, aerobic physical activities in this study were conducted only in one cycle (acute exercise), not continuous and not in long period of time. This condition influenced the synthesis of serotonin in brain tissues. As it was one cycle treatment, it was assumed that the rats could not adapt well to the treatments given. A study of Wang (2013) appealed that the levels of serotonin in rats induced with stress but not given physical activities decreased if compared with those in control group.

According to McArdle (2001), body adaptation response to physical exercises will appear at least four weeks after the treatments were applied. Adaptation response will appear only if exercises with adequate intensity and long duration were given. Theoretically, tissue adaptation occurs as a response to a physical stress. A study of Fiatarone *et al.*, (1994) stated that physical exercises in old men would give positive effects after doing ten-week physical exercises. It suggests that our body needs a certain period of time to adapt to physical exercises in order to get positive effects to our body.

The results of this study were in line with those of Chen's (2008). She used *Sprague-Dawley* rats and ran them for 9 m/min for 60 minutes. The study lasted four weeks. The results showed decreased serotonin levels in hippocampus. On the other hand, the results of this present study were

contradictive to Wilson's (1996). The subjects he examined were *lister hood* rats treated for 5-6 weeks on a treadmill for 20 m/min for sixty minutes a day. Microdialysis was used to measure the level of serotonin in ventral hippocampus. The results indicated that the level of serotonin in treatment groups increased if compared with control. The results of this present study were not also in line with a study of Malek's (2007) in which there was an increased expression of TPH2 genes in *raphe* nuclei of rats on voluntarily locomotor activities for six weeks.

Serotonin or *5-hydroxytryptamine* (5-HT) is a monoamine neurotransmitter in nerve cell synapses.^{1,12} The majority of serotonin, approximately 95%, can be found in the gastrointestinal tract (90% in *enterochromaffin* cells and 5% in enteric nervous system) and the remaining 5% is found in brain.¹³

Serotonin plays an important role in human physiology mainly in regulating such things as thermoregulation, eating, cardiovascular system, reproductive system, pain control, aggression, sleep cycle, memory, stress response, cognition, mood, and emotion.² Besides, serotonin is also important in the growth of new nerve cells (neurogenesis) in *dentate gyrus* of *hippocampus*. *Hippocampus* has very important role in memory.¹⁴

This study also indicated that there was no significant difference in serotonin level between treatment group given aerobic physical exercise three times a week and that given seven times a week ($p = 0.35$). It could be caused of the intensity of aerobic physical exercises in this study which were still in medium category. In addition, the exercises were not conducted in long period of time. Therefore, the adaptation response occurring in these two treatment groups caused the levels of serotonin in both groups decreased but were not different statistically. So, it can be said that there was no significant difference in both treatment groups. The decrease of serotonin levels in both treatment groups could be caused by the stress response coming from aerobic physical activities given. Moreover, aerobic physical activities conducted in this study were exercises not trainings or other repetitive exercises conducted for months. According to Supriadi (2000), trainings can make important changes in body. On the other hand, exercises only produce less important ones. The changes occurring while someone is doing exercises are called response, and those coming after training are called adaptation.

Conclusions. The level of serotonin in brain tissues in group given aerobic physical activities three times a week was 0.16 ± 0.15 ng/ml, and that given seven times a week was 0.11 ± 0.08 ng/ml. There was no significant difference in serotonin level of rat brain tissue between treatment group given aerobic physical exercise three times a week and that given seven times a week ($p > 0.05$). There was a decrease in the levels of serotonin in treatment groups if compared with control group.

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