POTENTIAL OF SOYBEAN (Glycine max) ON MUSCLE TISSUE STRUCTURE AND LACTIC ACID LEVELS OF WISTAR RATS

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ABSTRACT

Muscle fatigue is a condition resulting from prolonged muscle contractions. Synthetic materials are often used to restore muscle fatigue, although they can have side effects. Soybean is a natural product that contains complex compounds, including thiamine, which has the potential to replace synthetic compounds. The aim of the study was to analyze the potential of soybean (Glycine max) on the structure of muscle tissue for lactic acid levels in Wistar rats. The study used an experimental approach, the treatment of giving soybean powder and pure thiamine compounds to 24 male Wistar white rats aged 3 months totaling 24 rats, 150-200 g for 15 days. Data analysis techniques were carried out on the frequency of muscle fatigue through the struggling test, measurement of lactic acid levels in the blood, and structure of the biceps femoris muscle tissue in HE staining. The results showed struggling time, Fobs 16.398 > Ftable 3.554 and lactic acid levels Fobs 17.621 > Ftable 3.467 and changes in the muscle tissue structure of white rats. The conclusion that soybean seeds have the potential as a natural ingredient in overcoming muscle fatigue

Keywords: soybean, muscle tissue structure, lactic acid, struggling

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Muscle fatigue is a condition of strong muscle contractions for a long time. Muscle fatigue results in decreased oxygen levels and increased lactic acid levels (Lamb, G. D., & Stephenson, 2006);(Wan, J. J., Qin, Z., Wang, P. Y., Sun, Y., & Liu, 2017). Muscle fatigue occurs in anaerobic activities due to high-intensity activities, requiring fast energy and a short time. Muscle fatigue can result in a decrease in body capacity and condition (Pethick, J., Winter, S. L., & Burnley, 2021).

Reducing muscle fatigue can be done using vitamins B1, B6, and B12 (Wan, J. J., Qin, Z., Wang, P. Y., Sun, Y., & Liu, 2017); (Martel, J. L., Kerndt, C. C., Doshi, H., & Franklin, 2021). These vitamins are usually consumed in the form of synthetic compounds that are packaged and sold freely on the market.Various food and beverage supplements that are circulating in the market are often consumed by humans to reduce muscle fatigue. Synthetic chemicals are often used as dominant compounds, because they give a very fast effect and can restore conditions(Holdt, S. L., & Kraan, 2011);(Margot, J., Rossi, L., Barry, D. A., & Holliger, 2015);(Peng, H., Saunders, D. M., Sun, J., Jones, P. D., Wong, C. K., Liu, H., & Giesy, 2016); (Mardiana, M., Rachmawati, L., Sari, N. P., & Al Amien, 2022). Supplements are needed to reduce muscle fatigue because the mechanism of action is very short and immediately gives the expected effect (Peng, H., Saunders, D. M., Sun, J., Jones, P. D., Wong, C. K., Liu, H., & Giesy, 2016); (Bowtell, J., & Kelly, 2019);(Aćimović, M. G., Tešević, V. V., Smiljanić, K. T., Cvetković, M. T., Stanković, J. M., Kiprovski, B. M., & Sikora, 2020).

Various synthetic compounds of thiamine and pyridoxine are often used to treat muscle fatigue (Wan, J. J., Qin, Z., Wang, P. Y., Sun, Y., & Liu, 2017); (Titcomb, T. J., & Tanumihardjo, 2019). There are side effects caused by the use of synthetic materials, including tissue damage that can be caused by the use of synthetic materials, for example the occurrence of necrosis (Nisar, B., Sultan, A., & Rubab, 2018); (Huang, K., Hu, S., & Cheng, 2019). Various natural ingredients with thiamine and pyridoxine compounds are still not widely known, so they have not been widely used to treat muscle fatigue (Choi, S. K., Baek, S. H., & Choi, 2013); Wan, et al., 2017). The use of natural ingredients with various compound complexities in them can provide more moderate results (Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A., & Dzobo, 2018). Natural materials have a variety of complex compounds that have the potential to be used for health (Yuan, H., Ma, Q., Ye, L., & Piao, 2016);(Koparde, A. A., Doijad, R. C., & Magdum, 2019).

Thiamine is most commonly found in vegetable materials, especially legumes (Leguminoceae), one of which is soybeans (Annor, G. A., Ma, Z., & Boye, 2014); (Erbersdobler, H. F., Barth, C. A., & Jahreis, 2017). The value of thiamine in soybeans is quite large among other legumes, reaching 1.0 mg (Erbersdobler, et al., 2017). The use of soybeans to reduce lactic acid levels and muscle fatigue (Watanabe, N., & Sakuda, 2010); (Ding, J. F., Li, Y. Y., Xu, J. J., Su, X. R., Gao, X., & Yue, 2011);(Mardiana, M., Kartini, A., Sutiningsih, D., Suroto, S., & Muhtar, 2023). The aim of the study was to analyze the potential of soybean (Glycine max) on muscle tissue structure and lactic acid levels in Wistar rats.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

Treatment of experimental animals

Wistar rats, 3 months old, 150-200 g, 24 heads. Acclimatization was carried out for 1 week in the rearing cage. Mice were divided into 3 treatment groups. Eight control group rats (P1), eight group rats (P2) were given soybeans, and eight group rats (P3) were given synthetic thiamine. The treatment was carried out for 15 days. One hour after the treatment was continued with a swimming test (struggling).

Determine the dose of soy

Soybeans are washed and finely ground. 100 g of soybeans contains 0.93 g of thiamine. The thiamine value of 0.93 g per 100 g soybeans is adjusted to the normal thiamine requirement per day. The normal thiamine requirement dose is converted to the normal thiamine requirement for rats, body weight 150-200 g and 0.3 mg/day is obtained. Giving soy 3 mg/day for 15 days (P2).

The struggling test

Conducted a swimming test on the 16th day for all rat treatment groups. Data collection was carried out 2 times with an interval of 15 minutes. The strunggling test was carried out to analyze the swimming ability of the rats (Alver, 2014; Li, et al., 2016)

Surgery, and preparation

Surgery and removal of the biceps femoris was carried out on the 17th day Preparation of muscle tissue preparations stained with hematoxylin eosin (HE).

Data analysis

Data on lactic acid levels were analyzed using one way Anova SPSS 19 and changes in muscle tissue structure were analyzed using a dyno eye and optilab microscope.

3. RESULTS AND DISCUSSION

Analysis of the muscle contraction activity of the Wistar rats by the strunggling test after administration of soy and thiamine compounds showed changes in the structure of the biceps femoris muscle tissue (Figure 1).

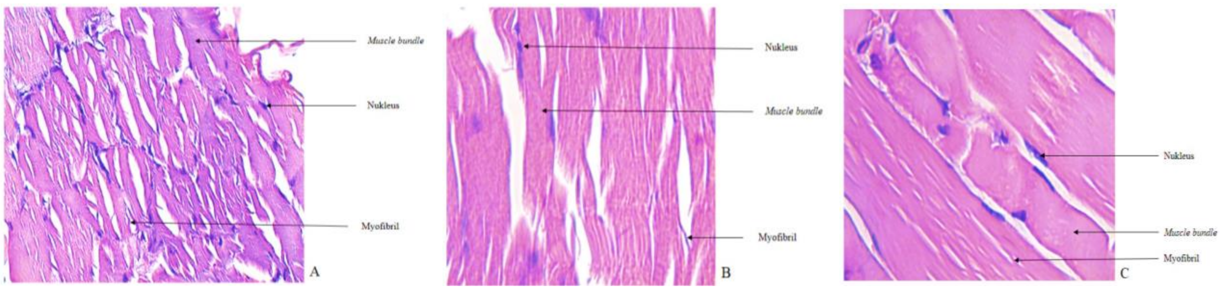

Figure 1. Myofibril structure of the biceps femoris muscle, HE, 400X

A. Mice after carrying out a strunggling test: Myofibrils are disorganized, the nucleus is at the edges, the arrangement is disorganized
B. Rats after carrying out the strunggling test were then treated with soybeans: Miofibrils arranged in an orderly manner, the nuclei at the edges in an orderly arrangement
C. After carrying out a strunggling test, rats are treated with synthetic thiamine: Myofibrils are in order, the nucleus is on the edge in an orderly arrangement

The results of observations of changes in the structure of muscle tissue in Figure 1 can be correlated with the results of lactic acid examination and strunggling time (Table 1).

Table 1. Lactic acid levels and struggling time

Table 1. variation of lactic acid levels according to different treatments

treatment	lactic acid levels		strunggling time
	before	After	
P_0	6.7	8.2	16.4
P_1	6.8	7.4	20.8
P_2	6.7	6.9	26.5

P_0 : Mice after carrying out a strunggling test

P_1 : Rats after carrying out the strunggling test were then treated with soybeans

P_2 : After carrying out a strunggling test, rats are treated with synthetic thiamine

Table 2. One way Anova test table

		Sum of squares	df	Mean square	F	Sig.
LACTIC_ACID	Between groups	6.947	2	3.474	16.398	.000
	Within groups	4.449	21	0.212		
	Total	11.396	23			
STRUNGG_TIME	Between groups	209.083	2	104.541	17.621	.000
	Within groups	106.790	18	5.933		
	Total	315.873	20			

Based on Table 2 shows Fobs > Ftable on lactic acid levels and struggling time, the Fobs value of lactic acid is 16.398 > Ftable 3.554 and the Fobs value of lactic acid is 17.621 > Ftable 3.467 with each value of Sig .000. Conclusion of the data in Table 2 that there is an effect of soy thiamine compounds on muscle fatigue and muscle tissue structure.

Muscle activity is a process of muscle contraction related to the use of adenosine triphosphate (ATP) as contraction energy (Li, J., King, N. C., & Sinoway, 2003); (Casas, M., Buvinic, S., & Jaimovich, 2014) ;(Sahlin, 2014). Contraction of muscle cells requires energy in the form of adenosine triphosphate (ATP), then ATP is hydrolyzed into adenosine diphosphate (ADP) and energy is used for contraction, this process can continue as long as the supply of intracellular ATP is still there.(Li, J., King, N. C., & Sinoway, 2003); (Casas, M., Buvinic, S., & Jaimovich, 2014); (Grassi, B., Rossiter, H. B., & Zoladz, 2015). The total supply of ATP in the body is very limited, further energy needs are obtained from ATP synthesis through the oxidative pathway of creatine phosphate, which is highly dependent on the availability of oxygen and glycogen reserves derived from glucose (Baker, J. S., McCormick, M. C., & Robergs, 2010); (Grassi, B., Rossiter, H. B., & Zoladz, 2015);(Hargreaves, M., & Spriet, 2020).

The energy obtained from creatine phosphate is only for the needs of muscle contraction for a few moments, and then ATP will be fulfilled through non-oxidative (anaerobic) phosphorylation processes (Li, J., King, N. C., & Sinoway, 2003); (Casas, M., Buvinic, S., & Jaimovich, 2014); (Grassi, B., Rossiter, H. B., & Zoladz, 2015); (Bartlett, 2019); (Fitzgerald, 2020). Anaerobic metabolism utilizes glucose and glycogen through the process of glycolysis without oxygen to produce ATP and metabolic waste in the form of lactic acid (Grassi, B., Rossiter, H. B., & Zoladz, 2015);(Hargreaves, M., & Spriet, 2020).

Continuous muscle contraction can cause muscle fatigue, because ATP supplies are very limited. Muscle fatigue is a condition where muscle cells are no longer able to contract due to a lack of ATP (Casas, M., Buvinic, S., & Jaimovich, 2014); (Hargreaves, M., & Spriet, 2020). The neuromuscular junction is unable to transmit impulses and is accompanied by an accumulation of lactic acid (Allen, D. G., Lamb, G. D., & Westerblad, 2008), (Girard, O., Millet, G. P., Micallef, J. P., & Racinais, 2012);(Mukund, K., & Subramaniam, 2020). Muscle fatigue will cause pain due to ischemia of muscle tissue (Queme, L. F., Ross, J. L., & Jankowski, 2017);(Wirth, K. J., & Scheibenbogen, 2021). Muscle fatigue occurs due to a decrease in the percentage of muscle strength, recovery time for muscle fatigue, and the time it takes for fatigue to occur (Ament, W., & Verkerke, 2009);(Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., & Rice, 2016); (Bartlett, 2019).

The strunggling activity carried out by rats is strenuous activity so that the energy required will greatly increase when compared to the resting state, this will activate anaerobic metabolism in muscle cells to produce energy and will increase intracellular lactic acid levels. Accumulation of lactic acid in muscle cells will cause intracellular acidosis and cause fatigue (Allen, D. G., Lamb, G. D., & Westerblad, 2008);(Mukund, K., & Subramaniam, 2020). Lactic acid in muscle cells will diffuse into the blood and increase blood plasma lactic acid levels (van Ginneken, V., Boot, R., Murk, T., van den Thillart, G., & Balm, 2004); (Seheult, J., Fitzpatrick, G., & Boran, 2017). The more strenuous physical activity will increase anaerobic metabolic processes, so that lactic acid levels also increase (Grassi, B., Rossiter, H. B., & Zoladz, 2015); (Bartlett, 2019);(Fitzgerald, 2020).

Thiamine is capable of carrying out oxidative decarboxylation of pyruvate to acetyl CoA and can cause the entry of substrates into the Krebs cycle to form energy (Depeint, F., Bruce, W. R., Shangari, N., Mehta, R., & O'Brien, 2006); (Tylicki, A., Łotowski, Z., Siemieniuk, M., 2018). Glucose deficiency can be overcome by administering vitamin B6 which plays a role in the form of pyridoxal phosphate (PLP) as a coenzyme, especially in decarboxylation and other reactions related to protein metabolism (McAllister, 2014); (Stach, K., Stach, W., & Augoff, 2021); (Calderón-Ospina, C. A., & Nava-Mesa, 2020); (Ito, T., Ogawa, H., Hemmi, H., Downs, D. M., & Yoshimura, 2022). As a coenzyme for phosphorylation, PLP helps release glycogen from the liver and muscles so that it can be broken down into glucose which can serve as an energy source during activities (Calderón-Ospina, C. A., & Nava-Mesa, 2020). Vitamin B1 (thiamine) which functions to convert carbohydrates into energy, strengthens the nervous system, where thiamin will stimulate neurotransmitter work (Campos-Bedolla, P., Walter, F. R., Veszelka, S., & Deli, 2014); (Mikkelsen, K., & Apostolopoulos, 2019); (Tardy, A. L., Pouteau, E., Marquez, D., Yilmaz, C., & Scholey, 2020). Thiamine is useful in helping to overcome symptoms of fatigue because this vitamin can improve carbohydrate metabolism which is used to produce energy and can reduce the buildup of lactic acid in muscles that are experiencing fatigue (Roosterman, D., & Cottrell, 2021).

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