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Antibacterial Effectiveness of Chloramphenicol Ophthalmic Hydrogel Against *Pseudomonas Aeruginosa* ATCC 9027 and *Streptococcus Pyogenes* ATCC 19615

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ABSTRACT

Sterile ophthalmic hydrogel is the development of conventional eye products that slowly releases the drug to increase its bioavailability, corneal permeability, and better drug retention time compared with eye drops as well as eye ointments. Chloramphenicol is a broad spectrum antibiotic that inhibits the growth of *Pseudomonas aeruginosa* and *Streptococcus pyogenes* causing conjunctivitis. The aim of this study was to determine the effectiveness of antibacterial activity of chloramphenicol in ophthalmic hydrogel preparations against *P.aeruginosa* ATCC 9027 and *S.pyogenes* ATCC 19615 comparing with eye drops dosage form. The results showed that the effectiveness of antibacterial activity of chloramphenicol ophthalmic hydrogel preparation compared with eye drops dosage form was 1 : 1.179 ppm for *P.aeruginosa* and 1 : 1.145 ppm for *S. pyogenes*. Minimum inhibitory concentration of ophthalmic hydrogel against *P. aeruginosa* was 156.25 to 312.5 and 78 to 156.25 µg/mL for *S. pyogenes*. Minimum bactericidal concentration of ophthalmic hydrogel against *P. aeruginosa* was 312.5 to 625 µg/mL and 78 to 156,25 µg/mL for *S.pyogenes*. The effectiveness of antibacterial preparations in chloramphenicol eye hydrogel was better compared to the form of eye drops preparations.

Keywords: ophthalmic hydrogel, chloramphenicol, *Pseudomonas aeruginosa*, *Streptococcus pyogenes,* minimum inhibitory concentration, minimum bactericidal concentration.

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INTRODUCTION

In the field of ophthalmology, eye disease most frequently found is conjunctivitis. According to The Indonesian Ministry of Health, from 135,749 visits to the eye hospitals, total cases of conjunctivitis and other disorders of the conjunctiva as much 99,195 cases [1]. Bacterial conjunctivitis causes, one of which was *S. pyogenes* [2], accordingly, *P. aeruginosa* is a rare etiological agent in bacterial conjunctivitis, but always be attentive eye care practitioner because of his relationship with keratitis that damage the cornea and cause a severe inflammatory response [3].

Treatment of eye infections is done by administering eye drug preparations containing antibiotic [4], such as chloramphenicol, that can overcome the acute conjunctivitis in the eye, caused by microorganisms. The chloramphenicol gives a good distribution of antibiotic drugs on the eye [5]. This medicine is very vague on the market in ointment preparations that form its use not comfortable.

For comfort in eye drug admistration, sterile eye gel formulation can be choosen. The comfort of such formulation is the same as eye drops form. The gel form gives a profit on an increased retention time of drug on the surface of the eye as well as increased permeability of drug in the cornea [6]. The drug release is slow moreover, can fix bioavaibilty value of drug in the eye [7].

The results of chloramphenicol eye hydrogel dosage form showed that the material meets the requirements of Indonesian Pharmacopoeia, such as organoleptic, pH, viscosity, sterility, assay and capacity of a gel [8]. Diffusion testing showed that levels of permeation were 1.513% for 8 hours [9], as well as antibacterial activities demonstrate against *S. aureus* [10].

MATERIALS AND METHODS

Instruments/equipment/apparatus

pH meter (Methrom™ USA), viscometer (Rion™ Japan), Laminar air flow cabinet (Esco™ USA), analytical balance (Mettler Toledo™ Canada), autoclave (All American™ USA), incubator (Memmert™ USA), oven (Memmert™ USA), membrane filters, cotton, muslin, eyedropper, micropipette (Biohit® Finland), perforator, ose, test tubes (Pyrex® Indonesia), racks of test tubes, petri dish (Pyrex® Indonesia).

Chemicals and reagents

Chloramphenicol (BioBasic© Canada, Batch: CB0118, ED: 09/2018), poloxamer 188 (Brataco® Indonesia), poloxamer 407 (Brataco® Indonesia), propylenglicol (Brataco® Indonesia), aquadestilata (Brataco® Indonesia), nipagin (Brataco® Indonesia), sodium tetraborate (E-Merck® USA), boric acid (E-Merck® USA), Phenyl mercury nitrate (E-Merck® USA), standard sol. pH 7 and pH 4 (E-Merck® USA), sodium chloride 0.9 % (IPHA® Indonesia), Trypticase Soy Agar (E-Merck® USA), Trypticase Soy Broth (E-Merck® USA), Mueller-Hinton Agar (E-Merck® USA), Mueller-Hinton Broth (E-Merck® USA), and blood.

Methods

Formulation of chloramphenicol ophthalmic hydrogel

The formulation was prepared according to Table 1.

Each of Poloxamer 188 and poloxamer 407 was weighed and dissolved with distilled water, then stored in the refrigerator for 24 hours. The chloramphenicol was dissolved with propylenglicol, and added with nipagin. The mixture of chloramphenicol was stirred until the entire dissolved and homogeneous. The materials were ready on each put in a bottle 100 mL size vial, then sterilized by autoclave for 15 minutes at 121 °C. The preparations hydrogel was done in aseptic condition at LAF cabinet.

Evaluation of chloramphenicol hydrogel ophthalmic preparations

Organoleptic test

Organoleptic hydrogel was checked by observing changes in color, odor and clarity. Clarity was checked visually by examination of the formulation against white and black background. Organoleptic observations were performed on the day-to-1, 3, 7, 14, 21 and 28.

pH measurement

pH was measured by using the pH meter tool calibrated with pH 4 and 7 of standard solutions. pH hydrogel ophthalmic preparations that have been made was measured by dipping the rod cathode pH meter into the preparation. pH call button was pressed, accordingly, the screen will appear on the pH of the preparation. pH measurement was performed on days 1, 3, 7, 14, 21 and 28.

Viscosity measurement

The viscosity measurement was done using Rion viscometer VT-04 by No.3 spindle to dip into a container containing hydrogel preparations up to the mark. Safety valve was released nevertheless the rotor turned until stable (± 2 min) that was appointed by needle pointer. A measurement was achieved during storage days 1, 3, 7, 14, 21 and 28.

Preparations of chloramphenicol eye drops

Eye drop was intended as comparison preparations to find out the effectiveness of chloramphenicol hydrogel. The preparations in accordance with the formula in Table 2.

Antibacterial effectiveness test of chloramphenicol opthalmic hydrogel and eye drops against *p. aeruginosa* ATCC 9027 and *s. pyogenes* ATCC 19615

The purpose of the stage was to compare the effectiveness of antibacterial preparations in the form of hydrogel chloramphenicol against the form of eye drops. The testing was performed by the method of diffusion in order. Testing was conducted during storage days in 0, 1, 3, 5, 7, 14, 21, and 28.

As many as 20 µL of bacterial suspension test with the level of turbidity that equivalent to Mc. Farland 0.5 (1.5 x 10^8/mL) were poured into 20 mL of agar medium at 45 °C. Mixture of medium with bacterial suspension was homogenized and cooled until it was solidified. Then solidified medium was perforated with perforating applications (9 mm) with 6 holes, which used as a reservoir for the concentration of each of the three preparations of hydrogel and eye drops. The medium test, then incubated at 37 °C for 18 h. The diameter of the clear zone formed drag was measured using a caliper. Drag the diameter data (y) each raw material was made the curve against the log concentration (x), moreover the value of the material of both the effectiveness of appeals, could be obtained by the equation of a line.

Determination of minimum inhibitory concentration (MIC) of chloramphenicol ophthalmic hydrogel with macro- dilution method

As many as 12 test tubes were prepared (2 tubes to the control and 10 tubes for treatment). Accordingly, 1 mL hydrogel preparations added in 1 mL of liquid growth medium with stratified so that dilution series made were 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.39%, 0.02%, and 0.01% by volume end of the tube was 1 mL. Then, as much as 1 mL of bacterial culture equal Mc. Farland 0.5 was added into the test tubes so that the final volume of the tubes was 2 mL. All test tubes were incubated at 37 °C for 18 h. Turbidity test was observed in the liquid medium conversely determined MIC value preparations.

Tube with negative results or was not indicate the presence of bacterial growth, then subcultured with solid growth medium as MIC assertion test of chloramphenicol preparations hydrogel. As many as 20 mL of solid growth medium was prepared, then given limit to variations of the sample concentration as a MIC determination with macrodillution method. With the method of scratch, the results of the saucer MIC

subculture incubated at 37 °C for 18 h. If there were scratches (+) then showed the presence of growth, when no scratches (-) then the growth was not occur. The data obtained was made in the table form.

RESULTS AND DISCUSSION

Preparations of chloramphenicol opthalmic hydrogel

Preparations of opthalmic hydrogel carried out in accordance with the formula in table 1. The formula was the best thing formula based on the parameter test such as organoleptic, pH, viscosity, sterility, test levels and capacity of the hydrogel compliant eye preparations based on Indonesian Pharmacopoeia 4th Edition [8].

The evaluation of chloramphenicol opthalmic hydrogel preparations

The evaluation of chloramphenicol opthalmic hydrogel was conducted to knew the changing of physical or chemical in the preparations may occur during storage, which would affect the stability and activity of the opthalmic hydrogel preparations. Physical observation preparations was done on day 1, 3, 5, 7, 14, 21 and 28.

Evaluation of chloramphenicol opthalmic hydrogel can be seen in Table 3, Figure 1 and 2. The results showed that the preparation was clear, colorless, odorless. Accordingly, pH test results evaluation of preparation demonstrated during 28 days of storage at room temperature were meets the requirements of the pH material of opthalmic hydrogel i.e. 5-7.4 [6]. Nevertheless the viscosity showed that the preparation was meeting the requirements i.e. 5-100 cps [11].

Antibacterial effectiveness test of chloramphenicol ophthalmic hydrogel

The purpose of the stage was to compare the antibacterial effectiveness of chloramphenicol preparations in the hydrogel against the eye drops dosage forms. The testing was achieved by the diffusion method, whereas conducted for 28 days of storage time. The results of the measurement of the diameter drags zone from opthalmic hydrogel and eye drops preparations can be seen in table 4 and figure 3.

Equations of lines curve of raw material of eye drops comparison preparations obtained as was y = 1.2908x – 2.0084, whereas for material of hydrogel was y = 1.3454x – 2.0704. This equation was used for comparison value testing of the both preparation by the bacteria *P.aeruginosa*. Diameter drag of antibiotic eye drops dosage form determined by entering values for the concentration of 1000 ppm (x = 3) in the equation of a line eye drops preparations so that the retrieved drag diameter y = 1.864 mm. The inclusion in the y values for the equation of a line preparation hydrogel then retrieved x = 2.9285. Antilog 2.9285 was 848.146 ppm. So, the value of comparative material hydrogel against eye drops was 848.146 : 1000. In conlusion the chloramphenicol power of 1 ppm in hydrogel preparations comparable to 1.179 ppm of chloramphenicol in eye drops preparations.

Value appeal for a testing bacteria test of *S.pyogenes was* obtained equations of lines curve of raw material for eye drops was y = 0, 77554x + 0.2865, accordingly, preparations hydrogel was y = 0, 80876x + 0.2443. Diameter drag the antibiotic eye drops was prescribed preparations at concentrations of 500 ppm (x = 2.699) moreover putted it in the equation of a line eye drops preparations so that the retrieved drag diameter was y = 2.3796 mm. The y value in the equation of the line included preparations hydrogel, retrieved x = 2.64. Antilog 2.64 was 436.80 ppm. The value of comparative material hydrogel against drops of the eye was 436.80 : 500. Then the power of 1 ppm of chloramphenicol in preparations hydrogel comparable to 1.145 ppm chloramphenicol eye drops in the preparations.

Preparations of ophthalmic hydrogel provide comfort as in preparations, eye drops gel form gives a profit on an increase in the retention time of the drug on the surface of the eye [6].

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Determination of Minimum Inhibitory Concentration (MIC) Preparations by the Macrodillution method

The stage aims to knew the smallest concentration of stocks to opthalmic hydrogel that inhibited the growth of bacteria test. The test was performed with the MIC macrodillution method with MHB media for *p. aeruginosa* and TSB for *s. pyogenes.* MIC test results can be seen in Table 5.

Based on Table 5, the results showed that the smallest concentration of chloramphenicol hydrogel preparations against test bacteria *p. aeruginosa* was at the range of 3.12 - 6.25% and the smallest concentration of chloramphenicol hydrogel preparations against bacteria test of *s. pyogenes* was 1.56 - 3.12%

To reaffirm the results of testing done MIC subculture with a method of macrodillution results scratch cup on solid medium. The result of the concentration macrodillution subculture MIC active chloramphenicol as antibacterial for bacteria *p.aeruginosa* and *s.pyogenes* can be seen in Table 5 and Figure 4.

The smallest concentration of chloramphenicol opthalmic hydrogel preparations inhibiting the growth of bacteria *p.aeruginosa* was 312.5 - 625 µg/mL, while *s.pyogenes* was 78 - 156.25 µg/mL.

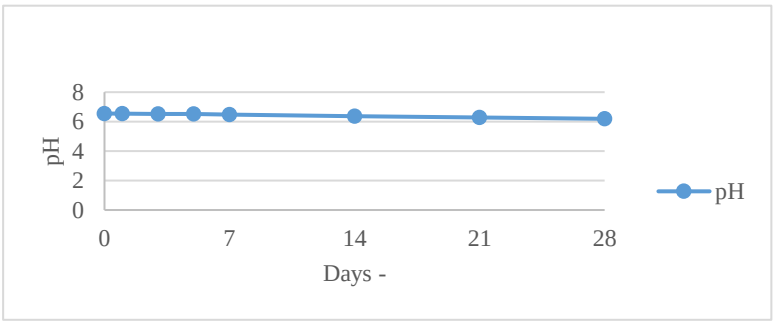


Figure 1. The pH observations graph of chloramphenicol opthalmic hydrogel for 28 days

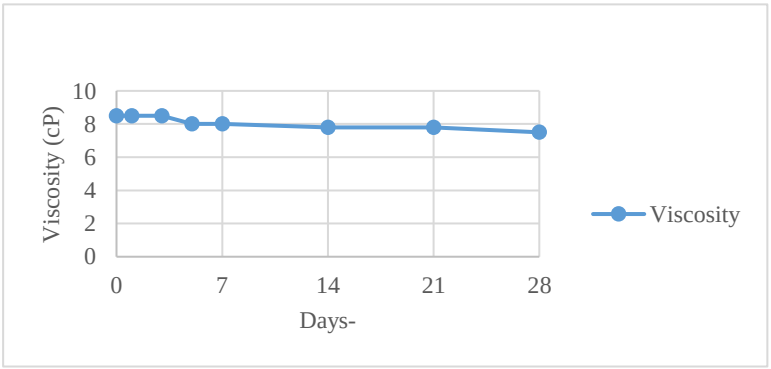
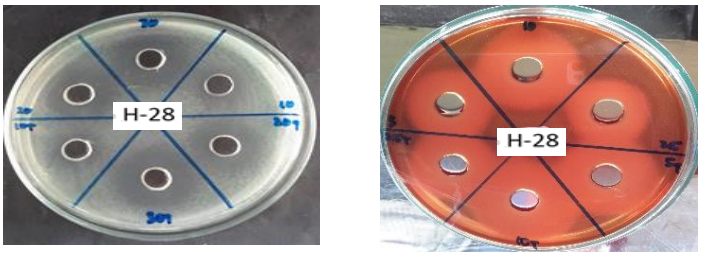


Figure 2. The viscosity observations graph of chloramphenicol opthalmic hydrogel for 28 days



(a) (b)

Figure 3. The effectiveness of preparations hydrogel against eye drops storage day-28 against bacteria (a) *P.aeruginosa* ATCC 9027 and (b) *S.pyogenes* ATCC 19215

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(a) (b)

Figure 4. MIC subculture test of chloramphenicol opthalmic hydrogel againts (a) *Pseudomonas aeruginosa* ATCC 9027 and (b) *Streptococcus pyogenes* ATCC 19215

Table 1. Formula of Hydrogel Ophthalmic Preparations

Ingredients (%)	Concentration (% b/v)
Chloramphenicol	0.5
Poloxamer 188	10
Poloxamer 407	10
Propylenglycol	10
Nipagin	0.2
Aquadestilata	ad 100

Table 2. Formula of Ophthalmic Eye Drop Preparations

Ingredients (%)	Concentration (% b/v)
Chloramphenicol	0.5
Acidum boricum	15
Sodium tetraborate	0.3
Phenyl mercury nitrate	0.02
Aquadestilata	ad 100

Table 3. Evaluation of Chloramphenicol Hydrogel Ophthalmic

Observation day	Evaluation of preparation		
	Performance	pH	Viscosity
0	Liquid, colorless, odorless	6.55	8.5
1	Liquid, colorless, odorless	6.54	8.5
3	Liquid, colorless, odorless	6.52	8.5
5	Liquid, colorless, odorless	6.51	8.0
7	Liquid, colorless, odorless	6.47	8.0
14	Liquid, colorless, odorless	6.36	7.8
21	Liquid, colorless, odorless	6.29	7.8
28	Liquid, colorless, odorless	6.19	7.5

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Table 4. Drags Zone Measurement Average of Chloramphenicol Eye Drops and Opthalmic Hydrogel Against The Storage Time

Days	Drags zone average (mm)					
	Opthalmic hydrogel			Eye drops		
***Pseudomonas aeruginosa* ATCC 9027**						
Preparation concentration (% v/v)	30	20	10	30	20	10
0	23,90	21,95	17,45	22,50	21,20	16,65
1	23,30	20,15	14,85	21,30	17,40	13,65
3	23,30	19,85	15,30	21,80	19,15	14,65
5	22,15	20,60	15,60	21,70	19,95	17,55
7	21,90	19,05	14,65	21,50	18,80	14,70
14	21,00	18,80	14,50	18,80	16,10	14,05
21	20,35	18,55	14,20	20,05	17,55	12,85
28	20,20	18,45	18,20	20,35	17,85	16,40
***Streptococcus pyogenes* ATCC 19615**						
Preparation concentration (%v/v)	**10**	**5**	**2,5**	**10**	**5**	**2,5**
0	26,15	22,90	20,80	26,75	24,65	22,90
1	25,55	24,30	21,20	24,95	22,70	20,10
3	25,05	23,35	21,00	24,90	22,30	20,05
5	24,50	22,75	19,80	24,35	22,65	20,00
7	24,30	21,50	18,50	23,00	17,40	16,50
14	23,40	22,35	20,35	23,10	22,25	19,95
21	22,90	20,10	18,40	22,80	20,00	17,30
28	21,50	19,10	14,35	20,95	18,90	16,65
Control +	+					
Control -	-					

Description :

\+ = Growth of microorganism

\- = No growth of microorganism

Table 5. MIC of Chloramphenicol Opthalmic Hydrogel Preparations

Concentration (% v/v)	*P.aeruginosa*	*S.pyogenes*
50	-	-
25	-	-
12,5	-	-
6,25	-	-
3,12	+	-
1,56	+	+
0,78	+	+
0,39	+	+
0,2	+	+
0,1	+	+
Control +	+	+
Control -	-	-

Description :

\+ = Growth of microorganism

\- = No growth of microorganism

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Table 6. Outcomes Subculture MIC of Chloramphenicol Opthalmic Hydrogel

Consentrasi (% v/v)	Result
P. aeruginosa	
50	-
25	-
12,5	-
6,25	+
3,12	+
S. pyogenes	
25	-
12,5	-
6,25	-
3,12	-
1,56	+

Description :

\+ = Growth of microorganism

\- = No growth of microorganism

CONCLUSION

The effectiveness of antibacterial preparations in chloramphenicol eye hydrogel was better compared to the form of eye drops preparations, where the minimum inhibitory concentration (MIC) against *p.aeruginosa* were 312.5 to 625 µg/mL and 78 to 156.25 µg/mL for *s.pyogenes*.

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