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THE EFFECTS OF KALIANDRA HONEY (*Calliandra Calothyrsus)* ON OXYGEN SATURATION (SpO_2) IN RATS EXPOSED TO PHYSICAL STRESS

Saila Azkiya.[1], Bambang Wiratmadji.[2], Bambang Purwanto.[3], Rita Ismawati [4]

[1]Department of Public Health, Faculty of Public Health, University of Airlangga.
[2]Department of Public Health, Faculty of Public Health, University of Airlangga.
[3]Department of Physiology, Faculty of Medicine, University of Airlangga.
[4]Department of Home Economics Education, Faculty of Engineering, State University of Surabaya.

**Corresponding author: Saila Azkiya, Faculty of Public Health, University of Airlangga (sailaazkiya@gmail.com)*

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ABSTRACT

Background: Physical stress in the form of increase in the maximum body workload lead to oxidative stress. Oxidative stress is the condition of imbalance between free radicals and antioxidants. This is indicated by decrease in oxygen saturation (SpO_2). Kaliandra honey is type of honey that is widely consumed in Indonesia. Kaliandra honey content of high antioxidant that is believed to prevent oxidative stress.

Materials and Methods: Twelve male rats (age 2 months: body weight 170-200 g) were recruited and assigned to perform physical stress model with keliandra honey consumption. Type experimental laboratories research using randomized post test control group design. There are four group of rats; normal variation (NC), physical stress (PC), giving honey 24 hours before physical stress (T1) giving honey 2 hours before physical stress (T2).

Result: The mean of SpO_2 rats was within under normal classification. The results showed that the mean SpO_2 level of NC was significant compared to the other group PC, T1 and T2 while there was no significant difference between PC, T1 and T2.

Conclusion: Physical stress success to create blocakge in the blood flow to the muscles. Proven with low oxygen saturation levels. The decrease in oxygen saturation to the muscles leads to an increase in free radical formation. The consumption of kaliandra honey and did not succeed in preventing oxidative stress.

Keywords: physical stress, oxygen saturation, honey, antioxidant, oxidative stress

1.0 Introduction

Physical stress is easy to find but can not be avoided in everyday life, therefore it necessary to find a solution. Physical stress phenomena are not only found in trained individuals such as athletes and the military army, but also in individuals not trained like communities. Communities often experience physical stress in unpredictable conditions such as chasing trains, climbing stairs to the third floor, wake-up work and workers pursued project completion targets.

Physical stress triggers oxidative stress, an imbalance between free radicals and antioxidants (Briben et al., 2012). Physical stress leads to the formation of free radical production like reactive oxygen species (ROS) and decreased production of endogenous antioxidants. During physical stress muscle contraction results in continuous rection oxygen species (ROS) (Martin, 2010). The oxidative stress encountered following physical stress is characterized by decreased oxygen saturation (SaO2), elevated levels of malondialdehide (MDA), and decreased superokside dismutase (SOD) activity (Olah, 2015).

During this time, physical stress is overcome by efforts to improve maximum work capacity through sports activities and through improved body response, both immediately and continuously. Maximum work capacity improvement is usually done by trained individuals such as athletes and military circles, whereas in individuals not ordinary telemas increase the body's response is done by consuming antioxidants. Consumption of antioxidants can be proteins, vitamins, minerals and nutritious natural ingredients (Purwanto, 2014).

Natural ingredients that have properties as antioxidants have the characteristics of easy to experience oxidation, causing the color changes to brownish and produces gas (bubbly). One of the natural ingredients that is suspected to have antioxidant character is honey. Honey is widely circulated in the market and is widely known by the community is honey Kaliandra because the tropics give suitable environtment to grow the plant. Kaliandra honey is cheeper than another kind of honey. Kaliandra honey has the highest total phenolic and flavonoid content compared to coffee, palm, randu and rambutan honey (Chayati et al., 2014). Phenolics and flavonoids are one type of exogenous antioxidant capable of against free radicals.

2.0 Materials and Methods

2.1 Participants

The population of experimental animals was male rats wistar strains aged 2-3 months with a weight of 170-200 grams. The sample size of each treatment group was determined using the formula (t-1) (n-1) $\geq$15, n $\geq$ 5 (rounding). The sample size of each treatment group is at least 5 rats. When a large estimate of 20% failure, the multiplier factor is determined by the formula 1/1-f, ie 1 / 1-0.2 = 1 / 0.8 x 5 = 6.25, so the number of samples is rounded to 7 rats per group. The total subjects of the study were 4 groups x 7 mice = 28 tars, of which 20 were used as observational objects, while 8 rats were used as reserves. The inclusion criteria were white rats *(Rattus norvegicus)* strain wistar age of rats ± 2-3 month with body weight 170-200 gram. On the visual observation of healthy looking rats, the fur looks shiny, clear eyes and move on. Microscopically no abnormalities were found. Never been the object of research. Exclusion criteria were sick mice, characterized by dull or falling feathers, non-active movements, and / or abnormal exudates, mice that experienced weight loss of more than 10% after adaptation in the laboratory, dead mice during the study (Powers, 2008).

2.2 Experimental Design

Type of research is experimental laboratories with randomized post test control group design. Start from April-June 2018. Rat observation done at Biokomia Laboratory Faculty of Medicine, University of Airlangga. The variables studied were SpO_2. Type of honey used in this study is kaliandara honey obtained from the center of honey farmers in Pare, Kediri, East Java. Antioxidants in honey are flavonoids.

The number of doses of flavonoids in honey refers to Grosso et al., (2017) that is 100 mg / day for adult humans. If converted to a dose of rats to 1.8 mg (Anroop, 2016). If in 100 g of honey potassium contains 44.46 mg of flavonoids (Chayati, 2008), honey is needed 1.8 mg / 44.46 mg x 100 g = 4.04 g flavonoids. If 1 ml of honey = 1.57 g, then required 4.04 /1.57 = 2.57 ml of honey kaliandra per sample. Honey is given orally and 4 hours before giving honey feeding is stopped to prevent mice vomiting.

The protocol refers to Lin et al., (2017), which is swimming stress with 3% load of rat body weight for 30 minutes. Time 30 minutes obtained from the results of preliminary research by swimming rats, than take 80% capacity of exhausted swimming stress. After swimming we observe the SpO_2 with Pulse Oximetry.

2.3 *Preliminary testing*

First rats we using digital scales for weight monitoring before and after the acclimation process. Weighing is done 3 times by the animal cage technician and then taken the average of the repetition. After weighing the mice in acclimatization for 1 week. Furthermore, the rats were weighed on the 7th day. On 7^{th} day T1 group was given honey 24 hours before physical stress, while T2 group was given honey 2 hours before physical stress on day 8th. In day 8th PC group, T1 and T2 were treated with physical stress with a maximum capacity of exhasusted swimming stress.

2.4 *Statistical analysis*

Data were statistically analysis using SPSS 2.4 software. Kolmogorov Smirnov Test for Normality, with significance value $p > 0,05$. Furthermore, homogenity test using Levene test at 95% confidence level ($\alpha = 0,05$), and using Anova to know difference between group. The analysis continued with the LSD test to see more clearly the differences between each groups.

3.0 Result

3.1 Oxygen Saturation Data

The mean of oxygen saturation are shown in Table 1.

Table 1. Oxygen saturation of rats

Group	Mean ± SD
Negative control (NC)	94 ± 1,58
Positive control (PC)	67,4 ± 6,46
Treatment 1 (T1)	71,6 ± 4,93
Treatment 2 (T2)	68,6 ± 5,68

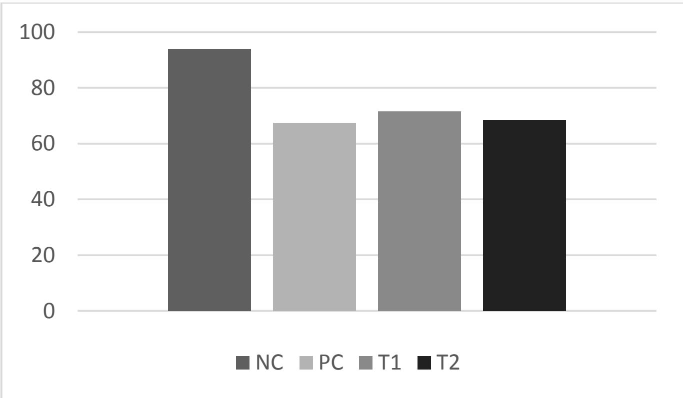

Figure 1. Reduced oxygen saturation of the rats during physical stress

4.0 Discussion

The mean of rats was within under normal classification of SpO_2 intake was 67,4 % (PC), 71,6 (T1) and 68,6 (T2). Our finding showed that the physical stress could reduce SpO_2 but the consumption of honey could not effective to protect the tissue muscle of rats during stress. Numerous studies have shown that physical stress could lead to oxidative stress. Physical stress is shown to cause oxidative stress. Some research results correlate physical stress caused by maximal physical activity hinga cause exhausted. It is generally known that physical activity increases the formation of free radicals. High intensity physical activity leads to oxidative stress in blood and organs in both rats and humans (Tanskanen et al., 2009).

Sports endurance exercises are known to increase DNA damage, athough with antioxidant supplementation (Alessandro et al., 2015).

Physical stress causes unbalanced ROS and antioxidant defenses, causing oxidative stress in myocardium. Increased ROS formation by the mitochondrial electron transport chain, NADPH and xanthine oxidase activate various variations of hypertophy signaling kinases and factor transcription that induce apoptosis by DNA and mitochondrial damage, also activate the pro-apoptotic kinase signal (Olah et al., 2014). In particular, it has been shown that physical exercise reduces antioxidant levels and increases lipid peroxidation markers in the blood and tissues. Therefore, antioxidant supplementation may protect against oxidative stress induced by exercise by forming less active radicals or free radicals and ROS (Yan and Hao, 2016)

Oxygen consumption in muscle increases 50 times when physical stress. This increase in the amount of oxygen consumption serves to tolerate stressful states (Boas et al., 2011). The amount of oxygen carried dependent on the hemoglobin in the blood is called as SpO_2 and this forms the main mechanism for the transport of oxygen to the cell. Oxygen saturation measurements provide information on hypoxia (Daglioglu et al., 2013). Hypoxia induces a mismatch between energy supply and demand that has the potential to cause cell death and organ failure. Studies have shown that hypoxia increases the production of reactive oxygen (O2) and nitrogen species, and limits the antioxidant capacity in red blood cells. In addition, cross-linked membrane proteins and extensive peroxidation of membrane lipids may occur, which may cause changes in fluidity, such as decreased membrane potential and increased permeability of different ions, leading eventually to haemolysis. A good systemic and cellular response will support global convective O2 delivery for progressive tolerance of hypoxia, as parallel increases in oxidative stress with hypoxia (Zhao et al., 2017).

Cell adaptation for hypoxia involves NO homeostasis involving the regulation of blood vessels to increase oxygenation and tissue supply with glucose and other substances. As signal transduction molecules, NO plays an important role in the execution of bodily physiological functions. Its main function is smooth muscle relaxation and stimulation of hypoxic dilatation and has other functions to inhibit platelet activation, neurotransmission, and inflammatory modulation (Zhao et al., 2018). Superoxide dismutase (SOD) help maintain NO homeostatis. Superoxide dismutase is an enzymatic antioxidant involved in superoxide anion cleaning and catalytically converts O2- into oxygen and H2O2. This reaction requires metal ion cofactors such as copper, zinc or manganese (Balasaheb, S and Pal, D., 2015).

The results showed that the mean SpO_2 level of negative control group (NC) was significant compared to the other treatment groups. This means swimming treatment successfully create barriers in the blood flow to the muscles. Proven with low oxygen saturation levels. The decrease in oxygen saturation to the muscles leads to an increase in free radical formation. The administration of kaliandra honey did not prevent oxidative stress, as there was no significant difference between positive control group (PC), treatment 1 (P1), treatment 2 (P2). This means that honey is not able to increase bioactivity and homeostasis NO to increase oxygenation and tissue supply with glucose and other substances.

5.0 Conclusion and recommendation

Physical stress treatment manages to block the flow of blood to the muscles characterized by low oxygen saturation levels. The decrease in oxygen saturation to the muscles leads to an increase in free radical formation. The consumption of kaliandra honey could not prevent the oxidative stress biomarkers. Its need to compare consumption with other natural food that contain minerals to be metal ion cofactors of SOD that can maintain NO homeostatis regulation. So it is necessary to consume complementary food as metal ion cofactor so SOD can clean ROS and prevent oxidative stress.

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Declaration

Author(s) declare that they have no conflict of interest regarding publication of this manuscript.

Authors contribution

Author 1:carried out the experiemental trials, performed the statistical analysis and drafted the manuscript

Author 2:participated in the design of the study and edited the manuscript

Author 3:participated in the design of the study and edited the manuscript

Author 4:participated in the design of the study and edited the manuscript

References

Alessandro, Pingitore. Giuseppina, P. P. L. Francesca, M. Quinones, A. Lervasi, G. Vassalle, C. 2015. Exercise and Oxidative Stress: Potential effects of Antioxidant Dietary Strategies in Sports. Vol 31, Issue 7-8, July-August 2015, Pages 916-922.

Anroop B, Nair. Shery, Jacob. 2016. Simple Practice Guide Dose Conversion Between Animals and Human. J Basic Clin Pharm, March 2016-May 2016: 7(2): 27-31

Boas, David A., Franceschini, Maria A. 2011. Haemoglobin Oxygen Sturation as a Biomarker: the Problem and a Solution. *Phil Trans R. Soc A*. 369, 4407-4424 doi:10.1098/rsta.2011.0250

Briben, e., Sahiner UM., Sackesen MD., Erzurum, S., Kalayci, O. 2012. Oxidative Stress and Antioxidant Defense. WAO Journal; 5;9-19.

Chayati, Ichda. 2008. Physicochemical Properties of Monoflora Honey from the Special Territory of Yogyakarta and Central Java. *Agritech Journal*. https://doi.org/10.22146/agritech.9779

Daglioglu, Onder., Mendes, Bekir., Bostanci, Ozgur., Ozdal, Mustafa., Demir, Tuncer. 2013. *Australian Journal of Basic and Applied Science* 7(10):446-449

Galvano, Fabio., Giovannucci, Edward L. 2017. Dietary Flavonid and Lignan Intake and Mortality in Prospective Cohort Studies: Systematic Review and Dose-Response Meta-Analysis. American *Journal of Eidemiology*. Volume 185, Issue 12, 15 June 2017, Pages 1304–1316,https://doi.org/10.1093/aje/kww207

Grosso, Giuseppe., Micek, Agnieszka., Godos, Justyna., Pajak, Andrzej., Sciacca, Salvatore.,

Lin, Chueh-Yu., Jhang, Yan-Shu., Lai, Shang-Chi., Chen, En-LI, Lin, I-Hsin., Chang, Tug-Wu., Tseng, Yu-Wei., Seenan, Vaishnavi., Shiung, Jane., Chen, Ming-Hua., Chiang, Yun-Chen., Chen, Tsu-I., Tzeng, Yin-Jeh. 2017. Antifatigure Properties of Tanshione IIA in Mice Subjected to the Forced Swimming Test. *Pharmaceutical Biology*. Vol 55. No 1, 2264-2269.

Martin, Ducos and Tabarinde, Antonie. 2010. Exercise, Training and the Hypothalamo-Piturity-Adrenal Axis. Chapter Hormone Use and Abuse by Athletes Volume 29 of the series *Endocrine Updates* pp 9-15.

Nimse, S B. Pal, Dilipkumar. 2015. Free Radicals, Natural Antioxidants and Their Reaction Mecanism. *Royal Society of Chemistry*. Page 27986-28006.

Olah, A., Nemeth, BT., Matyas, C., Horvath, EM., Hidi, L., Britalan, E., Kellermayer, D., Ruppert, M., Merkely, G., Szabo G., Merkely, B., Radovits, T. 2015. Cardiac Effect of Acute Exhaustive Exercise in a Rat Model. *International Journal of Cardiology* 258-266.

Powers, S.K and Jackson M. J. 2008. Exercise-induced oxidative stress:cellular mechanisms and impact on muscle force production, *Physiological Reviews*, vol.88, no.4, pp.1243–1276,

Purwanto, B., Harjanto., Sudiana, Ketut I. 2016. Curcuminoid Prevents Protein Oxidation but not Lipid Peroxidation in Exercise Induced. Muscle Damage Mouse. *Procedia Chemistry*. Volume 18, Pages 190-193.

Tanskanen, M., Atalay, M., Uusitalo, A. 2009. Altered Oxidative Stress in Overtrained Athletes. *Journal of Sport Science*. Vol 28, 2010, Issue 3

Yan, Feiwei., Hao, Haito. 2016. Effects of Laminaria Japonica Polysaccahides on Exercise Endurance and Oxidative Stress in Forced Swimming Mouse Model. *Journal of Biological Research-Thessaloniki*.

Zhao, W J. Yan, Y B. Increasing Susceptibility to Oxidative Stress by Catarac-causing Crystallin Mutation. *Int J Biol Macromol.* Epub 2017 Dec 6