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# A preliminary study of the effect of PPAR- $\gamma$ agonist from *Myristica fragrans* houtt seed extract on the biogenesis of rat infant's brain mitochondria and D1 dopamine receptor



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## ABSTRACT

**Background:** *Myristica fragrans* (nutmeg) seed extract (NuSE) has been reported as a ligand for Peroxisome Proliferator-Activated Receptor (PPAR)- $\gamma$  due to hypoglycemic and antidiabetic effects. The recent study also finds the role of PGC-1 $\alpha$  as the regulator of mitochondrial biogenesis and the co-activator of PPAR- $\gamma$  as well. Dopamine 1 receptor (D1DR) is generally distributed in the brain with a various function, such as cognitive function. As a preliminary study, we explore a possibility the current knowledge of the mechanism of NuSE enhance dopamine 1 receptor via neuronal mitochondrial biogenesis on infant rat brain tissue. Aim: This study was aimed to investigate whether

PPAR- $\gamma$  agonist from NuSE can induce expression brain PGC-1 $\alpha$  and D1DR (D1A and D1B). Methods: Seven male rats (4 weeks) as a control and another seven male rats (4 weeks) was giving 8.1 mg NuSE/day by gavage within 12 weeks. End of the day, the rats were sacrificed, and the forebrain was extraction. Expression mRNA PGC-1 $\alpha$  and D1DR were analyzed. Results: The result showed significantly higher relatives expression of mRNA PGC-1 $\alpha$  and mRNA D1DR in NuSE group ( $p < 0.05$ ). Conclusion: The findings provide preliminary support that PPAR- $\gamma$  agonist from NuSE may offer a novel for the study brain mitochondrial biogenesis related mechanism of signaling dopamine receptor.

**Keywords:** PPAR- $\gamma$  agonist, PGC-1 $\alpha$ , D1DR, brain mitochondrial biogenesis

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## INTRODUCTION

*Myristica fragrans* (nutmeg) seed extract (NuSE) has macelignan as an active compound which can activate PPAR- $\gamma$  (Peroxisome Proliferator-Activated Receptor). PPAR is an intranuclear receptor.<sup>1</sup> Recent studies used the NuSE as a ligand PPAR- $\gamma$  for treating metabolic disease including hyperlipidemia, cardiovascular disease, and diabetes mellitus type II.<sup>2</sup> A study using primary cultures of microglia demonstrated that macelignan inhibited lipopolysaccharide (LPS)-induced production of Nitric Oxide and might promote activation of microglia, which may contribute to its neuroprotective and neuroplasticity effect.<sup>3</sup>

Neuroplasticity is a term to describe an adaptive condition changes that occur in the structure and function of cells in the nervous system in response to physiological or pathological perturbations. Sprouting and growth of axons or dendrites, synapse formation, the strengthening of synapses in response to repeated activation, and neurogenesis are part of neuroplasticity. The biological basis of this capacity for structural and functional adaptation encompasses a diverse set of cellular and molecular mechanisms, including the pre- and post-synaptic apparatuses for neurotransmission, cytoskeletal remodeling, membrane

trafficking, gene transcription, protein synthesis and proteolysis.<sup>4</sup>

Mitochondria have several functions in neural outgrowth neuroplasticity not only supply the ATP. Mitochondria can move rapidly within and between subcellular compartments, undergo fission and fusion, respond to electrical activity and activation of neurotransmitter and growth factor receptors function as signaling outposts that contain kinases, deacetylases, and other signal transduction enzymes. Mitochondrial biogenesis is a process of growth and division of mitochondria. Mitochondria biogenesis is very abundant in neural progenitor cells and newly generated neurons in the embryonic and early postnatal brain.<sup>5</sup> PGC-1 $\alpha$  (peroxisome proliferator-activated receptor coactivator 1 $\alpha$ ) is a master regulator of mitochondrial biogenesis. Suggesting PGC-1 $\alpha$  has an important role in the dynamic processes of neuroplasticity.<sup>6,7,8</sup>

However, the potential neuro stimulant effect of PPAR- $\gamma$  agonist from NuSE to dopamine receptor (D1DR) via mitochondrial biogenesis pathway has not been investigated. Accordingly, we examine PGC-1 $\alpha$  as a regulator of mitochondria biogenesis from forebrain infant rat tissue and find out the relationship with the expression of D1DR.

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## MATERIAL AND METHODS

### Animals

This study was approved and carried out by the guidelines of the Animal Ethics Committee of Faculty Medicine Universitas Padjadjaran. Four-week-old male *Rattus norvegicus* 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 (CP551) and water were provided ad libitum.

### NuSE Treatment

Rats were randomized into two groups as follows: Group control without NuSE (6-7/group) and treatment group (T) with 8,1 mg/day (NuSE (6-7/group)), for 12 weeks period via gavage. NuSE were dissolved in distilled water just before the administration. This preliminary study using Glucopala caplet as NuSE. Glucopala is one of a natural patent product from Faculty Pharmacy of Universitas Padjadjaran (batch number FP08.A1604.001).

### Brain tissue Isolation

All rats were anesthetized with diethyl ether and sacrificed by cervical translocation. Whole brains were removed, washed in ice-cold PBS, were used for the detection of mRNA levels of PGC-1 $\alpha$  and dopamine receptor.

### mRNA Expression

For measurement of the PGC-1 $\alpha$  and dopamine receptors mRNA levels in the brain, Conventional semiquantitative RT-PCR was performed. RNA was extracted from the brain using 200  $\mu$ l 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  $\mu$ l of the reverse-transcribed cDNA, 0.5  $\mu$ l sense and antisense primers, 200  $\mu$ l dNTPs, and 0.125  $\mu$ l Taq polymerase (Roche) were added in a final volume of 25  $\mu$ l. To define the linear range for PCR amplification, the optimal number of PCR cycles was decided. Reverse transcription steps 30 min at 50°C. PCR initial activation 15 min at 95°C. Denaturation step 40sec at 94°C. annealing/ extension step for mRNA PGC-1 $\alpha$  was 90 sec at 62°C. Plates were amplified by 37 repeated cycles, mRNA D1A receptor annealing/ extension step 90 sec at 62°C, and for D1B receptor annealing/ extension step 90 sec at 57°C. Plates were amplified by 34 repeated cycles. All PCR products were detected and analyzed by the Electrophoresis

Documentation and Analysis System (Bio-rad; Australia). 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 a tiff. format) on the computer for digital image analysis using ImageJ software version 1.4.3u. Relative amounts of RNA from PGC-1 $\alpha$  and D1DR were determined by comparison kinetic amplification of  $\beta$ -actin as an endogenous control. Specific primers used for the Reverse Transcript-PCR are both a laboratory set of D1DR sense primer 5'-TATCTCCAGCCCTTTCCAGTATGA-3' and a unique set of antisense primer 5'-ATTCCACCAGCCTCTTCCTTCTTC-3'.

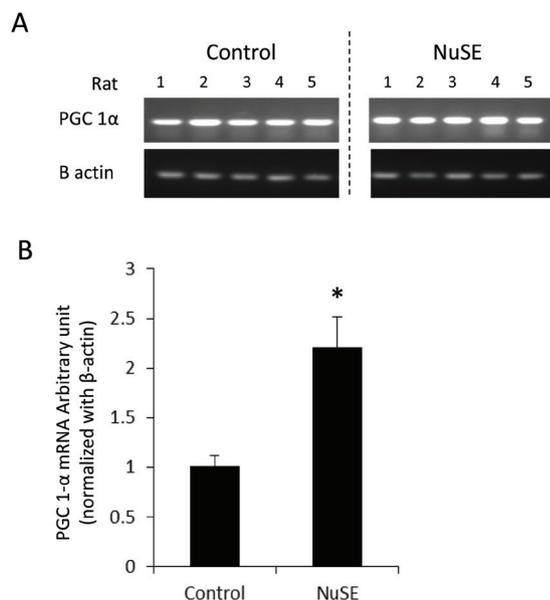
Electrophoresis of 5  $\mu$ l DNA molecular ladder was performed using 0.7-gram agarose gel 2% which adding 35 ml buffer tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer (pH - 8), 3.5  $\mu$ l *sybr green* and 0.5  $\mu$ l *loading dye*. Sample run at 80 Volt for 60 min. The size, thickness of the agarose gel, reagents, and other conditions were kept constant. The TAE buffer was not reused to avoid any additive effect of residual EtBr on the PCR band density. The band-size and DNA concentration of each PCR amplicon were determined by comparison to the corresponding band in the molecular weight ladder (Hyperladder-I). The DNA concentration in each band of Hyperladder-I is predetermined by the manufacturer. The amplicon images (PCR bands) in the gel were captured under ultraviolet (UV) light and documented using a Digital gel documentation system (Bio-rad; Australia).

### Statistical analysis.

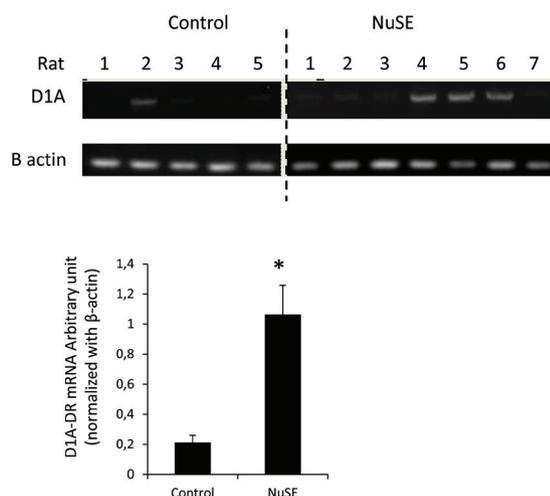
Results were analyzed using SPSS with one-way ANOVA. The sample size was determined based on previous experiments in our laboratory involving similar interventions. Statistical significance is shown as  $P < 0.05$

## RESULTS

8.1 gram NuSE feeding for 12 weeks resulted increasing expression mRNA PGC-1 $\alpha$  (Figure 1), and also both isoform D1A and D1B receptor (Figure 2 and Figure 3) in the infant rat brain tissue ( $p < 0.05$ ). We found approximately 2 times higher relative expression PGC-1 $\alpha$  in the treatment group. D1A dopamine receptor also increases 4 times higher relative expression than the control group. Although the rising of D1B dopamine receptor not higher than D1A, it's expression still higher than the control one.



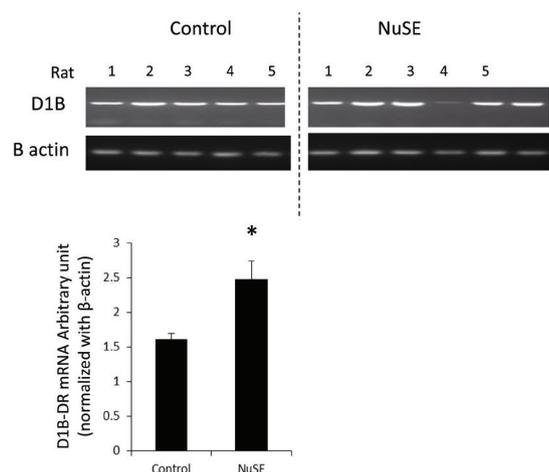
**Figure 1** NuSE increase relative expression of PGC-1α mRNA. NuSE was administered at 8,1 mg/day for 12 weeks. Data are means± SD (n=5). \*p<0.05 compared to the control group



**Figure 2** D1A-DR mRNA expression increase six fold in NuSE treated group compared with the control group (n=5). Data presented as mean ± SD. \*p<0.05

## DISCUSSION

NuSE is one of the natural polyphenolic flavonoid substances that are being investigated for their widespread health benefits recently. Macelignan as its active compound has generally been described to its combination of antioxidant and anti-inflammatory activity, according to the function as the ligand to PPAR-γ activity. But recent evidence suggests that increased mitochondria biogenesis could play an important role.<sup>8</sup> However,



**Figure 3** D1B-DR mRNA expression increase in NuSE treated group compared with the control group (n=5). Data presented as mean ± SD. \*p<0.05

the effects of NuSE on mitochondrial biogenesis in a dopaminergic neuron are unknown. This study examined the effect of long term effect (12 weeks) NuSE feedings on PGC-1α as a marker regulator of mitochondrial biogenesis in rat brain infant tissue.<sup>9</sup> The data indicate that long term feedings of the dietary NuSE can increase mRNA expression of PGC-1α in brain infant rat tissue. Furthermore, we determined if these changes in brain mitochondrial biogenesis were associated with increasing insensitivity of receptor postsynaptic neuron.

Brain mitochondria not only supply the energy ATP, but also regulated the velocity falls between of fast-moving small vesicles and slow-moving cytoskeletal proteins. Increasing the sensitivity of postsynaptic receptor indicate the higher exocytosis process of vesicle which carries out the neurotransmitter, such as dopamine.<sup>10,11</sup> Dopamine is one of excitatory neurotransmitter which involved in cognition processes like learning and memory, attention and reward system. The physiological actions of dopamine are mediated by G protein-coupled receptors (GPCRs), which divided into two major classes: D1-like receptor and D2-like receptor. This classification is generally based on the original biochemical function, which D-1 like receptor is able to modulate adenylate cyclase (AC). D1-class dopamine receptors [D1 and D5 (originally identified as D1B)] or D2-class dopamine receptors (D2, D3, and D4).<sup>12,13</sup> The result data from D1A and D1B as isoform from D1DR, increase significantly ( p < 0.05) in brain infant rat tissue which feeding by NuSE. Suggest the increasing sensitivity of DIDR contribute from increasing PGC-1α as a regulator of mitochondrial biogenesis and coactivator of PPAR-γ.

## CONCLUSION

The long-term feedings of dietary NuSE (PPAR- $\gamma$  agonist) can enhance infant rat brain mitochondrial biogenesis that was associated with an increasing dopamine receptor. Confirmation of these findings in large sample and various biomolecular variable would further support the need to study mitochondrial biogenesis related mechanism of signaling dopamine receptor in cognitive performance.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

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