Sys Rev Pharm 2020;11(8): 233-239
A multifaceted review journal in the field of pharmacy

Expression of Nutmeg Seed Extract in Integral Membrane Protein and Synaptic Vesicle: Younger Vs Aging

Fifi Veronica[a*], Lulu L. Fitri[b], A. Rizal Ganiem[c], Ambrosius P[a]., Hanna Gunawan[a,d], Unang Supratman[d], Ronny Lesmana[a,d]
[a]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjajaran, Bandung
[b]School of Life Sciences and Technology, Bandung Institue of Technology
[c]Department of Neurology, Dr. Hasan Sadikin General Hospital, Bandung
[d]Biology Activity Division, Central Laboratory, Universitas Padjadjaran, Bandung, Indonesia
*Corresponding email: fifiveronica2002@yahoo.com

Abstract

Introduction: Claudins and occludins proteins are the integral membrane protein which play as a critical role to maintain barrier properties of blood brain barrier (BBB). Large number of mitochondria in BBB regulate the presynaptic transport vesicle via endocytic and exocytic protein, synapsin and synaptophysin. Previous study stated, PGC-1α as central regulator of biogenesis mitochondria increased significanly in dopaminergic neuron. Based on our previous study, we discovered the potential effect of NuSE in BBB genes expression in younger and aging as aged changes. We also find out the NuSE effect to transport brain vesicle through Synapsin – Synaptophysin and BDNF genes expression. The role of NuSE to maintain integrity BBB may prove to be a great importance in brain drug delivery

Method: Twenty-eight rats, 11-12 weeks old male Rattus novergicus, randomly devide: 14 rats for younger rats and 14 for design to be older rats. Younger rats group divide into control group without NuSE and treatment group with 6,8 mg/day NuSE, for 12 weeks period via gavage. Meanwhile the rest rats (14 rats), continue carrying with normal feeding and drinking mineral water ad lib until they reach 80 weeks old, we called they are aging group. After 80 weeks old, all the aging rats were divide into control group without given NuSE (7 rats/group) and treatment group (7 rats/group) which given NuSE the same protocols with the younger group.

Result: In aging treatment NuSE group, Claudin and Occludin genes expression significantly higher. Claudin gene expression higher 2.4fold with p=0.00002. Occludin gene expression also higher 1.4 fold with p <0.05 . In younger treatment group, BDNFgene expression higher 1.2 fold, p <0.05. The NuSE enrichment also tended stimulate the level of genes expression Synapsin and synaptophysin in both young and aging, although not significant in statistic.

Conclusion: Nutmeg Seed Extract (NuSE) effected maintain integrity of Claudin and Occludin in aging blood brain barrier and also potentially play a role in modulating synaptic transmission both.

Keywords: Nutmeg Seed Extract, Claudin, Occludin, Synaptophysin, Synapsin

Corresponding Author: Fifi Veronica
Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia
Email: fifiveronica2002@yahoo.com

BACKGROUND

All aspects of cell biology has an integral membrane proteins with the features of lipid bilayers. Claudins, occludins and junctional adhesion proteins are the integral membrane protein forming tight junctions (TJ) in several epithelial tissue including in blood brain barrier. With endothelial cell of capillary vessel they play as a critical role to maintain barrier properties of blood brain barrier (BBB) 1.

Endothelial cells of BBB contain large number of mitochondria. Beside supply ATPs, brain mitochondria also regulated the neurotransmitter process. This process can be called as mitochondrial biogenesis. PPAR-γ as ligand-activated transcription factor has major role to regulate metabolism of cells. By triggering PPAR-γ with ligand activator, PPAR-γ later activates central regulator of mitochondrial biogenesis, PGC1-α. Activated PGC-1α causes mitochondria working more optimal. Mitochondria transmit the signal through presynaptic

transport vesicle via endocytic and exocytic protein, synapsin and synaptophysin. Synapsin and Synaptophysin are synaptic protein which have role in modulating synaptic transmission in presynaptic terminal. Accumulation synapsin in endocytocic zone maintains peripheral vesicle distribution in order to transmit the synapse signal while synaptophysin supports the process by continuing exocytosis activity on presynaptic vesicle.2, 3 Endothelial cell, astrocytes, pericytes neuron and TJ form neurovascular unit to support BBB provides homeostatis normal brain function. Occludin (60kDa), a tetraspan integral membrane protein, contribute TJ assembly and associated with redox regulation. Claudin (23kDa) play important as paracelular sealing with adjacent neighbouring endothelial cell to maintain integrity.1 Expresion brain tight junction protein such as Claudin and Occludin alters with age. Previous study stated that increasing of age-altered, followed by leakiness of BBB due to neurodegenerative process on cerebral tight junction protein.2,3 Pathogenesis of neurodegenative process associated with the impaired of mitochondrial function, as an organella that produce energy for cell to survive .4 Blood brain barrier disruption and impairment as functional decline in aging, impact in occludin and claudin as brain tight junction. Aging is one of the natural process which produce neuroinflamation. Several studies developed to find substance which can reduce the effect of neuroinflamation in aging.

Myristica fragrans seed (Nutmeg) is a popular spices in Indonesia. Several studies in vivo and in vitro models of the brain endothelium have been developed associated with the using Nutmeg Seed Extract (NuSE).5 NuSE has macelignan as an active compound which can activating PPAR-γ (Peroksisome Proliferator Activated Receptor).

Previous study by Keri Lestari et. al has also proven NuSE acted as a ligand at peroxisome proliferator activated receptor γ (PPAR-γ). 7 The later novelty study PPAR-γ adjusted the activity of biogenesis transcriptional co-activator PGC1-α in regulating mitochondrial structure in dopaminergic cells.The result show increasing relative expression of PGC-1α and also isoform of D1DR (dopamine 1A receptor). 8 Correlation between Dopamine Receptor (DA) with the regulation integrity of TJ in BBB and Brain Derived Neurotrophic Factor (BDNF) may also play a vital role in homeostatic regulation of tight Junction (TJ) in BBB. Based on our previous study, we will find out the potential effect of NuSE to maintain integrity Blood Brain Barier through brain TJ gene expression in younger and aging as aged changes through Synapsin – Synaptophysin and BDNF genes expression which maintaining transport brain vesicle in dopaminergic neuron. The role of NuSE to maintain integrity BBB may prove to be a great importance in brain drug delivery

MATERIALS & METHODS

Animals

This study was approved and carried out by the guidelines of the Animal Ethics Committee of Faculty Medicine Universitas Padjadjaran. Twenty eight rats,11-12 weeks old male Rattus novergicus were purchased from Biofarma Laboratories and were allowed to acclimate to our facility for at least 7 days before any experimental procedures. The rats were housed 4 per cage and were maintained on a 12:12-h light-dark cycle in a low-stress environment (22°C, 50% humidity, low noise). Food and water were provided ad libitum.

NuSE Treatment.

Rats were randomized into two groups as following: 14 rats for younger rats and 14 for design to be older rats. Younger group rats divide into control group without NuSE (7 rats/group) and treatment group with 6,8 mg/day NuSE (7 rats/group), for 12 weeks period via gavage. Meanwhile another rats (14 rats), continue carrying with normal feeding and drinking mineral water ad lib until they reach 80 weeks old, we called they are aging group rats. After 80 weeks old, all the aging rats were divide into control group without given NuSE (7 rats/group) and treatment group (7 rats/group) which given NuSE during 12 weeks period via gavage, as the same with the younger group. NuSE were dissolved in distilled water (which contain pulvis gum arabicum) just before the administration. This study using Glucopala caplet as NuSE to see integral membran protein in blood brain barrier. Glucopala is one of a natural patent product from Faculty Pharmacy of Universitas Padjadjaran (batch number FP08.A1604.001). The procedure for treatment of the animals were conducted according to the guide for the use and care of laboratory animals and were approved by research Ethics Committee of Universitas Padjadjaran with approval number 1223/UN6.KEP/EC/2019.

Tissue Preparation and PCR

Brain Isolation

After 12 weeks treatment, all rats were anesthetized with isoflurane flow rate (concetration to 5% or greater) and sacrificed by cervical translocation. Whole brains were removed, washed in ice-cold PBS, storage in -80º and were used for the detection mRNA levels of Occludin, Claudin and Brain Derived Neurotropic Factor (BDNF).

RNA Extraction and Semi-quantitative PCR

For measurement of the Claudin, Occludin and BDNF mRNA levels in the brain, semiquantitative Conventional RT-PCR was performed. RNA was extracted from the brain using 200 µl TRIzol Reagent (Qiagen). For reverse transcription, cDNA was synthesized from 500 ng of total RNA as described in the Transcriptor First Strand cDNA Synthesis kit (Takara Bio) using the oligo dT and random primers. In one reaction, 2.5 lL of the reverse-transcribed cDNA, 0.5 lM sense and antisense primers, 200 lM dNTPs, and 0.125 lL Taq polymerase (Roche) were added in a final volume of 25 lL. To define the linear range for PCR amplification, the optimal number of PCR cycles was decided. Reverse transcription step 30 min at 50○C. PCR initial activation 15 min at 950C. Denaturation step 40sec at 940C. annealing/ extension step and repeated cycles for mRNA BDNF, claudin and occludin shown as table 1 below. The PCR results for each sample were normalized by B-actin mRNA level as an internal control. All experiments were repeated three times to confirm the consistency of results. All the parameters and experimental conditions used were kept constant throughout the study. The image was saved (in tiff. format) on computer for digital image analysis using ImageJ software version 1.4.3u. Relative amounts of RNA from Claudin, Occludin and BDNF were determined by comparison kinetic amplification of β-actin as an endogenous control.

STATISTICAL DATA ANALYSIS.

Paired t-test were used to analyze the data. We used SPSS V.25.0 as analysis program. Statistical significance was designated at $p<0.05$. We expressed the data as mean ± standard error minimum (SEM).

Table 1. Primers used for Semi quantitative PCR Analysis

Gene Symbol	Primer sequence (5´ to 3´) Upper strand : sense Lower strand : antisense	Product SizeAnneling (0C) (bp)	Anneling (0C)	Cycle
BDNF	5'-CAAAAGGCACTGGAACTCGC-3' 5'-ACCGCCAGCCAATTCTCTTT-3	152	57,3	37
CLDN5	5′-GGCGATTACGACAAGAAGAACT3′ 5′-CCCGAACCCAACCTAACTT-3′	175	56	37
Occludin	5´-CCCAGGTGGCAGGTAGATTA-3´ 5´-AGGCCTGTTTTGTGAGCAC-3´	169	56	37
B actin	F 5'-TGG AGA AGA TTT GGC ACC A-3' R 5'-CCA GAG GCA TAC AGG GAC AA-3'	193	60	37
SYN	F 5'-GTG TCA GGG AAC TGG AAG ACC-3' R 5'-AGG AGC CCA CCA CCT CAA TA-3'	181	58	37
SYP	F 5'-GTG TAC TTT GAT GCA CCC TC-3' R 5'-TCT GCA GGA AGA TGT AGG TG-3'	177	55	37

Results

Effects of 12 Weeks NuSE On Cerebral Weight

Initial body weight of 2 group animal rats had similar (200 ± 50gr). Cerebral weight were recorded after termination. We found differences means cerebral weight to body weight ratio between control and treatment NuSE group (Fig. 1) Means ratio cerebral weight to body ratio tends to increase between NuSE group compared to control, in younger and adult rat. But statistically no differences between control and treatment group (t test, p >0,05).

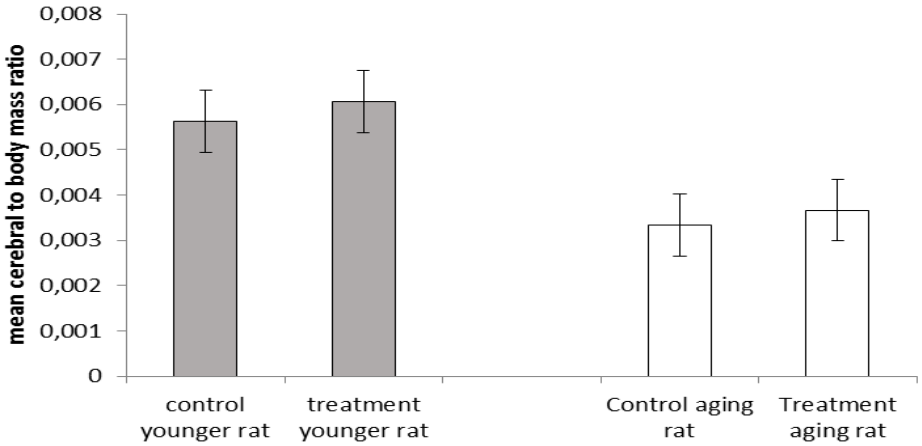

Figure 1: Mean cerebral mass to body mass ratio. Both grey and white-colored bar showed control compared to treatment group between younger and aging rats. Mean cerebral mass to body mass ratio in the treatment group higher than the control one, both of younger and aging rats, even not significant (t test, p>0.05).

Claudin mRNA Expression In Cerebral Cortex Of Wistar Rats

In order to confirm effect NuSE in brain tight junction, we examined Claudin gene expression using semi quantitative PCR. PCR bands of Claudin gene were normalized using b actin. The result is presented in Fig.2. In younger rats relative Claudin mRNA expresion were almost similar, but not in aging group. In aging group relative mRNA Claudin expression in treatment NuSE significantly higher compare to control (younger treatment NuSE 0.9 fold, p >0.05, aging rats treatment NuSE 2.4fold, p=0.00002).

Occludin mRNA expression in Cerebral Cortex Of Wistar Rats

We also examined occludin gene expression in rat cerebral cortex as part of brain tight junction using semiquantitative PCR. PCR bands of Occludin gene were normalized using b actin. The result is presented in Fig.3. In younger rats, relative expresion of Occludin almost similar between control and treatment NuSE. In aging group relative mRNA expression in treatment NuSE significantly higher compare to control (younger treatment NuSE 1.0 fold, p >0.05, aging rats treatment NuSE 1.4 fold, p <0.05).

BDNF mRNA Expression In Cerebral Cortex Of Wistar Rats

We examined brain neurotrophic factor using semiquantitative PCR. PCR bands of BDNF gene were normalized using b actin. The result presented is in Fig.4. In younger rats, relative expresion of BDNF younger group significantly increased compared to control. (younger treatment NuSE 1.2 fold, p <0.05, aging rats treatment NuSE 1.2 fold, p >0.05).

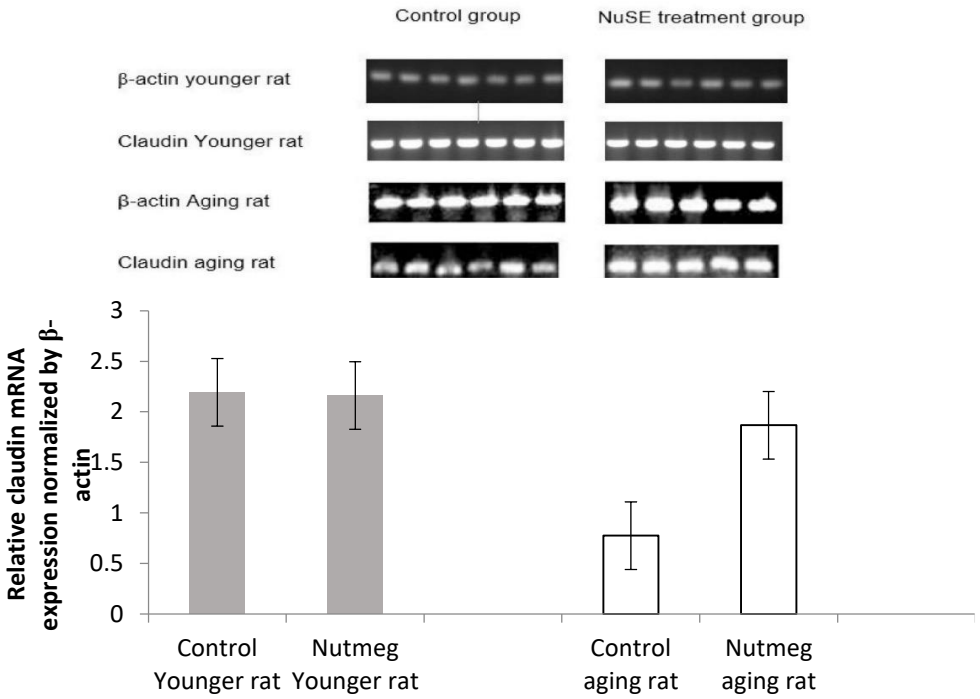

Figure 2: Relative claudin mRNA expression normalized by β-actin. Grey-colored bar showed relative claudin mRNA expression insignificantly between control and nutmeg in younger group. White-colored bar showed relative claudin mRNA expression significantly in nutmeg higher than the control within aging group. Note: *significant at p=0.00002 by paired t-test.

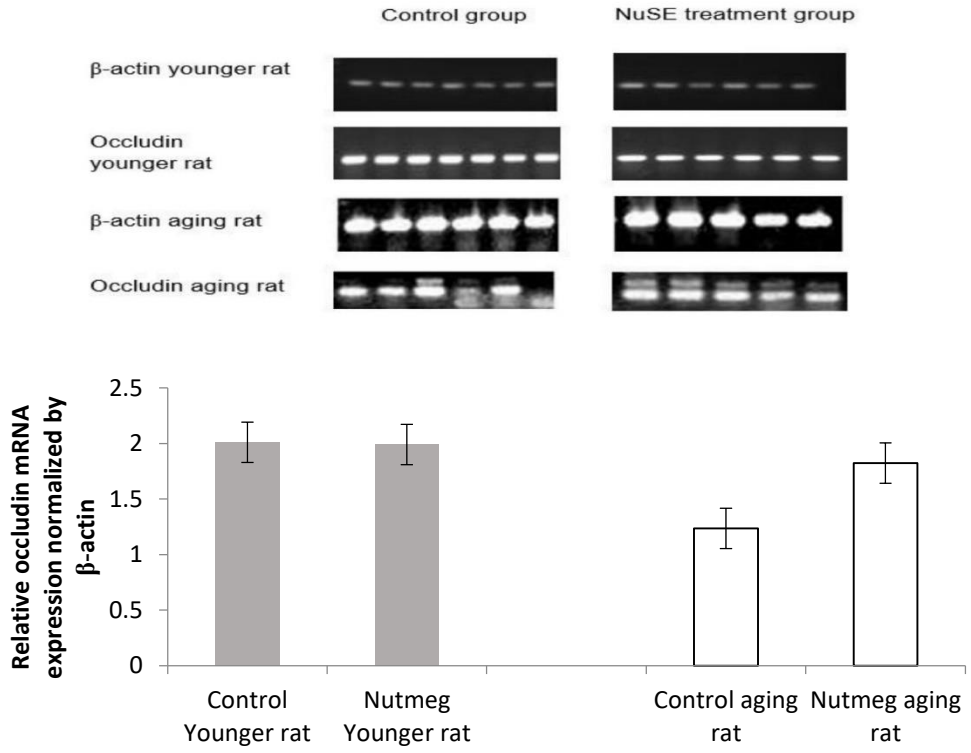

Figure 3: Relative occludin mRNA expression normalized by β-actin. Grey-colored bar showed relative occludin mRNA expression insignificantly between control and nutmeg in younger group. White-colored bar shown relative occludin mRNA expression significantly in nutmeg higher than the control one within aging group. Note: **significant at p<0.05 by paired t-test.

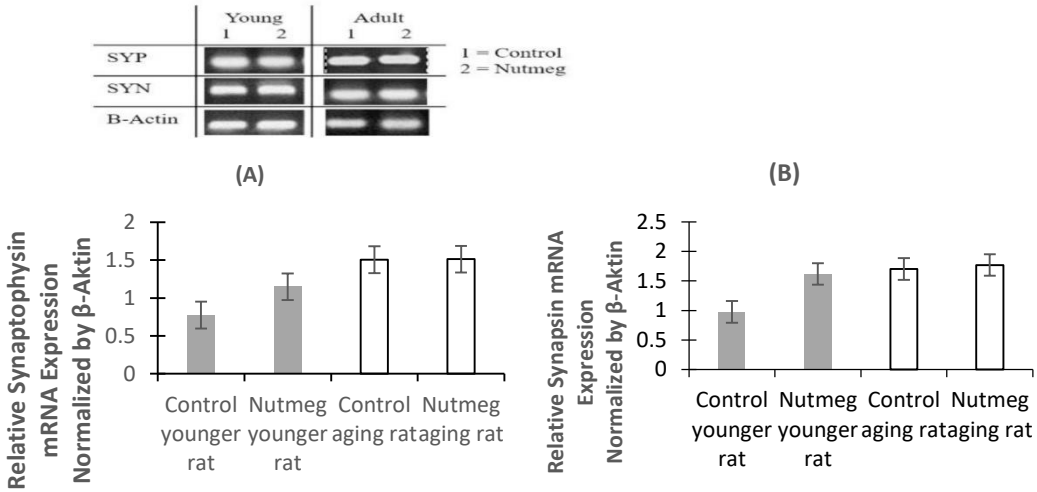

Figure 4. Relative BDNF mRNA expression normalized by β-actin. Grey-colored bar showed relative BDNF mRNA expression between control and treatment within younger group, where nutmeg significantly higher than the control one. White-colored bar showed relative BDNF mRNA expression in nutmeg was not significant as against the control within the aging group. Note: #significant at p<0.05 by paired t-test.

Synaptophysin and Synaphysin mRNA Expression In Cerebral Cortex Of Wistar Rats

We examined brain synaptophysin and Synapsin mRNA using using semiquantitative PCR. PCR bands of genes were normalized using b actin. The result Synapsin presented is in Fig.5. The NuSE enrichment tended to stimulate the level of Synapsin 1 in both young and aging nutmeg group compared into control group (Figure 5), although it is not stastically significant.. (younger treatment NuSE 0.263.fold, p >0.05, aging rats treatment NuSE 0.0671 fold, p >0.05 paired t-test). The expression of synaptophysin as synaptic plasticity protein, tended to be increased in nutmeg group (Figure 5'), however statistical calculation stated it is not significant (young rats 0.225±0.15, adult rats 0.061±0.235; p > 0.05; paired t-test). We did not find any interaction of nutmeg enrichment to the age of treatment (p > 0.05; Two Way ANOVA).

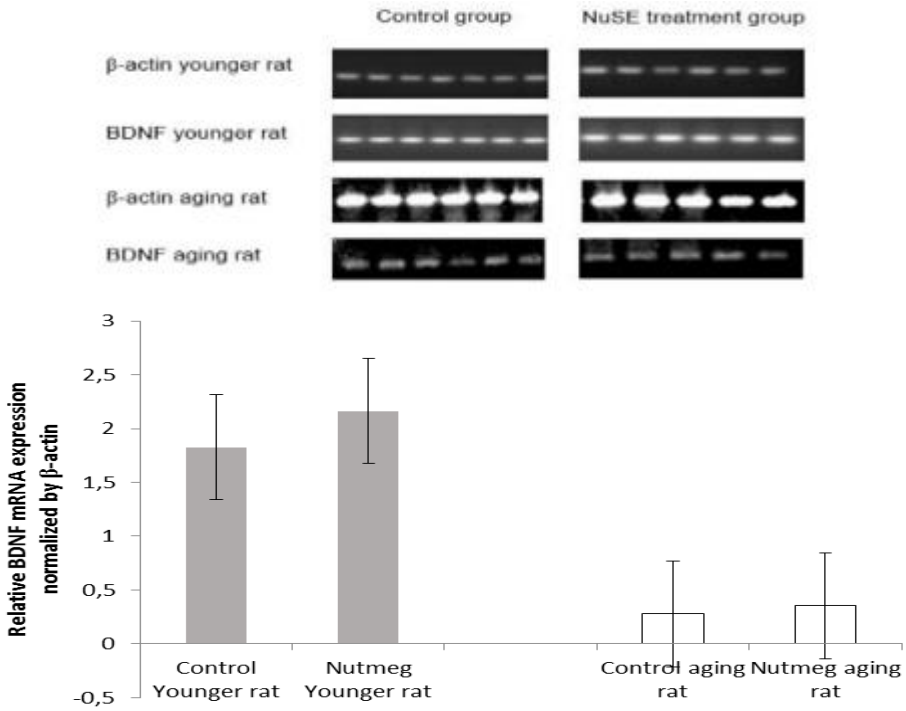

Figure 5. Relative synaptophysin (SYP) and synapsin (SYN) mRNA expression normalized by β-actin. Chart A and B showed relative mRNA expression, respectively SYP and SYN, within both younger (grey-colored bar) and aging groups (white-colored bar). In younger rats, both SYP and SYN tended to increase in the nutmeg group insignificantly rather than the control rats.

DISSCUSION

Effects of 12 Weeks NuSE On Cerebral Weight

Molecular and biochemical changes along age-dependent in brain corelation with the process of neurogenesis, synaptogenesis, gliogenesis and also maturation of oligodendrocyte. The changes also contribute to the growth and developmental brain in humans and rodents as animal research. Encephalization quotient is measurement of mean relative brain size from body weight value in the appopriate taxonomic group animal research.7 In this study, ratio brain weight to body weight either in younger and aging treatment group tends to increase compare to control group,although statistically not significant. The timing of the brain growth spurt, found to peak in 7 post- natal- day (pnd). The cortex reaches approximately 90% of its adult weight and had plateu phase after that in 20 pnd. 7 Several studies stated the role of PPAR γ agonist like thiazolidinedione, roziglitazone et.al in brain has neuroprotectif effect, which asscociated with mechanism anti inflamation, which aging is natural neuroinflamation, neurogenesis, angiogenesis and synaptogenesis. Treatment macelignan in NuSE as a natural ligand of PPAR γ agonist assumed contribution in increasing ratio brain weight compare to control group.9 Whether the better predictive of brain size is brain component architecture. Larger brain size predictable larger composed of cortex serebri, implication on high demand energy supply for the cortex, correlate with the large number of blood brain barier as an interface between vascular system and brain parenchyme. Gray matter has a higher density of synapses and higher levels of neural activity than white matter in cerebral cortex. This situation expected energy consumption larger from BBB 7,8

Claudin and Occludin mRNA Expression In Cerebral Cortex Of Wistar Rats

In this study in Fig.2 and Fig.3 aging group treatment shows increasing significantly expression mRNA claudin (2.4 fold) and occludin (1.4 fold) compare to control. Claudin is a member of family of TJ-associated marvel proteins (TAMP) which involved in oligomerization disulfide formation and contributes to the redox-dependency of TJ assembly. Occludin was found localizing to epithelial and endothelial TJ. Together with claudins play an importan role in posttranscriptional and posttranslational modification to enhance functional plasticity TJ in BBB. Macelignan in NuSE shows increase activity as ligand of PPAR γ agonist in cell line and animal experimental as antidiabetic agent. PGC1α as a coactivactor of PPARγ interact with the transcription factor and enhance mitochondrial biogenesis process. Increasing of brain Claudin 5 and Occludin in treatment group NuSE in this study suggested correlate with the increasing mitochondrial biogenesis process, which shown as increasing PGC1 in the same group in our previous study with the same protocols. 6 Although the sample in this study not specific taken from BBB, but we assumed that increasing PGC 1α also happened not only in brain parenchyme but also in whole mitochondria in endothelial cell in BBB. Need more confirmation study in the future.

The age-related alter BBB function such as decrease cholin transport , influx of brain glucose, narrowing capillary diameter and also decrease the number of mitochondria in endothelial cells. Decreasing mRNA expression of claudin5 and occludin in aging rats compare to younger rats related with the natural physiology process of aging in animal research. But interestingly, treatment NuSE group in aging rats shown level expression higher than control in the same group. We assumed that NuSE has potential protective effect in BBB to aging.

BDNF mRNA Expression In Cerebral Cortex Of Wistar Rats

Brain derived neurotropic factor serves neuroprotective and neurogeneration process. On dopaminergic neuron, BDNF also improve neuron transmission, not only in basal ganglia region but also in another subcortical region. BDNF has 2 isoform, proBDNF and mBDNF.ProBDNF is imature BDNF and may be converted into matur BDNF(mBDNF).9

A higher level of proBDNF exist during brain development, while mBDNF promotes neuroprotective activity and synaptic plasticity during adulthood. Neurodegeneration process reduce synthesis of mBDNF by producing non coding miRNA. PPAR gamma was the first proposed as a receptor for the thiazolinedione drugs (TZDs). The beneficial properties of TZD in different functions such as enhance learning and memory, decreading oxidative stress criteria, and upregulated brain BDNF. 10

Nutmeg Seed Extract (NuSE) has PPAR-γ as ligand-activated transcription factor has major role to regulate metabolism of cells, including cell biogenesis mithochondrial process. Intervention NuSE during12 weeks has potential effect increase expression mRNA brain BDNF in younger rats. It assumed corelated with the higher level of mRNA expression PGC1a in previous study. Vice versa with the aging rat

Synaptophysin and Synaphysin mRNA Expression In Cerebral Cortex Of Wistar Rats

Aging and cognitive level is frequently considered to be related. Therefore, during aging, alteration of neuron transmission highway in communicating information induced neurodegenerative process, both caused by altered processes or organelle damage.Memory and learning as example of important cognitive abilities will decrease significantly overtime.13 However, these degenerative process can be delayed by cell repair which induced by mitochondrial biogenesis. Beside supply ATPs, brain mitochondria also regulated the velocity of neurotransmitter process. PPAR-γ as ligand-activated transcription factor has major role to regulate metabolism of cells.14 By triggering PPAR-γ with ligand activator, PPAR-γ later activates central regulator of mitochondrial biogenesis, PGC1-α. Mitochondria transmit the signal through presynaptic vesicle via endocytic and exocytic protein, synapsin and synaptophysin. Our study revealed nutmeg enrichment into young and adult rat tended to modulate process of plasticity, even synapsin and synaptophysin mRNA expression are not significant by statistical calculation. Both of mRNA expression might be not significantly increased because of a few reasons. Inadequate dosage of treatment might influence quality of the results. The relative ratio were also affected by lack amount of samples and short duration of treatment.Nevertheless, previous study showed PPAR-γ can significantly sensitized dopamine as one of excitatory neurotransmitter which transmits signaling via synapse. This may show PPAR-γ can improve cognition processes like learning and memory by increase synaptic plasticity. In the other study, plasticity occurred by mitochondrial activity enhancement in biogenesis and maintaining cell performance, where as overexpressed PGC1-alpha increased synapsin and synaptophysin levels in

hippocampus neurons.11 Therefore, there are still limitations in this study. The treatment which have been done might be not specific into some areas

CONCLUSION

Nutmeg Seed Extract (NuSE) has potentially effect to maintain integrity of Claudin and Occludin as protein integral in aging blood brain barrier, through increasing activating PGC1a as marker of mitochondrial biogenesis. BDNF, Synaptophysin, and Synapsin as a marker of synaptic plasticity are also tend to increase even not significanly in statistical.

The limitation of this study is that we did not examine BBB in different region or specific region, as we know differences between brain structure might implication changes in the structure or function of BBB.

Future studies, it is suggested to study effects of NuSE on seminiferous epithelial cycle. Also NuSE effect on claudin and occludin in blood-testis barier (BTB).

ACKNOWLEDGEMENTS

Our research team thanks to Susianti SSi and Nurul Ihsani Assidiqiah, A.Md.Si and Canadia, Central Laboratory Analysts Assistants, Universitas Padjadjaran for assistance of this study. Gusti Muhammad Sena Arimadhyaksa, for support in completing this project.

FUNDING

This study was funded by funded by Indonesia Endowment Fund for Education (LPDP)

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS` CONTRIBUTIONS

Fifi Veronica, Lulu L. Fitri, Ambrosius Purba, Ahamd R Ganiem and Ronny Lesmana participated in the design of the research, supervised the experiment, and provided mentorship support. .Unang Supratman and Hanna Gunawan supervised and advised the experiments. All authors read and approved the final version of the article.

REFERENCES

1. Abbott NJ, Patabendige AK, Dolman DEM, Yuso SR, Begley DJ. Structure and function of the blood– brain barrier. Neurobiol Dis. 2010;37:13–25.
2. Orenbuch A, Shalev L, Marra V, Sinai I, Lavy Y, Kahn J, et al. Synapsin selectively controls the mobility of resting pool vesicles at hippocampal terminals. J Neurosci. 2012;32(12):3969–80.
3. Ashery U, Bielopolski N, Lavi A, Barak B, Michaeli L, Ben-Simon Y, et al. The Molecular Mechanisms Underlying Synaptic Transmission [Internet]. The Synapse: Structure and Function. Elsevier Inc.; 2013. 21–109 p. Available from: http://dx.doi.org/10.1016/B978-0-12-418675-0.00002-X
4. Mooradian AD, Haas MJ, Chehade J. Age-related changes in rat cerebral occludin and zonula occludens-1 (ZO-1). Mech Ageing Dev. 124(143_):146.
5. Lochhead J., Caffrey G., Quigley CE, Finch J, DeMarco KM, Nametz N, et al. Oxidative stress increases blood–brain barrier permeability and induces alterations in occludin during hypoxia–reoxygenation. J Cereb Blood Flow Metab. 30((9)):1625–1636.
6. Fischer F, Hamann A, Osiewacs H. Mitochondrial quality control: an integrated network of pathways. Trends Biochem Sci J. 37(7):284–92.
7. Adjene JO, Nwose E. Histological effects of long term consumption of nutmeg on the medial geniculate body of adult Wistar rats. N Am J Med Sci. 2((3)):134–137.
8. Abourashed EA, El-Alfy A. Chemical diversity and pharmacological significance of the secondary metabolites of nutmeg (Myristica fragrans Houtt.). Phytochem Rev. 15((6)):1035–1056.
9. Lestari K, Hwang J., Kariadi S., Wijaya A, Ahmad T, Subarnas A, et al. Screening For PPAR γ Agonist from Myristica Fragrans Houtt seeds for the Treatment of Type 2 Diabetes by in vitro and in vivo. Med Heal Sci J. 2012;12:7–15.
10. Veronica F, Lubis L, Soenggono A, Fitri L., Rizal A, Purba. A, et al. A preliminary study of the effect of PPAR- γ agonist from Myristica fragrans houtt seed extract on the biogenesis of rat infant's brain mitochondria and D1 dopamine receptor. Bali Med J. 2018;7(3):574–7.
11. Yang J, Siao CJ, Nagappan G, Marinic T, Jing D, McGrath K, et al. Neuronal release of proBDNF. Nat Neurosci. 2009;12(2):113–5.
12. Beheshti F, Hosseini M, Hashemzehi M, Soukhtanloo M, Khazaei M, Naser Shafei M. The effects of PPAR-γ agonist pioglitazone on hippocampal cytokines, brain-derived neurotrophic factor, memory impairment, and oxidative stress status in lipopolysaccharidetreated rats. Iran J Basic Med Sci. 2019;22(8):940–8.
13. Inda MC, Muravieva E V., Alberini CM. Memory retrieval and the passage of time: From reconsolidation and strengthening to extinction. J Neurosci. 2011;31(5):1635–43.
14. Tyagi S, Gupta P, Saini A, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. J Adv Pharm Technol Res. 2011;2(4):236–40.
15. Cheng A, Wan R, Yang J-L, Kamimura N, Son TG, Ouyang X, et al. Involvement of PGC-1α in the Formation and Maintenance of Neuronal Dendritic Spines. NIH Public Access. 2012;3:1250.