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Effect of Low Protein Diet Treatment for Six Weeks on The Brain Histopathological and Cognitive Function in The Wistar Rat

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***Abstract*— Malnutrition is a condition characterized by inadequate or excessive consumption of nutrients, resulting in decreased cognitive abilities, metabolic disorders, learning disabilities, growth retardation, a lowered immune system, and the risk of death. The prevalence of malnutrition in Indonesia was still relatively high. This research aims to identify the effect of low-diet protein treatment on the histopathology of the cerebral cortex, hippocampus, and cognitive function in Wistar rats. This study included two groups of male Wistar rats: the normal and low protein (12%) groups. Morphometric analysis was used to assess the growth. Cognitive tests included the Y-Maze, Novel Object Recognition Test (NORT), and Water Maze by Morris (MWM) tests, and histopathological examination of the brain sections was conducted on the cerebral cortex and hippocampus by hematoxylin and eosin stains. This study found that rats fed a 12% protein diet showed significant differences in body weight parameters, proportion of degenerated cells, and histopathological features of the cerebral cortex and hippocampus compared to the normal group fed a 20% protein diet, and this condition also affected the cognitive function of rats fed a low protein diet. The 6-week treatment with a 12% protein concentration left the rats malnourished.**

Keywords – Malnutrition; Body Weight; Cognitive Function; Low Protein Diet.

I. INTRODUCTION

Malnutrition is a condition in which an individual's nutritional intake is unbalanced due to insufficient or excessive consumption of nutrients. In 2020, an estimated 149 million children under five will have stunted growth, meaning they will be too short for their age. The preponderance of these cases occurred in low- and middle-income countries [1]. According to the Study on the Nutritional Status of Toddlers in Indonesia (SSGI) in 2022, the prevalence of stunting in Indonesia remained relatively high, at approximately 21.6% [2].

Malnutrition can cause cognitive problems, metabolic disorders, learning difficulties, mental retardation, and a weaker immune system, which makes it easier to get sick and more likely to die [3], [4], [5]. Malnutrition causes damage to tissues, slow growth, improper differentiation, a decrease in synapses and synaptic neurotransmitters, a delay in myelination, and a decrease in the complexity of dendritic growth in the growing brain. This condition changes the normal time frame for brain development, which in turn disrupts the formation of neural pathways [6]. Leenstra et al. (2004) found that malnutrition can cause long-term changes in brain function that could be tied to persistent cognitive impairment [7]. Alamy et al. (2005) and Bonatto et al. (2005) reported the effects of malnutrition on cognitive skills and how it affects neurotransmitter systems, protein phosphorylation and oxidative state in the brain [8], [9].

Brain growth and maturation are substantially impacted by chronic malnutrition. The stages of development involved in this process are intricate and multifaceted. These stages include the proliferation and migration of neural cells. The visual cortex, parietal neocortex, dentate gyrus, and cerebellum are among the brain regions whose thickness decreases significantly when dietary protein intake is insufficient [10]. Malnutrition-related cognitive impairments manifest as difficulties with memory retention, slower intellectual processing speed, and specific reading, writing, and mathematical learning difficulties [11].

Studies on the effect of low-protein diets on adult and pregnant rats are numerous, but those focusing on the effect of low-protein diets on 5-week-old growing rats have not been reported. This study aimed to examine the effect of feeding a diet with a protein level of 12% for 6-week to 5-week-old Wistar rats. The parameters seen are the impact on brain histopathology (cerebral and hippocampus) and associated with the cognitive function of rats treated with a low-protein diet compared to a normal group.

II. Materials And Methods

A. Animals and Research Design

Five-week-old male Wistar rats (Rattus norvegicus) were acquired from the animal housing in Muaropalam, West Sumatra, Indonesia. At least one week prior to the experiment, animals were housed in the laboratory under controlled environmental conditions: a constant temperature of 252°C, constant humidity of 6010%, and 12-hour light/dark cycles. A standard pellet diet and water were provided on an unlimited basis. Animal care and use, as well as experimental procedures, were carried out in accordance with the Declaration of Helsinki and the standard guideline for the care and use of animals for experimental purposes (No.985/UN. 16.2/KEP-FK/2022) established by the Research Ethics Commission of the Faculty of Medicine, Andalas University. During a six-week period, five male Wistar rats were fed a standard diet containing 20% protein (normal group) and five male Wistar rats were fed pellet feed containing 12% protein (protein diet 12% group). Rats were given food and water ad libitum.

B. Body Weight and Histopathological Analysis

Body weight measurements were conducted on a weekly basis using a laboratory animal scale [12]. Following the completion of a six-week treatment period, all animals were subjected to sacrifice. Subsequently, the brains of the dissected rats were preserved by immersion in a 10% formalin solution and subsequently embedded in paraffin. Tissue samples with a thickness of 5 µm were acquired from the hippocampus and cerebellar regions. These samples were subjected to staining using hematoxylin and eosin, and afterwards analysed using a light microscope for the purpose of histological investigation.

D. Cognitive Test

Before behaviour tests, the order in which the Y-Maze, Novel object recognition test (NORT), and Water Maze by Morris (MWM) tests were given was chosen at random. We used Go-PRO (HERO) to record how the mouse moved. Videos were tracked and scored with the help of ANY-Maze software or a blind spectator.

Morris's Water Maze (MWM)

The Morris water maze (MWM) was used to test rats' spatial learning and memory. Rat was put in a pool with a width of 116 cm and a height of 55 cm. The water temperature was between 21 and 23°C. The pool is split into four parts: North, South, East, and West. After the tests, the rats were warmed up and dried off gently before being put back in their box. For the test, a platform was put in the water, and the rats were timed on how long it took them to get there [13].

The Test of Y-maze

The Y-maze test is also used to check for spatial memory. This Y-maze has three arms 40 cm long, 8 cm wide, and 15 cm high. The arms are set at an angle of 1200 from the centre. During the test, two arms will be out in the open. One arm will be the starting place. Rat will have 5 minutes to look around with two open arms. After 2 hours of rest, the mice were looked at again. Kraeuter et al. (2019) found that rats with good spatial memory often go to new arms [14].

New Object Recognition Test (NORT)

Two identical items were put on opposite sides of the OFT box to test exploratory behaviours. The test has two parts: acclimatisation on the first day, training on the second and third days, and testing two hours after training. A blind person kept

track of how long people talked to each other. For this test, "interacting" meant that the rat sniffed the object or put its nose on it [15].

E. Statistical Analysis

Paired sample t-test was used for analysis of the differences data between Normal and Low Protein Diet group. ****p.0001, ***p.001, **p.01, *p.05, and ns = no significant correlation were used to denote statistical significance. Unless otherwise specified, analyses are expressed as the mean with SEM.

III. Result And Discussion

A. Body Weight and Histopathological Analysis

The average body weight of the rats increased significantly in the normal group and decreased significantly in the protein diet (12%) group ($p<0.05$). Figure 1 shows the average weight gain after six weeks of treatment with a normal diet (20%) of 98 grams and a protein diet (12%) of 90 grams to 80 grams. Compared to the control group (N), the Protein Diet (12%) group animals lost weight and had visible physical changes over six weeks.

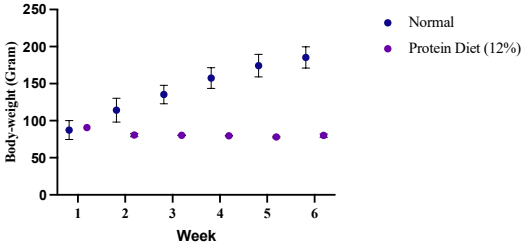

Figure 1. The Effect of normal (20%) and low protein diet (12%) on Body Weight of Malnourished Rats

Rats lose weight when they don't get enough protein, which makes them eat fewer calories. When each cell gets enough protein, the body breaks down each amino acid and turns it into energy or stores it as fat. But when the body doesn't get enough protein, specific amounts are broken down into amino acids. These amino acids can then be metabolized, which leads to weight loss. Essential dietary deficiencies or protein deficit might result in lower amounts of Insulin-like Growth Factor (IGF-1) and Growth Hormone (GH). Reduced levels of these hormones may impair linear growth, which can lead to height and body weight stagnation [16]. Leptin, a signaling molecule, disruption in malnutrition can lead to a decrease in Growth Hormone receptors during periods of caloric restriction, contributing to difficulties of inadequate weight [17]. In this study, rats with malnutrition lost significant weight after six weeks of treatment with a 12% low-protein diet. We hypothesize that this situation has impacted the control of growth hormones such as GH, IGF 1, and leptin, which causes the body weight of rats not to increase.

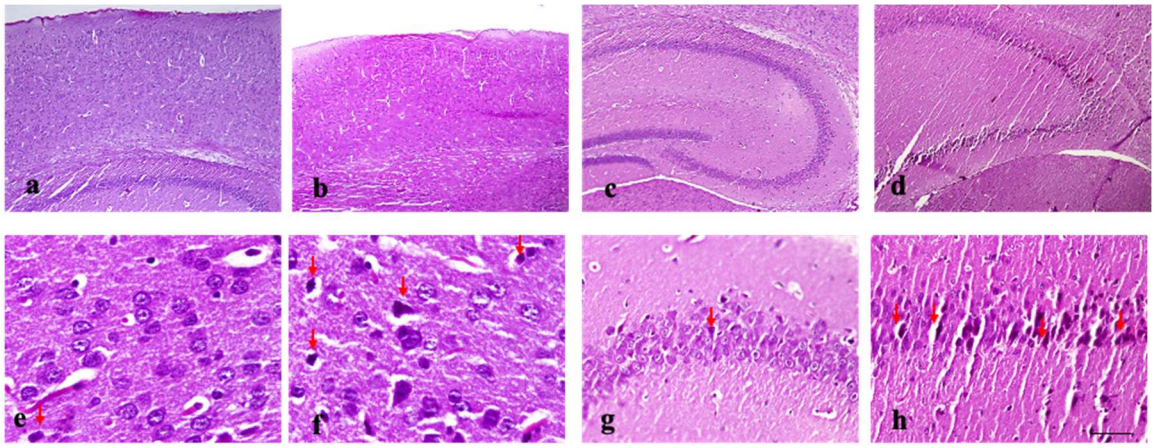

Figure 2. Histopathology of cerebral cortex (Normal group a and e; Protein Diet 12% group b and f) and hippocampus (Normal group c and g; Protein Diet 12% group d and h) using H&E staining at low magnification (top) and high magnification (bottom) Top panel objective: 10x; bottom objective: 100x (e, f); and 40x (g, h). Malnutrition induction shows signs of degeneration in some neuronal cells in the cortex and hippocampus area, characterized by pyknotic, shriveled cells or eosinophilic cytoplasm and cell lysis (↓).

The effects of a low-protein diet on experimental rat's cerebral cortex and hippocampus showed histopathological differences from the Normal group. The cerebral cortex and hippocampus of the normal control group had predominantly neuronal cells with typical characteristics such as big cells, granular cytoplasm, and smooth chromatin nuclei (Figure 2 a, e and c, g). Some cells showed signs of degeneration after six weeks of treatment with a 12% low-protein diet, such as increased eosinophilic cytoplasm, pyknotic nuclei, or shrinking cells (Figure 2 b, f and g, h). A 12% low-protein diet resulted in more degeneration of cells.

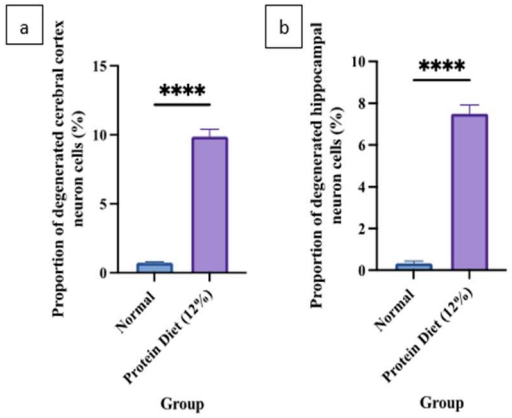

Figure 3. Data analysis of measurements of the proportion of degenerated neuron cells in the cerebral cortex (a) and hippocampus (b), The letters indicate significant differences based on the T test (P<0.0001).

The histopathological data analysis shows that the proportion of degenerated neuron cells in the cerebral cortex and hippocampus is lower than in the normal group (Figure 3). These results indicated that malnutrition impacts the rat's cerebral cortex and hippocampus. The effect on the cerebral cortex and hippocampus can vary depending on the duration and severity of malnutrition and the specific nutrient deficiencies involved (e.g., protein, vitamins, minerals). Severe malnutrition can lead to the loss of neuronal cells in the cortex, resulting in decreased cell density and disrupted cortical architecture. Long-term low-protein diets can lead to oxidative stress, producing free radicals such as superoxide and hydroxyl radicals, ultimately leading to cell damage [18]. Numerous nutrients play vital roles in the growth and development of neuronal cells, and This includes protein, iron, zinc, selenium, iodine, folate, vitamin A, choline, and long-chain polyunsaturated fatty acids [19], [20], [21]. Nutrition is essential not only for neurons but also for supporting glial cells. For specific brain regions, disruptions in early food can significantly impact cell proliferation, thereby influencing the total number of cells.

B. Cognitive Functions Analysis

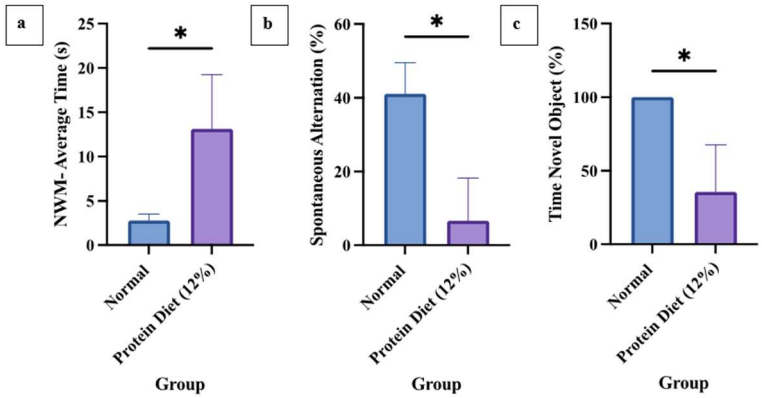

Figure 4. The effect of the normal and low-protein diet (12%) groups on cognitive tests, latency (a), spontaneous alternation (b), and novel object recognition (c).

The Morris Water Maze test was used to get the data in Figure 4.a about how long it takes to react. Compared to the normal group, the group that consumed a low-protein diet (12%) for six weeks spent the most time finding platforms, significantly different (P<0,05). The fact that it took the test animals a long time to find a platform shows that malnutrition may affect their ability to remember where things are and what they mean. The better the memory consolidation process in the test animal, the less time it takes for the test animal to find the platform. Memory

consolidation is how the hippocampus forms a memory through repeated use. The hippocampus will send messages to move short-term spatial memories into long-term spatial memories. Spatial memory is the process of encoding information in the brain about recognizing the environment in the form of position or navigation of a place. The data presented in the spontaneous alterations percentages from the Y-maze test indicates that the normal group demonstrated the highest spontaneous alterations percentage compared to the low-protein diet 12% group significantly (P<0,05) (Figure 4b). On the contrary, the low-protein diet 12% treatment exhibited the lowest spontaneous alternation. The Y-maze test assesses short-term memory in mice, as exemplified by the measure of spontaneous alternation. This evaluation of spatial working memory was achieved by allowing rats to explore all three arms of the maze. Their inherent curiosity drives them to explore areas they have not previously visited. A mouse with proficient working memory will recollect the arms of the maze it has explored and will tend to enter arms it has seldom visited. This process involves coordination among multiple brain regions, including the hippocampus and prefrontal cortex [14].

Figure 4c shows the data from the Novel Object Recognition test on the percentage of time spent with new objects. Compared to the normal group, the low-protein diet group had significantly (P0<005) less time spent with new objects than the normal group. The percentage of novel objects was used to test episodic memory, recognition memory, and semantic memory. Episodic memory functions to remember things that have happened in the past, and memory recognition functions to recognize previously known objects. The low percentage of novel object time in this test indicates decreased memory function in mice [22]. Chronic malnutrition significantly impacts the growth and maturation of the brain. The intricate phases of brain development encompass numerous complex processes: nerve cells must undergo proliferation, migration to precise locations, the establishment of appropriate connections, the creation of neurotransmitter receptors, and envelopment with myelin—a protective substance crucial for effective nerve message transmission. This intricate assembly of nerve cells is susceptible to environmental pressures, including malnutrition. Even after birth, brain development persists, marked by the migration and proliferation of cells [10]. Malnutrition will also affect brain maturation and cognitive function, resulting in abnormal behaviour and disruption of learning and memory processes [23]. Although malnutrition refers to a deficiency of one or more nutrients, Morgane et al. (2002) state that protein is the most crucial nutrient in brain maturation [24].

IV. Conclusion

Rats fed a 12% protein diet for six weeks showed significant differences in body weight parameters, proportion of degenerated cells, and histopathological features of the cerebral cortex and hippocampus compared to the normal group fed a 20% protein diet for six weeks, and this condition also affected the cognitive function of rats fed a low protein diet.

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