

EFFECTS OF ETHANOL EXTRACT OF PADANG TARO LEAVES (*COLOCASIA GIGANTEA*) ON THE WEIGHT AND MUSCLE THICKNESS OF MALNOURISHED MODEL MICE

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ABSTRACT

Malnutrition can cause impaired growth and muscle mass. Talas Padang (*Colocasia gigantea*) is one of the sources of natural ingredients with nutritional content. This study aims to analyze the effect of various doses of administration ethanol leaf extract of Talas Padang (ELETP) on the weight and thickness of skeletal muscle (soleus) in malnourished rat. Pure experimental research with Post-test Only Control Group design using 25 malnourished rat (Wistar strain). ELETP is obtained macerated using 96% ethanol. Treatment in 5 groups (n=5), K1: normal 56-day diet; K2: low-protein diet (LPD) 14 days + normal 42-day diet; K3, K4 and K5: 14-day LPD + normal diet and ELETP doses of 100, 200, 400/kgBB for 42 days. The soleus muscle is obtained by dissection in the posterior aspect of the mouse leg to measure weight and thickness. Muscle weight data were analyzed using Anova's one-way test and muscle thickness using the Wallis Kruskal test. The results showed average (gr) of muscle weight K3 (0.246 ± 0.087) > K5 (0.236 ± 0.098) > K4 (0.215 ± 0.092) > K1 (0.188 ± 0.060) > K2 (0.127 ± 0.025) and muscle thickness (mm) K4 (1 (1-1)) > K1, K3, K5 (1 (0,00-1)) > K2 (0,00 (0,00-1)). This study conclude that ELETP doses of 100mg/kgBB produce the largest muscle weight, while the largest muscle thickness is found at a dose of 200mg/kgBB.

Keywords: *Colocasia gigantea*, Malnutrition, Talas Padang.

INTRODUCTION

Stunting is a sign of chronic malnutrition experienced over a long period of time¹. According to UNICEF and WHO, in 2020 the global prevalence of *stunting* was 21.3%². Stunting measurement is measured using *z-score* by measuring height-for-age, if it is more than 2 standard deviations or below the median Child Growth Standard of the World Health Organization (WHO) then it is classified as *stunting*³. When compared to map information on various continents, the prevalence of *stunting* in Indonesia is still higher than the prevalence in Southeast Asia, which is 24.7%. This is evidenced based on data from the 2019 Nutritional Status Study of Toddlers in Indonesia (SSGBI) that the prevalence of *stunting* in Indonesia is 27.67%⁴.

There are several causes of *stunting* through a molecular approach, namely, congenital (familial) conditions, endocrine diseases, chromosomes, chronic diseases, malnutrition and a history of previous breastfeeding. Broadly speaking, *stunting* is divided into two, namely based on family history and pathological conditions⁵. Malnutrition affects the global population at several stages of life. In public health epidemiology, this condition can occur in everyone, but the most vulnerable are the poor, children, adolescents, the elderly, those who suffer from diseases and have a vulnerable immune system as well as breastfeeding and pregnant women⁶.

Dipasquale et al., stated that acute malnutrition is malnutrition due to insufficient energy or protein intake. Proteins derived from food are necessary for a source of amino acids for the synthesis of body proteins and other compounds that have various functional roles. Energy is

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Activated Protein Kinase). The role of AMPK as an energy sensor is very important in the grid which shows highly volatile energy cycling⁸. AMPK is heavily involved in controlling skeletal muscle metabolism through the regulation of many targets. Due to its effects on anabolic and catabolic cellular processes, AMPK plays an important role in controlling the development of skeletal muscle growth and regulates muscle mass and its regeneration, AMPK $\alpha 1$ is important in stimulating anabolism and regulating muscle cell dynamics during regeneration, while AMPK $\alpha 2$ regulates muscle degradation during atrophy⁹.

Malnutrition can be one of the causes of muscle atrophy or a condition of loss of skeletal muscle mass. Muscle atrophy causes muscle weakness and can lead to disability¹⁰. It is known that muscle atrophy results from decreased protein synthesis and accelerated proteolysis. In accordance with the pro-atrophic role of TNF α , it has been found that the cytokine decreases protein synthesis and tends to increase proteolysis in L6 myotubes¹¹. There was evidence that the weight and *cross-sectional area* (CSA) of plantaris muscle fibers in the malnourished group were lower than in the control group. However, the weight and CSA of soleus muscle fibers did not change much in malnourished conditions. This suggests that malnutrition induces a loss of plantaris muscle mass but not the soleus muscle¹². Meanwhile, according to Silva et al., the soleus muscle is proven to experience weight loss after giving a low-protein diet¹³.

There are many factors that affect the increase in muscle mass in each individual, one of which is the dietary factor. Consuming foods that contain high protein and reducing the consumption of foods that contain high fat will greatly affect the development of muscle mass of a person¹⁴. Taro leaves are one of the nutrient-rich plants because they contain a lot of various nutrients including carbohydrates and proteins. One of the species that is often consumed as a source of nutritious food is the species *Colocasia gigantea* or in Indonesia known as Taro Padang. Traditionally, *Colocasia* leaves show great potential for food as well as drug development¹⁵. In general, *Colocasia* leaves have good nutritional content, such as protein, carbohydrates, fats, Calcium, phosphorus, iron, and vitamins A, B, and C. The nutritional content of *Colocasia* leaves is 86.94% water content, 16.48% crude protein, 17.24% crude fiber, potassium 1.45%, phosphorus 0.4%, fat 4.3%, BETN 30.46% and gross energy 3966 kcal/kg¹⁶.

Until now, no one has studied the effect of *Colocasia gigantea* leaves on muscle conditions in malnutrition, especially on the soleus muscle. According to the USDA Agricultural Research Service, every 100 grams of *Colocasia* leaves contain 85.7 grams of water, 42 kcal of energy, 4.98 grams of protein, 0.74 grams of total fat, 6.7

0.167gr, lysine 0.246gr, tryptophan 0.048gr, phenylalanine 0.195gr and histidine 0.114gr. Therefore, with the potential of *Colocasia gigantea* leaves as a source of protein that can help improve nutrition in malnourished conditions, researchers are interested in finding out how the effect of administering ethanol extract of *Colocasia gigantea* leaves on the muscle weight and thickness of malnourished model mice.

MATERIALS AND METHODS

This study is purely experimental using an experimental animal model with a *Post-test Only Control Group research design* that has received permission from the USU Ethics Commission No.0150/KEPH-FMIPA/2023. The population of 6-8 week-old male white mice of the Wistar strain who were induced into malnourished model mice was 25 based on Federer's formula which was randomly divided into 5 groups. The sample of this study was the right and left soleus muscles measured on average weight and thickness from malnourished model mice. Inclusion criteria: intact soleus muscle and no deformity. Exclusion criteria: apparent soleus muscles with deformity. Provision of ELETP with Padang Taro leaf simplicia powder that has been macerated with 96% ethanol solvents. The K1 group was a group of normal rats who were given a normal diet for 56 days, K2 as a positive control was given DRP for 14 days then a normal diet for 42 days, K3 was given DRP for 14 days then a normal diet and ELETP at a dose of 100mg/kgBB orally once a day for 42 days, K4 was given DRP for 14 days then a normal diet and ELETP at a dose of 200mg/kgBB orally once a day for 42 days and K5 was given DRP for 14 days later, normal diet and ELETP dose of 400mg/kgBB orally once a day for 42 days.

Termination was carried out by giving concentrated chloroform by inhalation, then the soleus muscle sampling was carried out by dissection on the posterior aspect of the rat limb and then identifying the gastrocnemius muscle adjacent to the Achilles tendo and soleus muscle, then the soleus muscle was taken and immediately weighed using an analytical scale and then the muscle thickness was measured with a caliper. All data were analyzed to determine the difference in the average weight and thickness values of each treatment group with the Anova one-way test which had previously been tested for normality using the *Shapiro Wilk test* because the number of samples was <50 and the homogeneity test using the *Levene test*. In the results of the prerequisite tests that do not meet normality and homogeneity, the *Kruskal-Wallis test is carried out*.

RESULT

Effect of ELETP on Soleus Muscle Weight

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analytical scale and then after obtaining the two weights, the average weight is calculated in grams. In table 4.1, it can be seen that the average muscle weight of soleus muscle from each group, the average muscle weight in the low-protein diet group that was not given *Colocasia gigantea* leaf ethanol extract (K2) had the smallest average muscle weight (0.127 ± 0.025), while the average muscle weight was the largest in the K3 group, namely the low-protein diet group given *Colocasia gigantea* leaf ethanol extract with a dose of 100mg/kgBB (0.246 ± 0.087).

In the Shapiro-Wilk *normality test*, it was known that the *p-value* data in each group was greater than 0.05, thus, the data in each group was normally distributed. After that, a homogeneity test was carried out *Levene's Test* and a *p-value* of 0.466 was obtained which was greater than 0.05. This means that the data between groups has a homogeneous data variance. Because the prerequisite test has been fulfilled and then the *Anova one-way* test has been carried out, the treatment group that has the highest average is the K3 group. Meanwhile, for a *p-value* of 0.153 which is greater than 0.05, there was no significant difference in the average weight of the soleus muscle of each treatment group (Table 1).

Table 1. Effect of ELETP on Soleus Muscle Weight Information:

Group	N	Soleus Muscle Weight		<i>p-value</i>
		(gr)	(Mean \pm SD)	
K ₁	5	0,188 \pm 0,060		
K ₂	5	0,127 \pm 0,025		
K ₃	5	0,246 \pm 0,087		0,153
K ₄	5	0,215 \pm 0,092		
K ₅	5	0,236 \pm 0,098		

One way-Anova test with a significant value of *p*<0.05
K1: normal; K2 : positive control; K3: ELETP dose 100mg/kgBB; K4: ELETP dose 200mg/kgBB; K5: ELETP dose 400mg/kgBB.

Effect of ELETP on Soleus Muscle Thickness

The results of macroscopic measurements of the thickness of the soleus muscle in each group have been obtained. The thickness of the soleus muscle is measured at the distance between the anterior to posterior midpoint. The soleus muscles use a shovel. The average thickness of the soleus muscle in each group is, K1 group: 0.99mm, K2 group: 0.66mm, K3 group: 0.87mm, K4 group: 1mm and K5 group: 0.95mm. The K2 group has the smallest soleus muscle thickness, while the largest group K4 has. After

conducting the prerequisite test, it turned out that the data did not meet the assumptions of normality and homogeneity. Thus, a hypothesis test to find out the difference in average muscle thickness cannot be carried out using the *Anova one-way* test, but the *Kruskal-Wallis* test with a *p-value* of 0.233, greater than 0.05. This means that there was no significant difference in the mean thickness of the soleus muscle in each treatment group (Table 3.2).

Table 2 Effect of ELETP on Soleus Muscle Thickness

Group	N	Soleus Muscle Thickness (mm)	<i>p-value</i>
[Median (min-max)]			
K ₁	5	1 (0,00-1)	
K ₂	5	0,00 (0,00-1)	
K ₃	5	1 (0,00-1)	0,233
K ₄	5	1 (1-1)	
K ₅	5	1 (0,00-1)	

Information:

Kruskal-Wallis *test* with a significant value of *p*<0.05

K1: normal; K2 : positive control; K3: ELETP dose 100mg/kgBB; K4: ELETP dose 200mg/kgBB; K5: ELETP dose 400mg/kgBB.

DISCUSSION

Body muscle mass can be defined as total muscle weight¹⁷. In malnutrition, there is increased oxidative stress that decreases mitochondrial metabolic capacity thereby lowering anabolism and affects the decrease in muscle mass, so it can be considered a source of nutritious food from natural ingredients needed to help improve muscle mass in malnutrition. In this study, the average muscle weight in the low-protein diet group that was not given *Colocasia gigantea* leaf ethanol extract showed the smallest average muscle weight, while the average muscle weight was the largest in the low-protein diet group given *Colocasia gigantea* leaf ethanol extract at a dose of 100mg/kgBB. This can be associated with calorie restriction conditions on a low-protein diet, where reduced calories can reduce weight and skeletal muscle weight (gastrocnemius), as stated by Torrens-Mas et al., that it occurs in both sexes, but skeletal muscle weight is lower in female rats compared to male rats¹⁸.

Less than normal muscle mass falls into the criteria for diagnosing malnutrition¹⁹. However, muscle assessment is rarely performed in screening and nutritional assessment, due to the limited anthropometric and biochemical tools for the evaluation of muscle mass and function in malnourished patients²⁰. According to Prado et al., nutritional interventions are very important in spurring anabolism,

reducing catabolism and supporting the improvement of subnormal muscle conditions and malnutrition. In the assessment of muscle mass, an examination can be carried out to assess muscle mass in the process of improving nutrition.

When body composition tests are not available, physical examinations of muscles and anthropometry (upper middle arm circumference and calves) can be performed. In the calf muscle examination, it can represent muscle mass, including the gastrocnemius and soleus muscles²¹.

Malnutrition of protein energy, increased catabolism and inadequate nutrition lead to the loss of lean body mass with shrinking muscles accompanied by weak immune conditions²². In this study, the thickness of the soleus muscles in the group given a low-protein diet without being given *Colocasia gigantea* leaf ethanol extract had the smallest average thickness, while the group given a dose of 200mg/kgBB showed an average muscle thickness that exceeded that of the normal diet group, in addition, The greatest muscle thickness is also found at a dose of 200mg/kgBB. Based on this, malnutrition is related to decreased muscle function and mass as well as a decrease in health status. Protein supplementation is known to prevent muscle mass loss²³. Protein supplementation can increase body weight, lean muscle mass, bone area, mineral content and body density²⁴. Leucine supplementation diets have been shown to increase protein synthesis in muscles. Long-term supplementation can increase growth hormone resistance in mice with protein energy malnutrition. Leucine can spur skeletal muscle protein synthesis by regulating downstream anabolic signaling transduction²⁵.

Leucine is a branched-chain amino acid, which has been known recently as a therapeutic agent for the treatment of various conditions related to muscle wasting due to its ability to accelerate protein synthesis and reduce protein breakdown in the muscles. Leucine supplementation is also safe and effective for increasing muscle protein synthesis as well as reducing the loss of lean mass under catabolic conditions²⁶. L-leucine and its derivatives can regulate protein synthesis by direct or indirect activation of the mTORC1 kinase, which further improves muscle protein balance²⁷. The mTOR (*mechanistic Target of Rapamycin*) pathway regulates homeostasis by directly influencing protein synthesis, transcription, autophagy, metabolism, and biogenesis and organelle maintenance²⁸.

Research by Pertille et al., showed that skeletal muscle regeneration after malnutrition conditions showed a higher number of connective tissue and areas of inflammation in the MRI (*Malnourished Recovered and Injured group*). In addition, the CSA area of the regenerated myofiber decreased on MRI and there was a slow change in the process of anterior tibial muscle regeneration and slow muscle repair even after nutrient recovery²⁹. From this, additional protein supplements can be given to improve nutrition, in addition to being given adequate nutrition to improve malnutrition conditions due to

the provision of a low protein diet. In this study, we tried to Adlina Karimina Nurul Husna¹, Tri Widyawati^{1,2,3}, Dwi Rita Anggraini^{1,4}

(*Colocasia gigantea*). As mentioned by the USDA Agricultural Research Service, every 100 grams of *Colocasia* leaves contain 9 essential amino acids, of which leucine has the highest content, which is 0.392 grams. Although the level of essential amino acids in the ELETP has not been examined in this study, it can still be seen in giving a dose of 100 mg/kgBB that shows the greatest muscle weight and in a dose of 200mg/kgBB shows the greatest muscle thickness.

CONCLUSION

Based on the results and discussion of this study, it was concluded that there was no significant difference in the average muscle weight and soleus muscle thickness in each treatment group. However, ELETP at a dose of 100mg/kgBB produced the most muscle weight, while the one that showed the greatest muscle thickness was ELETP at a dose of 200mg/kgBB. The suggestion for the next study is to conduct a study with an intervention duration of more than 42 days, which is approximately 63 days to see the effect of the test material on the soleus muscle in malnourished model mice.

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