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Effect Of Application Of Mineral Trioide Aggregate (Mta) And Nanohydroxyapatite In Duck Egg Shell On Macrophages In Reversible Pulpitis

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ABSTRACT

Pulp disease is caused by bacteria that cause tooth decay in the form of caries. These bacteria can penetrate the pulp through the gaps in the dentine, and develop in the dentinal tubules so that the permeability of the dentinal tubules decreases. Reversible pulpitis is a type of pulp disease, which if left untreated will develop into irreversible pulpitis. Pulp capping treatment as a treatment for non medicament aims to maintain the pulp to remain vital by reducing the inflammatory process. Macrophages as a marker that can be used as a sign when inflammation occurs. Pulp capping treatment usually use calcium hydroxide and *Mineral Trioxide Aggregate* (MTA) as material agent. Duck egg shells contain calcium carbonate and *Mineral Trioxide Aggregate* has an effectiveness over calcium hydroxide. the purpose of this study is to determine the difference in the number of macrophages in reversible pulpitis using duck egg nanohydroxyapatite (NHA) and MTA. this research is an in vivo laboratory experimental post test only control design with 27 research samples which one control group and two experiment group. The independent variables are duck egg nanohydroxyapatite and MTA, and the dependent variable is differences in the number of macrophages. there were significant differences in the three groups, where the average value of macrophage formation was lowest in the group of rats that were applied to MTA and followed by the NHA group. Meanwhile, the control group, the reversible pulpitis rat group without medicament application, had the highest average score. The application of MTA as a pulp capping medicament can reduce macrophages in reversible pulpitis using rats *Sprague Dawley*.

1. INTRODUCTION

The pulp is a component of the tooth in the form of soft tissue that follows the shape of the tooth crown and the shape of the root canal of the tooth pulp. The surface of the dental pulp is used to form dentin [1]. The pulp has several functions, namely, 1) a formative function that plays a role in initiating the formation of dentin and enamel; (2 sensory functions that can respond to stimuli that occur directly or to email and dentin stimulation; (3) nutritive functions as a supply of nutrients to the formation of dentin and the integrity of the pulp tissue itself; and (4 protective functions, namely the ability of the pulp to process and identify foreign substances, such as toxins produced by bacteria that cause caries, and to cause an immune response to toxic substances produced by these bacteria [2].

Pulp disease is caused by bacteria that cause tooth decay in the form of caries. These bacteria can penetrate the pulp through the gaps in the dentine, then the bacteria develop in the dentinal tubules, causing the permeability of the dentinal tubules to decrease due to the irregular formation of peritubular dentine and reparative dentine. Toxins derived from bacteria will penetrate into the pulp and cause inflammation of the vital pulp [3].

American Association of Endodonthics (AAE) classifies pulp disease into four types, namely reversible pulpitis, symptomatic irreversible pulpitis, asymptomatic irreversible pulpitis, and pulp necrosis. Reversible pulpitis is a disease of the pulp that returns to normal when the irritant is removed. Inconvenience experienced when there is a stimulus such as cold or sweet and disappears in a few

seconds [4]. Reversible pulpitis that is not treated immediately will develop into irreversible pulpitis [5].

Pulp capping treatment as a treatment for reversible pulpitis aims to keep the pulp vital by reducing the inflammatory process which is characterized by a decrease in the number of macrophage cells, because the high number of macrophage cells damages healthy tissue around inflammation [6]. The treatment is divided into two, namely direct pulp capping by applying material directly to exposed pulp due to trauma or iatrogenic, and indirect pulp capping treatment, namely by applying material to healthy pulp but the pulp has not been opened [7]. Treatment of pulp caps can use calcium hydroxide and *mineral trioxide aggregate* (MTA), provided that the bleeding stops before applying the pulp capping material, if the procedure is not performed then treatment is not recommended [8].

Synthetic hydroxyapatite (HA) is a bioactive ingredient that can induce bone growth and osteointegration in orthopedic, maxillofacial and dental treatments. However, HA has drawbacks when used as a pulp capping medicament, because in certain cases HA can cause inflammation and necrosis of the pulp. HA was developed through nanotechnology to produce Nanohydroxyapatite in order to increase the effectiveness of HA medicaments. Nano-HA has excellent biocompatibility to the structure of human bones and teeth and contains molecules that have identical properties to dentine and enamel [9]. The nano-HA used in this study came from duck egg shells, because the manufacture of nanohydroxyapatite from natural ingredients is better than synthetic products so that it can increase its bioactive and biocompatible properties [10].

Duck egg shells contain calcium carbonate (CaCO3) is around 94-97% which can be utilized for synthesis as a source of calcium in the manufacture of hydroxyapatite crystals, and has the result of calcination of the purity level of calcium hydroxide Ca(OH)2, higher than the results of calcination of quail egg shells and chicken egg shells. For this reason, duck egg shells are used as the main compound for the synthesis of nanohydroxypatite because they are rich in calcium and phosphate compounds [11].

One of the ingredients of pulp treatment is *Mineral Trioxide Aggregate* (MTA) which has an effectiveness over that of calcium hydroxide. MTA contains components of bismuth oxide, dicalcium aluminate, dicalcium silicate, and tricalcium silicate [7]. MTA has the advantage of being more uniform and thicker in the formation of dentin bridges, less inflammatory response, and less necrosis of the pulp tissue [8]. Therefore the aim of this study is to determine the difference in the number of macrophages in reversible pulpitis using duck egg nanohydroxyapatite and MTA.

2. METHOD

This research is experimental laboratorium in vivo post test only control design. The research variables consisted of independent variables, namely duck egg nanohydroxyapatite and MTA, and the dependent variable namely differences in the number of macrophages.

The population in this study were rats *Sprague Dawley* which was bred in the Laboratory of Animal Physiology, Faculty of Medicine aged 3 months and weighing 250-300 grams. The research sample was divided into three groups which were taken randomly, namely 1 control group and 2 treatment groups. Each treatment group was given duck eggshell and nanohydroxyapatite medicaments *Mineral Trioxide Aggregate* (MTA), while the control group was not given any medicaments. Sampling using simple random sampling with sample calculations using the Feeder formula. The minimum total number of samples in the 3 groups is 27 samples, so that each group consists of 9 molar teeth.

The inclusion criteria in this study were rats *Sprague Dawley* male, mouse *Sprague Dawley* 3 months old, 250-300 gram body weight, maxillary left 1st molar element, healthy and active rat characterized by active movements for 7 days. Meanwhile, the exclusion criteria were that the rats died during the study, had physical disabilities, and the non medicament fillings on the teeth of the rats fell off. The research was conducted at the Laboratory of Animal Development, Faculty of Medicine, University of Muhammadiyah Surakarta.

The research step was that in the early stages 27 Sprague Dawley rats were adapted for 7 days. Then anesthesia was performed using ketamine HCL 0.1 ml/100 g of rat body weight. Occlusal surface of maxillary left 1st molar with diamond round bur diameter 008 to open pulp roof with a depth of 0.5 mm. Temporarily filled with glass ionomer cement. Then wait for 28 days. Next, decapitation and

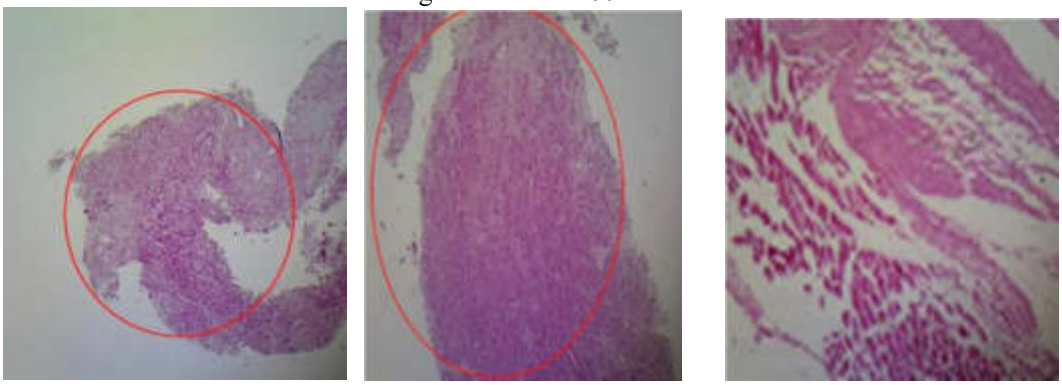
preparation of preparations were carried out, as well as macrophage cell counts. The final stage is data analysis.

Research tools in this study consists of pulp capping medicaments, rat treatment tools, tools for making histolonon medicamental preparations, and tools for observing macrophage cells. And the material consists of pulp capping medicaments, namely nanohydroxyapatite, duck egg shells and *Mineral Trioxide Aggregate* (MTA), rat treatment materials, and materials for making histolonon medicamental preparations.

Data analysis used the One Way Anova test to determine whether there was a significant difference in the number of macrophages in each group, then continued with the LSD (Least Significant Differential) to test the differences between each group. Previously the researchers conducted a normality test using the Shapiro-Wilk Test and homogeneity test using the Levene Test.

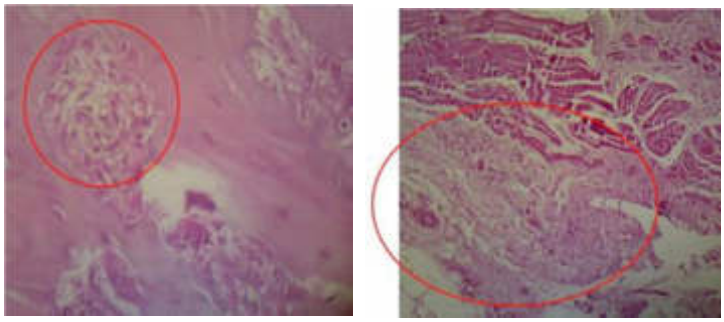
3. RESULTS AND DISCUSSION

Research on the effect of application of MTA and duck egg shell nanohydroxyapatite on macrophages in reversible pulpitis using rats *Sprague Dawley* has been done. The number of macrophages was counted using a microscope *Optilab Olympus* CX23 and *software image raster*. Macrophage counts were performed on the 7th day after treatment in rats *Sprague Dawley* by observing 5 visual fields randomly with a magnification of 200 times. Below are pictures of macrophage cells against NON MEDICAMENT with a magnification of 200 times.



a b c

Figure 1 Macrophage Cells Against The MTA with a Magnification of 200 Times



a b

Figure 2 Macrophage Cells Against the NHA with a Magnification of 200 Times

The table below is the average magrofave results.

Table 1. Average Macrophages

Group	Treatment	Mean ± SD
1	NON MEDICAMENT	26,11 ± 5,21
2	MTA	18,44 ± 2,96
3	NHA	19,33 ± 5,48

The results showed that the lowest average macrophage formation was in group 2, namely the group of rats that were applied to MTA and followed by the NHA group. Meanwhile, the non medicament group, which was a group of rats with reversible pulpitis without medicament application, had the highest average score.

Table 2. Results of Shapiro Wilk Test

Group	Treatment	Sig
1	Non Medicament	0.110
2	MTA	0,687
3	NHA	0,649

Based on the table it can be seen that groups 1, 2, and 3 show a significant value of >0.05 which interprets that the distribution of data is normally. Then researcher tested for homogeneity using the *Levene Test*. Test results of *Levene test* can be seen in the table.

Table 3. Results of Levene Test

Levene Test	Sig
2,312	0,121

The table above shows that the significant value is 0.121 ($p > 0.05$), so the variance of the data is homogeneous. Next, the One Way Anova parametric test was carried out.

Table 4. One Way Anova Test

Group	Sig
Non Medicament	
MTA	0,000
NHA	

The table above shows that the significant value obtained is 0.000 with a p value <0.05, so there is a significant difference between the three groups.

Table 5. LEAST Significane Different Test

Group	Non Medicament	MTA	NHA
Non Medicament	-	0,000	0,000
MTA	0,000	-	0,691
NHA	0,000	0,691	-

Based on LSD test results (LEAST Significane Different) significantly there were differences in the formation of macrophages with a p value <0.05 in the Non Medicament group against the MTA group, the Non Medicament group against the NHA group, the MTA group against the Non Medicament group, the NHA group against the Non Medicament group. While the other groups, namely the MTA group against the NHA group, the NHA group and the MTA group showed insignificant macrophage formation (p>0.05).

Discussion

This study aims to determine the difference in the number of macrophages in reversible pulpitis using duck egg shell nanohydroxyapatite and MTA by direct pulp capping treatment. The direct pulp cap is a protection against the pulp that is still vital with pulp cap medicaments in teeth with deep cavities, but the pulp has not been exposed or the pulp is exposed due to iatrogenic factors accompanied by signs of reversible pulpitis [2]. The occurrence of reversible pulpitis in this study was made by means of preparation using *diamond round bur* on the RA rat's left 1st molar tooth until the pulp roof was exposed. The exposed pulpal roof will cause odontoblasts to produce pro-inflammatory cytokines to activate macrophages.

Macrophages came because of the inflammatory process in reversible pulpitis, and macrophages were found in the injured area on the first day. Two to three days after an injury occurs, monocytes in the blood vessels migrate to the tissues and differentiate into macrophages to continue the process of

phagocytosis carried out by neutrophils. On the third day the number of macrophage cells increased and decreased on the seventh day, because the inflammatory phase had ended and entered the proliferative phase. Macrophages are in the injured area in the inflammatory phase until the wound healing phase ends, but in small numbers and macrophages function to initiate the proliferative phase by releasing anti-inflammatory cytokines and starting growth [12]. The process of reducing inflammation in reversible pulpitis is characterized by a decrease in the number of macrophage cells, a high number of macrophage cells results in damage to healthy tissue around inflammation [6].

Based on the results of the study, there was a decrease in the number of macrophages from the pulp of rats with reversible pulpitis that were treated with MTA which was lower than those given duck egg shell nanohydroxyapatite, because MTA is a pulp capping medicament that is close to ideal for connective tissue, and prevents bacterial leakage [13]. As an antimicrobial agent, biocompatible, low cytotoxicity, better mineralization, and lower solubility, induces more dentine bridges, and post-expansion setting so good that it is able to close all perforated pulp chamber roof defects [9].

Duck egg shell nanohydroxyapatite is also good for use as medicaments for direct pulp caps because HA has the same chemical structure as the mineral component of bone [14]. Nano has a scale from 1-100 nm but this material has weaknesses, namely its porous structure and poor mechanical properties. nHA can cause a reduction in inflammation and act as a modulator for macrophages which are responsible for the early inflammatory response [15]. The molecules found in hydroxyapatite have properties similar to dentin and enamel [9].

Hydroxyapatite can make reparative dentine in pulp cap treatment, toxic, and osteoconductive, nHA is able to form reparative dentin in pulp cap treatment [16]. Duck egg shells contain CaCO3 which can reduce inflammation and inhibit bacterial growth so that it can be used because it has a structure similar to the chemical components of teeth [10].

4. CONCLUSION

There were differences in the formation of macrophages with a p value <0.05 in the Non Medicament group against the MTA group, the Non Medicament group against the NHA group, the MTA group against the Non Medicament group, the NHA group against the Non Medicament group. While the other groups, namely the MTA group against the NHA group, the NHA group and the MTA group showed insignificant macrophage formation (p>0.05). The application of MTA as a pulp capping medicament was able to reduce macrophages in reversible pulpitis using rats *Sprague Dawley.* Further research is needed regarding the most effective dose of MTA and duck egg shell nano hydroxyapatite as pulp capping medicaments against macrophages formation. And further research also needs to be done to determine the peak time of macrophage formation in the pulp that was applied MTA and nanohydroxyapatite during that 7 day time span.

ETHICAL CLEARANCE

This research has received approval from the Health Ethics Committee of RSUD Dr. Moewardi with number 442 / III / HREC / 2023.

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