



RESEARCH ARTICLE

APPLICATION OF LUTEIN-ZEAXANTHIN OF EGG YOLK BASED HYDROGEL EYE MASK AS A PREVENTIVE EFFORT AGAINST COMPUTER VISION SYNDROME (CVS)

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Abstract



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Aims and objectives: Computer Vision Syndrome (CVS) is a collection of symptoms related to eye disorders due to the use of computer-based digital devices. So substantial protection is needed against damage caused by exposure to blue light by digital devices. Lutein and zeaxanthin are carotenoids that contain antioxidants so that they can overcome inflammation, have a calming effect and can maintain eye health. The purpose of this research is to produce a topical preparation in the form of a hydrogel eye mask from egg yolk which is useful for overcoming Computer Vision Syndrome(CVS).

Methods: This study formulated three formulas with the active ingredients of egg yolk extract 400 µg, 600 µg and 800 µg using 8.75 g of sodium alginate base and 3.75 g of xanthan gum. Evaluation of the preparations included organoleptic tests, weight and size, pH, swellability, shrinkage, homogeneity, viscosity, spreadability, adhesion and stability tests.

Results: of the evaluation of the preparations hydrogel eye mask in the three formulas showed good physical characteristics and stability. The results of the evaluation that have been carried out show that the organoleptic test has no changes in color, odor and shape. Test the appropriate weight and size under the eye area. The pH test of the three formulas before storage was 7.3±0.15, 7.1±0.05, 7.1±0.17 and after storage 7.5±0.1, 7.4±0.1, 7.2±0.2. The expansion and shrinkage test showed an increase in weight every hour and a decrease in weight every 10 minutes. The homogeneity test showed the three formulas were homogeneous. The viscosity test is in a good range. The dispersion test of the three formulas before storage was 5.13±0.2, 5.07±0.15 and 5.18±0.28 cm and after storage was 5.05±0.13, 5.20±0.32, and 5.11±0.22 cm. The adhesion of each formula before storage was 4.23±0.51, 3.27±0.84, 3.28±0.62 seconds and after storage 3.24±0.38, 3.26±0.15, 2.96±0.77 seconds.

Conclusion: The three formulas produced have good pharmaceutical characteristics and stability so that they can be used to treat Computer Vision Syndrome (CVS).

Keywords: Computer Vision Syndrome (CVS), egg yolk, hydrogel eye mask, lutein-zeaxanthin.

INTRODUCTION

Since COVID-19 pandemic has had a considerable impact on society. One of the major impacts that affect the community is the large-scale social restrictions that cause all community activities to be restricted. During the COVID-19 pandemic, the government imposed Work From Home (WFH) for workers and distance learning for students¹. So that children, teenagers and adults can spend most of their time in front of a computer or cell phone screen. According to Bhattacharya these devices can cause damage by

emitting high energy waves that can penetrate the eye and can eventually contribute to photochemical damage to retinal cells, which can make a person susceptible to various eye problems ranging from dry eyes to age-related macular degeneration². Eye strain caused by digital devices or also known as Computer Vision Syndrome (CVS) is the most common problem related to prolonged use of digital devices that can cause complaints such as dry eyes, itching, foreign body sensation, watering, blurred vision, and pain. Symptoms can be caused by poor lighting, glare and reflections on the screen, improper viewing distance,

poor posture, uncorrected vision problems, or a combination of these factors head³. The main cause of CVS is exposure to blue light sourced from LED lighting from digital devices so that exposure to blue light can have a negative effect on eye strain, psychological stress and overall health and sleep quality⁴. So substantial protection is needed against damage caused by exposure to blue light by digital devices. Lutein and zeaxanthin are known as carotenoids which contain strong antioxidants so that they can overcome inflammation, have a calming effect and can maintain eye health⁵. Lutein and zeaxanthin are carotenoids with antioxidant properties found in egg yolks⁶. Some researchers say that these carotenoids cannot be synthesized directly by humans. Therefore, it depends on the consumption of certain fruits, vegetables or animal products such as eggs⁷ and other food sources⁸.

The use of carotenoids lutein and zeaxanthin which contain antioxidants in overcoming Computer Vision Syndrome (CVS) is formulated into a preparation hydrogel eye mask. Hydrogel masks are one of the most commonly used mask ingredients because of their high water content. The hydrophilic base will form a strong matrix so that the active substance is easily delivered to the eye bags. Thus it can provide high effectiveness⁹. So in this study, a formulation was made hydrogel eye mask from egg yolk extract containing the carotenoids lutein and zeaxanthin. The focus of this research is to obtain a preparation hydrogel eye mask that can overcome Computer Vision Syndrome (CVS) with good pharmaceutical stability.

MATERIALS AND METHODS

Extract carotenoid compounds lutein-zeaxanthin chicken egg yolk, n-hexane (Intraco Indonesia), acetone (Sentana Indonesia), toluene (Sentana Indonesia), ethanol (Sentana Indonesia), Na₂SO₄ 10% (Intraco Indonesia), distilled water (Sumber Rejeki Indonesia), sodium alginate (Sentana Indonesia), xanthan-gum (Intraco Indonesia), glycerin (Intraco Indonesia), propyl paraben (Intraco Indonesia), methyl paraben (Intraco Indonesia), propylene glycol (Intraco Indonesia), calcium chloride (Intraco Indonesia), essence (Sumber Rejeki Indonesia).

Tools and materials of this research are prepared in accordance with the needs of the research to be carried out.

Preparation of Egg Yolk

Eggs were stored overnight for 7 days at 18°C. The yolks were separated from the egg whites manually and rolled on moistened filter paper to remove any remaining albumin and chalaza adherents. The egg yolks were then combined and homogenized in a blender at a speed of level 1 for 60 seconds¹⁰.

Carotenoid-Lutein Zeaxanthin extraction

Carotenoids were extracted from the prepared samples (1 g egg yolk homogenate or 2 mL serum) by adding 30mL of hexane: acetone: toluene: ethanol (10:7:7:6) and incubating overnight at room temperature. Then add hexane (30 mL) and 10% Na₂SO₄ to increase the total volume in a 100 mL volumetric flask. This

mixture was mixed and allowed to stand for 1 hour before the 10 mL aliquot of the hexane layer evaporated. The extracted carotenoids were then stored overnight in a dark room at 20°C¹¹.

Preparation of the hydrogel eye mask

Masks by preparing distilled water in a 1 liter beaker then adding sodium alginate while stirring at medium speed until a gel base was formed. Xanthan gum is mixed with glycerin, then put into a gel base. Then added lutein-zeaxanthin compound extract. The solution was stirred until dissolved and homogeneous. Then propyl paraben and methyl paraben were dissolved in propylene glycol, put into a beaker containing a gel base little by little. Then the gel was poured into a 10 cm diameter mold weighing 50 g. The mold is then immersed in an aquadest solution containing 0.5 g/100 mL (w/v) calcium chloride for 60 minutes. Then dried at room temperature. The hydrogel is molded according to the shape that is adapted to the under-eye area. The hydrogel mask is stored in a sealed package containing a small amount of propylene glycol solution¹².

Evaluation of hydrogel mask preparations

Organoleptic

The preparations were observed for size, shape, color, and odor as well as changes in color and odor changes¹².

Estimation of weight and size

Evaluation of the weight and thickness of the mask, hydrogel masks were taken and weighed one by one. The weight of the hydrogel mask was measured using a digital scale, while the length, width, and thickness of the hydrogel mask were measured using a caliper¹².

Estimation of surface pH

The surface pH of the hydrogel mask was measured using pH meter portable.

Estimation of expandability

The hydrogel mask was weighed and put into a beaker containing 30 ml of distilled water solution. Masks were weighed at 3, 9, 12, 24, 48, 72, and 1 week¹¹.

$$\text{Swelling power} = \frac{W_n}{W_o}$$

Where; W_n =Weight of hydrogel, W_o=Weight of dry mask before hydration

Estimation of shrinkage

The hydrogel mask that had solidified area and weight were measured every 10 minutes for 2 hours from the start of the experiment. The time the area shrinkage occurs is recorded.

Homogeneity test

All hydrogel preparations developed were tested for their homogeneity visually. The homogeneity test was carried out by applying the hydrogel eye mask formula on the object glass. The hydrogel was placed on a slide and then covered with a deg glass to see the clarity and presence of aggregates in the hydrogel preparation.

Viscosity test

The Brookfield Viscometer uses spindle number 6 to determine the viscosity of each formula. The speed was increased from 12 rpm (revolutions per minute), 30 rpm, to 60 rpm and the viscosity test results were recorded in mPa.s (millipascal-seconds), the viscosity

test was replicated three times and the average was calculated¹³.

Spreadability test

Spreadability was measured with two glass plates, one glass plate was given a millimeter block base for easy observation and measurement and the other plate was used as a cover. The measurement of the dispersion of the hydrogel was carried out by placing 1 g of the hydrogel in the middle of the glass. Cover the hydrogel with a cover slip and ballast with a total weight of 125 g for 1 minute, the diameter of the distribution area is calculated. Measurements were made 3 times of replication¹⁴.

Adhesion test

The adhesion test was carried out by weighing 1 gram of hydrogel placed on one glass plate and then covered with another glass plate. The glass plate was placed under 200 g for 5 minutes. Record the time when the adhesive was released by lowering the load to 200 g, the measurement was carried out 3 times.

Stability test

Evaluation of stability was carried out by the accelerated method, namely by placing the gel preparation at a freezing temperature of -10°C for 24

hours, then transferred again at room temperature around 25-29°C for 24 hours using a device climatic chambers. Parameter testing was carried out before and after the accelerated storage treatment. The gel is declared stable if there is no significant difference to the observed parameter results

Data analysis

This research is an experimental laboratory scale and uses statistical analysis ANOVA method.

RESULTS AND DISCUSSION

This research was started by extracting lutein and zeaxanthin from egg yolk. The egg yolk used is organic chicken egg yolk. Incubated chicken eggs in an incubator for 7 days at a temperature of 18°C before Carotene is extracted. Extraction of carotenoids using n-hexane, acetone, toluene and ethanol in the ratio (10:7:7:6) and incubated overnight at room temperature. Extraction was continued by adding n-hexane and 10% Na₂SO₄. Carotene is extracted is stored in a dark room at a temperature of 20°C before use.

Table 1: Hydrogel mask formulation.

Compoition	F1	F2	F3
Egg yolk caratenoid extract	400	600	800
Na-alginaet (grams)	8.75	8.75	8.75
Xanthan gum (grams)	3.75	3.75	3.75
Propylene glycol (grams)	7.5	7.5	7.5
Calcium chloride (grams)	0.5	0.5	0.5
Glycerin (grams)	13.75	13.75	13.75
Methyl paraben (grams)	2.5	2.5	2.5
Propyl paraben (grams)	1.25	1.25	1.25
Essence (drops)	3	3	3
Aquadest (mL)	ad 250	ad 250	ad 250

The carotenoid extracts lutein and zeaxanthin contain powerful antioxidants that can reduce oxidative damage indirectly by absorption of light contained in the pigment epithelium of the retina, lens, ciliary body, and iris. In addition, the presence of oxidized metabolites suggests that lutein and zeaxanthin may

protect against oxidative stress¹⁵. The carotenoid extracts of lutein and zeaxanthin are formulated in the form of hydrogel eye mask as much as 50 grams. Based on the preformulation, a formula was obtained using sodium alginate and xanthan gum as a base.

Table 2: Test results of weight, size, and pH of hydrogel eye mask.

Condi on	Formula	Observation				
		Weight (g)	Width (cm)	Length (cm)	Thickness (cm)	pH
Before	F1 (right and left)	2.29±0.46/ 2.44±0.39	2.23±0.40/ 2.30±0.36	6.26±0.45/ 6.23±0.05	0.23±0.03/0.23±0.03	7.5±0.1
	F2 (right and left)	3.10±0.24/ 3.02±0.04	2.20±0.10/ 2.033±0.23	6.33±0.25/ 6.23±0.40	3.28±0.13/3.25±0.13	7.13±0.05
	F3 (right and left)	2.30±0.12/ 2.52±0.32	2.03±0.15/ 2.20±0.43	3.28±0.13/ 3.25±0.10	2.23/0.05/2.16±0.05	7.1±0.1
After	F1 (right and left)	2.33±0.32/ 2.50±0.30	2.23±0.32/ 2.26±0.37	6.23±0.20/ 6.26±0.05	0.20±0.10/0.20±0.10	7.5±0.1
	F2 (right and left)	3.04±0.16/ 3.00±0.10	2.23±0.05/ 1.96±0.37	6.43±0.20/ 6.53±0.30	3.21±0.16/3.21±0.16	7.4±0.1
	F3 (right and left)	2.23±0.11/ 2.53±0.23	2.03±0.15/ 2.13±0.35	6.03±0.15/ 5.93±0.11	2.23±0.05/ 2.16±0.05	7.2±0.2

Table 3: Test results of the swelling power before and after storage.

Condition Before Storage			
Observation	Formula		
	F1	F2	F3
0 (Initial weight (g))	2.27±0.7	2.24±0.1	2.38±0.9
3 hours (%)	2.85±0.9	4.83±0.2	4.17±1.9
9 hours (%)	4.63±7.3	7.21±8.2	9.35±7.7
12 hours (%)	7.8±3.4	11.06±0.1	10.43±9.7
24 hours (%)	9.01±4.8	12.83±0.2	10.06±4.7
48 hours (%)	10.23±0.1	13.96±0.1	10.81±0.1
72 hours (%)	11.52±6.1	17.48±1.2	12.61±5.5
1 week (%)	17.57±9.5	26.67±2.4	17.37±8.2
Condition After Storage			
0 (Initial weight (g))	2.03±0.6	2.3±0.2	2.43±0.6
3 hours (%)	3.51±0.5	5.19±0.8	4.33±1.9
9 hours (%)	8.1±3.8	8.4±1.3	7.9±3.6
12 hours (%)	8.83±4.4	10.43±1.7	9.52±4.5
24 hours (%)	10.31±4.9	13.05±1.2	10.88±5.0
48 hours (%)	11.63±5.3	15.36±0.7	11.76±5.0
72 hours (%)	14.39±5.5	18.26±1.1	15.19±4.3
1 week(%)	18.48±6.0	25.23±1.2	19.04±5.7

At the time of optimization, sodium alginate mixed with distilled water did not form a thick preparation, so xanthan gum was added as an additional base and a hydrogel base was formed, then glycerin and propylene glycol were added as plasticizers, and calcium chloride as a crosslinking agent. Treatment with variations in the concentration of carotenoids with variations of 400 g, 600g and 800g in each preparation with three replications. This is done to determine the preparation with the best concentration. All components of the formula based on the preformulation were mixed using a mixer at medium speed for 60 minutes. The

homogenized hydrogel was immersed in a calcium chloride solution with a concentration of 0.5% for 60 minutes. After the immersion process, a solid structure was obtained from the hydrogel, the mask was then printed and stored in a container containing a propylene glycol solution as a humectant so that the water in the hydrogel did not evaporate quickly during the storage process. Evaluation of the preparation was carried out by means of organoleptic observation, weight and size, pH test, and swelling, shrinkage, homogeneity test, dispersion test and adhesion test which were formed on the three formulations.

Table 4: Test results of the shrinkage test before storage.

Condition Before Storage			
Observation	F1 (right and left)	F2 (right and left)	F3 (right and left)
10 minutes (g)	2.12±0.3/2.27±0.1	2.92±0.1/2.82±0.06	2.13±0.1/2.39±0.3
20 minutes (g)	2.04±0.3/2.12±0.1	2.82±0.1/2.82±0.06	2.01±0.8/2.33±0.3
30 minutes (g)	1.97±0.3/2.12±2.1	2.75±0.1/2.76±0.03	1.96±0.1/2.26±0.3
40 minutes (g)	1.88±0.3/2.05±0.2	2.45±2.5/2.67±0.05	1.85±0.1/2.09±0.3
50 minutes (g)	1.79±0.3/2.01±0.2	2.40±0.2/2.51±0.18	1.85±0.1/2.05±0.3
60 minutes (g)	1.74±0.3/1.98±0.2	2.38±0.2/2.36±0.07	1.82±0.1/2.05±0.3
70 minutes (g)	1.74±0.3/1.93±0.2	2.36±0.08/2.34±0.21	1.79±0.1/1.99±0.2
80 minutes (g)	1.71±0.3/1.89±0.3	2.35±0.08/2.30±0.21	1.76±0.1/1.97±0.2
90 minutes (g)	1.68±0.3/1.85±0.3	2.29±0.09/2.26±0.21	1.73±0.1/1.93±0.2
100 minutes (g)	1.63±0.3/1.82±0.3	2.24±0.09/2.17±0.1	1.89±0.1/1.89±0.28
110 minutes (g)	1.60±0.3/1.78±0.3	2.18±0.08/2.13±0.1	1.67±0.1/1.86±0.2
120 minutes (g)	1.55±0.3/1.74±0.3	2.05±0.08/1.72±0.4	1.61±0.1/1.82±0.2
Condition After Storage			
Observation	F1 (right and left)	F2 (right and left)	F3 (right and left)
10 minutes (g)	2.17±0.2/2.2±0.1	2.65±0.4/2.76±0.05	2.08±0.06/2.3±0.2
20 minutes (g)	2.11±0.3/2.1±0.1	2.65±0.4/2.69±0.06	2.04±0.05/2.3±0.3
30 minutes (g)	2.0±0.4/2.1±0.1	2.59±0.4/2.67±0.07	2.05±0.1/2.05±0.2
40 minutes (g)	1.94±0.4/2.06±0.1	2.49±0.4/2.60±0.09	1.95±0.1/1.95±0.3
50 minutes (g)	1.76±0.4/1.99±0.1	2.46±0.4/2.53±0.1	1.83±/1.83±0.2
60 minutes (g)	1.71±0.4/1.92±0.1	2.43±0.4/2.48±0.09	1.77±0.2/1.77±0.3
70 minutes (g)	1.66±0.4/1.86±0.2	2.37±0.4/2.43±0.1	1.73±0.2/1.73±0.3
80 minutes (g)	1.63±0.4/1.85±0.2	2.34±0.4/2.39±0.1	1.68±0.2/1.68±0.3
90 minutes (g)	1.60±0.4/1.81±0.2	2.31±0.4/2.19±0.1	1.65±0.2/1.65±0.3
100 minutes (g)	1.52±1.5/1.78±0.2	2.28±0.4/2.16±0.1	1.61±0.2/1.61±0.3
110 minutes (g)	1.41±0.4/1.75±0.2	2.20±0.2/2.12±0.1	1.58±0.2/1.58±0.3
120 minutes (g)	1.37±0.4/1.73±0.2	2.10±0.2/ 1.76±0.5	1.55±0.2/1.55±0.3

Table 5: Test results of viscosity and spreadability of hydrogel eye mask.

Formula	Viscosity		Spreadability	
	Before (cP)	After (cP)	Before (cm)	After (cm)
F1	25462±0.7	25016±0.6	5.13±0.2	5.05±0.1
F2	27096±0.2	21300±0.7	5.07±0.1	5.2±0.3
F3	21668±0.5	20000±0.7	5.18±0.2	5.11±0.2

In organoleptic testing, the dosage form, color and odor were observed. The results obtained for each of the three formulas with three replications before and after accelerated storage obtained the same results where the observations before accelerated storage until after accelerated storage did not experience significant changes in color, odor and shape. The three formulas have a pink color because the base used is sodium alginate. The resulting color is not too bright so it is still quite comfortable to use. Another physical appearance is the consistency of semi-solid and odorless so that this hydrogel is quite soft and gives a cooling effect when used. According to Harliatika and Noval, a good hydrogel is usually in the form of a semi-solid, not too thick and not too liquid¹³.

Evaluation of weight and size was carried out using three formulas before and after accelerated storage. The evaluation of the weight and size of the hydrogel eye mask from the three formulations has almost the same weight, length, width and thickness and is quite good. After accelerated storage, there was no significant change to the length, width, weight and thickness of the hydrogel eye mask. The weight and size are adjusted to the area under the eyes with a size of 6x2.5 cm (length and width)¹². pH testing was carried out on the three formulas before and after accelerated storage. Testing pH using a pH meter portable. The results obtained in each preparation showed that the pH of the preparation was in the range (7.1-7.3) before storage. While the pH of the preparation after storage was in the range (7.2-7.4) and there was an increase in pH but the pH of the preparation was still in the neutral pH range so that the preparation was hydrogel eye mask safe to use.

The power of the swelling hydrogel eye mask in the three formulas before and after storage was accelerated with three replications performed at 3, 9, 12, 24, 48, 72, and 1 week respectively. The test results obtained that the hydrogel mass tends to increase with increasing time both before and after accelerated storage. According to Okwani *et al.*, the increase in hydrogel mass describes the amount of water absorbed and indicates that the hydrogel can absorb water¹². The hydrogel was allowed to swell for one week to see the swellability profile of the hydrogel. The results of the swelling power measurement showed that the measurements from the 3rd hour to the first 1 week had an increase of up to 26% before storage, while after storage there was an increase of up to 25%. These results indicate that the resulting hydrogel is able to absorb water well. The shrinkage of the hydrogel eye mask was carried out on three formulas with each replication before and after accelerated storage and left in an open room for two hours. The hydrogel eye mask experienced shrinkage from the first ten minutes which was marked by a decrease in the weight of the hydrogel

eye mask±0.5grams every ten minutes and undergoes continuous shrinkage for up to two hours. According to Surini and Annisa shrinkage of hydrogel masks is caused by evaporation of water which indicates that hydrogel eye masks should be stored in closed container¹⁶. Based on the homogeneity test before and after accelerated storage which was carried out on the preparations hydrogel eye mask on the three formulas with three replications each, the results of the three formulas were homogeneous. Homogeneity was observed by looking at the clarity and there was no aggregate in the preparation hydrogel eye mask which was carried out with three tests on each formula so that the hydrogel produced had good homogeneity which was marked by the absence of aggregates in the preparation before and after accelerated storage.

Viscosity test hydrogel eye mask before and after storage is accelerated obtained test results on all three formulas with each of the three replication using a viscometer Brookfield at a speed of 12 rpm showed that the preparation hydrogel eye mask has a viscosity that is as good between the three formulas because they are within the range of viscosities good according to the literature. According to Edy *et al.*, the viscosity of a good hydrogel preparation is in the range of 50 dPa.S – 400 dPa.S¹⁴. The viscosity value will produce a hydrogel that is not too liquid and not too thick which is in dPa.S units (1 Poise=1 dPa.S).

Based on the results of the test results of the spreadability test of the preparations hydrogel eye mask before and after accelerated storage in the three formulas with three replications, it was found that the three preparations had spreadability that matched the standard range of a good hydrogel formula where the average spreadability of the three hydrogel eye mask preparations was before accelerated storage with a load of 125 g, 5.13±0.2 cm, 5.07±0.15 cm, and 5.18±0.28 cm respectively, while after accelerated storage with a load of 125 g the results were 5.05±0.13 cm, 5.20±0.32 cm and 5.11±0.22 cm. According to Edy *et al.*, the standard spreadability range of a good hydrogel formulation is between 5-7 cm so that the preparation obtained meets good dispersion¹⁴. The adhesion test was carried out by placing a 200g load on the three preparations hydrogel eye mask with three replications each. The results obtained from the adhesion test showed that the formula had good adhesion and met the standard of good adhesion range where before accelerated storage with a load of 200 g the results were 4.23±0.51 cm, 3.27±0.84 cm, and 3.28±0.62 cm respectively. while after accelerated storage with a load of 200 g the results were 3.24±0.38 cm, 3.26±0.15 cm, and 2.96±0.77 cm. According to Harliatika and Noval the range of good adhesion is in the range of 2.00-3.00 seconds¹³. Based on the stability test of the hydrogel eye mask preparation which was

carried out with the accelerated storage method in the climatic chamber, from the results of the organoleptic observation test, the homogeneity test after storage showed no significant changes occurred (stable). From the results of measuring the weight and size of the mask, pH test, swellability test, shrinkage test, viscosity test, spreadability test and adhesion test of hydrogel eye mask preparations after storage, there tends to be an increase or decrease from the number before storage. However, even so, the measurement standards of several tests that have been carried out are in the range according to the literature standards.

CONCLUSIONS

The formulation hydrogel eye mask containing lutein-zeaxanthin from egg yolk extract produced a fairly good preparation. Based on the tests that have been carried out, the three formulas obtained are good preparations and meet the quality parameters that have been set. The appearance of the hydrogel is subjectively comfortable to see and use because it has a pink color and is not too bright and has good physical stability so that it can be used as a preparation to overcome Computer Vision Syndrome (CVS).

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AUTHOR'S CONTRIBUTION

Hamsinah: writing original draft, literature survey. **Meylinda A:** methodology, conceptualization. **Khusnia:** formal analysis, review. **Mario R:** investigation, data interpretation. **Ummum A:** data curation, investigation. **Safitri D:** critical review, supervision. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work

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