Original Research Report

THE NEUROGENESIS EFFECTS OF *PASAK BUMI* (*Eurycoma longifolia* Jack) AND *SELUANG* FISH (*Rasbora* spp.) IN MALNUTRITION-INDUCED RAT MODELS

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ABSTRACT

Early developmental malnutrition exerts adverse effects on the structural, neurochemical, and neurophysiological maturation of cerebral cells by disrupting the process of neurogenesis. *Pasak bumi* (*Eurycoma longifolia* Jack) and *seluang* fish (*Rasbora* spp.), two indigenous natural resources of South Kalimantan, Indonesia, are believed to harbor nutritional components capable of mitigating these deleterious effects. We aimed to assess the impact of administering *pasak bumi*, *seluang* fish, and pure docosahexaenoic acid (DHA) on the neurogenesis process in malnourished rat models. The *Rattus norvegicus* specimens were partitioned into seven distinct cohorts, each consisting of five rats: healthy rats in the negative control group (KN), while malnourished rats in the positive control (KP) and treatment groups (P1, P2, P3, P4, and P5). Both the KP and KN groups received a placebo and a standard feed. The treatment groups received different interventions for five weeks: standard feed alongside *pasak bumi* extract for the P1 group, standard feed and DHA for the P2 group, standard feed in combination with *pasak bumi* extract and DHA for the P3 group, *seluang* fish for the P4 group, and *pasak bumi* extract and *seluang* fish for the P5 group. The doses determined for the *pasak bumi* extract and DHA were 15 and 1 mg/kg bw, respectively. The parameters evaluated consisted of the levels of brain-derived neurotrophic factor (BDNF), neural progenitor cell β-tubulin 3 (Tuj-1) expression, and peroxisome proliferator-activated receptor gamma (PPARγ). The data were subjected to analysis through the Kruskal-Wallis test and analysis of variance (ANOVA) at a 95% confidence level. A value of $p<0.05$ was considered significant. Statistically significant differences were observed in the BDNF levels ($p=0.00$) and Tuj-1 expressions ($p=0.01$) across all groups. In conclusion, the combined administration of *pasak bumi* and *seluang* fish demonstrates the capacity of enhancing neurogenesis in malnourished rats, as evidenced by elevated BDNF levels and Tuj-1 expressions.

Keywords: Malnutrition rat models; brain-derived neurotrophic factor (BDNF); neural progenitor cell (NPC); peroxisome proliferator-activated receptor gamma (PPARγ); health risk

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Highlights:
1. This study analyzed the effects of using locally sourced *pasak bumi* extract and *seluang* fish on the parameters of neurogenesis in malnourished rat models.
2. It promotes further exploration into modified treatments for malnutrition, emphasizing nutritional strategies that harness locally available natural resources.

INTRODUCTION

Human brain development initiates during the second trimester of gestation. The development undergoes rapid progression throughout the initial 1,000 days of life (Schwarzenberg et al. 2018, Gilmore et al. 2018). According to Urbã¡n & Guillemot (2014), neurogenesis primarily occurs in two specific regions: the subependymal zone in the

lateral ventricles (V-SVZ) and the subgranular zone (SGZ) located in the dentate gyrus of the hippocampal region. The initiation of neurogenesis involves the proliferative and multipotential neural stem cells (NSCs) and neural progenitor cells (NPCs), which subsequently undergo complete differentiation into new neuronal cells (Homem et al. 2015, Cho et al. 2019). The neural stem cells can also differentiate into neuroglia, such as oligodendrocytes and astrocytes. This mechanism contributes to the provision of physical and metabolic support to neurons (Gallo & Deneen 2014).

As demonstrated by Sanyoto et al. (2022) and Villapol (2018), the assessment of neurogenesis through NPC activity can be achieved by quantifying the expression levels of β-tubulin 3 (Tuj-1) marker and the peroxisome proliferator-activated receptor gamma (PPARγ) gene, specifically within the hippocampus. Tuj-1 exhibits specific localization within neurons, and its expression is closely associated with the initial stages of neuronal differentiation. Conversely, PPARγ regulates the expression of various enzymes, such as lipoprotein lipase and fatty acid transport proteins, which participate in lipid uptake processes crucial for neuron and oligodendrocyte differentiation (Krishna et al. 2021).

Neurogenesis is additionally triggered by a protein known as brain-derived neurotrophic factor (BDNF). The stimulation exhibits activity in key brain regions, such as the hippocampus, cortex, and basal areas (Ferreira et al. 2018, Miranda et al. 2019). Mature BDNF specifically interacts with tyrosine kinase receptors (TrkB), subsequently fostering cell survival, facilitating long-term potentiation (LTP), and enhancing spine complexity. These processes are crucial for cellular mechanisms involved in memory formation and retention through synaptic consolidation. BDNF may act as a mediator in the synaptic plasticity alterations that underlie both spatial and recognition memory processes (Mudjihartini 2021).

Neurogenesis, which occurs during early stages of life, is highly dependent on nutrition due to the fact that the largest portion of the neuron cell membrane structure is composed of macronutrients. The incomplete formation of neuron membranes can lead to disruptions in neuronal circuits, thereby affecting the morphological, neurochemical, and neurophysiological aspects of the brain (Rushmore et al. 2021, Sanyoto et al. 2021). It has been demonstrated that protein deficiency during the early stages of life results in diminished neurogenic activity within the CA1 region of the hippocampus. This deficiency manifests through various alterations in the structure and function of the brain. The changes include a reduction in brain volume along with decreased ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) content, a lower count of neurons, simplified dendritic and synaptic architecture, as well as decreased levels of neurotransmitters and growth factors (Gonçalves et al. 2016, Georgieff et al. 2018, de Guzman et al. 2018). Rat models with protein malnutrition have demonstrated a lower expression of Tuj-1, PPARγ, and BDNF markers compared to the control group, indicating a decrease in the neurons and neurogenic activity. Failure to address this specific condition can lead to severe consequences, including long-term impairments in attention and learning, decreased intelligence quotient (IQ) scores, and diminished visuospatial working memory. Ultimately, this can result in reduced academic achievement (Waber et al. 2014, Pérez-García et al. 2016).

The aforementioned argument encourages more research into modified therapy for malnutrition that focuses on nutritional interventions by using locally available natural resources. *Pasak bumi* (*Eurycoma longifolia* Jack) is a plant native to South Kalimantan, Indonesia, known for its abundance in flavonoid and alkaloid compounds. These constituents serve as potent anti-inflammatory and antioxidant agents (Triawanti et al. 2018, Cichon et al. 2020). Prior studies, such as those conducted by Bakoyiannis et al. (2019) and Minocha et al. (2022) have revealed the neuroprotective effects and ability of flavonoids to increase neuronal proliferation in Alzheimer's disease. Furthermore, it has been demonstrated that flavonoids stimulate the differentiation of progenitor cells into neurons and confer protection against cell death. These activities are enabled through the activation of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways (Ullah et al. 2020, Chen et al. 2022). In a study conducted by Sanyoto et al. (2022), it was observed that administering 15 mg/kg bw of *pasak bumi* root extract to rat models resulted in an increasing trend in neurogenesis activity compared to the negative control group. However, the increase was not statistically significant.

Another natural resource with potential to address malnutrition is *seluang* fish (*Rasbora* spp.), a species commonly consumed by the local community in South Kalimantan. The nutritional composition of *seluang* fish per 100 g contains a protein content of 40% w/w and a fatty acid content of docosahexaenoic acid (DHA) of 1.04% w/w. According to Basak et al. (2020), DHA is an omega-3 polyunsaturated fatty acid required for brain development. Sufficient levels of DHA within neural membranes are essential for the maturation of cortical astrocytes, as well as for facilitating

neurovascular coupling and promoting glucose uptake and metabolism. Several studies have shown that DHA possesses neuroprotective effects in animal models (Wang et al. 2018, Gómez-Soler et al. 2018, Lo Van et al. 2019). In a study conducted by Yunanto et al. (2015), rats fed with a *seluang* fish formula demonstrated a higher count of neuron cells in the hippocampus. The expression of PPARα and PPARγ was observed in mice from the treatment group, which was notably different from both the control group and the low-protein group. Meanwhile, the aim of this experimental study which employed rat models (*Rattus norvegicus*) with protein-energy malnutrition was to analyze the effect of both *pasak bumi* extract and *seluang* fish on neurogenesis activity in malnourished rats.

MATERIALS AND METHODS

This study received ethical clearance from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Lambung Mangkurat, Banjarmasin, Indonesia, under approval No. 061/KEPK-FK UNLAM/EC/II/2020 dated 25/02/2020. This study used an experimental design. A group of rats (*Rattus norvegicus*) were used as the experimental animals. The rats were induced into a state of malnutrition by being fed a low-protein diet. The experiment was conducted at the Faculty of Medicine and Health Sciences of Universitas Lambung Mangkurat from 2020 to 2021. The parameters used for assessing neurogenic activity were brain-derived neurotrophic factor (BDNF), β-tubulin 3 (Tuj-1) expression, and peroxisome proliferator-activated receptor gamma (PPARγ) from brain specimens (Fang et al. 2021).

The materials used in this study included *Rattus norvegicus*, *pasak bumi* extract, *seluang* fish, docosahexanoic acid (DHA), low-protein feed, standard feed with an energy range of 2,900–3,100 kcal, distilled water, 70% ethanol, 90% ethanol, rat tissue paraffin block, ether, PPARγ antibody, Tuj-1 antibody, immunostaining kit (mono), and BDNF enzyme-linked immunosorbent assay (ELISA) kit. The low-protein feed consisted of the AIN-76A Purified Rodent Diet (Dyets Inc., USA), 60 g/kg of casein, 183 g/kg of cornstarch, 50 g/kg of corn oil, 0.9 g/kg of DL-methionine, 609.1 g/kg of sucrose, 10 g/kg of AIN-76A Vitamin Mix #300050 (Dyets Inc., USA), 50 g/kg of cellulose, 35 g/kg of AIN-76A Mineral Mix #200000 (Dyets Inc., USA), and 2 g/kg of choline bitartrate. Meanwhile, the standard diet contained 20–22% protein, 5–7% fat, 5–7% cinder, 3–5% fiber, 0.6–0.8% phosphorus, and 9–11% calcium. The experiment and observation utilized several tools, including a digital analytical balance, measuring cup, rotary evaporator, blender, glass apparatus, cuvette, feeding tube, water bath, incubator, centrifuge, vortex, object glass, hot plate, staining jar, Leica 2125 RM microtome (Leica Biosystem, USA), and Olympus CX21 binocular microscope (Olympus Scientific Solutions, Japan).

The experiment started by producing a 70% ethanol extract from the root of *pasak bumi*. The root was first shaved and dried without direct sunlight exposure, then blended into a powder. Subsequently, 200 g of the powdered root were soaked in 1.5 L of 70% ethanol at room temperature for five days. The mixture was filtered to remove any solid particles. The dregs of the *pasak bumi* root powder mixture were subjected to re-maceration in 500 mL of 70% ethanol at room temperature for two days, subsequently followed by another filtration to obtain clean filtrates. The combined filtrates were concentrated using a rotary evaporator at 50 °C until a thick extract containing a small amount of solvent remained. Further evaporation in an oven at 40°C yielded a thick extract of *pasak bumi* roots (Sanyoto et al. 2022).

The preparation for developing the *Rattus norvegicus* malnutrition model was started by inducing the mice to be undernourished from birth. This was achieved by feeding their lactating mothers with low-protein feed (AIN76A, 6% protein) for a period of four weeks. Following weaning, the rat pups were kept on the same low-protein feed (AIN-76A) for an additional four weeks, totaling eight weeks (56 days) of low-protein feeding. The normal protein level in *Rattus norvegicus* is typically within the range of 4.7–5.2 g/dL (Khasanah et al. 2015). Blood samples were collected from the tail vein with a volume of up to 1 mL and thereafter subjected to centrifugation for analysis of serum protein levels. The rat pups were categorized as undernourished if their serum protein level fell below 4.7 g/dL.

Before administering the *pasak bumi* root ethanol extract, *seluang* fish, and DHA, the malnourished rats were divided into six groups, each containing five animals. These six groups were the positive control group (KP) and the treatment groups (P1, P2, P3, P4, and P5). Additionally, there was a negative control group (KN) consisting of healthy rats that were given a placebo and standard feed for five weeks. The positive control group (KP) comprised stunted rats administered with a placebo alongside standard feed. Different interventions were administered to the treatment groups for a period of five weeks. The P1 group received standard feed alongside 15 mg/kg bw of *pasak bumi* root ethanol extract. The P2 group was given standard feed along with 1 mg/kg of DHA. The P3 group was administered standard feed with a combined treatment of 15 mg/kg bw of *pasak bumi* root ethanol extract and 1 mg/kg bw of DHA. The P4 group was fed *seluang* fish, whereas the P5 group

received a combination of 15 mg/kg bw of *pasak bumi* root ethanol extract and *seluang* fish. The decision to administer *pasak bumi* root ethanol extract at a dose of 15 mg/kg bw was made according to a previous study that indicated its superior efficacy compared to a dose of 7.5 mg/kg bw (Triawanti et al. 2020).

After five weeks of treatment, the experimental animals were euthanized using anesthesia. Subsequently, the cranium was dissected to extract the brains. The BDNF levels in the brain serum were evaluated using the ELISA method. The brain tissues were fixed using a formalin buffer solution for the preparation of paraffin blocks. Immunohistochemistry staining was performed on the brain preparations using monoclonal antibodies targeting Tuj-1 and PPARγ. The stained tissues were then examined under a microscope to quantify the number of cells expressing Tuj-1 and PPARγ (Sanyoto et al. 2022).

The data obtained were analyzed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA). An assessment was conducted to determine the normality and homogeneity of the data distribution. If the data were normally distributed, an analysis of variance (ANOVA) test was conducted at a 95% confidence level, followed by the Tukey's honestly significant difference (Tukey's HSD) post-hoc test. However, if the data distribution was non-normal, a non-parametric Kruskal-Wallis test was performed, followed by the Mann-Whitney test with a 95% confidence level. A statistical significance was set at $p<0.05$ (Cleophas & Zwinderman 2016).

RESULTS

The activity of BDNF in the brain of malnourished rats after the administration of *pasak bumi* extract and *seluang* fish

Figure 1 depicts that the malnourished rats in the positive control group (KP) had significantly lower mean levels of brain-derived neurotrophic factor (BDNF) compared to both the normal control and treatment groups. The P1 group, which was administered 15 mg/kg bw of *pasak bumi* root ethanol extract, exhibited the highest mean BDNF level. The statistical analysis revealed that the data distribution was non-normal, necessitating the utilization of the Kruskal-Wallis test. The test results demonstrated significant differences among the treatment groups (p=0.000). The subsequent Mann-Whitney tests revealed significant differences between the KN group and the KP, P1, P3, and P4 groups. Additionally, there were significant differences observed between the KP group and the P1, P2, P3, and P5 groups, as well as between the P1 group and the P2, P3, P4, and P5 groups, and between the P2 group and the P4 group. However, no significant differences were observed among the P3, P4, and P5 groups. These results indicated that administering *pasak bumi* root ethanol extract at a dose of 15 mg/kg bw significantly increased BDNF levels compared to other interventions. Meanwhile, the rats that were given 1 mg/kg bw of DHA, as well as those receiving a combination of *seluang* fish and 15 mg/kg bw of *pasak bumi* root ethanol extract, exhibited lower BDNF levels relative to the normal control group.

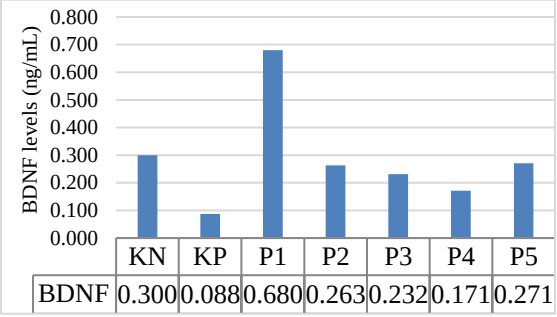

Figure 1. Mean levels of BDNF in the serum of rats after intervention.
Legends: KN=normal controls; KP=positive controls; P1=*pasak bumi* extract; P2=DHA; P3=DHA and *pasak bumi* extract; P4=*seluang* fish; P5=*seluang* fish and *pasak bumi* extract; p=0.000.

The expression of Tuj-1 in the brain of malnourished rats after the administration of *pasak bumi* extract and *seluang* fish

The malnourished rats in the KP group were administered a placebo and exhibited the lowest number of cells expressing Tuj-1 compared to the other groups. On the contrary, the normal control group (KN) displayed the highest Tuj-1 expression, as shown in Figures 2 and 3. Interestingly, in the P3 group, which consisted of malnourished rats receiving a combination of *pasak bumi* root ethanol extract and DHA, the Tuj-1 expression level was nearly equivalent to that of the normal control group. Following the ANOVA statistical analysis with a 95% confidence level, a significant difference was established among the treatment groups (p=0.01). Subsequent post-hoc tests revealed differences between the KN group and the KP, P1, and P2 groups. Similar differences were also noted between the KP group and all other treatment groups, as well as between the P1 group and the P3 group. However, there were no significant differences observed among the P2, P3, P4, and P5 groups.

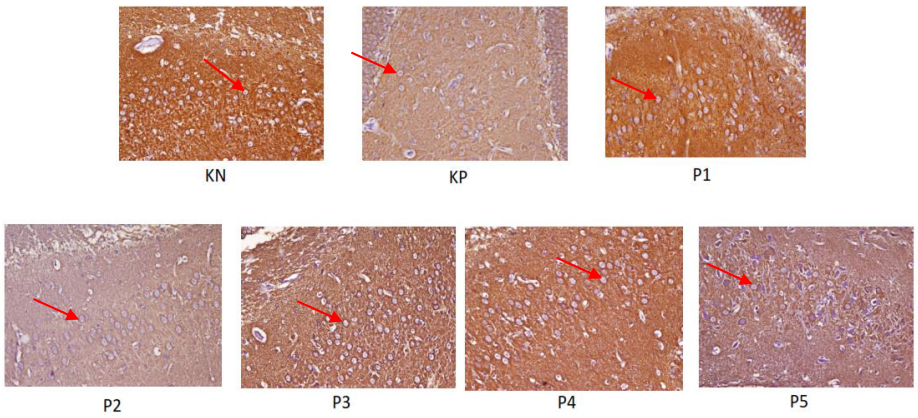

Figure 2. Immunohistochemical staining of Tuj-1 from the rats' brain after intervention.
Note: Red arrows indicate the marker. KN=normal controls; KP=positive controls; P1=*pasak bumi* extract; P2=DHA; P3=DHA and *pasak bumi* extract; P4=*seluang* fish; P5=*seluang* fish and *pasak bumi* extract.

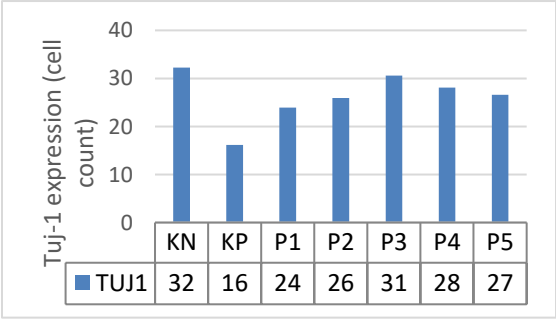

Figure 3. Mean Tuj-1 expression in the rats' brain tissue after intervention.
Note: KN=normal controls; KP=positive controls; P1=*pasak bumi* extract; P2=DHA; P3=DHA and *pasak bumi* extract; P4=*seluang* fish; P5=*seluang* fish and *pasak bumi* extract.

The expression of PPARγ in the brain of malnourished rats after the administration of *pasak bumi* extract and *seluang* fish

Figures 4 and 5 depict the expression of PPARγ in the neuron cells of rats across the control and treatment groups. The mean PPARγ expression in the normal control group (KN) was observed to be the highest compared to other groups. On the other hand, the malnourished rats in the KP group that were given a placebo exhibited the lowest PPARγ expression. However, the results of the ANOVA statistical test with a 95% confidence level revealed no significant difference among the groups (p=0.095).

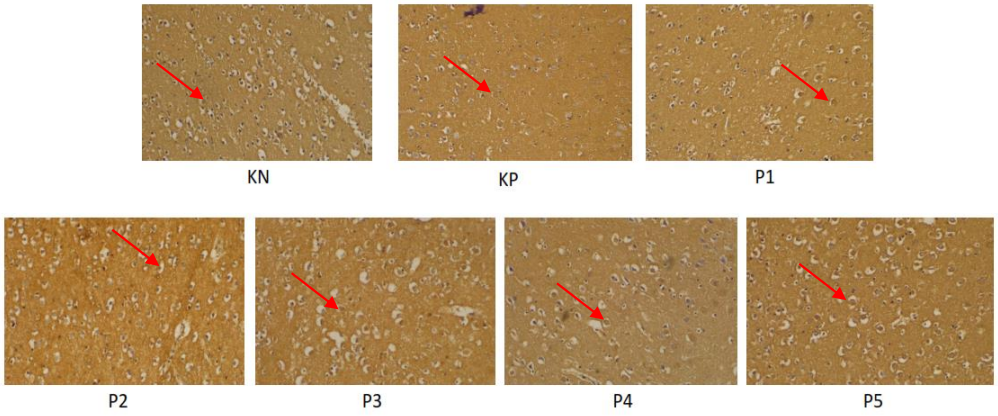

Figure 4. Immunohistochemical staining of the rats' PPARγ expression after intervention.
Note: Red arrows indicate the marker. KN=normal controls; KP=positive controls; P1=*pasak bumi* extract; P2=DHA; P3=DHA and *pasak bumi* extract; P4=*seluang* fish; P5=*seluang* fish and *pasak bumi* extract.

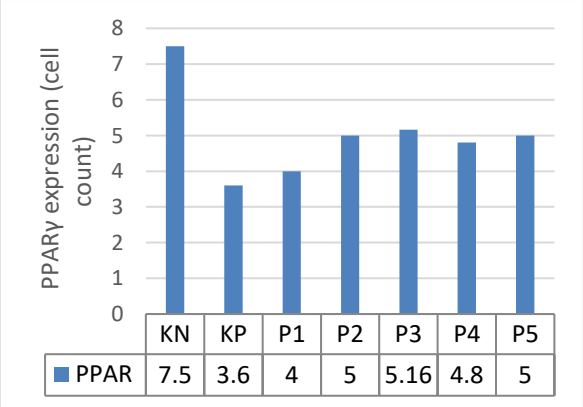

Figure 5. Mean expression of PPARγ in the rats' brain tissue after intervention.
Note: KN=normal controls; KP=positive controls; P1=*pasak bumi* extract; P2=DHA; P3=DHA and *pasak bumi* extract; P4=*seluang* fish; P5=*seluang* fish and *pasak bumi* extract; p=0.095.

DISCUSSION

Brain-derived neurotrophic factor (BDNF) is predominantly synthesized by neurons. Under normal physiological conditions, BDNF quickly enters neuron activity. Malnutrition experienced by the mother rats caused decreased levels of BDNF in the rat models' brains, as seen among the malnourished rats in the KP group as compared to the healthy rats in the KN group (Figure 1). The malnourished mother rats were prone to experience a downregulation in the activation of the cyclic adenosine monophosphate (cAMP)/protein kinase A/cAMP-response element binding protein (CREB) signaling pathway. The decrease in activity is linked to a notable reduction in the binding of CREB to the BDNF promoter region, consequently leading to decreased BDNF production. The regulation of BDNF expression is intricately managed through the transcriptional control exerted by CREB, in coordination with the signaling cascades initiated by cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) (Marwarha et al., 2017). According to the results, the BDNF levels in the P1 group, which received *pasak bumi* extract, were significantly higher compared to the KP group. It has been suggested that *pasak bumi* has a neuromodulatory function in the brain tissue. A study conducted by Kazi & Yaakob (2015) revealed that *pasak bumi* increases the expression of c-Fos, a neuromodulator marker in the neurons of the spinal cord. An increase in neuromodulators will increase BDNF upregulation, thereby leading to an increase in BDNF levels.

BDNF is synthesized not only by neurons but also by astrocytes. Astrocytes are recognized for their multifaceted functions in the central nervous system, including the expression of diverse growth factors such as vascular endothelial growth factor-C (VEGF-C), platelet-derived growth factor-AA (PDGF-AA), and transforming growth factor beta 1 (TGF-β1). These growth factors are pivotal in promoting neuronal resilience and memory formation, underscoring the integral role of astrocytes in neuronal homeostasis and cognitive processes (Liu et al. 2017). Malnutrition leads to a rise in the number of astrocytes as a reaction to neuronal injury. However, the astrocytes are premature, and the production of BDNF is also suboptimal. Prenatal protein malnutrition can accelerate astrocyte maturation, subsequently leading to compromised neuronal development (Wang et al. 2018). Protein malnutrition can exert effects on the activity of astrocytes. One of the effects includes a disruption in the uptake of glutamate. The decreased uptake of glutamate leads to an increased susceptibility of neurons to excitotoxicity. Glutamate neurotoxicity has implications for numerous neurological disorders. Astrocytes have been shown to protect neurons against excitotoxicity through extensive clearance of the extracellular space (Abbink et al. 2019). The administration of *pasak bumi* extract, which contains flavonoid compounds, is thought to be able to increase neurotrophic factor. In this study, BDNF levels were elevated in the astrocyte cells, as shown in the P1 group. The active compounds include quassinoids, canthin-6-one alkaloids, tirucallane-type triterpenes, carboline alkaloid, piscidinol A, squalene derivatives, scopoletin, and biphenyl-type neolignans (Triawanti et al. 2018). It has been demonstrated that various flavonoid compounds have the ability to stimulate BDNF production by astrocytes. The findings were summarized by analyzing 33 flavonoid compounds from various subclasses (Matias et al. 2016, Wang et al. 2018).

The measurement of Tuj-1 expression is a method to analyze the activity of neural progenitor cells (NPCs). The KP group had the lowest count of NPCs, as indicated by the Tuj-1 expression that was significantly distinct from the other groups. This implies that malnutrition could diminish neurogenesis. Although the Tuj-1 expression in the P1 and P2 groups differed from that of the KP group, it also exhibited differences in comparison to the KN group, indicating that the groups had not been able to reach the normal neurogenesis process. On the other hand, the P3, P4, and P5 groups did not show any significant differences when compared to the normal control group (KN). This indicated that the combination of *pasak bumi* extract with other nutrients could restore the neurogenesis process that was close to normal. The administration of DHA can significantly increase neurogenesis in the hippocampus, as evidenced by an increase in the number of neurons undergoing proliferation and

neuritogenesis. These processes can be observed by identifying an augmentation of dendritic spine density in the pyramidal neurons of the CA1 layer within the hippocampus (Phatnani & Maniatis 2015). According to Naik et al. (2017), DHA supplementation enhances the proliferation of neural stem or progenitor cells, indicating its ability to stimulate neuronal differentiation and growth of neurites. However, the single administration of DHA did not enhance neurogenesis in the P2 group.

DHA is present in the cell and will bind to intercellular fatty acid-interaction proteins, namely fatty acid-binding protein (FABP). FABP is believed to be involved in the uptake, transport, and targeting of long-chain polyunsaturated fatty acids (LC-PUFA) to specific intracellular organelles (Decara et al. 2020). Presently, there has been no research regarding FABP expression in states of protein malnutrition. We suspect that FABP is disrupted by protein malnutrition. The P3 group demonstrated that administering DHA combined with *pasak bumi* root ethanol extract improved neurogenesis to a level similar to that of normal conditions. The potential of *pasak bumi* root ethanol extract is believed to strengthen the effect of DHA in triggering neuronal cell differentiation and neurite growth. The administration of *seluang* fish was also able to trigger neurogenesis. This might be attributed to the fact that *seluang* fish contains amino acids and DHA, which are essential for neurogenesis, neuron cell differentiation, and neurite growth. Statistically, the administration of *seluang* fish either individually or in combination with *pasak bumi* root ethanol extract did not yield any significant difference in Tuj-1 expression compared to the normal control group. The findings regarding the PPARγ parameter indicated no notable distinctions among the groups. However, the malnourished rats in the positive control group had a fewer number of stained cells in comparison to the normal control group. Peroxisome proliferator-activated receptors (PPARs), a subgroup within the nuclear hormone receptor superfamily, are characterized by their transcriptional capabilities. The three known families (i.e., PPARα, PPARγ, and PPARβ/δ) share a common feature of binding to peroxisome proliferator response elements. Even so, their transactivating functions differ due to various factors, such as their distribution network and the specificity of coactivator ligands (Decara et al. 2020). In the brain, PPARγ exhibits a more localized expression pattern compared to PPARβ/δ, with a slight enrichment observed in the hippocampus. It is identified in various cell types within the central nervous system (CNS), including neurons and glial cells such as microglia (Falomir-Lockhart et al. 2019).

The classical pathway of peroxisome proliferator-activated receptors (PPAR) that regulates gene transcription involves a heterodimerization with retinoid X receptor (RXR) upon initial activation by ligand binding. The PPAR-RXR dimer then binds to a DNA response element known as the PPAR response element (PPRE), which was located within the promoter or intragenic region of target genes. Concurrently, nuclear receptor coactivators synergize with PPAR-RXR, enhancing and stabilizing the active transcriptional complex. Upon activation by ligands, PPARs form heterodimers with RXR. The resulting PPARγ/RXR heterodimer binds to the PPRE upstream of the target gene promoter, ultimately regulating target gene transcription. Ligands, typically fat-soluble molecules, initiate a biological response by modulating the expression of target genes upon binding to PPARs. This ligand binding induces a conformational change in PPARs, thereby promoting or inhibiting the expression of target genes (Wang et al. 2015). PPARγ acts as a transcription factor, influencing the expression of synaptic proteins that facilitate synaptic plasticity. This expression was found to result in an enhanced spatial memory in animals fed a diet rich in omega-3 fatty acids (Yunanto et al. 2015). Additionally, it suggests that protein malnutrition alters the synaptic plasticity by decreasing the number of PPARγ expression.

The P1, P3, and P5 groups demonstrated that *pasak bumi* root ethanol extract increased PPARγ-mediated gene expression, although the differences were not statistically significant. This effect might be attributed to the flavonoid content in *pasak bumi* (Beekmann et al. 2015). The administration of DHA and *seluang* fish also affected PPARγ expression in the malnourished rats, as shown by the P3 and P5 groups. DHA is a type of omega-3 fatty acid that acts as an endogenous ligand to the PPARγ. DHA has been demonstrated to physically bind to PPARγ, enabling it to regulate brain inflammation processes by suppressing the proinflammatory phenotype of activated microglia. This inhibition involves downregulating the expression of surface antigens and the synthesis of proinflammatory signals such as prostaglandins and nitric oxide (Falomir-Lockhart et al. 2019). Therefore, the consumption of DHA can lead to an increasing number of PPARγ expressions. It will overcome the neuroinflammation associated with malnutrition, which has a negative impact on the neurogenic process (Sung et al. 2020).

Strength and limitations

Our study offers insights into natural interventions for addressing neurogenesis deficits associated with malnutrition. *Pasak bumi* is known for its purported neuroprotective properties, while *seluang* fish has

contributed to the improvement of neurogenesis in malnourished models due to its abundance of essential nutrients. The positive effect of administering *seluang* fish on neurogenesis activity could pave the way for novel therapeutic approaches utilizing natural resources to combat malnutrition-related cognitive impairments. However, the study did encounter certain limitations. Firstly, the mechanisms underlying the effects of *pasak bumi* and *seluang* fish on neurogenesis activity might not be fully elucidated, potentially limiting the understanding of their precise therapeutic pathways. Secondly, variations in individual rat responses, environmental factors, and experimental conditions could introduce variability in the results, affecting the reproducibility and generalizability of the findings. Furthermore, extrapolating findings from animal models to human subjects requires caution, as biological differences exist between species. Despite these limitations, this study holds promise in expanding our understanding of potential interventions for addressing neurogenesis deficits associated with malnutrition.

CONCLUSION

The administration of *pasak bumi* root ethanol extract and *seluang* fish to malnourished rats has been proven to be able to improve neurogenesis, as indicated by the elevated levels of brain-derived neurotrophic factor (BDNF) serum and neural progenitor cell β-tubulin 3 (Tuj-1) expression in the murine brain.

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Conflict of interest

None.

Ethical consideration

The ethical approval for this study was issued by the Health Research Ethics Committee, Faculty of Medicine and Health Sciences, Universitas Lambung Mangkurat, Banjarmasin, Indonesia, under protocol No. 061/KEPK-FK UNLAM/EC/II/ 2020 dated 25/02/2020.

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Author contribution

DDS contributed to the conception and design, provision of study materials, obtainment of funding, experimentation, collection and assembly of the data, and critical revision of the article for important intellectual content. T contributed to the conception and design, experimentation, and collection and assembly of the data. MSN contributed to the experimentation as well as the collection and assembly of the data. DIA contributed to the analysis and interpretation of the data as well as drafting the article. All authors participated in the final approval of the article to be published and agreed to be accountable for all aspects of the work.

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