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# Effects of dietary edible bird's nest supplementation on hippocampal neurons of multigenerational mice

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## ABSTRACT

Edible bird's nest (EBN) is a natural food product rich in glycoproteins such as sialic acid, minerals and essential amino acids. Sialic acid from EBN most effectively absorbs in the brain, where it plays a fundamental role in the ganglioside structure involved in the brain development and synaptic transmission, particularly in preterm infants. Nevertheless, the impact of multiple generations of EBN on brain cellular variations is not yet fully understood. Therefore, the aim of this study was to investigate the impact of dietary EBN supplementation on brain neurons density and histology of multigenerational mice. A total of 40 females C57BL/6 mice as (F0) generation were equally distributed into four treatment and a control groups. Mice in the treatment groups were orally administered with four different sources of EBN for eight weeks. Subsequently, all mice were bred to produce the first generation (F1) followed by the second generation (F2). The sialic acid concentration in EBN samples was examined by High-Performance Liquid Chromatography (HPLC). The brain tissue of all mice was collected for histological study. The research found that dietary EBN significantly increased the number of neurons in the hippocampus of three generations (F0, F1, and F2) compared to the control. Histological study showed that the average number of neurons was significantly ($P<0.05$) higher in EBN-South and EBN-North groups compared to control in F0, F1 and F2 generations. However, the population of neurons was not significantly ($P>0.05$) higher in EBN-Commercial and EBN-Borneo groups compared to control. The highest neuron density was found in the mice supplemented with EBN contained higher concentration of sialic acid. In conclusion, it was suggested that the sialic acid from maternal EBN supplementation during pregnancy transmitted to the next generations of mice, where it influenced the development and functions of the fetal brain.

**Keywords:** Edible bird's nest (EBN), neuron density, hippocampus, histology, multigenerational mice

## INTRODUCTION

Edible bird's nest (EBN) is a medicinal substance produced during the breeding season by the salivary glands of swiftlets (Ma & Liu, 2012). Swiftlets are small insect-eating birds mostly located in Southeast Asia, including Malaysia, Indonesia, Thailand, Philippines, and Vietnam (Price *et al*., 2005). In Malaysia, two common species of swiftlets that produce EBN, are *Aerodramus maximus* and *Aerodramus fuciphagus* (Lundberg & McFarlane, 2012). Currently, Malaysia is the third-largest supplier of EBN globally, contributing about 9% of global demand, following Thailand (20%) and Indonesia (60%) (Hao & Rahman, 2016). In addition, EBN has been employed in traditional Chinese medicine and as a health-promoting substance to restore nutritional deficiency and supply the body with essential elements (Nabilah *et al*., 2018). Despite being one of the most costly food throughout the world, the precise composition of this substance is unclear to researchers (Marcone, 2005). Edible bird's nests principally contain sialic acid-rich O-glycosylproteins and represent a natural source of carbohydrate-rich substances (Wieruszeski *et al*., 1987). In fact, sialic acid comprises 9-12% of EBN, which is biologically

important for nerve health and brain function (Teh *et al*., 2018; Yu-Qin *et al*., 2000).

Sialic acid included in EBN is crucial for brain development and cognitive memory function (Yew et al., 2014; Rashed & Nazaimoon, 2010). It has been reported that EBN promotes the proliferation, regeneration and growth of neural cells (Khalid *et al*., 2019; Zainal Abidin *et al*., 2011). As a neuroprotective agent, EBN regulates the activity of antioxidant enzymes in the frontal cortex and hippocampus and prevents damage to the brain (Zhiping *et al*., 2015). Additionally, EBN supplementation in the diet significantly enhances cognitive function and exerts a potent neuroprotective effect in the hippocampus by inhibiting neuroinflammation and oxidative stress mechanisms (Careena *et al*., 2018). Physiologically, the hippocampus sections, such as CA1, CA2, CA3, and DG, contain numerous neurons that communicate with each other using neurotransmitters across synapses (Baptista & Andrade, 2018; Wheeler *et al*., 2015). Previous research has shown that the number of labelled neurons in the hippocampus of mice treated with EBN considerably increased compared to the control group. This led to an improvement in learning ability as the number of neurons increased (Xie et al., 2018; Yew *et al*., 2019). Thus, the increased quantity of new neurons can help to clarify the beneficial impact of EBN on enhancing the learning abilities of neonates.

The administration of EBN through dietary supplementation during prenatal and early postnatal stages resulted in an elevation of sialic acid levels in the brain ganglioside of the offspring, potentially playing a role in brain development (Xie *et al*., 2018). However, the exact effect of dietary EBN supplementation on neurological development across multiple generations is unknown. The considerable generation gap in humans continues to pose significant obstacles in accurately assessing and quantifying neuron development. Consequently, investigating the impacts of maternal EBN dietary supplementation on neonatal neuron development is a challenging task. In this study, mice were utilized because of the short gestation periods and reproductive cycles. This allows a comprehensive investigation of the neuronal development effects of EBN across generations using this animal model. Thus, the aim of this research was to examine the impact of dietary EBN supplementation over multiple generations on the density of neurons in the hippocampus of multigenerational mice.

## MATERIALS AND METHODS

### *EBN sample collection and preparation*

The edible bird's nests (EBN) were obtained during four months in 2018 from four locations in Malaysia, including Johor, Kedah, Borneo, and one sample from market (commercial). The edible bird's nests from Johor (EBN-S) were obtained from DesaBio Johor, edible bird's nests from Kedah (EBN-N) were obtained from Sungai Petani Kedah, edible bird's nests from Sabah (EBN-B) were collected from Tawau Sabah, and edible bird's nest from market (EBN-C) was purchased from Dingshen Imperial Birdnest, Petaling Jaya, Malaysia. The cleaned nests were ground into powder and 10 mg of the powder was soaked in distilled water for four hours. The soaked powdered nests were cooked in water for 30 minutes with a powder-water ratio of 1:10 (g/ml). The resulting solution was utilized to feed the mice individually. Sialic acid content in the nests was detected and analyzed by high-performance liquid chromatography (HPLC) as previously published by (Mahaq *et al*., 2020).

### *Animals and housing*

Four weeks old C57BL/6 breeder's mice consisting of 20 males and 40 females as generation 0 (F0) were purchased from a supplier (Biosystem Corporation Jerral Ding Weng Chuan, Singapore). The mice were placed at the Animal House Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). The mice were randomly placed into five groups, each consisting of eight individuals, and were housed individually. The mice were housed in a controlled environment within a conventional room that underwent 12 hours of light and

darkness. The room was air-conditioned, and the ambient temperature was regulated 22 to 26°C with a relative humidity of 40 to 60%. All mice were given unlimited access to standard pellets for food and drink. All the procedures were carried out in accordance with the guidelines of laboratory animals care and use committee approved by the Universiti Putra Malaysia (UPM/IACUC/AUP-R092/2017).

***Breeding program***

The F0 generation mice were utilized to generate the first (F1) and second (F2) generations. The mice were separated into four groups and received different sources of EBN for eight weeks using oral gavage (dosage 10 mg/kg); mice were supplemented with EBN collected from DesaBio Farm Johor (EBN-S; n=8), mice were supplemented with EBN collected from Swiftlet Farm, Kedah (EBN-N; n=8); mice were supplemented with EBN collected from Tawau Sabah (EBN-B; n=8) and mice were supplemented with commercial EBN (EBN-C; n=8). The control mice were given with normal saline (CTRL; n=8). After eight weeks of receiving a supplemented diet, all mice were bred to generate F1 and F2 offspring (Figure 1). Approximately, 8 female and male pups of the F1 generation were delivered in all groups. At 10 weeks old, female F1 mice were bred to generate F2 mice. Ten female individuals of the F2 generation were delivered in each group. At 16 weeks of age, all female mice from generations F0, F1, and F2 were euthanized, and brain tissues were prepared for histological analysis.

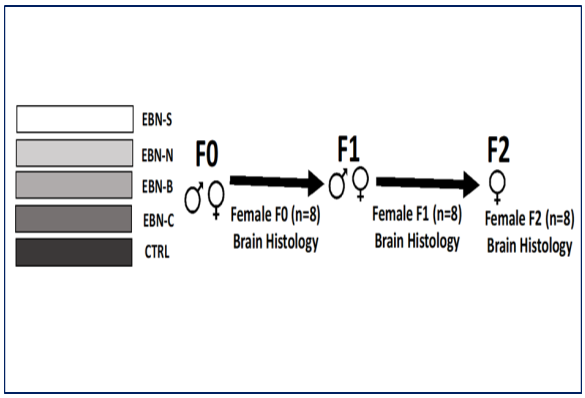


**Figure 1.** Shows the breeding procedure and experimental design of EBN dietary supplementation on brain neurons histology of maternal mice (F0), F1 and F2 generations.

***Histology and neurons density count***

A total of 60 female C57BL/6 mice (20 per each generation F0, F1, and F2) were euthanized using sodium phenobarbital 50 mg/kg IP, and the whole brain was harvested to determine the neuron population in the cornu ammonis-1 (CA1) area of the hippocampus. The harvested brain was kept in Bouin's solution for 20 hours. After dehydration, the whole brain was sectioned parallel with the coronal plane. Paraffin blocks were created, and sections of 3-5 µm in thickness were cut from the mid-dorsal hippocampus region using a rotary microtome. The tissues sections were placed on the slides and then stained with Hematoxylin and Eosin (H&E). The tissue sections were first deparaffinized using xylene and the dehydration was performed using different concentrations (70, 90 and 100%) of ethanol. After washing and staining with Hematoxylin and Eosin, the same concentrations of ethanol xylene were used for dehydration and clearance. Finally, the stained and dried slides were covered using cover-slips. The structure of the neurons was examined and counted in both the left and right brain hemispheres on each brain slide.

A total of six areas (three per each CA1) region in the hippocampus were selected and counted at each brain slide section using an image analyzer system (Motic Live Imaging Module) for light microscopy with 400x magnification. The number of neuron cells in the left and right CA1 regions of the hippocampus was quantified (Figure 2-a). To obtain an average neuron density in the CA1 region, the average number of neurons in the CA1 region of the hippocampus was calculated after counting the neuron population in three distinct areas per CA1 region. The overall number of neurons in a CA1 region was calculated based on the number of neurons counted per each selected area and sampling frequency (Figure 2-b) (Praag *et al*., 2002; Zhang *et al*., 2013; Koike *et al*., 2008).

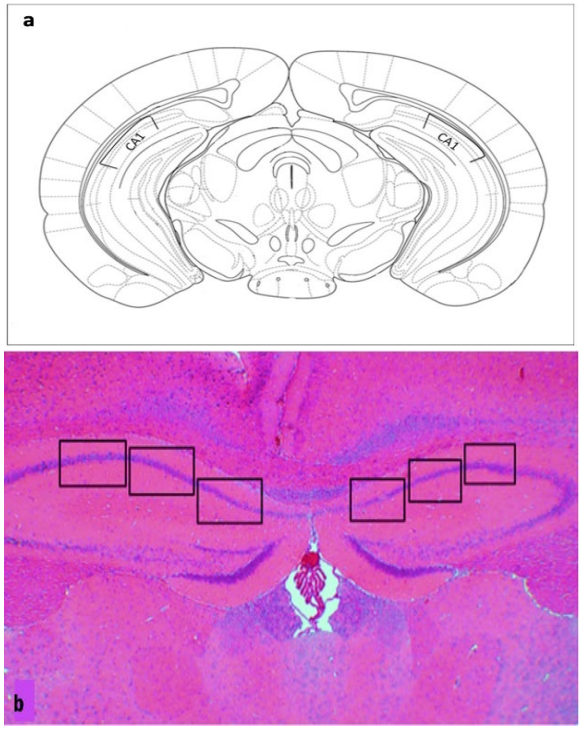


**Figure 2.** Indicates the area of interest. a) Shows the hippocampus and CA1 area (Rat Brain Atlas: https://labs.gaidi.ca/rat-brain-atlas/). b) Neuron counting at the three selected CA1 regions (left and right) of the brain hippocampus.

## RESULTS

### *Sialic acid level in EBN*

Table 1 displays the retention time and HPLC measurement of sialic acid levels in several EBN samples. N-acetylneuraminic acid was identified through HPLC analysis for a duration of 20 minutes, utilizing a standard compound. Comparatively, the concentrations of N-acetylneuraminic acid in 100 g of DesaBio Johor and Kedah EBN were 2.97 ± 1.63% and 3.15 ± 0.34%, respectively. These values were higher than those of EBN from Tawau, Sabah (2.02 ± 1.76%) and commercial EBN (1.17 ± 0.10%).

### *Number and density of neurons in the hippocampus*

Figure 3 illustrates the impact of dietary EBN supplementation on the number of neurons in mice across two generations. In the F0 generation, the dietary EBN from four different sources significantly increased brain neurons number in the CA1 region of the hippocampus ($F_{4, 15} = 4.039$, $P < 0.05$). The average number of neurons in the CA1 region of the hippocampus was significantly higher in EBN-S (31.725 ± 3.61; $P<0.048$) and EBN-N (32.40 ± 4.27; $P<0.05$) compared to CTRL (21.78 ± 5.27). However, the population of neurons in the hippocampus of the brain was not noticeable higher in EBN-C (27.84 ± 4.48) and EBN-B (24.69 ± 4.79) compared to CTRL (21.78 ± 5.27).

In F1 generation, the hippocampal neuron density in the CA1 region was significantly higher in groups maternally supplemented with EBN ($F_{4, 15} = 4.692$, $P<0.05$). The neuron population was significantly denser in EBN-S (33.22 ± 4.67) compared to CTRL (22.75 ± 5.26; $P<0.05$). Furthermore, the difference was also higher significantly between EBN-N (35.78 ± 4.72) and CTRL (22.75 ± 5.26; $P<0.05$). The quantity of neurons in EBN-C (30.89 ± 4.92), EBN-B (27.36 ± 5.69), and CTRL (22.75 ± 5.26) were not statistically significant.

In F2 generation mice fed with EBN, the average number of neurons in the CA1 area of the hippocampus was considerably higher ($F_{4, 15} = 3.567$; $P<0.05$). The highest neuron density was found in the mice supplemented with EBN-S (31.48 ± 3.57; $P<0.05$) and EBN-N (31.74 ± 4.80; $P<0.05$), which were significantly higher than CTRL (21.78 ± 2.86). The average number of neurons was not statistically higher in EBN-C (29.39 ± 5.35) and EBN-B (27.45 ± 4.52) compared to CTRL (21.78 ± 2.86).

**Table 1.** The relative retention time and results of HPLC from each edible bird's nest source.

| Edible bird nest (EBN) | Relative retention time (min) | HPLC parameters (Weight of EBN = 0.01015 gram) |
|---|---|---|

| | Chromatogr-am of sialic acid standard | Chromato-gram of EBN | Linearity | Correlation coefficient ($R^2$) | Content of sialic acid (%) |
|---|---|---|---|---|---|
| **EBN South (EBN-S)** | 6.40 | 6.33 | Y=2629.2x-7030.6 | 0.9998 | 3.1 ± 1.63 |
| **EBN North (EBN-N)** | 6.40 | 6.33 | Y=2629.2x-7030.6 | 0.9998 | 3.15 ± 0.34 |
| **EBN Borneo (EBN-B)** | 6.44 | 6.42 | Y=2599.6x+6756.5 | 0.9998 | 2.02 ± 1.76 |
| **EBN Commercial (EBN-C)** | 6.40 | 6.33 | Y=2629.2x-7030.6 | 0.9998 | 1.17 ± 0.10 |

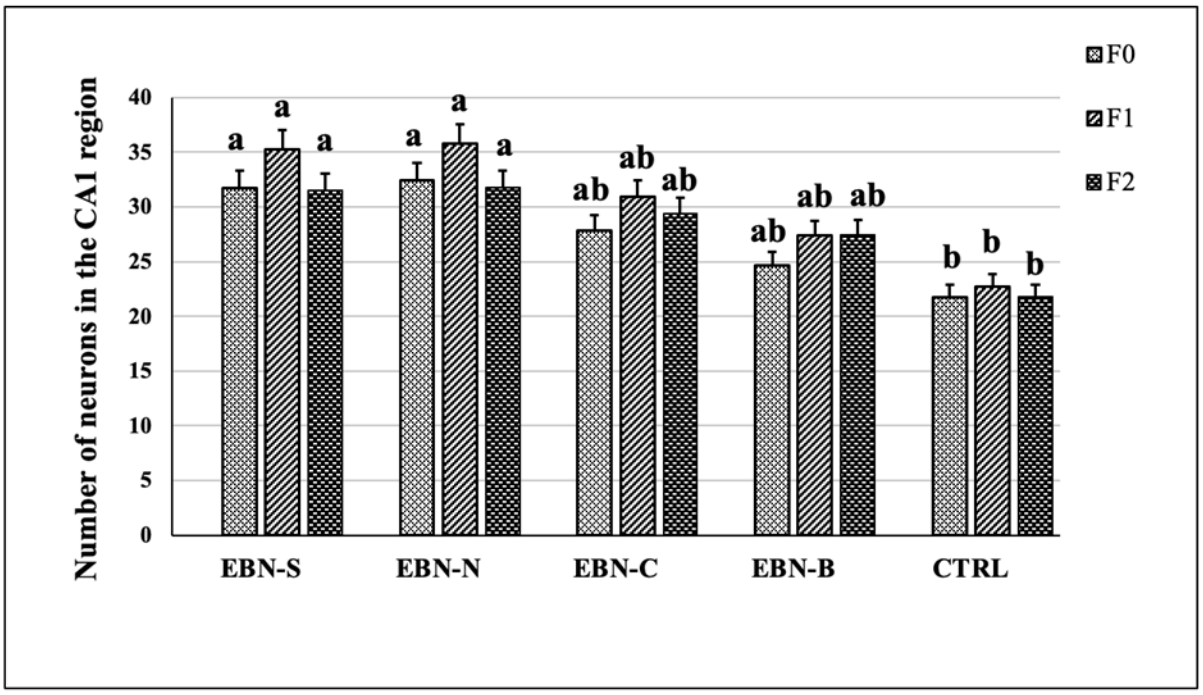


**Figure 3.** The average number of neurons in the CA1 region of the brain hippocampus in all experimental groups from F0, F1 and F2 generations. The bars with different letters show the significant differences between the treatment groups (P<0.05). EBN-S; mice received edible bird's nests from Johor, EBN-N; mice received edible bird's nests from Kedah, EBN-B; mice received edible bird's nests from Sabah and EBN-C; mice received edible bird's nest from Dingshen Imperial Birdnest. CTRL; mice received normal saline.

***Histological structure of the neuron***

Figure 4 indicates the comparison of neurons population in F0 generation among the five experimental groups. The hippocampus from the F0 generation showed that the neuron population was denser in all treated groups (e.g EBN-S, EBN-N, EBN-C, and EBN-B) in contrast to the control group (CTRL). Similarly, Figure 5 and Figure 6 compared the distribution of neurons in the F1 and F2 generations between the treatment and control groups using light microscopy 400x magnification, respectively. The hippocampus from the F1 and F2 generations showed that the neuron population was denser in all treated groups (EBN-S, EBN-N, EBN-C, and EBN-B) compared to the control group (CTRL).

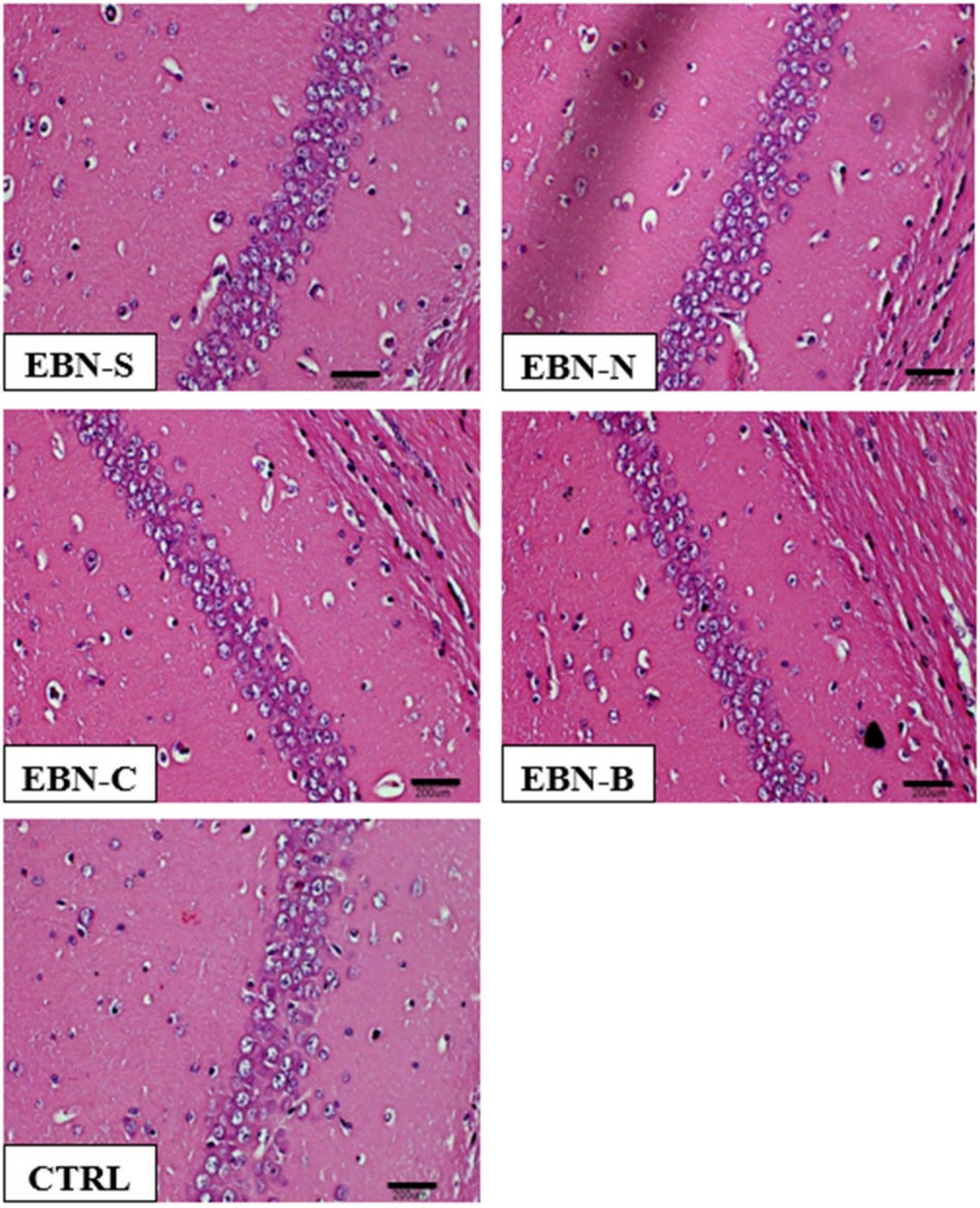


**Figure 4.** Hematoxylin and Eosin stains of neurons in CA1 region of the brain hippocampus from F0 generation. The neurons are densely populated in groups EBN-S, EBN-N, EBN-C and EBN-B compared to CTRL. Bar=200µm

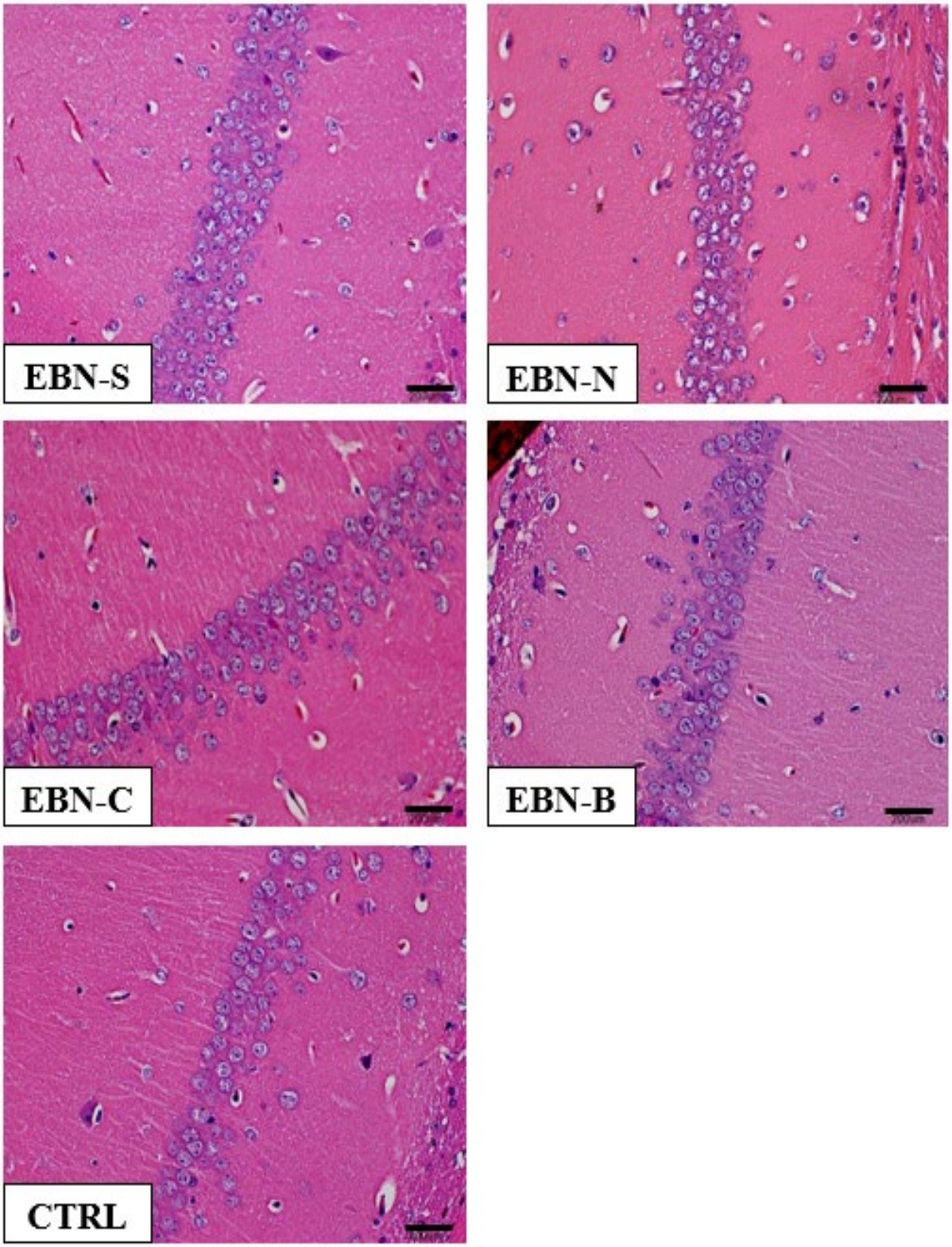


**Figure 5.** Hematoxylin and Eosin stains of neurons in CA1 region of the brain hippocampus from F1 generation. The neurons are densely populated in groups EBN-S, EBN-N, EBN-C and EBN-B compared to CTRL. Bar=200µm

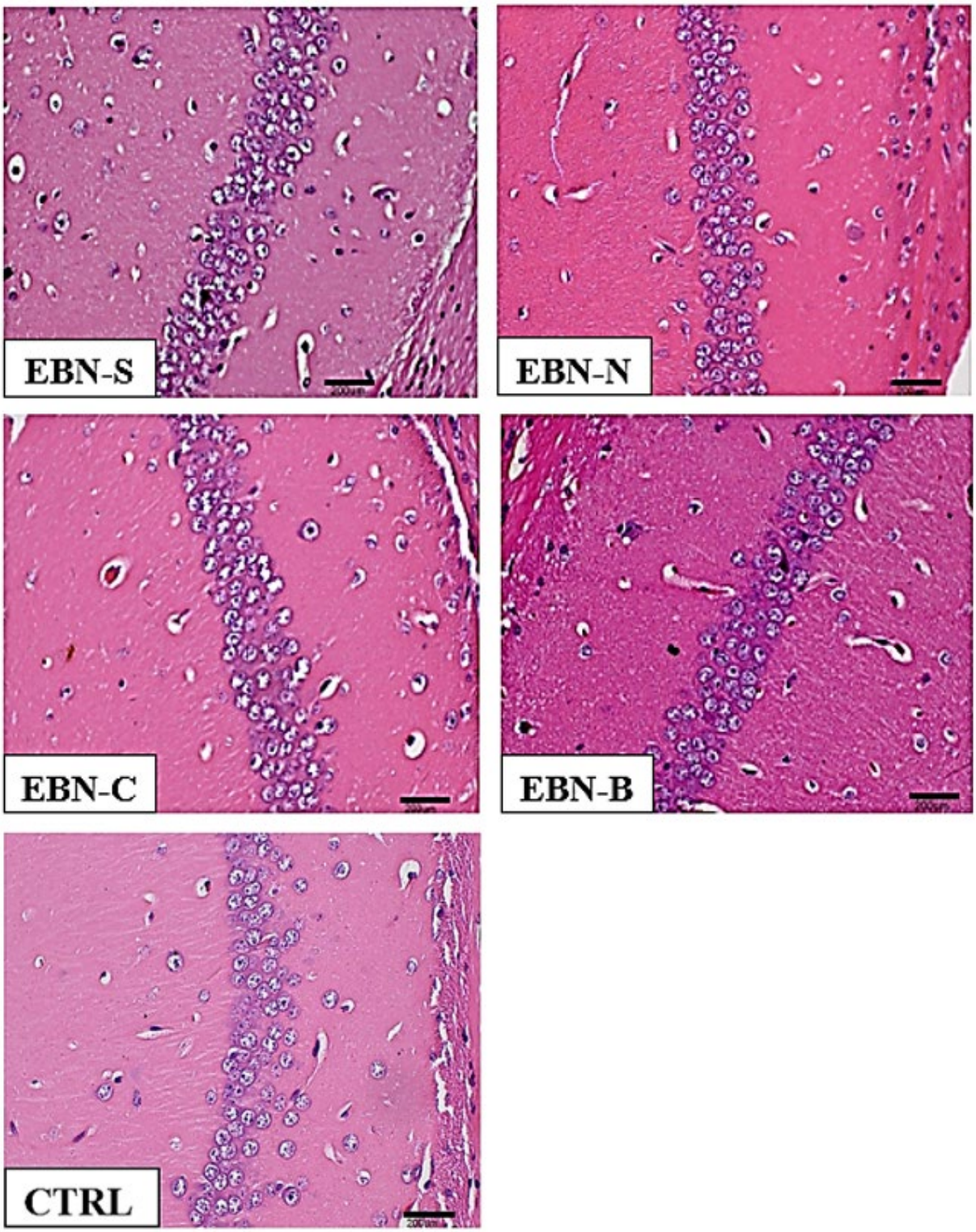


**Figure 6.** Hematoxylin and Eosin stains of neurons in CA1 region of the brain hippocampus from F2 generation. The neurons are densely populated in groups EBN-S, EBN-N, EBN-C and EBN-B compared to CTRL. Bar =200µm

## DISCUSSION

The study showed that edible bird's nest supplementation for eight weeks during pregnancy enhanced the density of neurons in the CA1 area in multigenerational mice. The findings of our study indicated that F2 mice, derived from

F0 and F1 generations, that were supplemented with EBN-S and EBN-N exhibited a considerably greater density and quantity of neurons in the CA1 hippocampus region in comparison to the control group that did not receive any EBN treatment. These findings correspond to previous studies, which reported a substantial increase in the quantity of neurons in the CA1, CA3, and DG regions in maternal mice with EBN supplementation (Xie *et al*., 2018).

The present investigation revealed that the sialic acid content in 100 g of EBN samples varied between 1.17% and 3.15%. Reportedly, the sialic acid content of EBN samples collected from the South and East Peninsular Malaysia varied significantly between 0.70% to 1.50% and 9% to 13.5% (Norhayati *et al*., 2010; Marni et al., 2014). Previous research has established that the levels of sialic acid in EBN are influenced by a variety of factors, including different breeding sites (Norhayati *et al*., 2010), types of habitats (Idris et al., 2014; Quek et al., 2018), and diet or food sources for *Aerodramus* (Ahmad *et al*., 2019). These variations in sialic acid levels are critical for neuronal transmission, ganglioside structure in synaptogenesis, and influence cellular function during neurodevelopment (Wang & Brand-Miller, 2003). In addition, various neuronal components (e.g., N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and N-acetylneuraminate) were generated through the metabolism of bioactive components derived from EBN, including sialic acid (Wang, 2009; Wang *et al*., 2006; Yu-Qin *et al*., 2000). As a result, the brain neuron population of mice in generations F0, F1, and F2 may have been influenced by the consumption of exogenous sialic acid derived from EBN, a micronutrient that is critical for optimal neurogenesis throughout pregnancy.

A comparison of the histological density of the brain neuron in the four treatment groups suggested that the number of neurons was influenced by the dietary EBN supplementation in mice. This study was designed to conduct a precise and systematic histological analysis of neuron populations in hippocampus regions on the effects of EBN dietary supplementation. In fact, pyramidal cells of the hippocampus, which were primarily formed prior to birth, were crucial for both learning and memory (Innis, 2000). Histological study revealed that the neuron distribution was greatly denser among the groups that received EBN. Mice from the control group had lower neuron density in their hippocampus regions compared to the EBN treated groups. The findings suggested that the number and population of neurons in the brain hippocampus grows and develops rapidly in mice with EBN supplementation compared to non-supplemented mice.

In this study, F1 and F2 generations were highly active periods for neuron development across multiple regions of the hippocampus. The hippocampus is one of the most essential region for brain development where neurogenesis occurs continuously (Stangl & Thuret, 2009). Previous evidence suggested that neurogenesis in the brain hippocampus has been directly related to the cognition and behavior of mammals (Praag et al., 2002; Zhao et al., 2008); Deng *et al*., 2009; Wu & Hen, 2014). This could indicate that dietary regulation at the hippocampus influence the neurogenesis not only in early development but also during adulthood as well as counteracting neurodegenerative effects (Gómez-Pinilla, 2008; Stangl & Thuret, 2009). However, neuron numbers decrease with age as unused neurons are allowed to regress progressively with age (Cao *et al*., 2005; Ziebell *et al*., 2018). Thus, this could suggest that EBN supplementation has potential to enhance neuronal growth across multiple generations particularly as their age decreases.

It was reported that the sialic acid product in neurons results in altered synaptic plasticity, which is required for memory consolidation (Foley *et al*., 2003). A significant proportion of exogenous sialic acid is confined to the synapse, where it has the potential to alter the morphology of the synaptic cleft, regulate the release of positive neurotransmitters, and impact transmitter movement (Morgan & Winick, 1981). In fact, when compared to other cell types in the body, the concentration of sialic acid is highest in

the membranes of neurons (Wang, 2012). Sialylated components such as brain gangliosides and polysialic acid are necessary for active learning processes (Wang, 2009; Palmano *et al*., 2015). Furthermore, it has been observed that polysialic acid forms a direct complex with brain-derived neurotrophic factor (BDNF) *(Kanato et al*., 2008; Sato & Kitajima, 2013). This complex plays a crucial role in various brain activities, including neuronal development, proliferation, and survival, which impact animal behaviours (Kanato *et al*., 2008; Sato, 2017). This explains how dietary supplementation, such as EBN high in sialic acid, affects gangliosides at the synapse and modifies neurotransmission through interactions with calcium ions (Palmano *et al*., 2015; Wang, 2012; Rahmann, 1995). Therefore, this could indicate that EBN promotes the growth and functionality of neurons in the hippocampus, thereby influencing neuronal density and synaptic transmission quality in the context of memory and learning.

## CONCLUSION

In conclusion, maternal EBN supplementation increased the number and density of hippocampal neurons across multigenerationes in mice through effects associated with neurogenesis. The increased number of neurons in the hippocampal region with different concentrations of EBN has been directly related to neurogenesis and sialic acid synthesis in the brain. Indeed, a large number of neurons are associated with increased branches of dendrites, underscores the importance on message transfer during synaptic transmission. Further research is needed to establish the metabolic and molecular pathway of sialic acid synthesis from EBN supplementation on neuron development especially neuroprotective properties of the sialic acid for more than three generations to explain how long the effects will influence the next generation.

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**AUTHORS CONTRIBUTIONS:**
Obaidullah Mahaq and Hafandi Ahamd designed and performed the experiments and contributed to the data management. The drafted manuscript was revised by Hasliza Abu Hassim, Mohd Hezmee Mohd Noor and Nurina Titisari. All authors provided the approval for publication of the manuscript.

## REFERENCES

Ahmad, H., Ong, S. Q., & Tan, E. H. (2019). The diet for edible-nest swiftlets: nutritional composition and cost of life stages of Megaselia scalaris Loew (Diptera: Phoridae) bred on 3 commercial breeding materials. *International journal of insect science*, *11*, 1-5.https://doi.org/10.1177/1179543318823533

Baptista, P., & Andrade, J. P. (2018). Adult hippocampal neurogenesis: Regulation and possible functional and clinical correlates. *Frontiers in Neuroanatomy*, *12*(6), 1–23.https://doi.org/10.3389/fnana.2018.00044

Cao, Q., Xu, X. M., DeVries, W. H., Enzmann, G. U., Ping, P., Tsoulfas, P., ... & Whittemore, S. R. (2005). Functional recovery in traumatic spinal cord injury after transplantation of multineurotrophin-

expressing glial-restricted precursor cells. *Journal of Neuroscience*, *25*(30), 6947-6957. https://doi.org/10.1523/jneurosci.1065-05.2005

Careena, S., Sani, D., Tan, S. N., Lim, C. W., Hassan, S., Norhafizah, M., … Lim, C. T. S. (2018). Effect of edible bird's nest extract on lipopolysaccharide-induced impairment of learning and memory in wistar rats. *Evidence-Based Complementary and Alternative Medicine*, *2018*, 1–7.https://doi.org/10.1155/2018/9318789

Deng, K., He, H., Qiu, J., Lorber, B., Bryson, J. B., & Filbin, M. T. (2009). Increased synthesis of spermidine as a result of upregulation of arginase I promotes axonal regeneration in culture and in vivo. *Journal of Neuroscience*, *29*(30), 9545–9552. https://doi.org/10.1523/jneurosci.1175-09.2009

Foley, A. G., Rønn, L. C. B., Murphy, K. J., & Regan, C. M. (2003). Distribution of polysialylated neural cell adhesion molecule in rat septal nuclei and septohippocampal pathway: transient increase of polysialylated interneurons in the subtriangular septal zone during memory consolidation. *Journal of Neuroscience Research*, *74*(6), 807–817.https://doi.org/10.1002/jnr.10820

Hao, Q., & Rahman, A. (2016). Swiftlets and edible bird's nest industry in Asia. *PJSRR Pertanika Journal of Scholarly Research Reviews*, *2*(1), 32–48.

Idris, A., Abdullah, A.-A., & Abd-Rehman, M. (2014). An overview of the study of the right habitat and suitable environmental factors that influence the success of edible bird nest production in Malaysia. *Asian Journal of Agricultural Research*, *8*(1), 1–16. https://doi.org/10.3923/ajar.2014.1.16

Innis, S. M. (2000). The role of dietary n–6 and n–3 fatty acids in the developing brain. *Developmental neuroscience*, *22*(5-6), 474-480.https://doi.org/10.1159/000017478

Kanato, Y., Kitajima, K., & Sato, C. (2008). Direct binding of polysialic acid to a brain-derived neurotrophic factor depends on the degree of polymerization. *Glycobiology*, *18*(12), 1044–1053.https://doi.org/10.1093/glycob/cwn084

Khalid, S. K. A., Abd Rashed, A., Aziz, S. A., & Ahmad, H. (2019). Effects of sialic acid from edible bird nest on cell viability associated with brain cognitive performance in mice. World Journal of Traditional Chinese Medicine, 5(4), 214.https://doi.org/10.4103/wjtcm.wjtcm_22_19

Koike, M., Shibata, M., Tadakoshi, M., Gotoh, K., Komatsu, M., Waguri, S., … Uchiyama, Y. (2008). Inhibition of Autophagy Prevents Hippocampal Pyramidal Neuron Death after Hypoxic-Ischemic Injury. *The American Journal of Pathology*, *172*(2), 454–469.https://doi.org/10.2353/ajpath.2008.070876

Lundberg, J., & McFarlane, D. A. (2012). National transformation programme (NTP) annual report 2017. *Geomorphology*, *157–158*, 153–168.https://doi.org/10.1016/j.geomorph.2011.04.043

Ma, F., & Liu, D. (2012). Sketch of the edible bird's nest and its important bioactivities. *Food Research International*, *48*(2), 559–567.https://doi.org/10.1016/j.foodres.2012.06.001

Mahaq, O., P. Rameli, M. A., Jaoi Edward, M., Mohd Hanafi, N., Abdul Aziz, S., Abu Hassim, H., ... & Ahmad, H. (2020). The effects of dietary edible bird nest supplementation on learning and memory functions of multigenerational mice. *Brain and behavior*, *10*(11), e01817.https://doi.org/10.1002/brb3.1817

Marcone, M. F. (2005). Characterization of the edible bird's nest the "Caviar of the East." *Food Research International*, *38*(10), 1125–

1134.https://doi.org/10.1016/j.foodres.2005.02.008

Marni, S., Marzura, M. R., Norzela, A. M., Khairunnisak, M., Bing, C. H., & Eddy, A. A. (2014). Preliminary study on free sialic acid content of edible bird nest from Johor and Kelantan. *Malaysian Journal of Veterinary Research*, *5*(1), 9–14.

Morgan, B. L. G., & Winick, M. (1981). The subcellular localization of administered N-acetylneuraminic acid in the brains of well-fed and protein restricted rats. *British Journal of Nutrition*, *46*(2), 231–238.https://doi.org/10.1079/BJN19810028

Nabilah, N., Mohamad, H., & Nawi, N. M. (2018). Consumer ' s perception on the quality of controversial contents in edible bird ' s nest products. *Scholarly Research Reviews*, *4*, 1–9.

Palmano, K., Rowan, A., Guillermo, R., Guan, J., & McJarrow, P. (2015). The role of gangliosides in neurodevelopment. *Nutrients*, *7*(5), 3891–3913.https://doi.org/10.3390/nu7053891

Praag, V., Af, S., Br, C., N, T., Td, P., & Fh, G. (2002). Functional neurogenesis in the adult hippocampus. *Nature*, *415*(2), 1030–1034.https://doi.org/10.1038/4151030a

Price, J. J., Johson, K. P., Bush, S. E., & Clayton, D. H. (2005). Phylogenetic relationships of the Papuan Swiftlet *Aerodramus papuensis* and implications for the evolution of avian echolocation. *Ibis*, *147*(4), 790–796.https://doi.org/10.1111/j.1474-919X.2005.00467.x

Quek, M. C., Chin, N. L., Yusof, Y. A., Law, C. L., & Tan, S. W. (2018a). Characterization of edible bird's nest of different production, species and geographical origins using nutritional composition, physicochemical properties and antioxidant activities. *Food Research International*, *109*, 35–45.https://doi.org/10.1016/j.foodres.2018.03.078

Rahmann, H. (1995). Brain gangliosides and memory formation. *Behavioural Brain Research*, *66*(8), 105 – 116.https://doi.org/10.1016/0166-4328(94)00131-X

Rashed, A. A., & Nazaimoon, W. W. M. (2010). Effect of edible bird's nest on Caco-2 cell proliferation. *Journal of Food Technology*, *8*(3), 126–130.https://doi.org/10.3923/jftech.2010.126.130

Sato, C. & Kitajima, K. (2013). Disialic, oligosialic and polysialic acids: distribution, functions and related disease. *Journal of biochemistry*, *154*(2), 115–136.https://doi.org/10.1093/jb/mvt057

Sato, C. (2017). Releasing mechanism of neurotrophic factors via polysialic acid. *Vitamins and Hormones*. Vol. 104, pp. 89–112.https://doi.org/10.1016/bs.vh.2016.11.004

Stangl, D., & Thuret, S. (2009). Impact of diet on adult hippocampal neurogenesis. *Genes and Nutrition*, *4*(4), 271–282.https://doi.org/10.1007/s12263-009-0134-5

Teh, Sue-Siang. Ma, Z.-F. (2018). Bioactive components and pharmacological properties of edible bird's nest. *International Proceedings of Chemical, Biological and Environmental Engineering*, *103*(7), 29–34.

Wang, B., & Brand-Miller, J. (2003). The role and potential of sialic acid in human nutrition. *European Journal of Clinical Nutrition*, *57*(11), 1351–1369.https://doi.org/10.1038/sj.ejcn.1601704

Wang, Bing. (2009). Sialic acid is an essential nutrient for brain development and cognition. *Annual Review of Nutrition*, *29*(1), 177–222.https://doi.org/10.1146/annurev.nutr.28.061807.155515

Wang, Bing. (2012). Molecular mechanism underlying sialic acid as an essential nutrient for brain development and cognition. *Advances in Nutrition*, *3*(3), 465-472.https://doi.org/10.3945/an.112.001875

Wang, Bing, Hu, H., & Yu, B. (2006). Molecular characterization of pig ST8Sia IV a critical gene for the formation of neural cell adhesion molecule and its response to sialic acid supplement in piglets. *Nutritional Neuroscience*, *9*(3–4), 147–154. https://doi.org/10.1080/10284150600903594

Wheeler, D. W., White, C. M., Rees, C. L., Komendantov, A. O., Hamilton, D. J., & Ascoli, G. A. (2015). Hippocampome. org : a knowledge base of neuron types in the rodent hippocampus. *eLIFE Tools and resources*, 4, 1–28. https://doi.org/10.7554/eLife.09960

Wieruszeski, J. M., Michalski, J. C., Montreuil, J., Strecker, G., Peter-Katalinic, J., Egge, H., … Vliegenthart, J. F. (1987). Structure of the monosialyl oligosaccharides derived from salivary gland mucin glycoproteins of the Chinese swiftlet (*genus Collocalia*). Characterization of novel types of extended core structure, Gal beta(1–3)[GlcNAc beta(1–6)] GalNAc alpha(1–3). *Journal of Biological Chemistry*, *262*(14), 6650–6657.https://doi.org/10.1016/S0021-9258(18)48291-9

Wu, M. V., & Hen, R. (2014). Functional dissociation of adult-born neurons along the dorsoventral axis of the dentate gyrus. *Hippocampus*, *24*(7), 751–761.https://doi.org/10.1002/hipo.22265

Xie, Y., Zeng, H., Huang, Z., Xu, H., Fan, Q., Zhang, Y., & Zheng, B. (2018). Effect of maternal administration of edible bird's nest on the learning and memory abilities of suckling offspring in mice. *Neural Plasticity*, *2018*, 1–13.https://doi.org/10.1155/2018/7697261

Yew, M. Y., Koh, R. Y., Chye, S. M., Othman, I., & Ng, K. Y. (2014). Edible bird's nest ameliorates oxidative stress-induced apoptosis in SH-SY5Y human neuroblastoma cells. *BMC Complementary and Alternative Medicine*, *14*, 391.https://doi.org/10.1186/1472-6882-14-391

Yew, M. Y., Koh, R. Y., Chye, S. M., Zainal Abidin, S. A., Othman, I., & Ng, K. Y. (2019). Neurotrophic properties and the de novo peptide sequencing of edible bird's nest extracts. *Food Bioscience*, *32*(9), 1–12.https://doi.org/10.1016/j.fbio.2019.100466

Yu-Qin, Y., Liang, X., Hua, W., Hui-Xing, Z., Xin-Fang, Z., & Bu-Sen, L. (2000). Determination of edible bird's nest and its products by gas chromatography. *Journal of Chromatographic Science*, *38*(1), 27–32.https://doi.org/10.1093/chromsci/38.1.27

Zainal Abidin, F., Hui, C. K., Luan, N. S., Mohd Ramli, E. S., Hun, L. T., & Abd Ghafar, N. (2011). Effects of edible bird's nest (EBN) on cultured rabbit corneal keratocytes. BMC complementary and alternative medicine, 11(1), 1-10.https://doi.org/10.1186/1472-6882-11-94

Zhang, Cheng, Li, Y., Wang, C., Lv, R., & Song, T. (2013). Extremely low-frequency magnetic exposure appears to have no effect on pathogenesis of alzheimer's disease in aluminum-overloaded rat. *PLoS ONE*, *8*(8), 1–8.https://doi.org/10.1371/journal.pone.0071087

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell*, *132*(4), 645–660.https://doi.org/10.1016/j.cell.2008.01.033

Zhiping, H., Imam, M. U., Ismail, M., Ismail, N., Yida, Z., Ideris, A., … Mahmud, R. (2015). Effects of edible bird's nest on hippocampal and cortical neurodegeneration in ovariectomized rats. *Food and Function*, *6*(5), 1701–1711.https://doi.org/10.1039/C5FO00226E

Ziebell, F., Dehler, S., Martin-Villalba, A., & Marciniak-Czochra, A. (2018). Revealing age-related changes of adult hippocampal neurogenesis using mathematical models. *Development,* *145*(1),

dev153544.https://doi.org/10.1242/dev.153544