

Dentin microhardness and porosity of wistar rats teeth following herbal-based devitalizing agent application



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Abstract

Objective: To evaluate the microhardness and porosity of the dentin of Wistar rats after pulp-out application.

Material and Methods: This was laboratory experimental research with posttest design with control group. Twelve teeth of wistar rats were allocated into 2 groups, the test group and the control group. In the test group, teeth were prepared on the labial surface until half thickness of the dentin, then pulpout was applied to the base of cavity, covered with GIC. On the 7th day, euthanasia and extraction were performed to test the dentin microhardness with Vickers Hardness Tester and dentin porosity with Laser Scanning Microscope (LSM).

Results: The mean value of tooth hardness after pulp-out application was 22.23 ± 3.51 HV while the mean value of the control group was 27.70 ± 1.70 HV, with p value = 0.31 at 95% significance level. There was a decrease in tooth hardness of treated sample which was not significantly different compared to the control group. CLSM photo shows white lines on the sample surface as the exposed dentinal tubules with a higher intensity compared to the control group.

Conclusion: Pulp-out decreased tooth microhardness and cause porosity of the teeth.

Keywords: Dentin Microhardness, Dentin Porosity, Pulp-out

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Introduction

Devitalizing agents are usually used in root canal treatment to devitalize the pulp when local anaesthesia is not effective to achieve painless during treatment.^{1,2} Commercial devitalizing agents usually used in dental practice are arsen trioxide and paraformaldehyde. However, some clinical cases reported necrosis of gingiva and alveolar bone following arsen trioxide application, while paraformaldehyde is slow-acting devitalizing agent with some drawbacks which causes irritation to soft tissues.³⁻⁶

Alternative of devitalizing agents are studied and some of them are derived from herbal extracts which showed potency to be developed as herbal-based devitalizing agent.⁷ Combination of jatropa sap, root of sidaguri and mellitin, namely Pulp-Out has been reported to cause pulpal cell death following its application into tooth cavity that showed similar cell death of commercial devitalizing agents.⁸ In previous in vitro study, a decreased of tooth hardness and erosion were reported which was in line with duration and dosage of Pulp-Out applied.⁹ It is assumed that the extracts are able to penetrate through the porosity formed and causing cell death. In addition, toxicity test of various doses of Pulp-Out showed no adverse effects occurred following its application both systemically and locally (unpublished data).

Of doses examined, 50-mg-dose of Pulp-Out was the adequate dose chosen for further study as herbal-based devitalizing agent. However, data of laboratory study should be supported with in vivo study to provide additional information prior to its application in humans.

This study is aimed to examine dentin hardness and dentin porosity following Pulp-Out application into tooth cavity of Wistar rats.

Material and Methods

Pulp Out® preparation

The Pulp Out® paste of a mixture sap of jatropa, root of sidaguri, and melittin was prepared as reported in the previous study.⁵

Animals use and care

Six healthy male Wistar rats (*Rattus norvegicus*) with average body weight of 135 to 150 g were used in this study. All animals were housed in the animal holdings of the Department of Pharmacology, STIFA Makassar, and bred locally in standard plastic cages for 7 days to allow for acclimatization under natural atmospheric conditions. They were fed with standard laboratory and water ad libitum. The procedure for the animal care was based on the "Guide for the Care and Use of Laboratory Animals – 8 Edition", as adopted by the Ethics Commission of the Dental and Oral Hospital, Faculty of

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Table 1. Average hardness value of control and treated sample

Sample	Hardness Value (HVN)			Mean
	Point			
	1	2	3	
C1	27.6	27.4	32.4	29.13
T1	32.1	25.4	28.4	28.63
C2	30.2	25.8	23.1	26.37
T2	19.6	17.8	18.1	18.50
C3	30.0	29.1	26.4	28.50
T3	19.8	18.7	21.9	20.13
C4	34.6	24.9	29.8	29.77
T4	22.5	18.3	25.1	21.97
C5	30.3	25.8	24.8	26.97
T5	21.5	23.8	24.8	23.23
C6	24.7	27.9	23.7	25.43
T6	22.8	17.7	22.5	21.00

C= Control, T= Test
Sample Group (Yellow) Control Group (Black)

Table 2. Normality and Homogeneity test of dentin hardness of each group

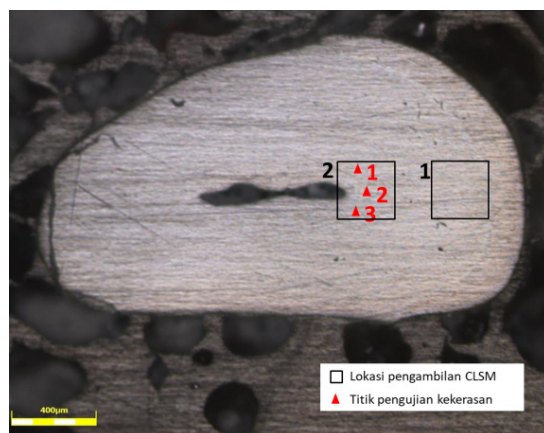
Treatment group	N	p	
		Shapiro wilk	Homogeneity
Control	6	0.78	0.32*
Pulp-out	6	0.39	

Table 3. Average value of dentin hardness following Pulp-out application

Treatment group	N	Mean \pm SD	p
Control	6	27.70 \pm 1.70	0.32*
Pulp-out	6	22.23 \pm 3.51	

*Shapiro-wilk; level of significance $p > 0.05$; CI 95%

** t-test; level of significance $p < 0.05$; CI 95%

**Figure 1.** Location of hardness and porosity test on samples

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Procedures of Pulp Out application

Six Wistar rats were anesthetized with ketamin injection i.m (PT Ethica-Indonesia). One of the anterior teeth was used as treated sample while the others were used as control sample with no preparation. The teeth were prepared with high-speed round-diamond bur (Diabur-Mani, Japan) until approaching the pulp. Pulp Out was applied into the base of cavity and covered with resin modified-glass ionomer cement (RM-GIC) (GC-Japan). All animals were caged for 7 days. On day 8, the animals were sacrificed using chloroform, and the teeth were extracted, fixed with formalin. All samples were cleaned from the attached soft tissue using periodontal currettes. Pulp out and RM-GIC were removed from the cavities, the root were separated from the teeth, as well the incisal part was cut horizontally from mesial to distal. All samples were embedded into self-cure acrylic mold of 1-inch-diameter, leaving the dentin was exposed for further manipulation. The exposed dentin surfaces of mounted specimens were manually grounded flat and smooth with a series of ascending grades of silicon carbide abrasive papers (1500, 2000 and 3000 grit) for further hardness test using Vickers hardness tester and porosity test using confocal laser scanning electron microscope (CLSM).

Results

Figure 2 and table 1 showed hardness value of all treated sample except 1 sample, decreased compare to control.

Average value of dentin hardness of treated sample is 22.23 HV which is lower than control. To examine statistically significant difference, normality test using Sapiro-Wilk test is carried out. The data was normally distributed ($p=0.32$). To examine the differences of dentin hardness between the group, t-test is carried out and no statistically significant difference of dentin hardness was observed ($p=0.32$).

The images of sample surfaces showed white lines which is assumed as opened dentinal tubules on all samples, however, treated samples showed greater intensity of opened dentinal tubules compare to control

Discussion

Lots of studies of herbal medicine for dental uses have been carried out, and mostly are used for oral

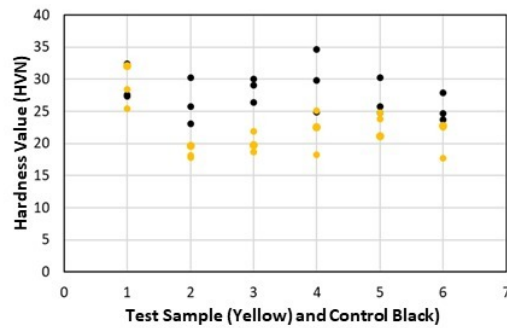


Figure 2. Value of dentin tooth hardness following pulp-out application

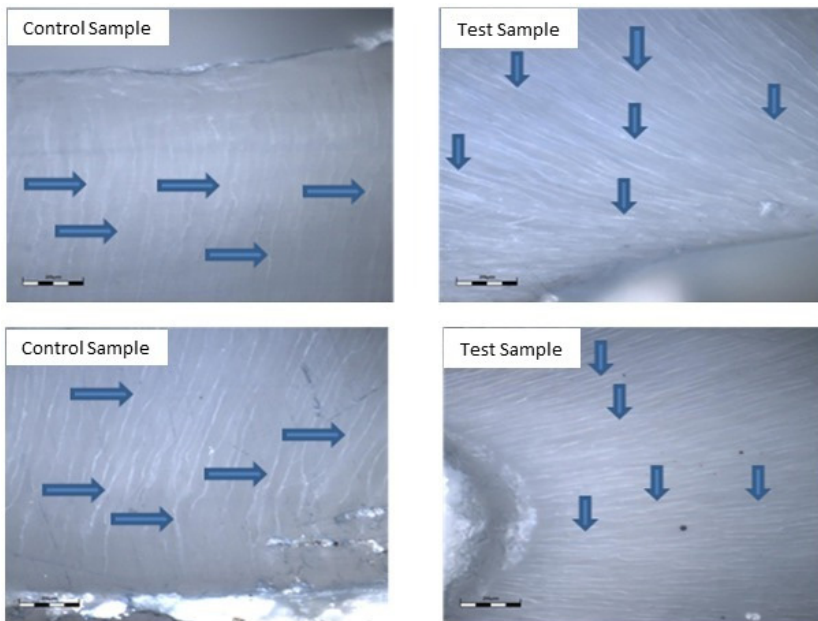


Figure 3. Visualization of sample surfaces

hygiene maintenance and antiseptics.¹⁰⁻¹² In this study, combination of herbal extracts, *Jatropha sap*, root of sidaguri, and mellitin, namely pulp out was performed. Pulp out is proposed to be developed as herbal-based devitalizing agent as they caused pulpal cell death following its application on exposed pulp. The image of cell death is similar to commercial devitalizing agent, arsenic trioxide.⁸ However the mechanism of pulpal cell death is not really understood. The finding of previous in vitro study on prepared human teeth showed erosion and a decrease of dentin tooth hardness following pulp out application. Level of erosion, as well as dentin hardness, is related to pulp out dosage and duration of application.⁹ This can be assumed that pulp out is able to penetrate through the pores formed.

In this in vivo study using Wistar rats, the results also detected a decrease of dentin tooth hardness although no significant difference was observed between treated and control sample. This may be

due to small sample size. Dentin microhardness was affected by dentinal tubules which decreases along with the increased density of dentinal tubules.¹³ Additionally, dentin microhardness was affected by tooth location that varies its peritubular and intertubular dentin. Decrease of dentin microhardness has been associated with dissolution of mineral.¹⁴ The components of pulp out tend to be acidic with pH below 6, pH of *Jatropha sap* 3.3-3.6, pH of mellitin is 4.5-5.5. pH of root of sidaguri is categorized as acidic.^{15,16} Using NMR (nuclear magnetic resonance), it is found that root of sidaguri has carboxyl group which is associated with acid group.¹⁷ According to Sampio et al.¹⁴ application of any agent on dentin can cause changing in the chemical component of dentin which can change permeability and dissolution of dentin.¹⁴ Tooth demineralization occurs when the pH is below 5.5.^{18,19} Erosion of samples in this study was observed as opened dentinal tubules and greater opened dentinal tubules was consistently observed following pulp out application. This is due to dissolution of minerals on peritubular dentin. It is known that the peritubular dentin is a highly calcified or hypermineralized tissue surrounding the dentinal tubules which has 2 times higher of the mineral/matrix contents/ratios than those in the intertubular dentin.²⁰ Surface roughness of the dentin will also increase when the dentinal tubules are exposed due to demineralization. This condition is related to the solubility of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) as crystal minerals in dentin due to acid.²¹

In line with this study, morphological changes of the dentin surface under SEM was also reported following application of 37% phosphoric acid for 10 seconds and 30 seconds.²²

Within the limitations of this study, it can be concluded that the application of pulp-out has the ability to penetrate into the pulp through the formed porosity. Pulp-out application also decreases dentin hardness due to the resulting mineral solubility. These can be assumed as one of the pulp-out mechanisms in causing pulpal cell death. Pulp-out is considered to be quite potential to be developed as an alternative herbal-based pulp devitalization agent.

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Conflict of Interest

The authors report no conflict of interest.

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