Journal of Medicinal and Chemical Sciences 7 (2024) 590-597

Journal of Medicinal and Chemical Sciences

Journal homepage: http://www.jmchemsci.com/

Original Article

The Effect of Administration of Honey on Physical Activity in Malondialdehyde, Glutathione, and Superoxide Dismutase Blood Levels in Male Rat

Makkasau Plasay[1,*], Lukman Muslimin[2]

[1]*Department of Nursing Pharmacology, STIKES Panakkukang, Makassar, South Sulawesi, Indonesia*
[2]*Department of Pharmaceutical Chemistry, Almarisah Madani University, South Sulawesi, Indonesia*

ARTICLEINFO

Article history

Receive: 2023-11-15
Received in revised: 2023-12-20
Accepted: 2023-12-24
Manuscript ID: JMCS-2311-2373
Checked for Plagiarism: **Yes**
Language Editor:
Dr. Fatima Ramezani
Editor who approved publication:
Dr. Mehrdad Hamidi

DOI:10.26655/JMCHEMSCI.2024.4.3

KEYWORDS

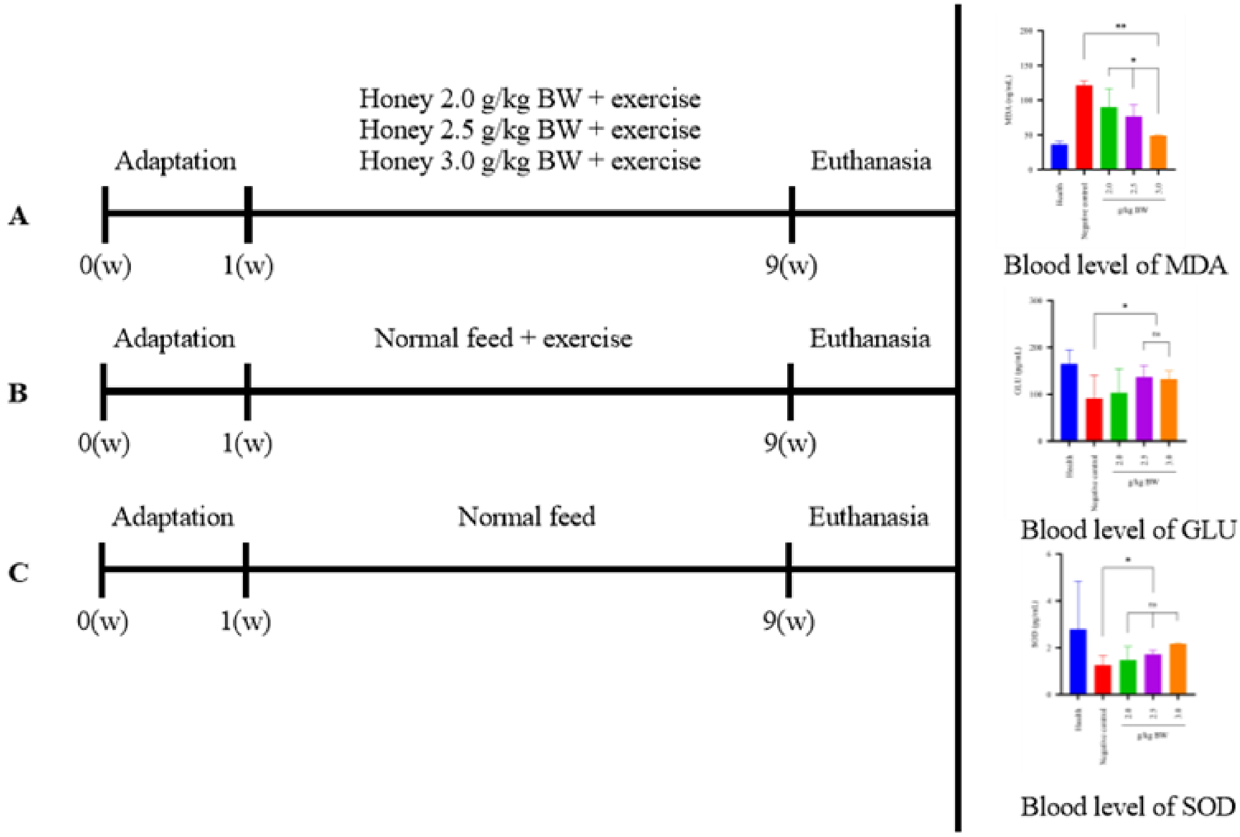
Antioxidant
Honey
Physical exercise

ABSTRACT

Physical activity creates an imbalance between the production of free radicals and the body's antioxidant defense system, known as oxidative stress. Physical activity increases the need for oxygen, improving free radical products. Thus, causing increased levels of malondialdehyde (MDA), glutathione (GLU), and superoxide dismutase (SOD) can be overcome by consuming honey that contains antioxidants. This study aimed to determine the effect of honey administration on the physical activity of MDA, GLU, and SOD blood levels in male Wistar rats. Rats were then divided into 5 groups: healthy control, negative control, and M1 to 3 (giving 2.0, 2.5, and 3.0 g/kg BW (gram per kilogram body weight) of honey twice weekly for 8 weeks). High-intensity swimming exercises were performed for 1 h/day, 5 days/week for 8 weeks. According to the manual kit, the blood levels of MDA, GLU, and SOD were carried out by the ELISA method. Administration of 3 g/kg BW of honey significantly decreased MDA blood level (49.18 ± 0.74 ng/mL) and increased SOD blood level (132.43±10.30 pg/mL) compared to all groups ($p < 0.05$). The blood level of GLU significantly increased in all honey compared to the negative control, but no significant ($p > 0.05$) difference existed between honey groups. It may be possible that honey supplementation following physical exercise would effectively attenuate the antioxidant status.

* Corresponding author: Makkasau Plasay
✉ E-mail: makkasau_mkes@yahoo.co.id

G R A P H I C A L A B S T R A C T



Introduction

Physical activity and exercise have positive and negative impacts on mental health. Physical activity carried out regularly and measurably can improve various components of physical fitness, increase performance for athletes, and reduce the risk of disease [1]. Moderate-intensity exercise can improve health and maintain cardiopulmonary fitness. Exercise also negatively impacts, causing an imbalance between reactive oxygen species (ROS) and antioxidants, leading to fatigue. Exercise increases the formation of oxidant compounds, followed by oxidative stress events.

Oxidative stress occurs due to an imbalance between the production of oxidants and antioxidants. Aerobic exercise can increase oxygen consumption in the body and skeletal muscles. Increased oxygen consumption during exercise increases the ROS formation and triggers oxidative stress [2-4].

One way to determine the degree of oxidative stress is by measuring plasma malondialdehyde (MDA) levels. The body has an endogenous antioxidant defense system and exogenous antioxidants to ward off free radicals [5]. Antioxidants are substances that can delay, prevent, or eliminate free radicals. The body's primary antioxidants, such as glutathione, will increase as blood MDA levels increase [6]. Glutathione, also called Glutathione Sulphydryl (GSH), is a protein naturally produced in the body, which plays a vital role in the immune system and cell regeneration and has antioxidant and anti-toxin properties. The molecule consists of 3 amino acids: glutamate, cysteine, and glycine. Glutathione also contains S – H groups, which can neutralize free radicals (hydroperoxides), namely radicals that can increase O_2 pressure in erythrocytes [7]. Apart from glutathione, an enzymatic antioxidant that is no less important is Superoxide dismutase (SOD).

SOD is one of the most effective intracellular enzymatic antioxidants and catalyzes the conversion of superoxide anion to dioxygen and hydrogen peroxidase. Accordingly, the role of SOD is needed to prevent organ damage [8, 9]. To prevent damage due to exposure to radicals during physical activity, you can increase your intake of food and drinks rich in antioxidants, such as honey. Honey is a sweet, thick food substance made by honeybees and several other insects. Bees produce honey from plant sugar secretions (flower nectar) or other insect secretions (such as honeydew or insect honey). The main content of honey is carbohydrates, especially fructose (around 38.5%) and glucose (approximately 31.0%) [10]. No less critical, honey also contains several compounds thought to function as antioxidants, including phenolic compound 26.9 mg GAE/100 g (gallic acid equivalent per hundred gram) and quercetin levels from 0.28 to 2.68 mg GAE/100 g [11]. Kaempferol, chrysin, pinobanksin, luteolin, apigenin, pinocembrin, genistein, hesperetin, p-coumaric acid, naringenin, gallic acid, ferulic acid, ellagic acid, syringic acid, vanillic acid, and caffeic acid are a few of the phenolic compound that has been found in honey [12]. Honey has been widely reported as a source of natural antioxidants to help improve stamina during exercise [13-15]. However, you need to know that many types of honey have different qualities. The quality of honey varies depending on the type of bee producing the honey, and geographical conditions will influence environmental conditions [16, 17].

This study will evaluate the effect of honey on blood levels of Malondialdehyde, Glutathione, and Superoxide Dismutase after physical activity using a male rat animal model. The honey used in this study is honey from Camba, Maros Regency, South Sulawesi. Camba is a green and shady area of hills and mountains. The Camba region is a medium plain area (around 340 meters above sea level) that has a cool climate.

Materials and Methods

Animals

Twenty-five 12-week-old Wistar rats with an initial body weight of 180-200 g were obtained from Almarisah Madani University. The Health Research Ethical Committee approved the experimental protocol, STIFA Makassar (Protocol No 212/EC.1.1.B/VIII/KEPK/2023). Standard chow in pellet form (commercial pet, Indonesia), and water were provided *ad libitum* to all rats in all groups throughout the study. The animals were provided with sufficient space and housed at room temperature, with a 12:12 light-dark cycle.

Honey preparation

Raw honey was harvested from wild honeybee species (Camba, Maros, Indonesia). The sample was collected in June 2023. The honey was stored in a glass jar at 4 °C until further use. The honey was diluted with distilled water at a 1:1 ratio upon administration.

Animal grouping and exercise protocol

The rats were divided randomly into 5 experimental groups, with 5 in each group. Group I served as healthy control (without physical exercise), while group II (negative control) was exposed to high-intensity swimming exercises. The exercise protocol was performed in a swimming pool following protocols as described previously with slight modification [18].

Groups III to V were study groups induced orally with 2.0, 2.5, and 3.0 g/kg BW (gram per kilogram body weight) of honey twice per week for 8 weeks and exposed to high-intensity swimming exercises. High-intensity swimming exercises mean the rats do swimming exercises for 1 h/day, 5 days/week for 8 weeks. All the rats of the exercise group swam simultaneously in separate water tanks with a calculated average of 300 cm^2 of water surface area for each rat and a depth of 60 cm at a water temperature of 35 °C ± 1 °C. An electric hair dryer was used to dry the body immediately upon removal from the water.

Blood collection and analysis

After 8 weeks of the experimental period, the rats were anaesthetized by ketamine (2.0 mg/100 g BW, ip). They were decapitated using a small guillotine. Blood was collected into the 10 mL test tube, allowed to clot completely, and subsequently centrifuged at 4 °C for 15 minutes at 4000 RPM (revolutions per minute). The serum was collected and stored at -80 °C for subsequent analysis.

Analysis of malondialdehyde, glutathione, and superoxide dismutase

Levels of serum malondialdehyde, glutathione, and superoxide dismutase were measured using commercial ELISA kits (Rat MDA ELISA Kit, ER1878; Rat Gpx1 ELISA Kit, ER0274; and Rat total SOD ELISA Kit, ER1950).

Statistical analysis

Statistical analysis was performed using the Statistical Package of Social Sciences (SPSS) Version 18.0. All the data are presented as mean ± SD. One-way analysis of variance (ANOVA) to determine the significance of the difference between groups and Duncan post hoc test was used to confirm the results. The p-value of < 0.05 was considered statistically significant and used for all the comparisons.

Results and Discussion

Physical activities such as exercise or high-intensity daily work can cause oxidative stress, especially in muscles. Increased MDA levels characterize oxidative stress. Elevated blood serum MDA levels in the long term will result in heart disease, diabetes, and permanent muscle damage [19]. Naturally, the body will produce natural antioxidants to neutralize MDA. However, if MDA levels exceed the body's ability to neutralize it, it will have fatal consequences for the body [20]. External antioxidants are needed to help neutralize these radicals, such as from food and drinks.

The MDA levels in the blood of rats after being measured are displayed in Figure 1. Blood MDA levels increased after heavy activity, as seen from the negative control with MDA levels of 121.50 ± 6.16 ng/mL. Administration of honey reduced blood MDA levels significantly compared to the negative control ($p < 0.05$). However, the most significant reduction was shown by the 3.0 g/kg BW dose of 49.18 ± 0.74 ng/mL, significantly different from the other doses. This shows that giving honey is significantly able to neutralize MDA radicals that are released during the process of physical activity.

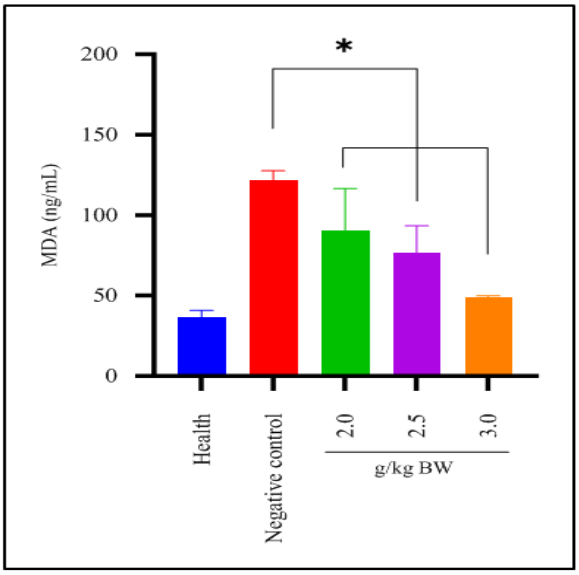


Figure 1: Effects of honey treatment on serum MDA concentration after high-intensity swimming exercises. The values are expressed as mean ± SD (n = 5). Values with superscript letters (*) are significant to negative control

This study investigated honey's ability to improve the body's antioxidant status after physical activity. During physical activity, there is an increase in oxygen in the body, and oxidative phosphorylation will occur in the mitochondria. Oxygen will enter the electron transport system in the mitochondria to be reduced, and then produce water and Adenosine Tri Phosphate (ATP). During the oxidative phosphorylation process, oxygen molecules will bind to single electrons that leak from the electron carrier in the respiratory chain and produce free radicals [21]. The free radicals formed are superoxide radicals (O^*_2), which will then form reactive hydroxyl (OH^*) and hydrogen peroxide (H_2O_2). The free radicals formed can later cause a lipid peroxidation process, which produces MDA [6]. A high increase in MDA levels was seen in this investigation after rats carried out physical activity. Giving honey can reduce blood serum MDA levels. It contains antioxidants from phenolic acid derivates such as kaempferol, chrysin, pinobanksin, luteolin, apigenin, pinocembrin, genistein, hesperetin, p-coumaric acid, naringenin, gallic acid, ferulic acid, ellagic acid, syringic acid, vanillic acid, and caffeic acid [12]. According to Stagos *et al*., honey obtained from Mount Olympus, Greece, has high levels of flavonoids and total polyphenols, which correlate with antioxidant and antibacterial activity [22]. This is in line with research published by Kamilatussaniah *et al.* (2015), which states that honey can reduce MDA levels and increase the body's antioxidant status after exposure to the heavy metal lead [23]. As the body's natural antioxidant, the role of GLU cannot be ignored. The body will produce natural antioxidants such as GLU and SOD in response to increasing MDA levels. This can be seen from the negative control, which experienced an increase in GLU levels of 65.99 ± 9.34 pg/mL, which was significantly different ($p<0.05$) from all treatment groups (Figure 2).

Giving honey 2.0, 2.5, and 3.0 g/kg BW showed increased GLU levels compared to the negative control. However, the study found that giving honey 2.5 to 3.0 g/kg BW was not significantly different in their effect on GLU level. Nevertheless, in general, honey can increase GLU levels after physical activity. GLU is the most essential enzyme as an endogenous antioxidant that is able to capture free radicals [20]. Free radicals have high affinity and reactivity towards the GLU [24]. When the sulfhydryl group is bound to free radicals, it will cause glutathione to lose its ability as an antioxidant. In their research, Sani *et al.* (2014) showed that gelam honey combined with ginger increased antioxidant status in animal models of stress metabolites. As with GLU, SOD antioxidant levels were also determined in this study. The results showed that giving honey increased SOD levels, compared to the negative control. SOD levels after administering honey at doses 2.0, 2.5, and 3.0 g/kg BW of 1.71 ± 1.40; 1.92 ± 1.71; and 2.07 ± 2.14 pg/mL and when compared with the negative control, had low SOD levels of 0.80 ± 0.95 pg/mL (Figure 3).

Even though there was an increase, there was no significant difference between the dose of honey and SOD levels. In this study, it was also revealed that gelam honey also increased SOD levels, which is in line with the results of this study [25]. We assumed that high-intensity exercise generates free radicals, resulting in increases in lipid peroxidation, glutathione oxidation, and oxidative protein damage which cannot be neutralized by honey.

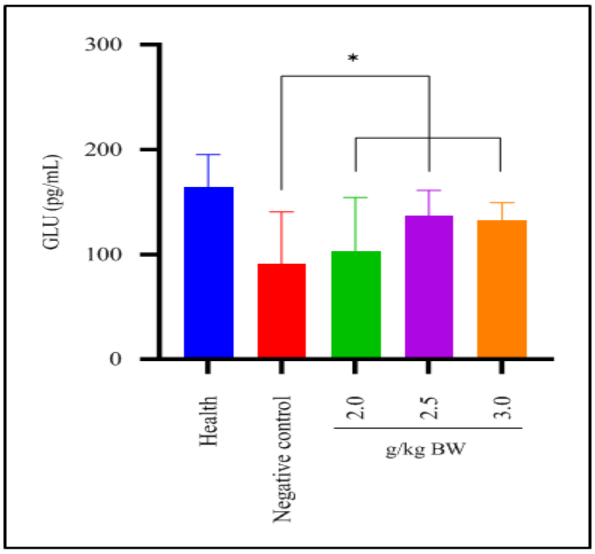


Figure 2: Effects of honey treatment on serum GLU concentration after high-intensity swimming exercises. The values are expressed as mean ± SD (n = 5). Values with superscript letters (*) are significant to negative control

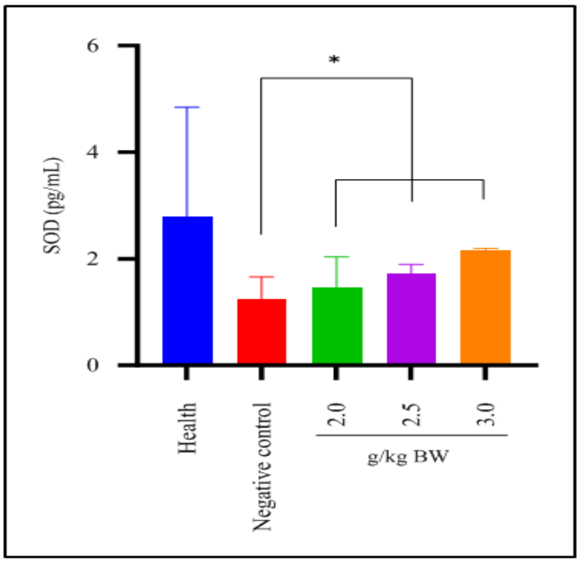


Figure 3: Effects of honey treatment on serum SOD concentration after high-intensity swimming exercises. The values are expressed as mean ± SD (n = 5). Values with superscript letters (*) are significant to negative control

Honey is a natural food rich in energy but has a low glycemic index value and various biological activities [26, 27]. In addition, it is reported that honey can improve muscle pathology in diseased conditions. Muscles are critical structures involved in physical activities such as swimming. Honey improves physical performance at moderate activity levels, reducing inflammatory cytokine production. Apart from that, honey can also maintain performance and reduce fatigue after strenuous exercise [15].

Therefore, the use of honey as an additional therapy may have an impact on increasing exercise capacity or hard work. Well-designed studies are needed to evaluate these possibilities.

Conclusion

Honey can improve the body's antioxidant status after physical activity. Animals that only did physical activity without receiving honey supplements had high MDA levels and reduced GLU and SOD levels. Animals treated with honey had lower MDA levels and showed increased GLU and SOD levels compared to negative controls. Further research will be carried out on its effect on muscles, especially concerning the model used, namely strenuous activity by swimming. Overall, honey can be used as a supplement to restore the body's performance during physical activity.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

ORCID

Makkasau Plasay
https://orcid.org/0000-0002-4277-312X
Lukman Muslimin
https://orcid.org/0000-0002-5703-3714

References

[1]. Malm C., Jakobsson J., Isaksson A., Physical activity and sports—real health benefits: a review with insight into the public health of Sweden, *Sports*, 2019, **7**:127 [Crossref], [Google Scholar], [Publisher]

[2.] Powers S.K., Deminice R., Ozdemir M., Yoshihara T., Bomkamp M.P., Hyatt H., Exercise-induced oxidative stress: Friend or foe?, *Journal of sport and health science*, 2020, **9**:415 [Crossref], [Google Scholar], [Publisher]

[3]. Wang F., Wang X., Liu Y., Zhang Z., Effects of exercise-induced ROS on the pathophysiological functions of skeletal muscle, *Oxidative Medicine and Cellular Longevity*, 2021, **2021**:1 [Crossref], [Google Scholar], [Publisher]

[4.] Matei B., Winters-Stone K.M., Raber J., Examining the Mechanisms behind Exercise's Multifaceted Impacts on Body Composition, Cognition, and the Gut Microbiome in Cancer Survivors: Exploring the Links to Oxidative Stress and Inflammation, *Antioxidants*, 2023, **12**:1423 [Crossref], [Google Scholar], [Publisher]

[5]. Alvarez-Mon M.A., Ortega M.A., García-Montero C., Fraile-Martinez O., Lahera G., Monserrat J., Gomez-Lahoz A.M., Molero P., Gutierrez-Rojas L., Rodriguez-Jimenez R., Differential malondialdehyde (MDA) detection in plasma samples of patients with major depressive disorder (MDD): A potential biomarker, *Journal of International Medical Research*, 2022, **50**:03000605221094995 [Crossref], [Google Scholar], [Publisher]

[6]. Martemucci G., Costagliola C., Mariano M., D'andrea L., Napolitano P., D'Alessandro A.G., Free radical properties, source and targets, antioxidant consumption and health, *Oxygen*, 2022, **2**:48 [Crossref], [Google Scholar], [Publisher]

[7]. Di Giacomo C., Malfa G.A., Tomasello B., Bianchi S., Acquaviva R., Natural compounds and glutathione: Beyond mere antioxidants, *Antioxidants*, 2023, **12**:1445 [Crossref], [Google Scholar], [Publisher]

[8]. Rosa A.C., Corsi D., Cavi N., Bruni N., Dosio F., Superoxide dismutase administration: A review of proposed human uses, *Molecules*, 2021, **26**:1844 [Crossref], [Google Scholar], [Publisher]

[9]. Wang Y., Branicky R., Noë A., Hekimi S., Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling, *Journal of Cell Biology*, 2018, **217**:1915 [Crossref], [Google Scholar], [Publisher]

[10]. Khan F., Abadin Z.U., Rauf N., Honey: nutritional and medicinal value, *International journal of clinical practice*, 2007, **61**:1705 [Crossref], [Google Scholar], [Publisher]

[11]. Alshammari G.M., Ahmed M.A., Alsulami T., Hakeem M.J., Ibraheem M.A., Al-Nouri D.M., Phenolic compounds, antioxidant activity, ascorbic acid, and sugars in honey from ingenious hail province of Saudi Arabia, *Applied Sciences*, 2022, **12**:8334 [Crossref], [Google Scholar], [Publisher]

[12]. Ahmed S., Sulaiman S.A., Baig A.A., Ibrahim M., Liaqat S., Fatima S., Jabeen S., Shamim N., Othman N.H., Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action, *Oxidative Medicine and Cellular Longevity*, 2018, **2018** [Crossref], [Google Scholar], [Publisher]

[13]. Hills S.P., Mitchell P., Wells C., Russell M., Honey supplementation and exercise: a systematic review, *Nutrients*, 2019, **11**:1586 [Crossref], [Google Scholar], [Publisher]

[14]. Apriyanto K.D. Giving honey before moderate intensity physical activity on plasma malondialdehyde levels in Wistar rats. Medikora: *Jurnal Ilmu Kesehatan Olahraga*, 2018, **17**:73 [Crossref], [Google Scholar], [Publisher]

[15]. Ali A.M., Ali E.M., Abou Mousa A., Ahmed M.E., Hendawy A.O., Bee honey and exercise for improving physical performance, reducing fatigue, and promoting an active lifestyle during COVID-19, *Sports Medicine and Health Science*, 2021, **3**:177 [Crossref], [Google Scholar], [Publisher]

[16]. Mohamadzade Namin S., Ghosh S., Jung C., Honey Quality Control: Review of Methodologies for Determining Entomological Origin, *Molecules*, 2023, **28**:4232 [Crossref], [Google Scholar], [Publisher]

[17]. Mehdi R., Zrira S., Vadalà R., Nava V., Condurso C., Cicero N., Costa R., A preliminary investigation of special types of honey marketed in Morocco, *Journal of Experimental and Theoretical Analyses*, 2023, **1**:1 [Crossref], [Google Scholar], [Publisher]

[18]. Abdullahi H., Atiku M., Abdulmumin Y., Sadiya W., Effect of honey and intensity of swimming exercise on semen parameters of male albino Wistar rats, *Tropical Journal of Obstetrics and Gynaecology*, 2019, **36**:258 [Crossref], [Google Scholar], [Publisher]

[19]. Simioni C., Zauli G., Martelli A.M., Vitale M., Sacchetti G., Gonelli A., Neri L.M., Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging, *Oncotarget*, 2018, **9**:17181 [Crossref], [Google Scholar], [Publisher]

[20]. Jena A.B., Samal R.R., Bhol N.K., Duttaroy A.K., Cellular Red-Ox system in health and disease: The latest update, *Biomedicine & Pharmacotherapy*, 2023, **162**:114606 [Crossref], [Google Scholar], [Publisher]

[21]. Nolfi-Donegan D., Braganza A., Shiva S., Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement, *Redox biology*, 2020, **37**:101674 [Crossref], [Google Scholar], [Publisher]

[22]. Stagos D., Soulitsiotis N., Tsadila C., Papaeconomou S., Arvanitis C., Ntontos A., Karkanta F., Adamou-Androulaki S., Petrotos K., Spandidos D.A., Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece, *International Journal of Molecular Medicine*, 2018, **42**:726 [Crossref], [Google Scholar], [Publisher]

[23]. Kamilatussaniah K., Yuniastuti A., Iswari R., Pengaruh suplementasi madu kelengkeng terhadap kadar TSA dan MDA tikus putih yang diinduksi timbal (Pb), *Indonesian Journal of Mathematics and Natural Sciences*, 2015, **38**:108 [Crossref], [Google Scholar]

[24]. Kurutas E.B., The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state, *Nutrition journal*, 2015, **15**:1 [Crossref], [Google Scholar], [Publisher]

[25]. Abdul Sani N.F., Belani L.K., Pui Sin C., Abdul Rahman S.N.A., Das S., Zar Chi T., Makpol S., Yusof Y.A.M., Effect of the combination of gelam honey and ginger on oxidative stress and metabolic profile in streptozotocin-induced diabetic Sprague-Dawley rats, *BioMed research international*, 2014, **2014** [Crossref], [Google Scholar], [Publisher]

[26]. Bobiş O., Dezmirean D.S., Moise A.R., Honey and diabetes: the importance of natural simple sugars in diet for preventing and treating different type of diabetes, *Oxidative Medicine and Cellular Longevity*, 2018, **2018** [Crossref], [Google Scholar], [Publisher]

[27]. Meo S.A., Ansari M.J., Sattar K., Chaudhary H.U., Hajjar W., Alasiri S., Honey and diabetes mellitus: obstacles and challenges–road to be repaired, *Saudi journal of biological sciences*, 2017, **24**:1030 [Crossref], [Google Scholar], [Publisher]

HOW TO CITE THIS ARTICLE

Makkasau Plasay*, Lukman Muslimin, The Effect of Administration of Honey on Physical Activity in Malondialdehyde, Glutathione, and Superoxide Dismutase Blood Levels in Male Rat. *J. Med. Chem. Sci.*, 2024, 7(4) 590-597.

DOI: https://doi.org/10.26655/JMCHEMSCI.2024.4.3

URL: https://www.jmchemsci.com/article_187228.html