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ATCC25923 dan Escherichia coli
ATCC25922

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Activity of Cyprofloxacin from implant with cross link agent genipin in composites to *Staphylococcus aureus* ATCC25923 dan *Escherichia coli* ATCC 25922**Esti Hendradi, Dewi Melani Hariyadi, Muhammad Faris Adrianto**

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Abstract- The Research on effectiveness of cyprofloxacin against *Staphylococcus aureus* ATCC25923 and *Escherichia coli* ATCC 25922 bacteria was done. Composite mixture used was Bovine Hydroxyapatite-Chitosan which cross link with genipin as the carrier Ciprofloxacin. The ratio between Ciprofloxacin: BHA: Chitosan = 10:30:60. As Cross linker was genipin 0.7% , the concentration of Ciprofloxacin as active ingredient was 10%. The results showed that the implant can release preparation Cyprofloxacin by sustained released system in vitro and to inhibit bacterial growth *Staphylococcus aureus* ATCC25923 and also *Escherichia coli* ATCC 25922.

Keywords: implant, ciprofloxacin, anti bacteria *Staphylococcus aureus* ATCC25923, anti bacteria *Escherichia coli* ATCC 2592

Introduction

Complications of bone diseases and bone disorders caused by traumatic accidents may result in a gap (defect) on the bone. The healing process of damage or fracture is determined by the level of trauma and soft tissue damage (Strobel et al., 2011). Some cases of damage or injury to the bone cannot undergo natural recovery (Porter et al., 2009). The release of antibiotics on the target network is expected to last continuously for a certain time and achieve a greater concentration than the minimum inhibitory concentration (MIC). Drug delivery systems in a controlled manner (controlled release system) can help increase the bioavailability of antibiotics in target tissues. The system is designed to release the drug at the expected location at a rate that is appropriate for a certain time period (Mourino et al., 2010). In previous research on the optimization of composite test results showed good composite composition was Ciprofloxacin: BHA: Chitosan = 10:30:60. And adding genipin 0.7% as crosslinker had potential effect to retard ciprofloxacin release from Bovine Hydroxyapatite-chitosan-ciprofloxacin implant for 30 days in vitro (Hendradi et al, 2015). The purpose of this research is to make the preparation of bone implants with Bovine Hydroxyapatite composite Chitosan with Cross-linker genipin with Cyprofloxacin active ingredients can inhibit the bacteria *Staphylococcus aureus* ATCC 25923 (SA-GE) and *Escherichia coli* ATCC 2592 (EC-GE)

Materials and Methods

1) Materials Cyprofloxacin (Shangyu Jingxin Pharmaceutical Co. Ltd) ; Bovine Hydroxyapatite (BHA) from Bank Jaringan RSUD DR Soetomo Surabaya; Chitosan (Biotech Indonesia); Genipin was obtained from Challenge Bioproduct Co., Ltd., Taiwan. Acetic acid glacial p.a (Merck), KBr IR (for Spectroscopy) Na₂HPO₄ p.a (Merck), K₂HPO₄ p.a, KH₂PO₄ p.a, NaCl p.a (Merck-German), Nutrient Agar (Oxoid), and Aquabidest

2) Method 1: Formulation of Bovine Hydroxyapatite-chitosan ciprofloxacin implant The implant produced by compression method. Cyprofloxacin were dissolved in aquabidest, Bovine Hydroxyapatite added gradually and mixed until homogen with cyprofloxacin. Chitosan powder were added to cyprofloxacin-Bovine Hydroxyapatite blend and mixed until homogen. Aquabidest were added gradually with continuous stirring until form wet granules mass. Wet granules mass were sieved using 1 mm sieve and dried overnight (24 hours) at 40 °C to obtain dried granules. Dried granules were immersed in genipin solution (0.3%, 0.7% concentration) for 24 hours until the colour was change. Granules were washed with aquabidest to remove the residual genipin. At the final stage, granules were washed with phosphate buffer saline (PBS) pH 7.40. Granules were dried in oven at 40 °C for 24 hours. Dried granules were weighed 100 mg, pressed using tablet press machine with 4.0 mm diameter and the compression pressure was 2 tons (Hendradi et al, 2015).

3) Method 2: Test potential dilution method of antibiotic Firstly, the release study of cyprofloxacin from implant was done as sample for test potential. Implant was placed in a vial containing 5 ml of phosphate buffer saline (PBS) pH 7.4. Vial was placed in a shelf and incubated in waterbath at 37 °C ± 0.5 °C. Sampling was conducted by pipetting 1 ml of elution fluids at predetermined time intervals (1st, 3rd, 5th, 7th)h and replaced with fresh buffer to maintain sink condition. Appropriate dilution was prepared using phosphate buffer saline (PBS) pH 7.4. The release of ciprofloxacin HCL from the implants was assayed in triplicate. Secondly, test antibiotic potential dilution method prints holes (wells)

design 3-3. A total of 10 mL of inoculum of *Staphylococcus aureus* ATCC 25923 or *Escherichia coli* ATCC 25 922 was inserted into the tube containing the seed layer 8 mL media Nutrient Agar that had thawed and then allowed to stand up to a temperature of 45 - 50°C. Homogenized with a vortex, then poured evenly over the surface of the base layer has been solidified in a petri dish, allowed to solidify. Hole was made in order to use the printer for sterile. Each hole was filled with the test solution and standard solution as 50,0µL for each hole. Petri dish and then incubated at 37°C for 24 hours. Diameter of inhibition zone formed at each hole was measured by using a caliper. The resulting inhibition zone diameter compared with the border of the effective inhibition zone, the minimum range of 14-16 mm (Depkes RI, 2014).

Results

The result of potency cyprofloxacin from implant was divided potency the standard (ciprofloxacin) and times 100%. The results can showed in figure1. In this figure showed that potency of cyprofloxacin from implant against *Escherichia coli* ATCC 25922(EC-GE) was higher compared *Staphylococcus aureus* ATCC25923 (SA-GE).

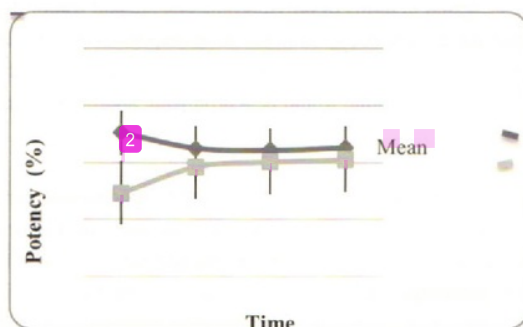


Figure1. Potency cyprofloxacin profil vs time (hour) released from implant BHA-chitosan-ciprofloxacin 0.7% genipin in phosphate buffer saline for 7 hours. Each value represents the mean \pm S.D. of 3 determinations

Conclusion

The results obtained from this study indicated that implants with Bovine Hydroxyapatite composite Chitosan with Cross-linker genipin 0.7% with Cyprofloxacin active ingredients could inhibit the bacteria *Staphylococcus aureus* ATCC 25923 (SA-GE) and *Escherichia coli* ATCC 2592 (EC-GE).

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