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THE EFFECTIVENESS OF *MORINGA OLEIFERA* EXTRACT AND ENDURANCE TRAINING AGAINST TNF- α IN THE MUSCLES OF RATS

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Abstract

The purpose of this research is to determine the effect of *Moringa oleifera* and endurance training on TNF-α in the muscles of Wistar strain rats. The method used in this research is True Experimental by The Randomize Posttest-Only Control Group Design. In this research, we used a sample of 16 Wistar rats. Analysis of rat organs using Western Blot. The analysis used is a one-way ANOVA analysis using SPSS software. The results of the research indicated that the water extract of Moringa oleifera leaves had a significant effect on reducing TNF-α levels. This research is beneficial for all athlete, coach, and others ,regarding the advantage of *Moringa oleifera* as ergogenic aids in increase sports performance.

Keywords: Ergogenic aids, *Moringa oleifera*, TNF- α.

1. Introduction

Exercise is an activity that is carried out systematically and planned to improve body function [1, 2]. In simple terms, training can be interpreted as all efforts to increase the overall physical state with a systematic and repeated process with increasing the amount of load, time, or intensity of exercise. Someone does the exercise because it is a form of effort to achieve a goal [3].

Many athletes train hard to succeed in the sport they play. Athletes have spent a lot of time training [4, 5]. This decrease is caused by a condition called overtraining [6, 7]. Overtraining is the process of exercising too much without adequate rest, resulting in a decrease in the athlete's condition in the middle of the training program process which is characterized by reduced body ability to perform exercise [8, 9]. Thus, athletes experience excessive fatigue and easily increase the incidence of injury more quickly. They are encouraged to achieve the highest achievement that can be achieved. However, too much exercise can lead to decreased performance [10]. Overtraining is a condition in which the number of muscle cells in the body that have been damaged due to physical activity, such as exceeding the maximum capacity of the body [11]. Although physical activity is important, for some cases, it needs to be considered [12].

It cannot be managed again (recovery) instantly by the body. There will be stress that attacks the body in the long term. It can be concluded that Overtraining is a term for excessive fatigue that attacks the body because it uses energy massively from the body's maximum strength limit [13, 14].

Endurance training is classified as a mode of exercise carried out over a long period with a low workload, or swimming [1]. Endurance training is characterized by repeated isotonic contractions of skeletal muscles and can be divided according to the intensity of doing low, medium, and high-intensity exercises [15, 16]. Endurance training without sufficient portions can lead to overtraining. Overtraining can be defined as a state of athletes who are forced to train with excessive loads (overloads) in a training program to the point where rest is no longer sufficient [17, 18]. Because of this, overtraining causes hormonal, immunological (immune), muscle, mental or emotional imbalances and can cause fatigue, depression, injury, and decreased performance [19, 20].

Therefore, overtraining shows signs and symptoms depending on the individual. Inflammation is a natural protective response of the body against tissue injury because of chemical, mechanical or thermal stimulation, trauma, microbial or auto-immune disease [9].

Under certain conditions, physical exercise can become a stressor that will stimulate damage or injury to the muscles caused by local inflammation. Thus, the muscles experience degeneration and regeneration around the connective tissue [1]. Giving repeated physical stimulation to the body can cause an adaptation process that reflects an increase in functional ability but if the amount of stimulation is not enough for the loading process, then the body will not experience an adaptation process. Conversely, if the stimulus is too great and cannot be tolerated by the body, it will cause tissue damage and disturb the homeostasis state of the body's system. In athletes who are given the maximum load during physical exercise which results in severe fatigue, it is found that there is a change in the number of leukocytes in the blood and causing an increase in the incidence of respiratory tract infections,

due to depression of immune system function, resulting in a decrease in body resistance and causes inflammation in certain tissues [9]. In carrying out healing and overcoming overtraining food consumption greatly affects healing. Recovery by consuming foods that contain ergogenic substances such as glucose, salt, anabolic drugs, liver extracts, and anti-inflammatories.

Regular exercise can create an anti-inflammatory atmosphere or environment in the body [10]. Irregular and strenuous exercise can result in overproduction of pro-inflammatory cytokines, due to imbalance in the production of anti-inflammatory cytokines and proinflammatory. One of the pro-inflammatory cytokines is TNF-α [21].

TNF- is a type of pro-inflammatory cytokine made by macrophages as an I inflammatory response. At low levels, TNF- acts on leukocytes to induce acute inflammation. TNF- at moderate levels plays a systemic inflammatory role, while at high levels TNF- causes a drop in blood pressure, severe metabolic disorders (sugar levels drop to levels that are not possible to live), intravascular thrombosis, and complications occur in septic shock [22].

In several previous studies, it has been known that the methanol extract of Moringa oleifera has an anti-inflammatory effect. The 1,3-dibenzyl urea molecules aurantiamide acetate and aurantiamide acetate in the methanol extract of Moringa leaves are said to have anti-inflammatory effects by inhibiting the expression of TNF- α [23].

In this study, there was evidence of the effect of *Moringa oleifera* in inducing inflammation. The results showed that the most effective treatment group in reducing TNF-α levels was the control group at a dose of 20mg/kgBW/day. Researchers are interested in knowing the effectiveness of *Moringa oleifera* extract to TNF- α in the muscles of Wistar strain rats [24].

Judging from the results of previous studies regarding the effect of *Moringa oleifera* as an anti-inflammatory, as well as many considerations that seem to be very rare in studies testing *Moringa oleifera* and its effect on inflammation, it makes researchers feel the need to conduct a study in this regard. Therefore the researcher wanted to know "The effect of giving *Moringa oleifera* and endurance training on muscle inflammation in Wistar rats".

2.Method

The method used in this research is Experimental with The Randomize Posttest-Only Control Group Design. In this research, researchers used a sample of 16 Wistar rats. Judging from the results of previous studies regarding the effect of *Moringa oleifera* as an anti-inflammatory, as well as several considerations, it seems that there are still very few research about *Moringa oleifera* and its effect on inflammation, making researchers feel the need to make a study related to this.

Analysis of rat organs using Western Blot. Western Blot is a technique that has been widely used in the field of molecular biology to find particular proteins from a complex mix of proteins extracted from tissues cells or tissues. Synthetic antibodies, or antibodies of animal origin, are made to react with special target proteins. The analysis used is a one-way ANOVA analysis using SPSS software.

3. Results and Discussion

After experimenting, data was obtained in the form of the average weight of the rats in Table 1. From Table 1, the average body weight of the rats after treatment in 1 month for the control group with an average rat weight of 234.1 g, the Moringa group with an average rat weight of 231 g, the exercise group with an average rat weight of 226.3 g, and the exercise + moringa group with an average rat weight of 231.2 g. After experimenting, data was obtained in the form of the average organ weight of the rats in Table 2.

Table.1 Average rat weight.

No.	Group	Average Rat weight (g)
1	Control	234.1
2	Moringa	231
3	Exercise	226.3
4	Exercise + Moringa	231.2

Table.2 Average rat muscle body weight.

No.	Group	Average Muscle Body Weight (mg)
1	Control	1.432
2	Moringa	1.340
3	Exercise	1.385
4	Exercise + Moringa	1.295

Table 2 shows the average organ muscle weight after treatment in 1 month for the control group with an average organ weight of 1,432 mg, the moringa group with an average organ weight of 1,340 mg, the exercise group with an average organ weight of 1,385 mg, and the exercise + moringa group with an average organ weight of 1,295 mg. After all the data is obtained, after that, an analysis is carried out to look for the significance contained in Fig. 1 and Table. 3.

Figure 1 shows a bar chart of the mean and standard deviation of Moringa's effect on TNF-α. It can be seen that the Moringa group has a better score than the control group because it has the lowest average score. The Moringa group had a significant difference in effect from the control group on TNF-α, marked by an asterisk (*) in Fig. 1.

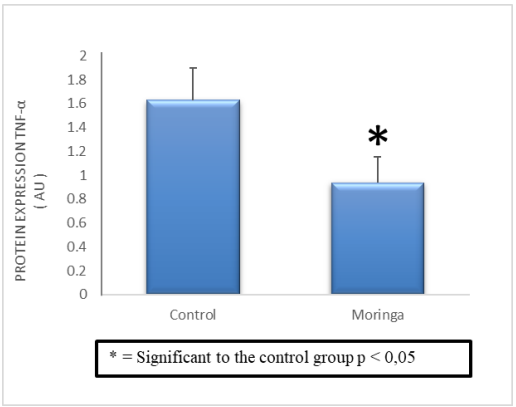

Fig. 1 Average rat muscle body weight.

From Table 3, the average TNF-α value from rats in the control group was 1.6275, and the average TNF-α level in white rats in the Moringa group was 0.9375. After being analysed, it got a significant value of 0.007 < 0.05, which could mean that there was a significant effect of moringa administration on TNF-α in experimental rats of rats.

Table. 3 Effect of moringa on tnf-α.

No.	Group	Mean	Std. Dev.	Sig.
1	Control	1.8675	0.456	0.022
2	Moringa	1.1400	0.090	

Based on this, it can be concluded that the aqueous extract of Moringa (*Moringa oleifera*) leaves has a significant effect on reducing TNF-α levels). This is following a study conducted, Moringa leaf flour (*Moringa oleifera*) can reduce levels of Tumor Necrosis Factor-α (TNF-α) rat serum (Rattus nonvegans) strain [25].

Quercetin also has anti-inflammatory properties. The mechanism of Moringa leaf water extract (*Moringa oleifera*) as an anti-inflammatory that can lead to a reduction in TNF-α levels is through inhibition of Nuclear Factor Kappa B (NF-κB) [26]. NF-B becomes activated under the influence of stimuli from agents such as ROS that cause endothelial dysfunctional, exposure to pathogen, DNA damage, and physical stress. NF-B functions to manage the expression of genes sign proinflammatory cytokines and chemokines such as TNF-, IL-1β, IL-6, and other proteins [27].

The content of Moringa leaves (*Moringa oleifera*) has an important role in antioxidant action, namely flavonoids. The main phenol bioactive component of Moringa leaves which is a class of flavonoids is quercetin which can bind atoms to free radicals so that excess free radicals are not formed, thereby inhibiting the modification of LDL to ox-LDL so that atherosclerosis will not form. Quercetin also has the ability as an anti-inflammatory [28]. The mechanism of extract of Moringa leaves (*Moringa oleifera*) as an anti-inflammatory that can lead to a reduce in TNF-α levels is through retardation of Nuclear Factor Kappa B (NF-κB). NF-κB becomes active due to the influence of stimuli from agents such as Reactive Oxygen Species (ROS) which cause endothelial dysfunctional, exposure to pathogen, DNA damage, and physical stress.

In unstimulated cells, NF-κB resides in the cytoplasm and interacts with Ik-B. The stimulus can activate Ik-B kinase which can phosphorylate IkB, so that IkB undergoes degradation and NF-κB translocation in the nucleus. In the nucleus, NF-κB binds to target genes and stimulates the transcription of inflammatory mediators such as TNF-α, IL-1β, and IL-6 [29]. Quercetin plays a role in blocking Ik-B kinase resulting in decreased IkB phosphorylation. Thus, quercetin can reduce NF-κB activation. This decrease in IkB phosphorylation is a direct mechanism for quercetin's inhibition of NF-κB activity, thereby reducing the production of TNF-α) [30].

The results also showed significant changes after being given Moringa oleifera extract by showing a significant decrease in TNF-α to be lower than normal levels. The decrease in the average expression of TNF-α is due to the antioxidant content (flavonoids) in the methanol extract of moringa oleifera which can counteract free radicals thereby preventing chronic inflammation in liver cancer cells of Wistar rats [28]. Investigated the cytotoxic effect of *Moringa oleifera* extract methanol on human multiple myeloma cell lines. This extract was shown to reduce cell viability up to 2% at a dose of 0.2 mg/mL [21].

Based on several studies on the effect of physical training on TNF-α carried out in humans, we still get different results, wherein a study reported that giving light and moderate-intensity aerobic exercise can reduce TNF-α levels and insulin levels, but the effect of the decreased was more significant in moderate intensity aerobics than light intensity [30]. who were given aerobic exercise for 8 weeks also obtained results that showed a significant effect of physical training on TNF-α levels.

The effect of regular physical exercise can reduce inflammatory cytokines but the effect on TNF-α is still not clear because there are studies that get results of the effect of physical exercise on TNF-α levels but there are also studies that do not find the effect of physical exercise on TNF-α levels [26]. Factors that can influence physical exercise on TNF-α levels are the intensity of regular physical exercise and the characteristics of training. Regular physical exercise intensity and training characteristics can decrease single cytokine responses.

TNF-α is an inflammatory cytokine produced by various cells so to see the effect of physical exercise (exercise) on TNF-α levels is very difficult. Expression of cytokine genes in muscle comes from muscle, such as TNF-α serum concentration has no effect after physical exercise, but the mRNA of these cytokines is reduced in muscle as a result of physical exercise [31].

4. Conclusion

Based on the results of the research that has been done, there is a significant effect of giving Moringa oleifera to Tnf-α in experimental rats of rats. From the analysis that has been done, it shows that there is a significant difference in the average of the control group and the moringa group. Furthermore, there was a significant effect of resistance training against Tnf-α in the rat test. From the analysis that has been done, it shows that there is a significant difference in the average of the control group and the exercise group. For further indications, there is an effect of the combination of Moringa oleifera and endurance training on Tnf-α in experimental animals of rats. From the analysis that has been carried out, it shows that there is a significant difference in the average of the control group with the other groups.

References

1. Werner, C.M.; Hecksteden, A.; Morsch, A.; Zundler, J.; Wegmann, M.; Kratzsch, J.; and Laufs, U. (2019). Differential effects of endurance, interval, and resistance training on telomerase activity and telomere length in a randomized, controlled study. *European Heart Journal*, 40(1), 34-46.
2. Shibaguchi, T.; Ishizawa, R.; Tsuji, A.; Yamazaki, Y.; Matsui, K.; and Masuda, K. (2017). Fermented grain beverage supplementation following exercise promotes glycogen supercompensation in rodent skeletal muscle and liver. *Indonesian Journal of Science and Technology*, 2(1), 1-7.
3. Masuda, K.; Jue, T.; and Hamidie, R.D.R. (2017). Mitochondrial biogenesis induced by exercise and nutrients: Implication for performance and health benefits. *Indonesian Journal of Science and Technology*, 2(2), 221-229.
4. Villa, E.C.; Guerrero, J.; and Calixtro Jr.; V.L. (2022). Educating and construing the lived experiences of athletes of south cotabato in the new normal: Proposed intervention. *Indonesian Journal of Educational Research and Technology*, 2(1), 75-80.

5. Bakinde, S.T. (2022). Physical fitness status of male, and female non-athlete in Nigeria. *Indonesian Journal of Educational Research and Technology*, 2(2), 123-132.
6. Bakinde, S.T. (2022). Educating aerobic exercise and skill-related fitness of athletes in University of Ilorin. *Indonesian Journal of Multidiciplinary Research*, 2(1), 195-202.
7. Singh, A.; and Keur, H. (2022). Educational perspective: An analytical study of coping skill of track and field medalist and non-medalist male athletes. *ASEAN Journal of Community Service and Education*, 1(2), 89-94.
8. Suherman, A.; Yassin, A.; Rahayu, N.I.; and Sartono, H. (2022). A critical success factors model for golf athletes' talent development in Malaysia and Indonesia. *ASEAN Journal of Physical Education and Sport Science*, 1(1), 27-34.
9. Salehi, O.R.; Hosseini, S.A.; Farkhaie, F.; Farzanegi, P.; and Zar, A. (2019). The effect of moderate intensity endurance training with genistein on brain-derived neurotrophic factor and tumor necrosis factor-α in diabetic rats. *Journal of Nutrition, Fasting and Health*, 7(1), 44-51.
10. Rothschild, J. A.; and Bishop, D. J. (2020). Effects of dietary supplements on adaptations to endurance training. *Sports Medicine*, 50(1), 25-53.
11. Samson, A.B.; and Agboola, M.A. (2022). Correlates of physical activity and sedentary behaviour of academic and non-academic staff in University of Ilorin, Ilorin, Nigeria. *Indonesian Journal of Multidiciplinary Research*, 2(2), 381-388.
12. Coyoca, M.B.P.; Del Rosario, M.A.B.; Lazo, J.R.L.; Calixtro Jr, V.L.; and Edaño, L.B. (2022). Impact of gardening on physical and mental health in the midst of covid-19 pandemic. *Indonesian Journal of Community and Special Needs Education*, 2(2), 99-102.
13. Saodat, O. (2023). Organization of extracurricular physical education at school and its role in the physical and mental improvement of students. *Indonesian Journal of Community and Special Needs Education*, 3(1), 73-84.
14. Apriyatna, F.; Nandiyanto, A.B.D.; Kurniawan, T.; and Bilad, M.R. (2023). Analysis of physical fitness level elementary school students during the covid-19 pandemic. *ASEAN Journal of Science and Engineering Education*, 2(1), 103-110.
15. Marcaida, J.P. (2022). Physical and mental health struggles during the time of pandemic: An overview of domestic setting. *ASEAN Journal of Community and Special Needs Education*, 1(1), 23-28.
16. Hidayatullah, W.; Muktiarni, M.; and Mupita, J. (2022). Analysis comparative of physical fitness of students with disabilities and normal students. *ASEAN Journal of Community and Special Needs Education*, 1(2), 69-74.
17. Ilhomovich, I.A. (2022). Boxing training technology based on the level of physical development of children. *ASEAN Journal of Physical Education and Sport Science*, 1(1), 1-8.
18. Sultanto, M.A.; Al Afghani, R.I.; Meisya, S.D.; Salsabila, I.A.; Rohimat, S.S.; and Stephani, M.R. (2023). Physical education online class for students with hearing impairment during covid-19 pandemic. *ASEAN Journal of Community and Special Needs Education*, 2(1), 17-26.

19. Laroza, A.; Piral, L.; Palcis, M.J.; and Calixtro, V.L. (2021). Zumba preference and its cardiovascular endurance intensification. *Indonesian Journal of Teaching in Science*, 1(2), 153-158.
20. Firdaus, I.R.; Febrianty, M.F.; Awwaludin, P.N.; Ilsya, M.N.F.; Nurcahya, Y.; and Sultoni, K. (2023). Nutritional research mapping for endurance sports: A bibliometric analysis. *ASEAN Journal of Physical Education and Sport Science*, 2(1), 23-38.
21. Cuellar-Núñez, M.L.; De Mejia, E.G.; and Loarca-Piña, G. (2021). Moringa oleifera leaves alleviated inflammation through downregulation of il-2, il-6, and tnf-α in a colitis-associated colorectal cancer model. *Food Research International*, 144, 110318.
22. Cui, C.; Chen, S.; Wang, X.; Yuan, G.; Jiang, F.; Chen, X.; and Wang, L. (2019). Characterization of Moringa oleifera roots polysaccharide mrp-1 with anti-inflammatory effect. *International Journal of Biological Macromolecules*, 132, 844-851.
23. Cuellar-Núnez, M. L.; Loarca-Pina, G.; Berhow, M.; and de Mejia, E. G. (2020). Glucosinolate-rich hydrolyzed extract from Moringa oleifera leaves decreased the production of tnf-α and il-1β cytokines and induced ros and apoptosis in human colon cancer cells. *Journal of Functional Foods*, 75, 104270.
24. Romanowska-Próchnicka, K.; Felis–Giemza, A.; Olesińska, M.; Wojdasiewicz, P.; Paradowska-Gorycka, A.; &Szukiewicz,D. (2021). The role of tnf-α and anti-tnf-α agents during preconception, pregnancy, and breastfeeding. *International Journal of Molecular Sciences*, 22(6), 2922.
25. Xiong, Y.; Rajoka, M.S.R.; Mehwish, H.M.; Zhang, M.; Liang, N.; Li, C.; and He, Z. (2021). Virucidal activity of moringa a from Moringa oleifera seeds against influenza a virusesby regulating tfeb. *International Immunopharmacology*, 95, 107561.
26. Rajanandh, M.G.; Satishkumar, M.N.; Elango, K.;& Suresh, B. (2012). Moringa oleifera lam. a herbal medicine for hyperlipidemia: a pre–clinical report. *Asian Pacific Journal of Tropical Disease*, 2(Supplement 2,), S790-S795.
27. Nieman, D.C.; Henson, D.A.; Davis, J.M.; Angela Murphy, E.; Jenkins, D.P.; Gross, S.J.;& Mayer, E.P. (2007). Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mrna. *Journal of Applied Physiology*, 103(5), 1728-1735.
28. Mahajan, S.G.; Mali, R.G.; and Mehta, A.A. (2007). Protective effect of ethanolic extract of seeds of Moringa oleifera lam. against inflammation associated with development of arthritis in rats. *Journal of Immunotoxico*logy, 4(1), 39-47.
29. Parvathy, M.V.S.; and Umamaheshwari, A. (2007). Cytotoxic effect of Moringa oleifera leaf extracts on human multiple myeloma cell lines. *Trends in Medical Research*, 2(1), 44-50.
30. Salehi, O.R.; Hosseini, S.A.; Farkhaie, F.; Farzanegi, P.; and Zar, A. (2019). The effect of moderate intensity endurance training with genistein on brain-derived neurotrophic factor and tumor necrosis factor-α in diabetic rats. *Journal of Nutrition, Fasting and Health*, 7(1), 44-51.
31. Wadee, S.A.; Sarhat, E.R.; and Najim, R.S. (2018). Effect of Moringa oleifera extract on serum glucose and interleukin-1, interleukin-2 and tumor necrosis factor α in streptozotocin-induced diabetic rats. *Tikrit Medical Journal*, 24(1), 61-68.