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The Influence of Tween 80 in Characteristics and Stability of Self-Nanoemulsifying Drug Delivery System (SNEDDS) of VCO-Nutmeg Seed Oil

Rizky Dwi Larasati[1*], Anisa Fitria[2], Diantinofriani[3]

[1]Pharmacy Department, Universitas Sebelas Maret, Surakarta, Indonesia
[2-3]Pharmacy Department, Muhammadiyah Bandung University, Bandung, Indonesia
**Corresponding authors at: rizkydl@staff.uns.ac.id*

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ABSTRACT

Nutmeg seed oil is hydrophobic and does not penetrate the skin easily. A Self-Nanoemulsifying Drug Delivery System (SNEDDS) produces a nanoemulsion with droplet sizes of 20-200 nm. This improves the stability and skin penetration of nutmeg seed oil. This study aims to investigate the ratios of VCO, Tween 80, and PEG 400 that affect the characteristics and stability of SNEDDS. The Tween 80 ratios in the nutmeg seed oil SNEDDS formulas were F1 (1:4:1), F2 (1:7:1), and F3 (1:8:1). All SNEDDS had an O/W emulsion type. The pH was ≤ 7.5, the % transmittance ranged from 95% to 100%, and viscosity was ≤ 2000 cPa.s with non-Newtonian pseudoplastic rheology. Therefore, particle sizes were 23.71 ± 2.41, 7.4 ± 2.73, and 21.27 ± 0.07 nm. Zeta potentials were 189.3 ± 0.85, 190.0 ± 0.00, and 189.9 ± 0.00 mV, while PDI values were 1.861 ± 1.23, 3.80 ± 1.85, and 0.128 ± 0.09. Among these, F3 showed greater stability and better characteristics than F1 and F2. Increasing Tween 80 concentration improves stability and uniformity, as shown by reduced particle size, higher zeta potential, and more uniform size distribution after testing.

INTRODUCTION

Nutmeg seed oil contains several compounds, including methyl eugenol, isoeugenol, α-pinene, β-pinene, and myristicin. Nutmeg seed oil has been shown to have anti-inflammatory, antioxidant, and antibacterial activity (Nurjanah et al., 2017). In recent studies, nutmeg seed oil has been formulated as shampoo (Sari et al., 2021), cream (Luhulima et al., 2021), microemulsion (Shabrina et al., 2021), and nanoemulgel (Lailah et al., 2024). Nutmeg seed oil is volatile, with a relatively short shelf life and skin absorption; it is unstable during storage and insoluble in water. To maintain the stability and increase the penetration, a Self-Nanoemulsifying Drug Delivery System (SNEDDS) is needed (Lailah et al., 2024).

A Self-Nanoemulsifying Drug Delivery System (SNEDDS) is a homogeneous complex system consisting of oil, surfactants, and cosurfactants that are thermodynamically stable and form nanoemulsions spontaneously, without the need for energy input, upon contact with water (Annisa et al., 2023; Pratiwi et al., 2018). The SNEDDS has a clear, transparent, or slightly opaque appearance because the dispersed-phase droplets are small. SNEDDS is preferred because it can form nanometer-sized droplets, thereby increasing the bioavailability, solubility, dissolution rate, and absorption of insoluble active substances, such as nutmeg seed oil (Annisa et al., 2023).

The droplet size, solubility of the active ingredients, and stability of the nanoemulsion will be affected by the concentration and composition of oils, surfactants, and cosurfactants. VCO, as a carrier oil, has high solubility properties and is resistant to oxidation. It will generate the oil-phase properties required for a self-nanoemulsifying drug delivery system. Tween 80, as a surfactant, may lower the surface and interfacial tensions between immiscible phases. PEG 400, as a cosurfactant, can help the surfactant increase the spontaneous formation of nanoemulsions. The droplet size can be maintained at the nanoscale by reducing the interfacial tension between the two liquids when a surfactant-cosurfactant layer is present on the droplet surface (Annisa et al., 2023). Thus, the formulation of SNEDDS of nutmeg seed oil (*Myristica fragrans* Houtt) using VCO as an oil carrier with a combination of Tween 80 surfactant and PEG 400 cosurfactant. Then, a characteristic test was carried out to meet the standards of a good SNEDDS preparation, including organoleptic evaluation, homogeneity, emulsion type, pH measurement, transmittance determination, particle size determination, zeta potential determination, polydispersity index determination, viscosity, rheology, and stability testing (freeze-thaw).

METHODS

Materials

Nutmeg seed oil was obtained from CV. Ratu Aroma. Other materials included aquadest, acetic anhydride (Kimia Market), hydrochloric acid (Merck), sulfuric acid (Merck), Butylated Hydroxy Toluene (BHT) (Kimia Market), diethyl ether (Kimia Market), ethanol 90% (Kimia Market), ferric chloride III (Merck), polyethylene glycol 400 (PEG 400) (Kimia Market), tween 80 (Kimia Market), and virgin coconut oil (VCO) (Kimia Market).

Determining the Characteristics of Nutmeg Seed Oil (*Myristica fragrans* Houtt.)

The characteristics of nutmeg seed oil were evaluated in accordance with the Indonesian National Standard. Tests included color, weight compared to water, refractive index, residue after evaporation, and ethanol miscibility.

Formulation of Nutmeg Seed Oil Nanoemulsion

For the SNEDDS formulation, a 100 mL preparation was made using several components. The formulation included nutmeg seed oil (6.5%), VCO oil, tween 80, and PEG 400. The ratios are shown in Table 1.

SNEDDS was made by mixing nutmeg seed oil with VCO, tween 80, and PEG 400 in various ratios. The mixture was homogenized by vortexing for 15 minutes, followed by sonication for 30 minutes at 37ºC (Ujilestari et al., 2018). The mixture was left for 24 hours. Physical stability was observed visually (Zubaydah et al., 2023).

Table 1. Formulation of SNEDDS

Material	Formula (ratio)		
	F1	F2	F3
VCO	1	1	1
Tween 80	4	7	8
PEG 400	1	1	1

Evaluation of Nutmeg Seed Oil Nanoemulsion

Homogeneity Test

For the homogeneity test, a thin layer of the nanoemulsion was placed on an object glass (a microscope slide) and examined visually. The sample was considered homogeneous if no coarse (large) particles were seen under the microscope.

Emulsion Type Test

The emulsion type was checked using the color indicator methylene blue, a chemical dye. If the emulsion is oil-in-water (O/W), the mixture appears evenly blue and homogeneous. If it is water-in-oil (W/O), the methylene blue color appears nonhomogeneous and forms clumps. SNEDDS typically produces an oil-in-water (O/W) emulsion.

pH Test

pH was measured with a pH meter at room temperature. The target pH range is 4.5–7.5, matching skin pH.

Transmittance Test

A UV-visible spectrophotometer at 650 nm was used to measure transmittance, with distilled water as the blank. For SNEDDS, 95%-100% is required.

Particle Size and Polydispersity Index Test

Dilute 100 μL of SNEDDS in 100 mL of distilled water, then analyze with a Particle Size Analyzer. The acceptable size range is 20–200 nm.

Zeta Potential Test

Zeta potential was measured using a Zeta Potential Analyzer. Dilute 1 mL of the sample to 250 mL with water, and measure at 25°C.

Viscosity and Rheology Test

Viscosity was measured with a Brookfield viscometer, using 100 mL of SNEDDS and spindle no. 4 at 100 rpm for 1-minute intervals. Good nanoemulsions have a viscosity between 10 and 2000 cPa·s. Rheology was measured using a Brookfield viscometer with spindle 4S and shear rates of 50, 62.5, 75, 87.5, and 100 rpm at 25°C.

Stability Test

SNEDDS stability was tested using a freeze-thaw method. SNEDDS was stored at 4°C for 24 hours, then at 40°C for at least 24 hours. This represented one cycle. After each cycle, phase separation was observed. Stability testing continued for 6 cycles over 12 days. Evaluations covered organoleptic, homogeneity, pH, emulsion type, particle size, zeta potential, polydispersity index, viscosity, and rheology tests (Shabrina & Khansa, 2022).

RESULT AND DISCUSSION

Characteristics of Nutmeg Seed Oil

Indonesian National Standard (SNI) 06-2388-2006 is used as a reference in the characterization of nutmeg seed oil. Characterization includes organoleptic tests, specific gravity tests, refractive index tests, optical rotation tests, residual evaporation tests, and solubility tests in 90% ethanol (Badan Standardisasi Nasional, 2006). The results of the test to determine the characteristics of nutmeg seed oil are shown in Table 2.

Table 2. Result of Nutmeg Seed Oil Characteristics

Parameters	Result	Standard (SNI 06-2388-2006)
Organoleptic	Yellowish and smell like nutmeg seed oil	Colorless to yellow and smell like nutmeg seed oil
Specific gravity (g/mL)	0.8999	0.880 - 0.910
Refractive index (nD^{20})	1.479	1.470-1.497
Optical rotation	(+) $23,1^0$	(+) 8^0 - (+) 25^0
Residual evaporation (%)	0.7	Max 2
Solubility tests in 90% ethanol	1:3 clear	1:3 clear

Based on the results of the nutmeg seed oil characteristic test (*Myristica fragrans* Houtt.), the six tests were conducted in accordance with the Indonesian National Standard (SNI) 06-2388-2006, which serves as a reference for the characterization of nutmeg seed oil (Table 2). Therefore, it can be concluded that the nutmeg seed oil used in this study is genuine, high-quality nutmeg seed oil in accordance with SNI 06-2388-2006.

Evaluation of Nutmeg Seed Oil Nanoemulsion

Homogeneity Test

The results of the homogeneity test on SNEDDS showed that F1, F2, and F3 remained homogeneous before and after freeze-thaw (6 cycles). SNEEDS formulas 1, 2, and 3 remained clear in color, there were no coarse grains, and no separation after freeze-thaw. These results are consistent with the literature, which states that the preparation is considered homogeneous if there are no coarse grains (Fahrezi et al., 2021). Homogeneity will ensure that the SNEDDS formed have a uniform particle size, thereby maintaining uniform bioavailability of the active ingredient. Homogeneity also affects the stability of SNEDDS. Homogeneity reduces the likelihood of phase separation, thereby maintaining SNEDDS stability for a long time.

Emulsion Type Test

The results of the emulsion type test on SNEDDS showed that F1, F2, and F3 before and after freeze-thaw had an oil-in-water (O/W) type. This is indicated by the homogeneity and non-clumping of methylene blue as a dye indicator to determine the emulsion type in SNEDDS. Methylene blue is polar, allowing it to spread evenly throughout the SNEDDS preparation, which has a polar external phase (Ma'arif et al., 2023). SNEDDS has an oil-in-water (O/W) emulsion type and is intended to form an oil-in-water (O/W) emulsion type (Dyah Ayu Nurismawati & Sani Ega Priani, 2021). Generally, the oil-in-water (O/W) emulsion type will be formed if the HLB value of the surfactant and cosurfactant is 8-18, while the water-in-oil (W/O) emulsion type will be formed if the HLB value is 3-6 (Nirmalayanti, 2021). The SNEDDS of nutmeg seed oil had a mixed HLB above 10.

This was due to the HLB of the Tween 80 surfactant and PEG 400 cosurfactant, resulting in an oil-in-water (O/W) emulsion. The oil-in-water (O/W) SNEDDS type is preferred because it is more comfortable to use, easy to wash, has high penetration power, and is easy to apply to the skin.

Table 3. Result of Evaluation Tests for Nutmeg Seed Oil SNEDDS

Formula	Before Freeze Thaw	After Freeze Thaw	Requirements
Result of pH Test ± SD			
F1	6.71 ± 0.04	6.64 ± 0.09	4.5-7.5 (Rismarika et al., 2020).
F2	6.73 ± 0.04	6.73 ± 0.02	
F3	6.77 ± 0.06	6.73 ± 0.15	
Result of Particle Size Test ± SD (nm)			
F1	23.71 ± 2.41	489.00 ± 2.84	20 –100 nm (Pratiwi, 2021).
F2	7.40 ± 2.73	21.64 ± 2.98	
F3	21.27 ± 0.07	390.00 ± 2.83	
Result of Polydispersity Index Test ± SD			
F1	1.861 ± 1.23	0.961 ± 0.68	≤ 0,3 or close to 0 (Catur Adi et al., 2019).
F2	3.800 ± 1.85	1.421 ± 0.75	
F3	0.128 ± 0.09	1.150 ± 0.06	
Result of Zeta Potential ± SD (mV)			
F1	189.3 ± 0.85	35.7 ± 0.00	> 30 mV (Ujilestari et al., 2018).
F2	190.0 ± 0.00	33.2 ± 9.19	
F3	189.9 ± 0.00	45.5 ± 1.63	
Result of Viscosity Test ± SD (cPa.s)			
F1	364 ± 3.46	352 ± 3.46	10 – 2000 cPa.s (Zubaydah et al., 2023).
F2	366 ± 20.78	364 ± 19.29	
F3	368 ± 9.16	368 ± 9.16	

Transmittance Test

Based on the results of the transmittance test on SNEDDS, the percentage transmittance of F1, F2, and F3 were 99.788%; 97.687%; and 99.387%. The SNEDDS preparations must have a transmittance of ≥ 95% or be close to that of aquadest. All formulas have a high transmittance, indicating that the SNEDDS in each formula already have a small particle size (Pratiwi, 2021). The particle size is related to the percentage of transmitted light; the smaller the particle, the greater the light transmission, so the higher the percentage transmittance. The transmittance value in the SNEDDS test indicates that the SNEDDS formulation has entered the nanometer droplet size range, with a clear, transparent appearance (Annisa et al., 2023). The highest SNEDDS transmittance percentage is in F1 with a composition of oil (VCO): surfactant (Tween 80): cosurfactant (PEG 400) of 1: 4: 1. The formulation with the highest Tween 80 surfactant (F3) with a ratio of 1: 8: 1, should produce the highest transmittance percentage among other formulations, because the higher the Tween 80 surfactant used, the smaller the droplet size, the clearer the preparation, and the higher the transmittance percentage produced (Pratiwi, 2021). This shows that the transmittance percentage is influenced not only by the Tween 80 surfactant ratio but also by the PEG 400 cosurfactant and VCO carrier oil ratios, which affect the preparation's clarity and the resulting transmittance percentage.

pH Test

The pH test results for SNEDDS F1, F2, and F3 before and after freeze-thaw showed that the pH of the three formulations was within the normal human skin pH range of 4.5 to 7.5 (Table 3). Based on the results of the one-way ANOVA test, the significance value of pH before and after freeze-thaw was ≥0.05. It can be concluded that the SNEDDS nutmeg seed oil has a good pH, as the statistical results do not show a significant difference. The pH of the SNEDDS preparation must be within the skin pH range to avoid the risk of skin irritation and scaling upon application (Pratiwi, 2021).

Particle Size

The results of the SNEDDS particle size test before freeze-thaw showed that the droplet sizes in F1, F2, and F3 were 23.71 ± 2.41, 7.4 ± 2.73, and 21.27 ± 0.07 nm (Table 3). These results indicate that the SNEDDS particle size must be within the required range of 20-200 nm (Pratiwi, 2021). According to Table 3, a small particle size was observed in F3. These results fall within the range of SNEDDS particle sizes and align with the findings of Shabrina et al., (2021). In the results of particle size from F1 to F3, there was a decrease in particle size. That was caused by the concentration of the Tween 80 surfactant. The high concentration of Tween 80 surfactant will decrease particle size. Tween 80, as a surfactant, reduces the interfacial tension between the oil and water phases during conversion of SNEDDS into nanoemulsions. Tween 80 will adsorb onto the oil-phase droplets, forming micelles that can reduce interfacial tension, resulting in a good nanoemulsion with a small particle size. In addition to the Tween 80 surfactant, the PEG 400 cosurfactant also affects the final result of the particle size. PEG 400 cosurfactant will help in preventing phase separation from recombining. The data in Table 3 show an increase in particle size in SNEDDS after freeze-thaw. This occurs due to decreased surfactant stability, which is influenced by storage time, extreme temperatures, and surfactant concentration (Shabrina & Khansa, 2022). The longer the freeze-thaw test is carried out, and the lower the concentration of the surfactant used, the more potential SNEDDS can experience Ostwald Ripening (OR) that causes the resulting small droplets to merge into larger droplets due to unstable surfactant bonds. High-temperature conditions during freeze-thaw test storage will increase the kinetic energy of small particles in SNEDDS, enabling them to easily merge and increase particle size (Koroleva & Yurtov, 2021).

Polydispersity Index

Based on Table 3, the polydispersity indices of SNEDDS F1, F2, and F3 before freeze-thaw varied. F1 and F2 showed a polydispersity index of more than 0.7. The polydispersity index indicates that the particle sizes in F1 and F2 were not uniform, had different shapes, and exhibited a broad particle distribution, making them physically unstable and prone to aggregation (Taurina et al., 2017).

While in F3, the polydispersity index value obtained was 0.128; this indicates monodispersity, with particles of uniform, homogeneous shape and a particle size distribution not too wide. SNEDDS must have a polydispersity index close to 0. However, the polydispersity index of F1 and F2 was ≥ 0.3. This is due to the Tween 80 surfactant used to stabilize the particle size of SNEDDS in F1 and F2, as its concentration is lower than that in F3 (Satrialdi et al., 2023). One approach to improve surfactant stabilization is to increase surfactant and cosurfactant concentrations, thereby reducing surface tension. In Table 3, the SNEDDS polydispersity index values increased after freeze-thaw. This occurs because the surfactant's ability to maintain particle size decreases. The diversity of particle distribution and particle size in SNEDDS is shown in Figure 1.

Zeta Potential

Based on Table 3, the results of the determination of the zeta potential of SNEDDS F1, F2, and F3 before and after freeze-thaw, the zeta potential values obtained are within the required range, namely above ±30 mV (Ujilestari et al., 2018). The zeta potential values of F1, F2, and F3, respectively, before the freeze-thaw process were 189.3 ± 0.85 mV, 190.0 ± 0.00 mV, and 189.9 ± 0.00 mV. These results are within the zeta potential range of≥ 60 mV, indicating that F1, F2, and F3 before freeze-thaw had good stability. The high zeta potential will prevent aggregation by repelling dispersed particles, ensuring stability of the preparation. After freeze-thaw, the zeta potential values of F1, F2, and F3 were 35.7 ± 0.00 mV, 33.2 ± 9.19 mV, and 45.5 ± 1.63 mV, respectively. These results fall into the zeta potential range of ≥ 30 mV, indicating that the preparation has good stability. Zeta potential is influenced by the components in SNEDDS, especially surfactants located at the interface of two immiscible liquids. This study used Tween 80, a nonionic surfactant that tends to reduce zeta potential. This is because most particles dispersed in water become negatively charged due to adsorption that prefers hydroxyl ions. Zeta potential has practical applications in the stability of systems containing dispersed particles, as it regulates the degree of repulsion between particles of the same charge that are close together (Handayani et al., 2018).

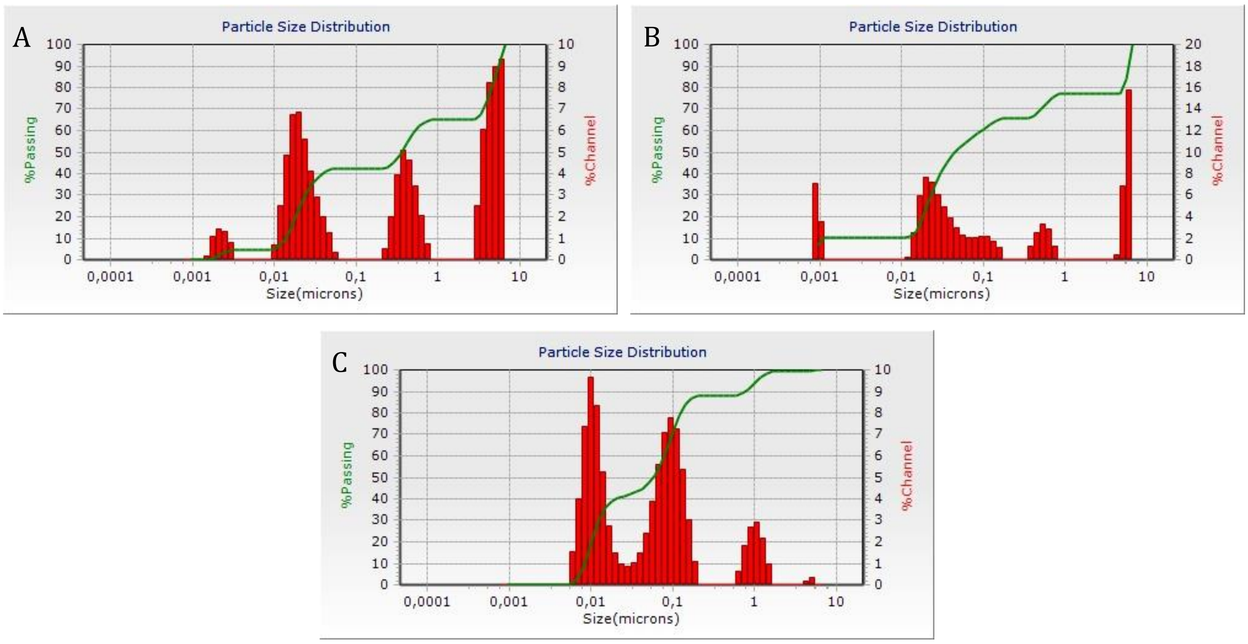

Figure. 1. Particle Size Distribution: (A) Formula 1 (B) Formula 2 (C) Formula 3

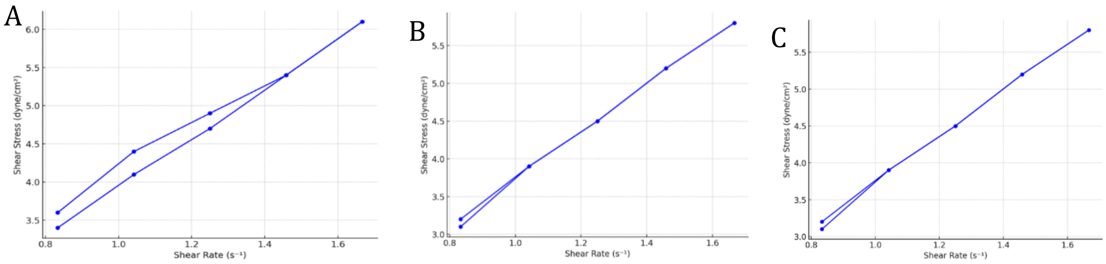

Figure 2. Rheology of Nutmeg Seed Oil SNEDDS: (A) Formula 1 (B) Formula 2 (C) Formula 3

Viscosity and Rheology

The results of the viscosity test of SNEDDS before and after freeze-thaw were within the required range of 10 - 2000 cPa.s. The viscosity data in Table 3 show an increase in viscosity between the formulas. The increase in viscosity was due to differences in the concentration of the Tween 80 surfactant used. Meanwhile, the viscosity decreased between the pre- and post-freeze-thaw states. This decrease in viscosity was caused by the influence of extreme temperatures during freeze-thaw. In this process, temperature is increased during SNEDDS preparation, and the effect of temperature on fluid viscosity is inversely proportional. The higher the temperature, the lower the viscosity (Zubaydah et al., 2023). The statistical analysis of viscosity results shows no significant difference ($p > 0.05$) between before and after freeze-thaw.

The rheology results for nutmeg seed oil SNEDDS preparations showed that the SNEDDS exhibited a pseudoplastic flow behavior (Figure 2). Pseudoplastic flow is a type of non-Newtonian flow that is not affected by time. Pseudoplastic exhibits a non-linear curve, lacks a yield value, and generally yields a curved rheogram. The rheogram results are closely related to viscosity; the viscosity of pseudoplastic substances decreases with increasing shear rate (Umeyor et al., 2016).

CONCLUSIONS

The variation in Tween 80 surfactant concentration affects the characteristics of nutmeg seed oil SNEDDS, including particle size, zeta potential, polydispersity index, and viscosity. The higher the concentration of Tween 80 used, the smaller the droplet size, the higher the zeta potential and viscosity, and the lower the polydispersity index. Variation of surfactant concentration, Tween 80, affects the stability of SNEDDS nutmeg seed oil. The higher the Tween 80 used, the more stable the resulting preparation. Tween 80 will increase the viscosity of the preparation, and the high viscosity will prevent phase separation from recombining.

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AUTHORS' CONTRIBUTIONS

RDL: conception, research design, data analysis and manuscript preparation. AF: experimental work, manuscript preparation and data analysis. D: data analysis and manuscript preparation.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

ETHICAL CONSIDERATION

Ethical issues including plagiarism, data fabrication, falsification, and double publication have been completely observed by the author.

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