Asih Rahaju, Histological Response of Pulp to Belimbing Wuluh (*Averrhoa bilimbi L.)* Gel Compared to Carbamid Peroxide as Tooth Whitening (*in vivo)*: Journal of Health and Dental Sciences.e-ISSN 2807-3126 Vol. 03 No. 02: pp. 125-134

HISTOLOGICAL RESPONSE OF PULP TO BELIMBING WULUH (*Averrhoa bilimbi L.*) GEL COMPARED TO CARBAMID PEROXIDE AS TOOTH WHITENING (*IN VIVO*)

*(*RESPON HISTOLOGIS PULPA TERHADAP GEL BELIMBING WULUH (*Averrhoa bilimbi L.*) DIBANDINGKAN KARBAMID PEROKSIDA SEBAGAI PEMUTIH GIGI (*IN VIVO)*

Asih Rahaju[1*], Henri Hartman[2], Ris Kristiana[3], Tiara Putri[4]

[1]Department of Conservative Dentistry, Faculty of Dentistry, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
[2]Department of Pedodontics, Faculty of Dentistry, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
[3]Department of Anatomical Pathology, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
[4]Faculty of Dentistry, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

*Corresponding author
asih.rahaju@lecture.ac.unjani.ac.id

JHDS.unjani.ac.id/jite
Doi: 10.54052/jhds.

Article History
Received:20/06/2023
Accepted: 05/07/2023

ABSTRACT

A teeth whitening procedure is an action to get naturally bright teeth color. The teeth whitening process often has side effects on the tooth structure and its surroundings. Teeth whitening ingredients generally contain carbamide peroxide. One of the natural ingredients that can whiten teeth is *Averrhoa bilimbi Linn.* This study aimed to determine the effect of *Averrhoa bilimbi L.* gel and carbamide peroxide on the histological response of the pulp. The research method was carried out by processing *Averrhoa bilimbi Linn* into extracts through the

maceration method and then making a gel with a concentration of 50%. The study was conducted on 27 rabbit incisors and divided into three groups (negative control group, 10% carbamide peroxide, and 50% *Averrhoa bilimbi L. gel*). Each group was exposed for 4 hours for 14 days, then the teeth were extracted, and the pulp was taken with K-file number 10-40 and continued to look at the histological picture. The data was processed using a one-way ANOVA statistical test; the statistical test results showed significant differences for the three groups with a p-value of 0.000 ($p < 0.05$). It can be concluded that there is an effect of 50% *Averrhoa bilimbi L*. gel on the histological response of the pulp, there is an effect of 10% carbamide peroxide on the histological response of the pulp, and there is a significant difference between 10% carbamide peroxide and 50% *Averrhoa bilimbi L*. gel.

Keywords: *Averrhoa bilimbi Linn* gel; carbamide peroxide; pulp inflammation

ABSTRAK

Prosedur pemutihan gigi merupakan tindakan untuk mendapatkan warna gigi yang cerah alami. Proses pemutihan gigi seringkali memberikan efek samping kepada struktur gigi dan sekitarnya. Bahan pemutih gigi umumnya mengandung karbamid peroksida. Bahan alami yang mampu memutihkan gigi salah satunya adalah belimbing wuluh. Penelitian dilakukan dengan tujuan untuk mengetahui pengaruh gel belimbing wuluh dan karbamid peroksida terhadap respon histologis pulpa. Metode penelitian dilakukan dengan mengolah belimbing wuluh menjadi ekstrak melalui metode maserasi kemudian dibuat gel dengan konsentrasi 50%. Penelitian dilakukan pada 27 gigi insisif kelinci dan dibagi menjadi 3 kelompok (kelompok kontrol negatif, karbamid peroksida 10% dan gel belimbing wuluh 50%). Setiap kelompok dilakukan pemaparan selama 4 jam dalam 14 hari kemudian gigi diektraksi dan diambil pulpa dengan K-file nomor 10-40 dan dilanjut melihat gambaran histologis. Data diolah dengan uji statistik ANOVA

satu arah (one-way ANOVA), hasil uji statistik memperlihatkan adanya perbedaan signifikan untuk ketiga kelompok tersebut dengan nilai p-value 0,000 (p<0,05). Dapat disimpulkan bahwa terdapat pengaruh gel belimbing wuluh 50% terhadap respon histologis pulpa, terdapat pengaruh karbamid peroksida 10% terhadap respon histologis pulpa dan terdapat perbedaan yang bermakna antara karbamid peroksida 10% dengan gel belimbing wuluh 50%.

Kata kunci: *gel belimbing wuluh; inflamasi pulpa; karbamid peroksida*

INTRODUCTION

Dental and oral health is the primary key to getting the appearance of white teeth and a healthy oral cavity so that you can appear more confident.[1,2] The teeth whitening procedure is brightening the teeth to obtain a natural tooth color and have an aesthetic effect.[3,4] There are side effects to using teeth whitening agents, which can reduce enamel hardness, damage to the enamel surface, tooth hypersensitivity, and reduce the amount of phosphate, fluoride, and calcium.[3,5] Teeth whitening ingredients have hypertonic properties, which are very sensitive, so they can cause cell damage in the human dental pulp tissue.[1,6] The ingredients commonly used in teeth whitening products are hydrogen peroxide and carbamide peroxide.[3]

Generally, two kinds of chemicals containing peroxide in teeth-whitening ingredients are hydrogen peroxide and carbamide peroxide. Hydrogen peroxide with the chemical formula H_2O_2 includes a powerful oxidizing agent and is usually used as a bleaching agent and a disinfectant. Hydrogen peroxide has a pH of 4.5, is a colorless, odorless, clear liquid thicker than water.[1]

Apart from chemicals, teeth whitening can also use natural ingredients, namely *Averrhoa bilimbi Linn*.[7] Carambola wuluh is a tropical fruit plant commonly grown in the yard.[8] The *Averrhoa bilimbi L*. tree is easy to reproduce and grow by planting seeds or grafts. The number of fruits that can be produced by one tree is 1,500. *Averrhoa bilimbi L*. has a rich water content and a sour but fresh taste.[9] The content of *Averrhoa bilimbi L*. in the form of carboxylic compounds (oxalic acid) and peroxide compounds can whiten teeth that experience discoloration and have a pH of 1.5 – 4.7 because they contain oxalic acid.

[7,10,11]

The use of natural ingredients in dentistry has developed as more research has been conducted, such as watermelon extract and belimbing wuluh extract, which has the effect of whitening teeth.[12,]13The community uses Belimbing wuluh as an herbal medicine to prevent and treat dental and oral diseases, canker sores, cavities and bleeding gums.[14,15]

The results of a study conducted by Vaz et al. (2016) in the in-office treatment with 38% hydrogen peroxide (applied for 45 minutes every three visits) caused pulpal damage, the number of macrophages was higher than teeth whitening at home with 15% carbamide peroxide (applied for 16 days, 2 hours a day).[6] Kintan (2018) conducted a study using *Averrhoa bilimbi L*. extract gel with a concentration of 50% in an in vivo study of increasing the brightness of tooth color in the teeth of New Zealand rabbits, the results proved that there was an increase in the degree of brightness in the color of the teeth of rabbits.[16] The results of Fadhil's research (2022) stated that the application of 50% *Averrhoa bilimbi L*. gel did not affect reducing tooth enamel hardness compared to the application of 10% carbamide peroxide on the teeth of New Zealand rabbits *in vivo*.[17] Until now, no researcproves inflammation's effect on the dental pulp by applying 50% *Averrhoa bilimbi L*. gel to rabbit teeth *in vivo*.

Based on this background, the authors were interested in researching inflammation in the dental pulp of rabbits using *Averrhoa bilimbi L*. gel as a natural ingredient and carbamide peroxide which was carried out for 14 days for treatment. They continued to see the results whether there were signs of inflammation or not in the dental pulp.

METHOD

The design of this study used an experimental laboratory with a research focus on conducting trials to determine the effect of 50% Averrhoa bilimbi L. gel and 10% carbamide peroxide on the histological response of pulp *in vivo* in rabbit teeth. The object of this research is the upper incisors of rabbits. The study was carried out on rabbits' teeth and an experiment was carried out by making a personal impression spoon from shellac to accommodate 50% *Averrhoa bilimbi L.* gel so that it would make contact with the maxillary incisors. First, impressions of the upper jaw of the rabbit teeth with alginate, then the working model is prepared and shaped, and the base plate/red wax is placed on the entire surface of the maxillary incisors to maintain a space with a thickness of 4 mm for 50% *Averrhoa bilimbi L*inn. Then, it was reprinted using

the Laginat and made a plaster model again. Finally created a personal cast spoon based on the new plaster model [18]

50% *Averrhoa bilimbi Linn* gel and 10% carbamide peroxide were applied to each group of maxillary incisor teeth in rabbits, and then the personal printing spoon was filled with 50% *Averrhoa bilimbi Linn* gel and 10% carbamide peroxide, make sure *Averrhoa bilimbi Linn* gel and carbamide peroxide had covered the teeth with Good. The application lasts 4 hours and is repeated for 14 days. After 4 hours, the personal printing spoons of *Averrhoa bilimbi Linn* gel and carbamide peroxide were removed and the rabbits were fed carefully in individual cages then *Averrhoa bilimbi Linn* gel and 10% carbamide peroxide were prepared for the next day.[18]

Perform general anesthesia in rabbits prior to injury using ketamine, a general anesthetic at a dose of 20 mg/kg BW intramuscularly in the upper thighs of rabbits to provide an anesthetic effect. After being anesthetized, the rabbit was slaughtered using a scalpel and blade, followed by extraction of the maxillary right incisor moved using child extraction pliers.[15,19] Teeth that have been extracted are followed by pulp extraction. Maxillary incisors were prepared with round burs until perforation was reached. Pulp collection using k-file no. 10 to k-file no. 40 sequentially with a working length of 4 mm.[20]

After taking the pulp and storing it in formalin, then make preparations. The preparation stages are fixation, trimming, dehydration, embedding-blocking, cutting, staining, and mounting.[21,22]

Histological evaluation was carried out in this study using a light microscope with a magnification of 400x – 1000x, with the results showing the presence of inflammatory cells in the form of macrophages, lymphocytes, neutrophils, and the presence of vasodilatation of blood vessels. Data on the number of inflammatory cells found through microscopy were tabulated and then analyzed using the One Way ANOVA test to compare group means. If the test results are not normal, then proceed with the Willcoxon test.[23,24]

RESULT

The sample used in this study consisted of 27 upper incisors in rabbits, which were divided into three groups, namely nine teeth in the negative control group, nine teeth in 50% *Averrhoa bilimbi L.* gel, and nine teeth in 10% carbamide peroxide, which were exposed for 4 hours for 14 days.

Histological evaluation was carried out in this study using a light

microscope with a magnification of 400x – 1000x, with the results showing the presence of inflammatory cells in the form of macrophages, lymphocytes, neutrophils, and the presence of vasodilatation of blood vessels. Data on the number of inflammatory cells found through microscopy were tabulated and then analyzed using the One-way ANOVA test to test group mean comparisons. If the test results are not normal then proceed with Willcoxon test.

Table 1. Average Dental Pulp Inflammation

Group	n	Mean
Negative Control	9	1.33
Carbamide Peroxide 10%	9	5.22
Averrhoa bilimbi Linn gel 50%	9	3.42

Table 1 shows that the 10% carbamide peroxide group obtained the highest mean inflammation in the pulp in the carbamide peroxide group at 5.22 and the lowest in the negative control group, 1.33.

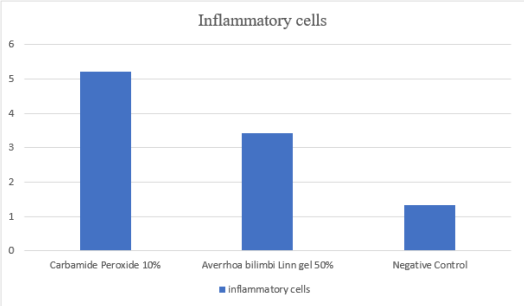

Figure 1. Graph of Average Dental Pulp Inflammation

In Table 2 the results of the three groups using the Kruskall Wallis test showed significant differences in dental pulp inflammation, the average difference for 50% *Averrhoa bilimbi L*. gel and 10% carbamide peroxide.

Table 2. Effect of Negative Control, 10% Carbamide Peroxide and 50% Carambola Gel

Test Group	Mean ± St. Deviation	P value
Negative Control	1.37 ± 1.35	
Carbamide Peroxide 10%	5.22 ± 4.18	0.000
Averrhoa bilimbi Linn 50%	3.42 ± 3.69	

The results of the analysis of the effect of the negative control, 50% *Averrhoa bilimbi L*. gel and 10% carbamide peroxide on in vivo pulp inflammation in rabbit teeth using the Kruskall Wallis test showed that there were significant differences for the three groups. The result was continued with the Wilcoxon test.

In Table 3, calculations using the

Wilcoxon test showed a pairwise comparison; the results showed that there was a significant difference between 50% *Averrhoa bilimbi L.* gel and negative control (p=0.000<0.05), there was a significant difference between 10% carbamide peroxide and negative control (p =0.001<0.05) and there was a significant difference between 10% carbamide peroxide and 50% *Averrhoa bilimbi L*. gel (p=0.001<0.05).

Table 3. Paired Comparison Test

Group	Negative Control	Carbamide Peroxide 10%	*Averrhoa bilimbi Linn* 50%	P-Value
Negative Control	-	5.22	3.42	0.001
Carbamide Peroxide 10%	1.37	-	3.42	0.001
Averrhoa bilimbi Linn 50%	1.37	5.22	-	0.000

DISCUSSION

Medical histology is the microscopic study of tissues and organs by cutting, staining, and examining these parts under a microscope. Further interpretation of histology slides combined with a history of treatment in experimental animals, in this study, using rabbit teeth by applying 50% *Averrhoa bilimbi L*. gel and 10% carbamide peroxide, which can enforce the prognosis of a histological reaction.[25]

The teeth used in this study came from New Zealand rabbits with a total of 27 maxillary incisors, so there were significant histological results.

Microscopic observations made on the results of this study showed the presence of inflammatory cells due to the use of 10% carbamide peroxide and 50% *Averrhoa bilimbi L*. gel, which had been carried out for 4 hours in 14 days. Inflammatory cells in the carbamide peroxide group 2.22 were more than 50% 3.42 *Averrhoa bilimbi L*. gel. Using tooth-whitening ingredients in the form of hydrogen peroxide can cause destructive effects that can deactivate the pulp's enzymes, disrupting the pulp's normal cell activity and damaging the pulp; the number of macrophages is higher.[26]

Previous reports said there were no side effects to using low concentrations of teeth whitening agents. However, in vitro evidence shows that at low concentrations (5% and 10%), carbamide peroxide damage from hydrogen peroxide to free radicals can be detected throughout the dentine and pulp chamber.[27]

Hydrogen peroxide can dissociate into water, active oxygen, and free radicals such as hydroxyl radicals (OH-). The reaction of tooth whitening agents can be

carried out by hydrogen peroxide free radicals, breaking down chromophore dentin chromogenic molecules into smaller molecules with lower optical or non-absorbing properties. However, hydrogen peroxide does not remain in the dentine, so it can penetrate the pulp chamber; most of it diffuses through the dentinal tubules. After reaching the pulp chamber, hydrogen peroxide will cause decreased cell proliferation, viability, metabolism, reduced pulp reparative capacity, tissue necrosis, and finally pulp pain.[27]

The use of *Averrhoa bilimbi L*. gel 50% as a tooth whitener can affect the degree of brightness of the teeth because it contains peroxide compounds, which can affect the mucosa, hard tissue, and tooth sensitivity.[16] The pH content of *Averrhoa bilimbi Linn* is 4.7-1.5 because it contains oxalic acid.[7,10,11]

This study was carried out on the dental pulp of New Zealand rabbits with 10% carbamide peroxide and 50% *Averrhoa bilimbi L*. gel, which was carried out in vivo within 4 hours 14 days; the results of dental pulp inflammation showed a significant difference because the Willcoxon test results p=0.00 (p<0.05).

CONCLUSION

Based on the results of the research that has been done, there is an effect of 50% *Averrhoa bilimbi L*. gel, and there is an effect of 10% carbamide peroxide on the histological response of the pulp. There is a significant difference between Carbamide Peroxide 10% and Belimbing Wuluh Gel 50%.

CONFLICT OF INTEREST

The authors reported no potential conflict of interest.

ACKNOWLEDGEMENT

This study supported by the Community Services Research Institute (LPPM), Universitas Jenderal Achmad YaniIndonesia. The funder had no role in study design, data collection and analysis, decision to publish, or manuscript preparation.

REFERENCES

1. Alma R, Adang F, Suprastiwi E, Usman M. Pemutihan gigi teknik home bleaching dengan menggunakan karbamid peroksida. Jakarta; 2022.
2. Syafriadi M, Risa R. Efek bahan pemutih gigi (bleaching) terhadap jaringan lunak dan jaringan keras rongga mulut. Jember; 2013 Sep.
3. Syahland MR, Setyawati A. The effectiveness grape (*Vitis vinifera L*.) as an in-gredient for tooth whitening (bleaching) based on concentration

difference. IDJ. 2013;2:51–2.

4. Meizarini A, Devi R. Bahan pemutih gigi dengan sertifikat ADA/ISO (Tooth bleaching material with ADA/ISO certificate). Dent J. 2005;38:73–5.
5. Rahaju A, Dewi ZY, Alatas F, Putri K, Shafarkiani N. The effect of *Averrhoa bilimbi L.* (belimbing wuluh) gel extract on increasing degree of teeth brightness (in vivo). Gel Extract On. 2021;1(1):81–4.
6. Vaz MM, Lopes LG, Cardoso PC, de Souza JB, Batista AC, Costa NL, et al. Inflammatory response of human dental pulp to at-home and in-office tooth bleaching. Journal of Applied Oral Science. 2016 Sep 1;24(5):509–17.
7. Selvia EY, Muharraran F, Anggie Adrisya Lubis P, Aldi Lumbantobing D, Rahmadani L. Pengaruh jus belimbing wuluh (*Averrhoa bilimbi L.*) terhadap perubahan warna stain ekstrinsik pada resin komposit nanohibrida. JODS. 2021;4(1):23–6.
8. Eko Purwaningsih. Multiguna belimbing wuluh. Bekasi: Ganeca; 2007. 1–2 p.
9. Putriana A. Ekstrak buah belimbing wuluh (*Averrhoa bilimbi L.*) sebagai ovisida keong mas (*Pomacea canaliculata L.).* Universitas Islam Negri Raden Intan; 2018.
10. Renatami RK, Noerdin A, Soufyan A. Pengaruh perbedaan konsentrasi ekstrak belimbing wuluh (*Averrhoa bilimbi L.*) dalam gel terhadap kekasaran permukaan email. 2014.
11. Abidin T, Nainggolan M. The effect of bilimbi (belimbing wuluh) extract (*Averrhoa bilimbi L*) against dental remineralisation and enamel microstructure (in vitro research). DDJ. 2017;20:21–4.
12. Setyawati A, Nur SNFF. The effectiveness differences between watermelon (*Citrullus lanatus*) extract 100% and carbamide peroxide gel 10% in tooth whitening (ex vivo). IDA J. 2020 Feb 20;3(1):31.
13. Chasanah N, Rosandi Prakosa B, Andina DT, Bhakti. Pemanfaatan buah belimbing wuluh (*Averrhoa bilimbi L.*) terhadap diskolorisasi gigi pasca perendaman kopi the utilization of belimbing wuluh (*Averrhoa bilimbi L.*) on post Coffee Immersion Dental discolorization. PPIKJ. 2021;2(1):9–17.
14. Sumardilan SFRYFAS. Uji karakteristik fisis, pH dan organoleptikasari buah belimbing wuluh. PJ. 2015;5:71–2.
15. Yuslianti ER, Khaerunnisa R, Puti RS I, Herawati H, Rahaju A, Ichwana DL, et al. Peningkatan pengetahuan bahan alam untuk kesehatan gigi mulut

melalui program merdeka belajar kampus merdeka. IPI J. 2022 Mar 5;10(1):82–91.

16. Kintan PNS. Pengaruh gel ekstrak belimbing wuluh (*Averrhoa bilimbi L.*) terhadap peningkatan derajat kecerahan warna gigi (kajian in vivo). Universitas Jendral Achmad Yani; 2020.
17. Fadhil AM. Pengaruh gel belimbing wuluh (*Averrhoa bilimbi L.*) sebagai bahan pemutih gigi alami terhadap kekerasan email gigi (secara in vivo). Universitas Jendral Achmad Yani; 2022.
18. Han M, Li QL, Cao Y, Fang H, Xia R, Zhang ZH. In vivo remineralization of dentin using an agarose hydrogel biomimetic mineralization system. SR. 2017 Feb 7;7:6–7.
19. Thomas B, Bhat K, Mapara M. Rabbit as an animal model for experimental research. DRJ. 2012;9(1):111.
20. Li M, Tian J, Xu Z, Zeng Q, Chen W, Lei S, et al. Histology-based profile of inflammatory mediators in experimentally induced pulpitis in a rat model: screening for possible biomarkers. IEJ. 2021 Aug 1;54(8):1328–41.
21. Citra H, Dan P, Manan A. The basic histology technique of gouramy fish (*Osphronemus gourami*). JIPK . 2015;7:154.
22. Berata K. Teknik pembuatan preparat histopatologi. 2018.
23. Budi HS, Soesilowati P, Imanina Z. Gambaran histopatologi penyembuhkan luka pencabutan gigi pada makrofag dan neovaskular dengan pemberian getah batang pisang ambon. MKGI. 2017 Dec 29;3(3):3.
24. Kirana IM. Pengamatan histopatologis pulpa gigi M. nemestrina yang terpapar getah J. curcas. DJ. 2008;7:20–1.
25. Gurina TS, Simms L. Histology, Staining [Internet]. Statpearls; 2022 [cited 2023 Feb 15]. 1 p. Available from: https://t.ly/LCOO
26. Fatimatuzzahro N. Perubahan histologis jaringan pulpa sebagai respon terhadap aplikasi bahan etsa. JKG Unej. 2015;12:7–8.
27. Redha O, Mazinanian M, Nguyen S, Son DO, Lodyga M, Hinz B, et al. Compromised dental cells viability following teeth-whitening exposure. SRJ. 2021 Dec 1;11(1).