International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Special Issue 2, 2025

Original Article

EVALUATION OF ETHANOL EXTRACT OF TARO LEAVES (*COLOCASIA GIGANTEA*) AS MALNUTRITION THERAPY: *IN VIVO TEST*

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Received: 15 Mar 2025, Revised and Accepted: 15 May 2025

ABSTRACT

Objective: Skeletal muscle mass and small intestine function are two areas of the body that are severely impacted by malnutrition. Many communities rely on *Colocasia gigantea* (Talas Padang) as a common food source to address this nutritional challenge.

Methods: The design of this study included male white rats of Wistar strain 6-8 w old induced into 25 malnourished model mice with a Post-test Only Control Group involving 5 experimental groups. Group K1 (normal control) was given normal diet for 56 d, while Group K2 (positive control) was given a Low Protein Diet (LPD) for 14 d followed by a normal diet for 42 d. As for Groups K3, K4, and K5 received 14 d of LPD followed by a normal diet combined with Ethanol Extract of Taro Leaves (EETL) at doses of 100, 200, and 400 mg/kgBW orally for 42 d, respectively. The expression of AMPK-α1 in soleus muscle and PepT1 expression in small intestine tissues were examined with immunohistochemistry. The analysis was done using Shapiro-Wilk test for normality, Levene's for homogeneity, one-way ANOVA for parametric data, and Kruskal-Wallis non-parametric test for comparison. Post-hoc analysis was carried out by Mann-Whitney test.

Results: The administration of ethanol extract of taro leaves (EETL) appeared to affect the expression of AMPK-α1 and PepT1 in Wistar rats. While group K4 (200 mg/kgBW) showed the highest AMPK-α1 expression (median: 18, IQR: 9.00), no statistically significant differences were observed among the groups ($p>0.05$). For PepT1 expression, group K4 also demonstrated elevated and more consistent values (median: 15, IQR: 3.00), with a statistically significant difference between treatment groups ($p = 0.028$).

Conclusion: Although the expression of AMPK-α1 did not increase statistically, the treated rats showed an increase in expression, which was usually seen in association with the improved PepT1 expression in the treated animals. Therefore, EETL at a dose of 200 mg/kgBW is potentially beneficial in modulating the malnutrition-relevant parameters, albeit just showing trends for increased expression at that dose. Further studies should continue to be done to confirm the effects and mechanisms behind them.

Keywords: AMPK-α1, *Colocasia gigantea*, Malnutrition, PepT1, Talas padang

DOI: https://dx.doi.org/10.22159/ijap.2025.v17s2.03 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Stunting is a marker of chronic malnutrition experienced over a long period [1]. According to UNICEF and WHO, the global stunting prevalence in 2020 was 21.3% [2]. This medical condition is assessed using a z-score by measuring height-for-age, where values greater than 2 standard deviations or below the median of the World Health Organization (WHO) Child Growth Standards are classified as stunting [3]. Compared to information on maps of various continents, the prevalence of stunting in Indonesia is still higher than in Southeast Asia, which is 24.7%. This is evidenced based on data from the 2019 Study of Nutritional Status of Toddlers in Indonesia (SSGBI), where an incident rate of 27.67% was recorded.

Several causes of stunting that can be understood through a molecular method include inherited condition (familial), endocrine disease, chromosomal, chronic disease, malnutrition, and history of previous breastfeeding. Moreover, stunting can be categorized into familial and pathological conditions [4]. Malnutrition is a widespread problem, affecting the global population at several stages of life. In public health epidemiology, malnutrition is a general problem, predominantly affecting the most vulnerable. This includes poor groups, children, adolescents, the elderly, individuals suffering from diseases, and those with compromised immune systems, such as breastfeeding and pregnant women. Malnutrition includes both undernutrition, such as wasting, stunting, underweight, and mineral- and vitamin-related, as well as overnutrition, including overweight, obesity, and diet-related non-communicable diseases [5].

Acute malnutrition, according to Dipasquale *et al.*, is caused by inadequate protein or energy intake [6]. As a source of amino acids for the body's protein synthesis and other molecules with diverse functional roles, protein is typically obtained from food. All of the body's physiological and biochemical processes also depend on energy. Moreover, AMPK (Adenosine Monophosphate-Activated Protein Kinase) is the intracellular energy status system that maintains energy stores by refining anabolic and catabolic pathways. In networks with extremely variable energy turnover, AMPK is an important energy sensor [7].

AMPK regulates multiple targets and affects muscle growth, development, mass, and regeneration as part of its role in controlling the metabolism of skeletal muscle. In particular, during regeneration, AMPK α 1 stimulates anabolism and controls the dynamics of muscle cells, whereas during atrophy, AMPK α 2 regulates degradation [8]. Malnutrition results in muscle weakness and disability and may be a contributing factor to muscle atrophy or loss of skeletal muscle mass in the musculoskeletal or nervous system [9]. According to a prior study, the soleus and plantaris muscles of the malnourished group showed higher levels of AMPK expression. This phenomenon led to increased oxidative stress-induced AMPK-independent SIRT1 inhibition, which in turn reduced the mitochondrial metabolic capacity in both fast and slow muscles [10].

Intestinal peptide transporter PepT1 is the main source of providing amino acids in large quantities to epithelial cells. PepT1 belongs to very important solute transport metabolism as it belongs to the SLC15 solute carrier family. Its transport capacity is high but has low affinity. It is located in the apical membranes of enterocytes within the small intestine and in the distal colon [11]. PepT1 is a sodium-independent symporter or cotransporter with a large capacity that

catalyzes the sequential and independent electrogenic transport of in-and tri-peptide L-enantiomers. The driving force is provided by transmembrane electrochemical proton gradient and peptide translocation in conjunction with H+ movement. In addition to absorbing in-and tripeptides released during the digestion of endogenous proteins from the small intestine or dietary proteins, PepT1 controls the body's supply of nitrogen [12].

Moreover, malnutrition brought on by insufficient protein intake is connected to absorption in the small intestine. This demonstrates that in order to meet energy and protein needs, nutritional improvements are required. To promote nutritional improvements, it is necessary to take into account the availability of additional therapy for treating malnutrition conditions. The prevalence of malnutrition can be reduced by obtaining locally grown plants that are rich in different nutrients, such as proteins and carbohydrates. One of the most well-known plants is talas; specifically, the leaves of the *Colocasia gigantea* species, which is referred to locally as talas padang, are a rich source of nutrients. These leaves have historically demonstrated significant promise for the development of foods and pharmaceuticals [13] because they are high in nutrients, including protein, carbs, fat, calcium, phosphorus, iron, and vitamins A, B, and C.

Colocasia leaves have 86.94% water content, 16.48% crude protein, 17.24% crude fiber, 1.45% potassium, 0.4% phosphorus, 4.3% fat, 30.46% Non-Nitrogen Free Extract, and 3966 kcal/kg [14] gross energy. Despite all of these advantages, no research has looked at how Talas Padang (Taro) leaves affect malnutrition in general or in the skeletal muscles and small intestine in particular. Thus, the purpose of this work was to examine how Ethanol Extract of Taro Leaves treatment affected the expression of AMPK-α1 in the skeletal muscle and PepT1 in the small intestine in a model of malnourished Wistar strain rats.

MATERIALS AND METHODS

Preparation of extracts

Colocasia gigantea from Medan area was verified by Herbarium Medanense, Universitas Sumatera Utara (No.031/MEDA/2022). The further process was carried out using an ethanol extract at the Pharmacology Laboratory of the USU Medan Faculty of Pharmacy. The weight of the fresh leaf sample was 14.750 g, and it was gradually dried using a drying cabinet. The dried simplicia was milled into simplicia powder, weighing 2100 g. Ethanol Extract of Taro Leaves (EETL) was obtained by using the maceration method, which involves a 1:10 (21 l) ratio of 96% ethanol solvent, the powder is extracted. After soaking the simplisia powder in 75 parts of the solvent (15.75 l) for five times as long at room temperature with an aluminum foil lid and periodic stirring, the powder is filtered through filter paper to produce filtrate and residue.

The residue was re-soaked for two 24-hour periods (remaceration) using the remaining 25 parts of the solvent (5.25 l), covered with aluminum foil, and stirred every two hours. The residue and filtrate were then filtered again. After combining the filtrates 1 and 2, the resulting extract is vaporized using a vacum rotary evaporator to produce a thick extract. The thick extract weighed about 120 g, resulting in a yield percentage of 5.71%. Following investigational ranges used in previous animal studies involving plant extracts, the doses of 100, 200, and 400 mg/kg used in this study were selected.

Acclimatization

White rats of the 6-8 w old Wistar strain were Acclimatized for 7 d in a laboratory environment with a temperature of 22–25 °C, humidity of 50–70%, and a light cycle of 12 h of light-dark. Animals are kept in small groups with good ventilation. During acclimatization, animals are given standard feed and ad libitum water. Regular monitoring is carried out to ensure the health of the animals before being given an experimental diet.

Experimental groups

The experimental groups included K1, which was normal rats given a normal diet for 56 d, K2 served as positive control fed with Low Protein Diet (LPD) for 14 d followed by a normal diet for 42 d, and K3 was given LPD for 14 d, with normal diet, and EETL dose 100 mg/kgBW orally once a day for 42 d. Furthermore, K4 was given LPD for 14 d, normal diet, and EETL dose 200 mg/kgBW orally once a day for 42 d, K5 received LPD for 14 d, normal diet, and EETL dose 400 mg/kgBW orally once a day for 42 d.

Tissue sampling

Rats were terminated using inhalation anesthesia with concentrated chloroform; after rats were unconscious, surgery was performed to collect samples. Rat limbs' posterior aspects were dissected in order to sample skeletal muscles (soleus). The achilles tendon and gastrocnemius muscle were found, which made it easier to determine which muscle to cut: the soleus. The small intestine was then sampled by longitudinally cutting the proximal portion of the duodenum to the distal ileum. In addition, the midpoint of the incision was measured from proximal to distal, and measurements of 5 cm were taken to the left and right to symbolize the ileum and jejunum.

A 10 cm sample of the small intestine was extracted as a result of this procedure. To facilitate histopathological preparations of the small intestine and skeletal muscle (soleus), as well as immunohistochemical (IHC) procedures for AMPK-α1 skeletal muscle (soleus) and PepT1 small intestine, all samples were placed into a pot with a 10% formalin buffer solution.

AMPK-α1 skeletal muscle (soleus) Immunohistochemistry procedure

AMPK-α1 immunohistochemistry procedure of skeletal muscle (soleus) using Soleus muscle tissue paraffin blocks were cut to 4 µm thickness using a microtome, then deparaffinized with xylol. AMPK-α1 polyclonal antibody (BIOSS, bs-5551R) was used and detected using Powervision (DAKO A/S, Denmark) as well as visualization with peroxidase-DAB. Sample evaluation was carried out by the researcher and one pathologist. Examination of soleus muscle cells was carried out in every 20 fields of view; images of each slide were photographed with an Olympus CX22 microscope and a 5 MP microscope camera (OPTILAB ADVANCE) to see areas stained by reagents marked with brown in the nucleus and cytosol. The size of the stained area was examined using 40x magnification (10x ocular lens and 4x objective lens) with an assessment of 0-4% being given a score of 1, 5-20% being given a score of 2, 21-40% being given a score of 3, 41-60% being given a score 4, 61-80% are given a score of 5 and 81-100% are given a score of 6. Then calculate the color intensity with 400x magnification (10x ocular lens and 40x objective lens) which is given a score of 0 if there is no brown color, if the intensity of the brown color is light = 1, moderate = 2 and strong = 3, then the final assessment of each slide is carried out by calculating Area x Intensity for statistical analysis.

PepT1 small intestine immunohistochemistry procedure

PepT1 immunohistochemistry procedure of small intestine using small intestine tissue paraffin blocks were cut to 4 µm thickness using a microtome, then deparaffinized with xylol. PepT1 polyclonal antibody (BIOSS, bs-0689R) was used and detected using power vision (DAKO A/S, Denmark) as well as visualization with peroxidase-DAB. Sample evaluation was carried out by the researcher and one pathologist. Examination of small intestinal cells was carried out in every 20 fields of view; images of each slide were photographed with an Olympus CX22 microscope and a 5 MP microscope camera (Optilab Advance) to see the area stained by the reagent marked with brown at the apical membrane of the villi. The size of the stained area was examined using 40x magnification (10x ocular lens and 4x objective lens) with an assessment of 0-4% being given a score of 1, 5-20% being given a score of 2, 21-40% being given a score of 3, 41-60% being given a score 4, 61-80% are given a score of 5 and 81-100% are given a score of 6. Then calculate the color intensity with 400x magnification (10x ocular lens and 40x objective lens), which is given a score of 0 if there is no brown color, if the intensity of the brown color is light = 1, moderate = 2 and strong = 3, then the final assessment of each slide is carried out by calculating Area x Intensity for statistical analysis.

Statistical analysis

Data collected were examined to see how the means of PepT1 and AMPK-α1 expression for each treatment group were compared to

the others. Following the validation of normality by using the Shapiro-Wilk test, the Levene test for homogeneity was performed when there were less than fifty samples. The Kruskal-Wallis test was then carried out to analyze AMPK α1 expression in skeletal muscle (soleus) and PepT1 expression in the small intestine. Following the above procedure, Mann-Whitney tests were used for treatment groups with different dose variations, as shown in fig. 1. Descriptive statistics were used to report the interquartile range (IQR) and the 95% confidence intervals (CIs), which helped in assessing the dispersion, variability, and precision of expression values across the groups.

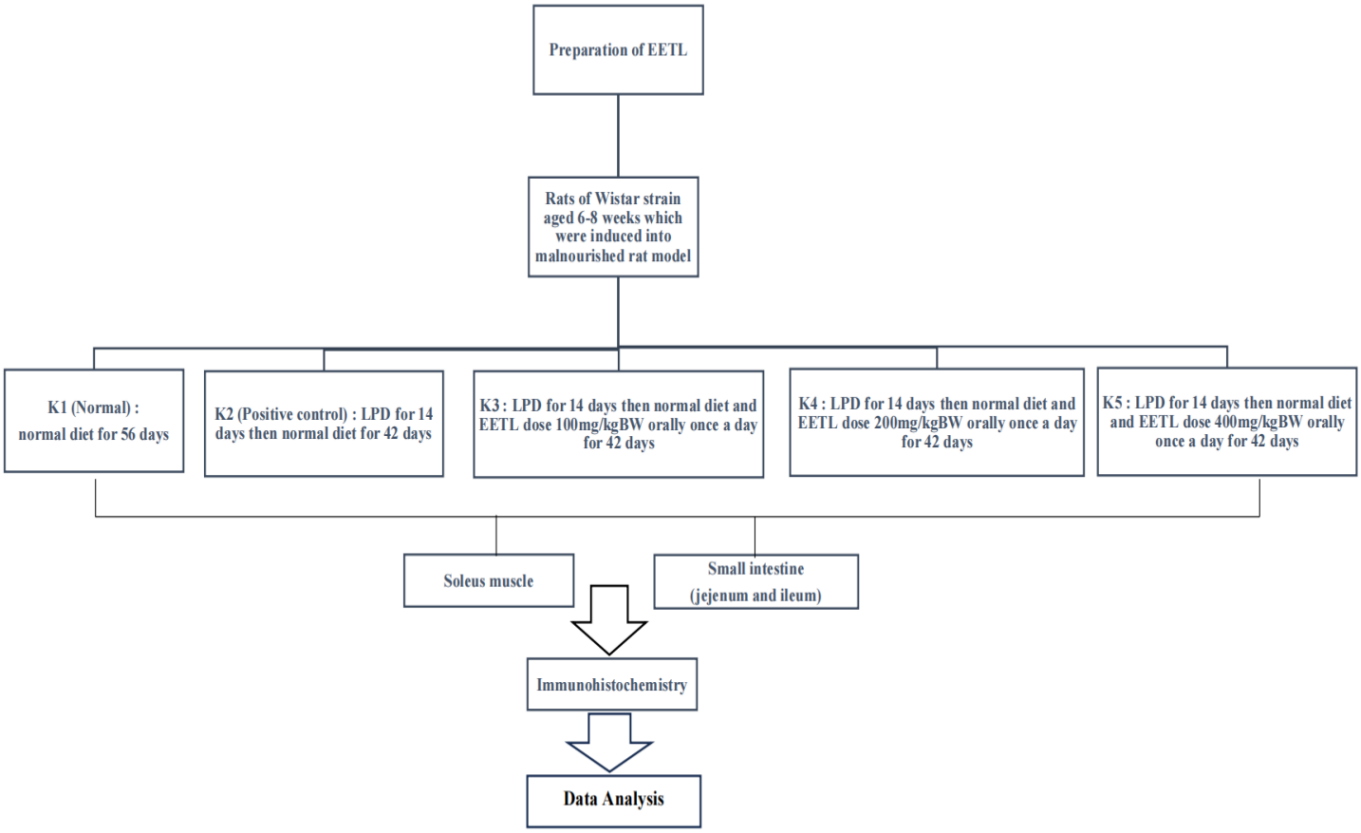

Fig. 1: Research flowchart

Methods

This study used animal models and a Post-test Only Control Group design, which is a true experimental methodology. Ethical approval was received from the USU Ethics Commission No. 0150/KEPH-FMIPA/2023. The study population consisted of Wistar strain male white rats that were 6–8 w old. Using the Federer formula, a total of 25 rats were made into malnourished model rats and then randomly assigned to 5 groups. In contrast to rats with a normal diet who were given feed (pellets) that already filled with protein, carbohydrates and fats according to their needs, mouse models malnutrition is obtained by giving a Low Protein Diet (DRP) during 14 d. The sample was made up of small intestine and skeletal muscle tissue from rats raised in a malnutrition model, as shown by histopathology of paraffin blocks. The absence of damage, such as mold growth, and the ability to read the histopathological results of paraffin blocks were requirements for inclusion. In the meantime, the intestinal lumen's histopathological paraffin block results showing a parasitic picture served as the exclusion criterion.

RESULTS AND DISCUSSION

Effect of EETL on skeletal muscle (Soleus) AMPK-α1 expression

Muscle mass-related anabolism and muscle regeneration activity are indicated by AMPK-α1 expression. For KI and K2 group, there was a significant difference, as shown in fig. 2.

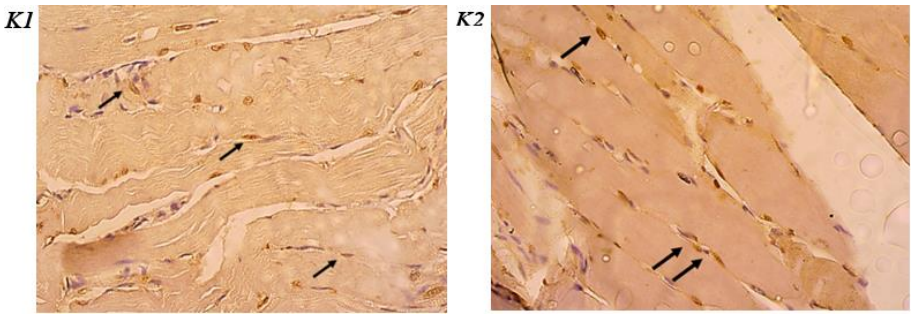

Fig. 2: Microscopic images of muscle tissue by staining AMPK-α1 polyclonal antibodies (brown colour in nucleus, arrow in the figure) in normal (K1) and positive control (K2) rats. IHC, 400x

In contrast to the K1 group (normal), the K2 group, acting as a positive control and lacking EETL dose intervention, exhibited a greater intensity of α1 AMPK expression. The K4 group, which was administered 200 mg/kgBW of *Colocasia gigantea* leaves and was malnourished, exhibited the highest intensity of AMPK-α1 expression. When comparing the nucleus and cytosol of brown skeletal muscle cells to the group that received dose variations of 100 mg/kgBW and 400 mg/kgBW (K3 and K5), this effect was primarily seen in these areas. Meanwhile, table 1 and fig. 3 demonstrates that the K5 group had the lowest intensity.

Table 1: Effect of EETL on intergroup skeletal (Soleus) muscle α1 AMPK expression

Group	n	AMPK-α1 expression *Score* [Median (min-max)]	*IQR*	*Mean±SD*	95% CI	*p-value*
K_1	5	12 (8-18)	6.00	12.00±3.74	7.35–16.65	
K_2	5	12 (8-18)	6.50	13.00±3.74	8.35–17.65	
K_3	5	12 (8-15)	5.00	12.40±2.88	8.82–15.98	0.693
K_4	5	18 (8-18)	9.00	14.40±4.98	8.22–20.58	
K_5	5	12 (6-15)	6.50	10.60±3.58	6.16–15.04	

(Kruskal-Wallis test: p>0.05 K1 (normal); K2 (positive control); K3 (EETL dose 100 mg/kgBW); K4 (EETL dose 200 mg/kgBW); K5 (EETL dose 400 mg/kgBW).

The AMPK-alpha-1 expression median was 18 for group K4 (IQR: 9.00), while the mean was 14.40±4.98 and the 95% CI ranged from 8.22 to 20.58, making it higher than other groups. For example, K1 had a median of 12 (IQR: 6.00), mean of 12.00±3.74, and a 95% CI that is 7.35-16.65-hence it could be inferred that there is stimulation of anabolic activity by EETL treatment at 200 mg/kgBW. The fact that group K4's CI is found to be very wide reflects variability of individual response that could result from differences among individuals in extract uptake or metabolism. On the contrary, narrower CIs in the remaining groups suggest that they produced either uniform effects or much lesser biological variability.

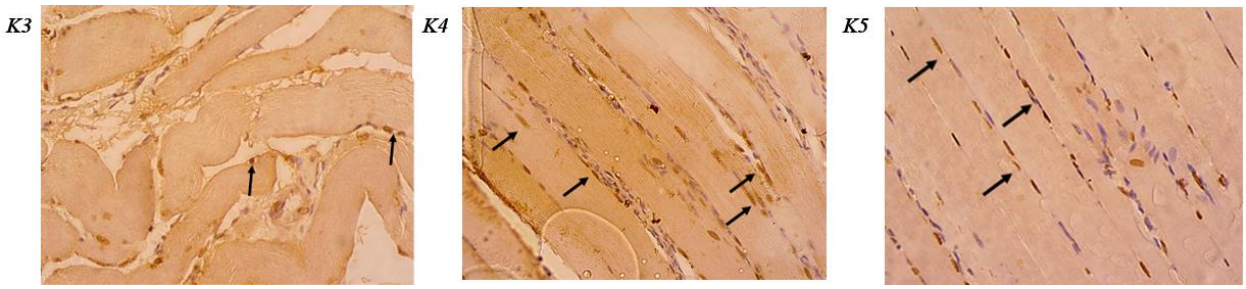

Fig. 3: Microscopic image of muscle tissue by staining polyclonal antibody AMPK-α1 (brown colour in nucleus, arrow in the figure) in malnourished rats with EETL doses of 100 mg/kgBW (K3), 200 mg/kgBW (K4) and 400 mg/kgBW (K5). IHC, 400x

Differential expressions of AMPK-α1 were found in every LPD group in this investigation. The 200 mg/kgBW dose group demonstrated significant expression, according to the results. Heterotrimeric protein kinases, specifically the α, β, and γ subunits, can be grouped into about 12 distinct isoforms that make up the essential AMPK subunits, each contributing to the vital role of AMPK in energy regulation. Because of its variety, AMPK in skeletal muscle tissue is extremely complex. The complexity stems from the heterogeneity of skeletal muscle tissue, which includes different types of blood cells, endothelial and adipose cells, fibroblasts, and myofibers, or muscle fibers. These additional cells provide AMPK heterotrimers that are absent from the myofibers, albeit in small quantities [15, 16]. This demonstrates that the expression of AMPK-α1 soleus muscle can be determined by immunohistochemical analysis without homogenizing skeletal muscle tissue, providing specificity to each treatment group. Additional thought is necessary because the computation is based on the staining seen during the preparation.

AMP-activated protein kinase (AMPK) is an enzyme composed of three subunits, which can include either an α1 or α2 catalytic subunit. It functions as an energy sensor that helps maintain cellular balance [17]. The ATP to AMP/ADP ratio decreases in the context of malnutrition, which is defined by inadequate energy or protein intake. This is due to an energy-stress condition. This activates AMPK kinase activity, upregulating ATP-producing catabolic pathways and reducing catabolic pathways. AMPK is a heterotrimeric complex that serves as a regulatory center for energy homeostasis [18]. The process of mitochondrial turnover and dynamics is fundamental to a healthy neuromuscular system, with the regulation of AMPK being an important mechanism. AMPK stimulates mitochondrial biogenesis as well as take part in organelle dynamics and mitophagy [19]. Natural antioxidants may perform an important role in reducing oxidative stress. The combination may be the means by which specific antioxidants together reduce oxidative damage and enhance cellular defense and physiological stability [20]. To address malnutrition, nutritional improvements are essential, considering the provision of natural ingredients from plants with antioxidant activity such as (-)-epicatechin in *Colocasia gigantean.* Muscle mass and mitochondrial metabolic capacities were found to be significantly reduced as a result of the elevated oxidative stress associated with malnutrition. This suggested that in cases of malnutrition, antioxidants may help to improve nutritional status.

The average score of the highest soleus muscle AMPKα1 expression calculation showed that the LPD group given a dose of 200 mg/kgBW had the highest expression. Compared to the normal diet group, the average expression was lower. The findings were consistent with Hirabayashi *et al.* 10's finding that the malnutrition group receiving an LPD and daily feeding had AMPK expression levels limited to a 50% increase in soleus and plantaris muscle. Nevertheless, in the malnutrition model of wistar rats, there was no discernible variation among the different EETL doses concerning the expression of AMPK-α1 soleus muscle.

Effect of EETL on small intestine

A histopathological analysis was performed to ascertain the general status prior to analyzing PepT1 expression in the small intestine, as

illustrated in fig. 4-5. In the microscopic image of jejunum-ileum histopathology in K1, a normal structure was observed from the Muscle Layer to the Villous which, showing the absence of pathological conditions or collapse. In K2 as a low-protein diet group without intervention doses of EETL, Villous collapse and necrosis showed atrophy, as presented in fig. 4. For K3, the picture showed cell exocytosis, while K4 was almost similar to the normal diet group. Furthermore, the K5 group had features that led to parasitic infection (worm), classified among the exclusion criteria, as shown in fig. 5.

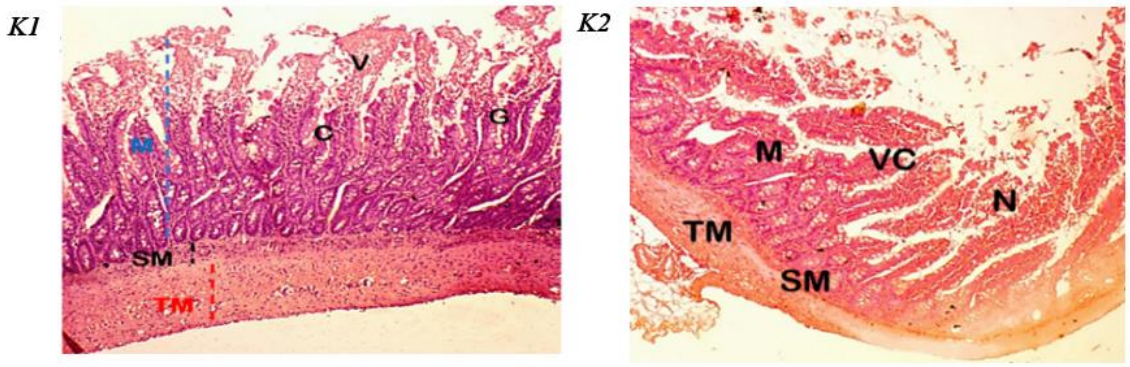

Fig. 4: Histopathology of the small intestine in the normal rats' group (K1) and the positive control group (K2). TM: Tunica Muscularis (red line), SM: Sub Mucosa (black line), M: Mucosa (blue line), V: Villi, C: Crypts, G: Goblet Cells, N: Necrosis, VC: Villous Collapse. HE, 100x

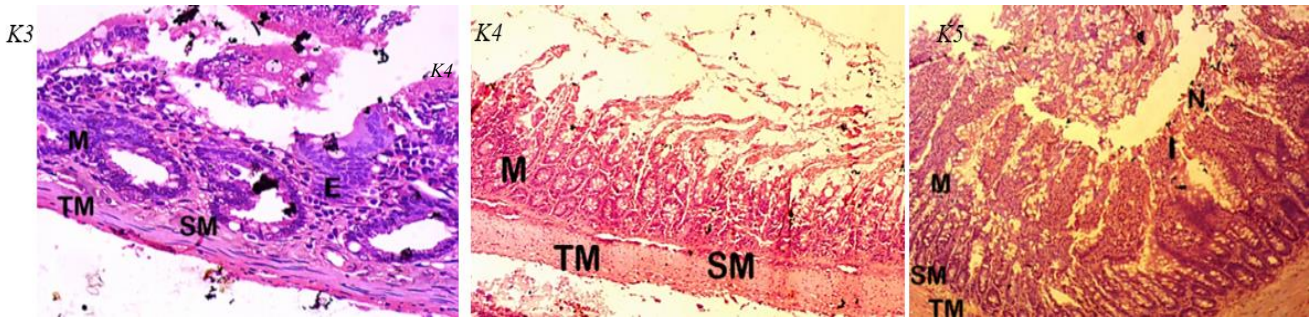

Fig. 5: Histopathology of the small intestine in the malnutrition model group with EETL doses of 100 mg/kgBW (K3), 200 mg/kgBW (K4) showing a normal picture, 400 mg/kgBW (K5). TM: Tunica Muscularis, SM: Sub Mucosa,, M: Mucosa, E: Exocytosis, I: Infiltrate, N: Necrosis. HE, 100x

Without EETL intervention, microscopic images of the small intestine histopathology representing the jejunum-ileum of LPD groups revealed the presence of necrosis and villous collapse, which indicate intestinal tissue damage or atrophy. In the meantime, the group administered 200 mg/kgBW of LPD displayed a picture that was nearly identical to that of a group on a typical diet. PepT1 plays a critical role in maintaining intestinal homeostasis because it is responsible for the absorption of dipeptides and tripeptides and regulates certain microRNAs and proteins along the crypt-villus axis. PepT1-depleted mice were observed to have diminished body weight, shortened intestinal microvilli, and disturbed expression patterns of miRNAs and proteins in the jejunum. This suggests PepT1 may play a critical role in maintaining the structure and function of the small intestine [21].

Using histopathological preparations and IHC staining, which showed up as a brown color on the villi's apical membrane, the expression of small intestinal PepT1 was evaluated in each group. The K3 group appears to have the lowest level of PepT1 expression. In the meantime, K1 and K2, as shown in fig. 6-7, were the groups that demonstrated high intensity.

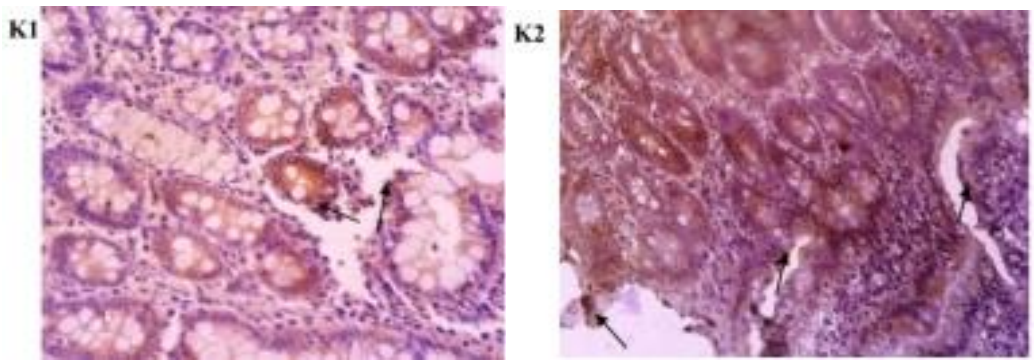

Fig. 6: Microscopic lamination of small intestinal tissue (jejunum-ileum) by staining using PepT1 polyclonal antibodies (brown colour in cells, arrow ini the figure) in normal rats' group (K1) and positive control group (K2). IHC, 400x

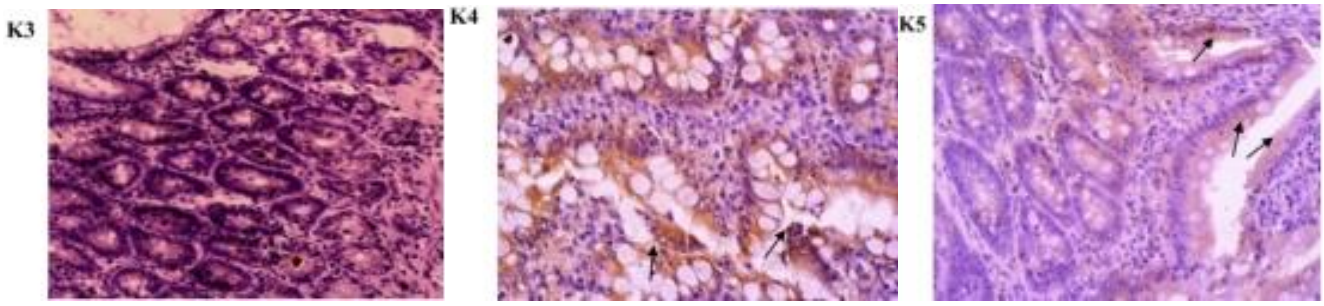

Fig. 7: Microscopic image of small intestinal tissue (jejunum-ileum) by staining using PepT1 polyclonal antibodies (brown colour in cells, arrow ini the figure) in the malnutrition group with EETL doses of 100 mg/kgBW (K3), 200 mg/kgBW (K4) and 400 mg/kgBW (K5). IHC, 400x

Analysis of PepT1 immunohistochemistry was conducted to find out how the administration of different doses of EETL and LPD intervention affected the expression of PepT1 on the apical membrane of enterocytes. Comparing the LPD group to the other groups, the results indicated that the group receiving a dose of EETL 100 mg/kgBW had the least intensity of small intestinal PepT1 expression. In the meantime, the group with the highest intensity belonged to the groups on a normal diet and the LPD group that did not receive an EETL. PepT1, a proton-dependent oligopeptide transporter, plays a vital role in maintaining intestinal homeostasis. Primarily expressed in enterocytes of the small intestine, it facilitates the uptake of di-and tripeptides derived from dietary proteins [22]. Thus, the potential health benefits of this plant extract may depend on dosage and duration of use, highlighting the need for further research [23].

The Kruskal-Wallis test was carried out due to the non-normal distribution of two groups, namely K1 and K2. The resulting p-value was 0.028, which was smaller than 0.05. This showed that there were significant differences in various doses of EETL on small intestinal PepT1 expression in Wistar strain rats model of malnutrition. As shown in table 2, the K5 was excluded due to the indication of microscopic features of parasitic infections in the intestinal lumen. Subsequently, the Mann-Whitney test was used to assess the comparison between each dose of EETL. Based on the results presented in table 3, significant differences in small intestinal PepT1 were observed between K1 and K3, K2 and K3, as well as K4 and K3.

Table 2: Differences of PepT1 expression between treatment groups

Group	n	PepT1 expression of the small intestine [Median (min-max)]	*IQR*	*Mean±SD*	95% CI	*p-value*
K_1	5	18 (12-18)	4.50	16.20±2.68	12.87–19.53	
K_2	5	18 (10-18)	5.50	15.80±3.49	11.46–20.14	0.028*
K_3	5	10 (8-12)	8.00	13.60±4.10	8.51–18.69	
K_4	5	15 (12-18)	3.00	16.80±1.64	14.76–18.84	

(*p<0.05 with Kruskal-Wallis test; K5 is excluded because parasites are found in the intestinal lumen; K1 (normal); K2 (positive control); K3 (EETL dose 100 mg/kgBW); K4 (EETL dose 200 mg/kgBW).

Among all groups, the group that showed the least IQR on PepT1 expression was represented by K4 (IQR: 3.00) whose mean value is 16.80±1.64 and has a CI95% of 14.76–18.84 signifying a more consistent and precise treatment response. The highest IQR can be seen in group K3 (IQR: 8.00) which is also said to have a mean 13.60±4.10 and a CI95% of 8.51–18.69; thus, it reflects higher variability. This result indicates that the 200 mg/kgBW dose not only enhances PepT1 expression; it also does so at lower interindividual variability and strengthens the case as the optimal therapeutic dose.

Table 3: Differences of PepT1 expression inter-treatment group

Groups	[Median (min-max)]	*p-value*
K_1 and K_2	18 (12-18) dan 18 (10-18)	0.906
K_1 and K_3	18 (12-18) dan 10 (8-12)	0.013*
K_1 and K_4	18 (12-18) dan 15 (12-18)	0.371
K_2 and K_3	18 (10-18) dan 10 (8-12)	0.041*
K_2 and K_4	18 (10-18) dan 15 (12-18)	0.435
K_3 and K_4	10 (8-12) dan 15 (12-18)	0.013*

(Mann Whitney test, significant value *p<0.05; K1 (normal); K2 (positive control); K3 (EETL dose 100 mg/kgBW); K4 (EETL dose 200 mg/kgBW).

Reduced PepT1 leads to a deficiency of various dietary proteins or peptides absorbed from the intestinal lumen, resulting in malabsorption syndrome [24]. The administration of ethanol extract of *Colocasia gigantea* leaves, containing 9 essential amino acids is expected to overcome the continuation of malnutrition conditions. The process also aimed to prevent the worsening of malabsorption in the intestine due to chronic villous atrophy, hyperplasia crypts, and inflammatory cell infiltration leading to necrosis in the small intestine image in the malnutrition group. In this study, there were significant differences in various doses of EETL on the expression of small intestinal PepT1 in wistar rats malnutrition model. The group with significant differences in the ethanol extract was revealed by the Mann-Whitney test. These consist of LPD receiving an EETL dose of 100 mg/kgBW and a group following a typical diet. Furthermore, a noteworthy distinction was noted between K1 and K3, K2 and K3, and K4 and K3.

Structural atrophy of the mucosa during non-administration of the diet, as needed, was shown to worsen in two distinct metabolic phases. These included the mobilization phase of fat stores as body

fuel and increased catabolism of proteins for energy expenditure, leading to increased apical expression of PepT1. However, there is a phase that allows complete restoration of the jejunum morphology in 3 d after re-feeding the diet. In this study, the role of EETL is intended as a treatment for malnutrition conditions to enhance the intake of nutritious foods, particularly diets that meet nutritional content according to energy and protein needs. After re-feeding, the experimental animals improve their image in the jejunum by giving extract. This demonstrates that in cases of malnutrition, the administration of extract can hasten recovery and improvement. The histopathological image of the group administered 200 mg/kgBW (K4) along with LPD shows an almost normal morphology.

To enhance clinical relevance, the doses assessed in this study can be converted to human equivalent doses (HED) using body surface area scaling. The conversion factor for rats to humans (according to FDA guidelines) is 0.162. Thus: 100 mg/kgBW in rats is about equal to 16.2 mg/kgBW in humans; 200 mg/kg body weight in rats is approximately 32.4 mg/kg body weight in humans. The equivalent dose of 400 mg/kgBW in rats is about 64.8 mg/kgBW in humans. For a 60-kg adult, the amounts correspond approximately to 972 mg, 1,944 mg, and 3,888 mg per day, respectively. The 200 mg/kgBW dose (human equivalent ~2 g/day) may thus represent a therapeutically feasible and safe range for future translational studies. The future exploration into the pharmacokinetics, safety profile, and dose-optimization of *Colocasia gigantea*, in conditions of malnutrition or similar clinical situations should be guided by suitable and relevant considerations.

Some limitations in this present study involved the absence of physiological measurements (i. e., body weight, muscle mass, and serum albumin) and lack of handling of markers ATP or PGC-1α, or performing Western blot/qPCR validation. Whereas elaborate phytochemical analysis (such as HPLC for (-)-epicatechin) was not included as parameters along with fasting duration or humane endpoints, these are liminally considered in further studies to allow mechanistic and clinical relevance. Related to phytochemical binding to its target, in pharmacology, particularly in the areas of drug design and pharmacokinetics, the hypothetical tool of computing technology is extremely helpful [25]. Binding interactions between the drug candidates and their target proteins would explain, thus affect, their therapeutic efficacy and safety; such are also complemented by molecular docking studies with computational approaches [26].

CONCLUSION

The malnutrition model has shown that there was no statistically significant effect of EETL on the expression of AMPK-α1 in skeletal muscle in Wistar strain rats. However, group K4 (200 mg/kgBW) showed the highest median and mean value with a relatively wider confidence interval, suggesting that there might be a positive effect but with a variable response. However, EETL at a lower dose of 100 mg/kgBW was found to have a significant suppressive effect against PepT1 expression in the small intestine, whereas 200 mg/kgBW was accompanied by an increased expression accompanied by narrower IQR and CI, pointing toward a more stable and consistent biological effect. Thus, based on the statistical significance and biological consistency, the 200 mg/kgBW dose would appear to be the best treatment; however, further studies are needed to determine its long-term effects and underlying mechanisms, including the dose-response observed during this 42-day intervention period.

ACKNOWLEDGEMENT

The authors are grateful for the supportive grant received from the Ministry of Research and Technology and the Higher Education Republic of Indonesia, Research and Community Service, Universitas Sumatera Utara Grant (No.9417/UN5.1. R/PPM/2022).

FUNDING

Nil

AUTHORS CONTRIBUTIONS

AKNH: carried out the concept, design, and drafting of the article. TW: supervise the concept, editor, statistical analysis, critical revision, supervision, and final approval. DRA: interpretation of data, critical revision, and final approval.

CONFLICT OF INTERESTS

The authors declare there is no conflict of interest

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