



The Effect of Decyl Glucoside on Stability and Irritability of Nanostructured Lipid Carriers-Green Tea Extract as Topical Preparations

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Abstract

Background: Green Tea Extract (GTE) is a natural antioxidant compound that can protect the skin from photocarcinogenesis (DNA damage due to ultraviolet exposure). GTE has low stability, which needs a delivery system such as Nanostructured Lipid Carriers (NLC) with decyl glucoside (DG) as a natural surfactant that at the right concentration can produce a significantly small particle size which can improve the stability of the NLC.

Objective: To determine the effect of DG usage on the characteristics, physical stability, and irritability of NLC-GTE preparation. **Methods:** NLC-GTE preparation used the High Shear Homogenization (HSH) method with three formulas, which contained DG 2%, 2.5%, and 3% consecutively. Afterwards, the characteristic and physical stability tests were conducted using the thermal cycling method for three cycles with two different temperatures (48 hours/cycle, 2 - 8°C and 40°C). The irritability test used Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) method. **Results:** Characteristic test of organoleptic showed that all formulas were white, odorless, and had a semi-solid consistency. However, the pH, particle size, and polydispersity index values from all formulas were within the normal range of values. The physical stability test result showed that 3% DG was the most stable formula. This formula was within the non-irritating range of values in HET-CAM. **Conclusion:** NLC-GTE with an increased concentration of DG as a surfactant can improve the characteristics and physical stability of the preparation. F3 (3% DG) is the best formula compared to other formulas and indicates non-irritating in the HET-CAM test.

Keywords: nanostructured lipid carriers, green tea extract, decyl glucoside, thermal cycling, Hen's Egg Test on the chorioallantoic membrane

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INTRODUCTION

Green tea extract (GTE) is obtained from the tea plant or *Camellia sinensis* (Wang *et al.*, 2020). GTE has the highest phenolic and flavonoid content compared to other tea extracts, with an IC₅₀ value of 0.487 µg/mL (Widowati *et al.*, 2015). In addition, GTE can also be used for UVA and UVB protection (Saini *et al.*, 2019) and anti-photoaging (Siyahpoosh *et al.*, 2022). Katiyar *et al.* (in Aaron & Robert, 2009) explained that GTE could increase penetration and absorption into the skin when used topically. This statement is supported by Dal Belo *et al.* (2009) who conducted an *in vitro* penetration test on GTE cosmetic preparations, using the Franz diffusion cell method. The method used in this test, namely the Franz diffusion cell method, uses fresh human skin (Caucasian) as the sample application medium, then measured using the High-Performance Liquid Chromatography (HPLC) instrument. Based on these tests, the results showed that this preparation has good skin penetration and retention capabilities. It is characterized by detecting GTE in all skin layers, especially in the dermis layer (9.2%). In addition, epigallocatechin gallate (EGCG) the main phenolic compound in GTE, turns out to have poor stability because it is very susceptible to oxidation due to several factors (exposure to light, temperature, pH, and others), so it must be overcome by using a suitable delivery system (Sugihartini *et al.*, 2016; Shi *et al.*, 2018).

NLC or nanostructured lipid carriers is a delivery system suitable for active antioxidant ingredients (Chen-yu *et al.*, 2012) and topical use (Souto *et al.*, 2020). Manea *et al.* (2014) stated that green tea extract could be developed in topical preparations using an NLC delivery system. In addition, Tamjidi *et al.* (2013) explained that NLC consists of solid lipids and liquid lipids that can form a matrix and control the entrapment of active substances to increase the stability of a preparation. In addition to the various advantages described above, the preparation stage of NLC has several problems. During the crystallization process, the surface area of the particles will increase rapidly; thus, the whole system is unstable. Some literature states that it can be overcome by adding surfactants (Han *et al.*, 2008) which are needed to improve the quality of the nanoparticle interface (Han *et al.*, 2008). The quality of the nanoparticle interface can affect the system's characteristics and ability to absorb the active substance. Furthermore, the surfactant will also affect the formed nanoparticles' physical stability (Averina *et al.*, 2009). Besides that, surfactants also have disadvantages that can cause irritation and various skin disorders by

damaging the lipid membrane, which is the external protective layer on the skin, to disrupt its function (Kosswig, 2012). This problem can be solved by selecting a surfactant that has low irritability.

Decyl glucoside (DG) is a type of natural surfactant derived from plants, which is produced by the reaction of glucose (from corn starch) and fatty alcohol decanol (from coconut). Mehling *et al.* (2007) conducted the tests for DG irritability. It showed that this surfactant did not show irritation in various irritability testing methods. Furthermore, based on the research of Chaiyana *et al.* (2020), surfactant DG can produce significantly small particles in NLC preparations containing an active substance in the form of an extract, namely *Ocimum sanctum* Linn. In contrast, the small NLC particle size can affect various aspects, including increasing the stability of the preparation (Müller-Fischer *et al.*, 2007). The problem lies in the concentration of DG used. By the explanation, Lasoń *et al.* (2018) stated in their research article that too high a DG concentration could result in foam formation during the NLC preparation process using forskolin as the active substance. Based on these considerations, several DG concentrations were compared to determine their effect on the characteristics (organoleptic, pH, particle size, polydispersity index), physical stability, and irritability of the NLC-GTE preparation. It aims to obtain the most optimal preparation.

MATERIALS AND METHODS

Materials

The materials used in this research were *green tea extract* (PT. Angler BioChemLab, Surabaya, Indonesia), *cetyl palmitate* (BASF, Indonesia), *glyceryl stearate* (BASF, Indonesia), *grape seed oil* (NHR Organic Oils, UK), *decyl glucoside* (Dow Chemical Pacific, Singapore), *synperonicF68* (PT Megasetia Agung Kimia, Jakarta, Indonesia), and *lecithin* (Solae™, Amerika) were cosmetic grade. *Aquademineralisata* (Indonesia) was a technical grade and fertile chicken eggs (chicken farm, West Java & East Java, Indonesia).

Method

Preparation of NLC-GTE

The method used in the preparation of NLC-GTE was a modification of the high shear homogenization (HSH) method by Manea *et al.* (2014). This method was carried out by preparing two different phases: the oil and water phases (Table 1). The oil phase consists of cetyl palmitate, glyceryl stearate, and grape seed oil.

Table 1. Design formula of NLC-GTE

Materials	Function	Formula (%)		
		F1	F2	F3
Green tea extract (GTE)	Active substance	0.1	0.1	0.1
Lipid phase	Cetyl palmitate	3.5	3.5	3.5
	Glyceryl stearate	3.5	3.5	3.5
	Grape seed oil	3	3	3
Water phase	Decyl glucoside	2	2.5	3
	Lecithin	0.5	0.5	0.5
	Synperonic F68	0.5	0.5	0.5
	Aquademineralisata	Ad 100	Ad 100	Ad 100

Description:

F1 = NLC-GTE + DG 2%

F2 = NLC-GTE + DG 2.5%

F3 = NLC-GTE + DG 3%

Meanwhile, the aqueous phase consisted of surfactant DG, synperonic F68, lecithin (1%, 1:1, w/w), and aquademineralisata. Furthermore, both were heated with Thermo Scientific Cimarec⁺ at the same temperature, namely 70°C for 30 minutes, then GTE (which had been dissolved in 10mL aquademineralisata) was added in the oil phase. Before mixing the two phases, the aqueous phase was stirred using Ultra-Turax IKA@T25 Digital for 2 minutes at a speed of 15,000 rpm. Then the two phases were mixed for 7 minutes at a speed of 15,000 rpm thus the NLC-GTE preparation was obtained.

Characteristics test of NLC-GTE

Organoleptic

This organoleptic test was performed visually with several aspects of observation, in the form of smell, color, and consistency of the NLC-GTE preparation (Loreta, 2015; A'yun, 2019; Indrajaya, 2021).

pH

The pH values of the NLC-GTE preparations were measured using a pH meter SI Analytics Lab 865 electrode calibrated and inserted into the preparation. The value shown on the screen was recorded as the pH value and replicated three times (A'yun, 2019; Indrajaya, 2021). Furthermore, statistical analysis was carried out using the One Way ANOVA method.

Particle size dan polydispersity index

Particle size (PS) and polydispersity index (PI) measurements were conducted by diluting the NLC-GTE preparation first and analyzing using the Beckman Coulter® DelsaTMnano C Particle Analyzer instrument. This measurement was replicated three times, and the particle size and polydispersity index values were recorded on the monitor screen (Mayangsari, 2021). The data obtained from this test

were then analyzed statistically using the One Way ANOVA method.

Physical stability test of NLC-GTE

The stability test of NLC-GTE was performed using the accelerated method, namely thermal cycling. According to Rohmah (2020), this method test procedure can be conducted with a total of three cycles at two different temperatures (48 hours at a temperature of 2 - 8°C and 40°C, respectively). Moreover, the preparation characteristics such as organoleptic observations, pH, particle size (PS), and polydispersity index (PI) would be monitored. The results of these tests (pH, PS, and PI) were then analyzed using a statistical method, namely the Paired T-test.

Irritability test of NLC-GTE

This method's test procedure began with incubating and periodically rotating chicken eggs in an incubator at 37°C. On the 10th day, the eggs were binoculars to distinguish and select fertile eggs, marked the air cavity with a pencil, and then opened using sterile scissors. The outer egg membrane was moistened with warm sterile 0.9% NaCl solution and incubated for 5 - 20 minutes to facilitate the removal of the outer egg membrane. Ensure the chorioallantoic membrane (CAM) conditions were still in good condition and place 0.2 mL NLC-GTE (the best formula based on the physical stability test) in the CAM and let stand for 20 seconds (Hagino *et al.*, 1999). As an irritant control (+C), 1% sodium lauryl sulfate (SLS) and sterile 0.9% NaCl solution were used as negative controls (-C). Then the CAM was cleaned using a sterile 0.9% NaCl solution. Further observations began after the CAM was cleaned from the sample for 300 seconds. Observed and recorded the time of hemorrhage, lysis, and coagulation in the presence of CAM (Yuliani, 2016).

RESULTS AND DISCUSSION

Characteristics test of NLC-GTE

Organoleptic

NLC-GTE preparations with varying concentrations of DG as a surfactant showed that all formulas were white, odorless, and had a semi-solid consistency (Figure 1).



Figure 1. Preparation of NLC-GTE with variations in DG concentration (F1: 2%; F2: 2.5%; F3: 3%)

pH

The pH value of the NLC-GTE preparation can be determined by measuring it using a pH meter. The following results were obtained (Table 2). The results of the measurement of the pH of the NLC-GTE preparation described in Table 2 showed that all formulas were at a normal pH range of 4 - 7 (Souto & Müller, 2008). Furthermore, statistical analysis was carried out and the results showed that the difference in DG concentration did not affect the pH value of the NLC-GTE preparation (sig. > 0.05).

Table 2. pH value of NLC-GTE preparations (n = 3)

Formula	Average ± SD (CV)
F1	6.19 ± 0.00 (0.02)
F2	6.20 ± 0.00 (0.10)
F3	6.20 ± 0.00 (0.05)

Particle size (PS)

The results of characteristic testing in the form of determining the particle size of NLC-GTE can be seen in the histogram (Figure 2). Based on the histogram in Figure 2, it can be seen that the PS values of all formulas

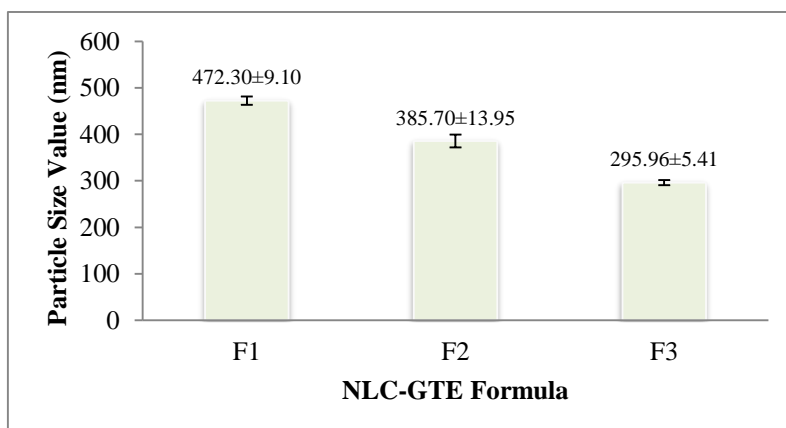


Figure 2. Histogram of the particle size value (average ± SD) of the NLC-GTE preparation (n = 3)

meet the NLC particle size requirements, which were in the range of 10-500nm (Sharma & Baldi, 2018) and 10-1000nm (Faizatun *et al.*, 2020). The value of each formula showed that F3 has the smallest size compared to F2 and F1. These results proved that increasing the concentration of DG can reduce the PS value. This statement was in line with Zirak & Pezeshki's (2015) opinion that a high surfactant concentration can lower surface tension. Hence, the energy obtained (stirring) would make breaking up oil droplets (melted lipids) easier, which can stabilize the newly formed surface to produce a larger size. The results of statistical analysis showed that there was a significant difference between each formula (sig. < 0.05), so it can be said that an increase in DG concentration could affect the PS value of NLC-GTE.

The polydispersity index (PI)

According to Avadi *et al.* (2010), this measurement aimed to determine several aspects of the colloidal solution, particle size and homogeneity distribution. The PI value of each formula can be seen in Table 3. Table 3 showed that all formulas have a PI value of less than 0.3 which meant that all formulas had a uniform and homogeneous particle size distribution. This result is similar to Oliveira *et al.* (2021) in that PI value of < 0.3 a homogeneous particle size distribution. F3 produces a value of 0.23 ± 0.00, the smallest PI value compared to F1 and F2, which produced a PI value of 0.26 ± 0.01 and 0.25 ± 0.00, respectively. This series of values can prove that variations in DG concentration can affect the PI value in NLC-GTE (Subramaniam *et al.*, 2020).

Table 3. The polydispersity index value of NLC-GTE preparations (n=3)

Formula	Average ± SD (CV)
F1	0.26 ± 0.01 (4.19)
F2	0.25 ± 0.00 (3.16)
F3	0.23 ± 0.00 (1.51)

By Lullung & Suprapti's (2012) explanation, the smaller the surfactant concentration, the greater the PI value obtained, and vice versa. It can be caused by the DG concentration of 3% being more optimal for covering the lipid and water interface and reducing the surface tension of the new particles formed during the homogenization process so that uniform nanoparticles can be created (Witayaudom & Klinkesorn, 2017). In addition, an increase in surfactant concentration can affect the energy produced in the stirring process so that the nanoparticles formed can be distributed more efficiently (Zirak & Pezeshki, 2015). Based on the statistical analysis carried out, it showed that there was no significant difference between F1 and F2 (sig. > 0.05). At the same time, for F1 and F3, there was a significant difference between the two (sig. < 0.05). Likewise, F2 and F3 showed a significant difference (sig. < 0.05). Based on these data, it can be said that the PI value in F3 was significantly different when compared to F1 and F2. While F1 and F2 have the same PI value, there was no significant difference between the two.

Physical stability test of NLC-GTE

Organoleptic

Physical stability testing of NLC-GTE was carried out using the thermal cycling method, and several things were observed, including organoleptic analysis, the results of which can be seen in Figure 3. Based on the thermal cycle test results of the NLC-GTE organoleptic preparation, which has been described in Figure 3, it can be seen that all formulas have the same color and odor, both before and after the cycle. The difference that can be seen from this observation was phase separation in F1 and F2. However, there was almost no phase separation at all in F3. These results are the opinion of Zirak & Pezeshki (2015) who state that low surfactant concentrations can cause instability and recrystallization due to insufficient amounts in the formation of nanoparticle structures. This case will trigger the formation of git, which are large particles that cannot be stabilized by surfactants and break into non-uniform particles and cause phase separation in the NLC-GTE preparation (Lullung & Suprapti, 2012). Based on these results, F3 (3% DG) showed better phase stability than F1 (2% DG) and F2 (2.5% DG). Furthermore, measurements of PS and PI were carried out in each formula to strengthen this statement.



Figure 3. Physical stability test results of NLC-GTE [(a) before cycle; (b) after cycle]

pH

The pH value was a measurement that was implemented in this physical stability test. The following was the data for each pH value before and after treatment (Table 4). The pH value of the physical stability test (thermal cycling) in all formulas was within the range of normal skin pH values, namely 4 - 7 (Souto & Müller, 2008). The pH value data were then analyzed using statistics and showed no significant difference between the pH values before and after treatment [sig.(2-tailed) > 0.05]. Thus, it can be said that the pH value of all NLC-GTE preparations remained stable after testing the physical stability using the thermal cycling method.

Table 4. The pH value of NLC-GTE preparations from physical stability tests (n=3)

Formula	Average ± SD (CV)	
	Before	After
F1	6.19 ± 0.00 (0.02)	6.20 ± 0.00 (0.05)
F2	6.20 ± 0.00 (0.10)	6.20 ± 0.00 (0.13)
F3	6.20 ± 0.00 (0.05)	6.21 ± 0.00 (0.07)

Particle size (PS)

Another measurement was applied, namely the particle size of the NLC-GTE preparation. The following are the PS values measured before and after the test cycle (Figure 4).

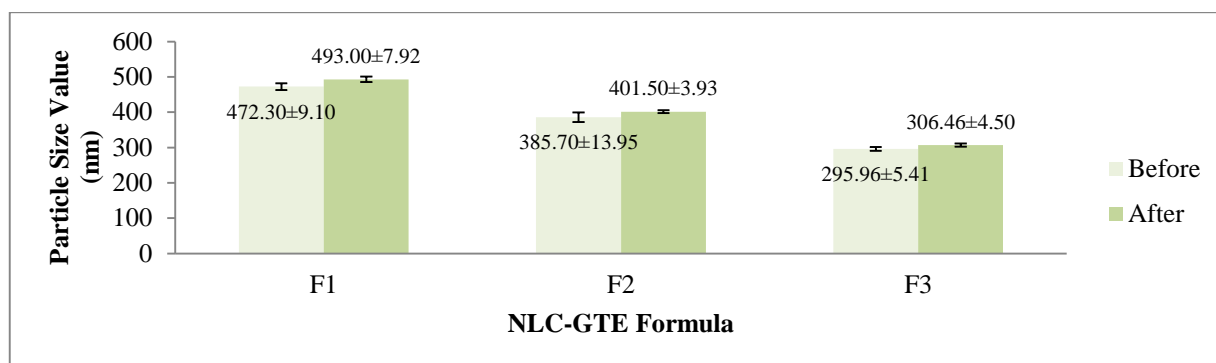


Figure 4. Histogram of particle size values (average ± SD) for NLC-GTE preparations (n = 3) from physical stability tests

PS data from each NLC-GTE formula, both before and after the cycle, showed that all formulas had particle sizes that were still within the normal range of size values, namely the range of 10-500 nm (Sharma & Baldi, 2018; Faizatun *et al.*, 2020). Furthermore, statistical analysis was carried out and obtained different results for each formula. F1 showed a significant difference between before and after the cycle (sig (2-tailed) < 0.05). Meanwhile, F2 and F3 showed that there was no significant difference between before and after the cycle (sig (2-tailed) > 0.05). Based on the results of this analysis, it can be said that after testing the physical stability using the thermal cycling method on NLC-GTE preparations, F2 and F3 had PS values that were more stable than F1. These results proved that increasing the surfactant concentration of DG can affect the physical stability of NLC-GTE. Following the opinion of Han *et al.* (2008) opinion, surfactants can improve the nanoparticle interface's quality, affecting the preparation's characteristics and physical stability (Müller-Fischer *et al.*, 2007).

The polydispersity index (PI)

PI values of each NLC-GTE formula obtained before and after the treatment cycle can be seen in Table 5. The results indicated an increase in the PI value when compared before and after the treatment cycle. In F1, it showed that the PI value after the cycle was more significant than 0.3, so it can be ascertained that the PI value in F1 was unstable. While F2 and F3 still produce PI values in the normal range even after testing, which was < 0.3. The data were analyzed statistically and can be interpreted that the PI values of F1 and F2 experienced a significant change (sig (2-tailed) < 0.05). Meanwhile, F3 did not change significantly between before and after treatment (sig (2-tailed) > 0.05). This analysis proved that increasing the concentration of decyl glucoside can affect the PI value in the physical stability test of NLC-GTE.

Table 5. Polydispersity index values of NLC-GTE preparations from physical tests (n = 3)

Formula	Average ± SD (CV)	
	Before	After
F1	0.26 ± 0.01 (4.19)	0.32 ± 0.00 (0.94)
F2	0.25 ± 0.00 (3.16)	0.28 ± 0.00 (0.92)
F3	0.23 ± 0.00 (1.51)	0.23 ± 0.00 (3.00)

Based on the results of the physical stability tests (organoleptic, pH, PS, and PI) described above, it can be said that 3% DG (F3) is the most optimal concentration to obtain stable NLC-GTE. These results are by the explanations of Lullung & Suprapti (2012) and Han *et al.* (2008), namely, the optimal amount of surfactant can cover the surface of nanoparticles formed in the homogenization process so that it can produce and maintain a small particle size, prevent the formation of git and can increase the stability of the nanoparticle interface in the NLC-GTE preparation. Based on these results, the test will be continue with an irritation test to determine the safety of NLC-GTE with 3% DG as a topical preparation.

Irritation Test of NLC-GTE

The Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) method tests the irritation of preparation, one of which was a topical preparation. According to Chaiyana *et al.* (2020), this method did not require ethical approval because it only used animal embryos for less than half the total incubation period, making it quite convenient and easy. Based on this test, it can be seen that the highest irritation score was seen in +C, which used sodium lauryl sulfate as an irritant, which was 8.37±0.10. While the irritation scores -C (sterile NaCl 0.9%) and F3 (best formula) have the same value, namely 0.00. These values indicate that F3 can be classified as non-irritant preparation (Table 6). In contrast, the results of the +C value were classified as moderate irritation (Yuliani, 2016). The results of this test can be seen in Figure 5, namely hemorrhage

(bleeding) and lysis (loss of blood vessels) in the CAM after the application of SLS. At the same time, the -C (sterile NaCl) and F3 (best formula) groups did not show any difference between before and after the sample was applied.

Table 6. Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) test results (n = 3)

Treatment Group	Average ± SD (CV)
+C	8.37±0.10 (1.18)
-C	0.00±0.00 (0.00)
F3	0.00±0.00 (0.00)

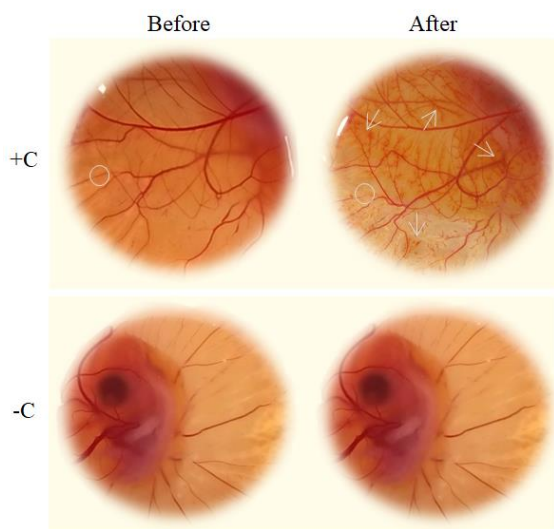


Figure 5. Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) test results

Based on the irritability test result used in the HET-CAM method, the irritability of the NLC-GTE preparation in F3 (3% DG) can be the best formula. Hence, NLC-GTE, which contained 3% DG, was considered safe to use and developed in various topical cosmetic preparations.

CONCLUSION

Based on all the tests that have been carried out, it can be concluded that increasing the surfactant DG concentration in the NLC-GTE preparation does not affect the pH of each formula. However, it can decrease PS and PI and increase the NLC-GTE preparation's physical stability. Furthermore, based on the irritability test results, F3 (3% DG) as the best formula were also proven safe because it did not show irritation characteristics in the HET-CAM test.

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AUTHOR CONTRIBUTIONS

Conceptualization, R. A., N. R., W. S.; Methodology, R. A., N. R., W. S.; Validation, R. A., N. R., W. S.; Formal Analysis, R. A.; Investigation, R. A.; Resources, R. A., N. R., D. A. P.; Data Curation, R. A., N. R., W. S.; Writing - Original Draft, R. A., N. R.; Writing - Review & Editing, R. A., N. R., W. S.; Visualization, R. A., N. R., W. S.; Supervision, N. R., W. S.; Project Administration, N. R., W. S., Funding Acquisition, R. A.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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