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Original Research Article

The Effect of Cocoa Pod Husk and Green Tea on Smad3 and FGF2 Expression in Exposed Dental Pulp

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ABSTRACT

Background: *Direct pulp capping employing calcium hydroxide has been used to maintain the health and vitality of the pulp and encourage pulp cells to form reparative dentin. Calcium hydroxide was recommended as a material of direct pulp capping because it has beneficial properties. However, calcium hydroxide has also several weakness. Cocoa pod husk and green tea contain high polyphenols which are useful as antibacterial, anti-inflammatory, and antioxidant properties. Calcium hydroxide combined with cocoa pod husk extract and green tea extract is expected to increase the effectiveness of calcium hydroxide as a pulp capping material.* ***Purpose:*** *This research aims to prove the effect of cocoa pod husk and green tea extract on the Smad3 and FGF2 expression in mice perforation dental pulp.* ***Methods:*** *A total of 54 rats were used in this study, then the study sample was divided into three groups consisting of group given calcium hydroxide treatment with distilled water, next group given calcium hydroxide with cocoa pod husk extract and last group given calcium hydroxide with green tea extract, then cavities are restored. On days 3rd, 7th and 14th, experimental animals from each treatment group were killed to see the Smad3 and FGF2 expression.* ***Result:*** *Data analysis with Tukey HSD test showed no significant difference for Smad3 and FGF2 expression on two test group.* ***Conclusion:*** *Cocoa pod husk and green tea extract have the same ability to increase the Smad3 and FGF2 expression in exposed dental pulp.*

Keywords: *antioxidant, cocoa pod, green tea, Smad3, FGF2*

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INTRODUCTION

Dental pulp is a connective tissue consisting of nerves, blood vessels, basic substances, interstitial fluid, odontoblasts, fibroblasts, and other cell components.[1] Pulp tissue can be affected by various stimuli such as deep carious and trauma affected by accidental preparation techniques that used during tooth restoration.[2] Perforation in the pulp can cause a homeostatic imbalance of the pulp, so that the pulp will respond histologically, which is characterized by inflammation, synthesis of new collagen tissue, and formation of dentine bridges that end with the final formation of reparative dentin.[3]

Fibroblast Growth Factor-2 (FGF2) is a growth factor that has a significant role in the regeneration of the dentin-pulp complex. FGF2 plays a role in regulating tooth morphogenesis by controlling cell proliferation and differentiation. FGF2 is also a strong angiogenic factor which stimulates the formation of new blood vessels in the dental pulp along with PDGF and VEGF. FGF2 also induces the migration of dental pulp cells and stimulates the proliferation of tooth pulp cells without differentiation, whereas FGF2 in combination with TGFβ1 induces differentiation of dental pulp cells into odontoblast-like cells, and synergistically increases the effect of TGFβ1 on odontoblast differentiation. FGF2 stimulates the formation of reparative dentin or dentin particles in exposed pulp.[4] Smad is a protein involved in TGFβ1 signaling. Smad3 is one of the Smad groups involved in TGFβ1 signaling.[5] Functional signaling from Smad3 is required for FGF2 mRNA-induced TGF2 and protein expression in fibroblasts.[6]

Pulp capping direct is a treatment for vital pulp that is exposed due to mechanical or chemical factors by placing dental material over an open area to facilitate barrier formation and maintain pulp vitality.[7] The vitality of the dentin-pulp complex needs to be maintained because it is fundamental to the functional life of the teeth.[8] The success of the pulp closure procedure is highly dependent on the capacity of the covering material to completely cover the tubules, does not cause irritation, protects the pulp from mechanical, chemical and bacterial irritants, induces fibroblast and odontoblast cells to form perfect dentin and dentin bridges.[9]

Calcium hydroxide [$Ca(OH)_2$] is recommended as a direct pulp capping material because it has beneficial properties which can stimulate mineralization (recovery process) and can inhibit bacterial growth.[10] However, calcium hydroxide also has some drawbacks, including: dissolves clinically within 1-2 years, susceptibility to dissolution by acids and tissue fluids causes problems during acid etching resin restoration, 89% of the dentin bridge formed has tunnel defect which is at high risk of causing micro- leakage, which leads to re-infection of bacteria, continued pulp inflammation and necrosis.[11]

Cocoa is a natural ingredient, it contains polyphenols which consist of condensed tannins

that have the potential to be antioxidants, anti-inflammatory, and antimicrobial properties.[12] The effectiveness as an antibacterial has been proven against the *enterococcus faecalis*.[13] Cocoa pod husks have a much higher total phenolic content and have a higher antioxidant effect than other parts of cocoa.[14] Proantocyanidin is the most polyphenol in cocoa, the amount reaches 58% - 65% of the total polyphenols in cocoa pods.[15,16] This substance acts as an anti-inflammatory agent by inhibiting the release of arachidonic acid and lysosomal enzymes by blocking the cyclooxygenase and lipoxygenase pathways in the process inflammation. The suppression of prostaglandins and leukotrienes as inflammatory mediators causes the migration of inflammatory cells to the wound area to be reduced, so that it can suppress the inflammatory response.[17] In addition, proanthocyanidin works as an antioxidant by interfering with one of Fenton's reactions thereby inhibiting oxidation.[18] Previous research has shown that the application of proanthocyanidin in direct contact with the pulp can increase cell metabolism and collagen synthesis and act as a biostimulator and protect dental pulp cells.[19] Other research has proven the effectiveness of cocoa pod husk on reparative dentin formation.[13]

Green tea is a source of antioxidants that are rich in polyphenols which have anti-inflammatory, antioxidant, antibacterial, antiviral and anti-fungal benefits.[20] EGCG is the main polyphenol of green tea, accounting for about 59% of total catechins.[21] Several studies have shown that EGCG polyphenols are active flavonoid compounds that have good antioxidant properties and play a role in shortening the duration of inflammation by inhibiting the production of inducible nitric oxide synthase (iNOS) and reducing levels of Nitric Oxide so that inflammatory cells in the wound area will be reduced and the inflammatory reaction will lasts shorter then starts the proliferation phase so that the healing and tissue repair process will occur more quickly.[22] The ability of EGCG to scavenge free radicals by providing stable phenol radicals is due to the presence of galloyl groups in the B and D rings that can simultaneously capture OH and O_2 free radicals.[23] One of the cells that play a role in tissue repair is macrophages which play a role in starting the proliferation phase by releasing growth factors that stimulate repair and healing. In previous studies it was found that EGCG with hydrogel dosage form has good biocompability and has the ability to regenerate pulp because it can induce the release of FGF-2 and induce tissue regeneration.[22] Other research has proven the effectiveness of green tea on reparative dentin formation.[24]

This research aims to prove the effect of cocoa pod husk and green tea extract on the Smad3 and FGF2 expression in mice perforation dental pulp.

MATERIAL AND METHODE

The total sample size of this research was 54 male Rattus novergicus aged 12-16 weeks with a body weight of 250-300 grams. After that, the samples were randomly divided into three groups. Each group consisted of 18 male rats. Meanwhile, the concentration of cocoa pod husk extract used of this study was 3.125% and green tea extract was 0.8%. This research has received approval from the Ethics Commission of the Faculty of Dentistry, Airlangga University with the license number 212 / HRECC.FODM / IV / 2020.

Positive control group (group 1) were given calcium hydroxide with distilled water in a ratio of 1:1, the next group given calcium hydroxide with cocoa pod husk extract in a ratio of 1:1 (group 2) and last group given calcium hydroxide with green tea extract in a ratio of 1:1 (group 3). The material was placed on the pulp surface and the cavity was restored. The mice were returned to the cages after being tagged.

To see activation of Smad3 and FGF2, eighteen mice were killed on the 3rd day. The other 18 mice were killed on the 7th day, and the other 18 mice were killed on the 14th day. Each day consisted of 6 mice for each treatment group. Then after taking or cutting, the jaw bones in the interdental area of the right maxillary first molar were taken to carried out the process of making histopathology. Histological preparations were obtained with a thickness of ± 4µm parallel to the long axis of the tooth. Each specimen obtained two slides to be observed.

Immunohistochemical (IHC) observations on Smad3 and FGF2 activation were performed using primary antibodies (monoclonal mouse) Smad3 and FGF2 (Santa Cruzz B) so that protein and antibody binding could be seen. The enzyme was then reacted with the chromogen substrate, then observed with an Olympus BX51 light microscope with a magnification of 1000 times and a calculation of 20x the field of view.

The data obtained from this study are presented in the form of mean and standard deviation. To determine the distribution of data and data homogeneity, the Kolmogorov- Smirnov Lilliefors test was used, followed by a homogeneity test using the Levene test. The ANOVA test was used to see if there were significant differences in the treatment group, the Tukey HSD test was used to assess the differences with a significance level of α 0.05.

RESULT

Immunohistochemical (IHC) detection of Smad3 on day 3rd, 7th, and 14th can be seen on figure 1-3. The arrows show activated Smad3 on fibroblast cells in each group. A. Group 1; B. Group 2; C. Group 3. While The average of Smad3 expression in each treatment group was shown in graph 1. The highest mean value of Smad3 expression in each group was on day 14th. The average was 8.50 in group 1; 14 in group 2; and 15 in group 3.

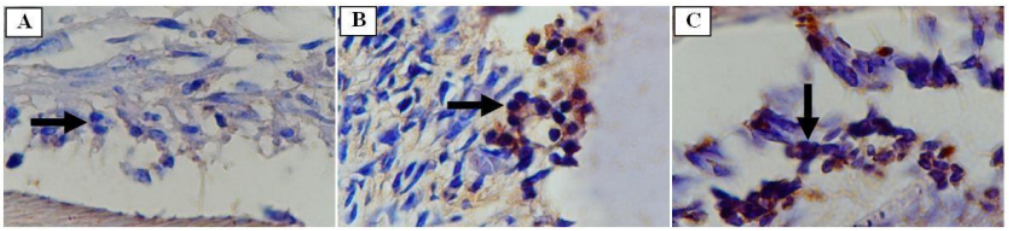


Figure 1. IHC detection of Smad3 on day 3rd. The arrows show activated Smad3 on fibroblast cells. A. Group 1; B. Group 2; C. Group 3.

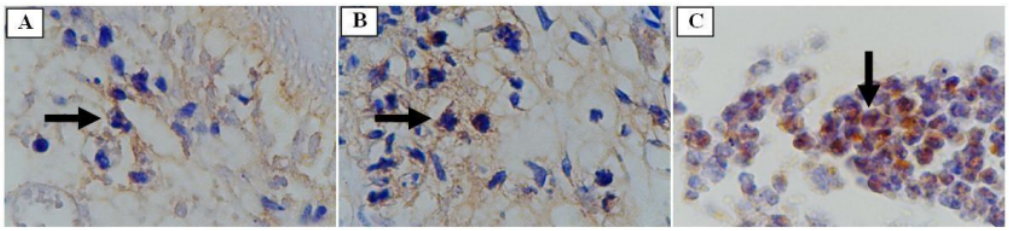


Figure 2. IHC detection of Smad3 on day 7th. The arrows show activated Smad3 on fibroblast cells. A. Group 1; B. Group 2; C. Group 3.

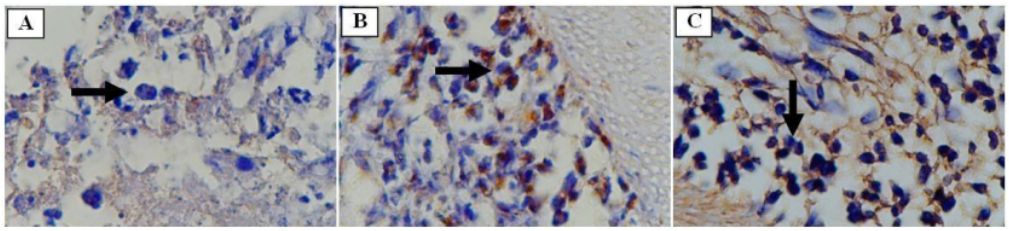
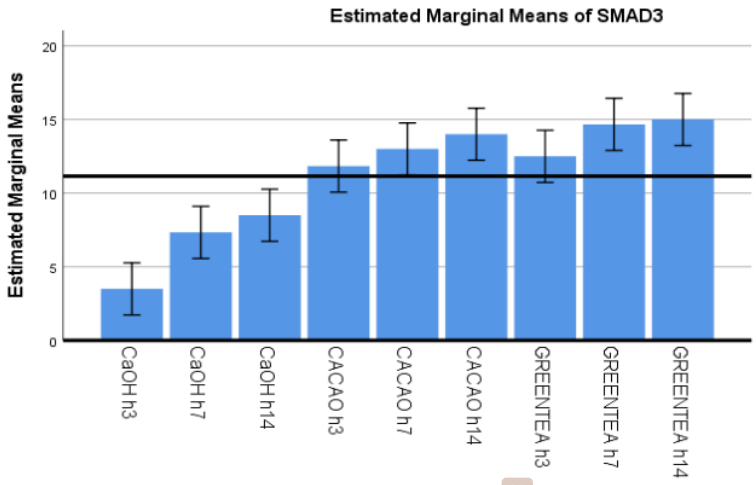


Figure 3. IHC detection of Smad3 on day 14th. The arrows show activated Smad3 on fibroblast cells. A. Group 1; B. Group 2; C. Group 3.



Graph 1. The mean value of Smad3 activation on day 3rd, 7th, and 14th

IHC detection of FGF2 on day 3rd, 7th, and 14th can be seen on figure 4-6. The arrows show activated FGF2 on fibroblast cells in each group. A. Group 1; B. Group 2; C. Group 3. While The average of FGF2 expression in each treatment group is shown in graph 2. The highest mean value of FGF2 expression in each group was on day 14th. The average was 9 in group 1; 14,83 in group 2; and 15 in group 3.

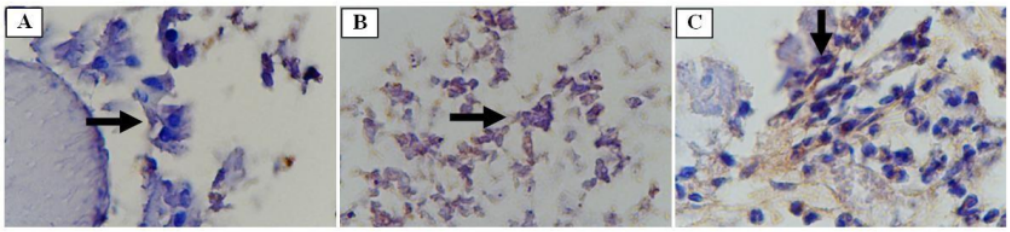


Figure 4. IHC detection of FGF2 on day 3rd. The arrows show activated FGF2 on fibroblast cells. A. Group 1; B. Group 2; C. Group 3.

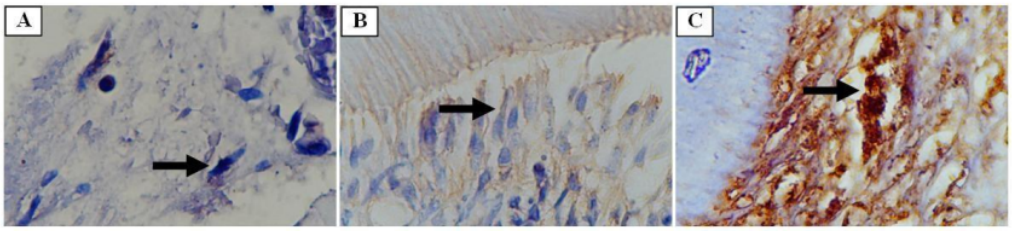


Figure 5. IHC detection of FGF2 on day 7th. The arrows show activated FGF2 on fibroblast cells. A. Group 1; B. Group 2; C. Group 3.

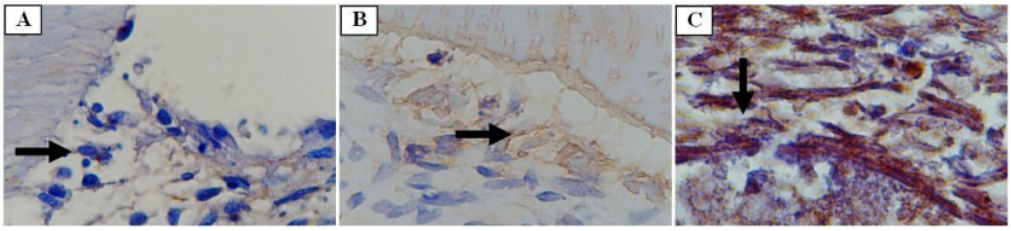
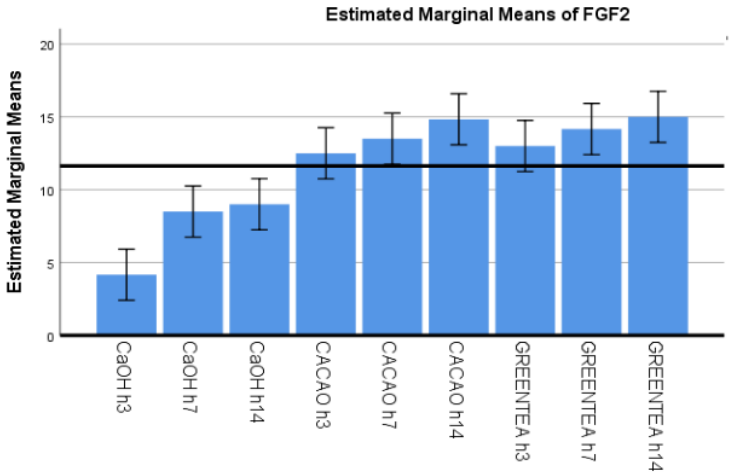


Figure 6. IHC detection of FGF2 on day 14th. The arrows show activated FGF2 on fibroblast cells. A. Group 1; B. Group 2; C. Group 3.



Graph 2. The mean value of FGF2 activation on day 3rd, 7th, and 14th

Based on normality and homogeneity test of Smad3 and FGF2 activation, data were obtained with normal and homogeneous distributions. Furthermore, the *ANOVA* test was carried out to determine the effect of the treatment group on Smad3 and FGF2 activation.

Table 1. *ANOVA* test for Smad3 expression

Sum of Squares		df	Mean Square	F	Sig.
Between Groups	726.815	8	90.852	19.655	.000

Table 2. *ANOVA* test for FGF2 expression

Sum of Squares		df	Mean Square	F	Sig.
Between Groups	645.148	8	80.644	17.688	.000

In this study, the result of the ANOVA on Smad3 activation (Table 1 and Table 2) revealed that the F value between groups had a significance value of 0.000 (P <0.05). So it can be concluded that there was a significant effect between the treatment groups on Smad3 and FGF2 expressions.

Based on the activation of Smad3, on day 3rd, 7th, and 14th the sample of group 1 has significant difference compared to group 2 and III on day 3rd, 7th, and 14th with *p* value <0,05. But there was no significant difference between the sample of group 1 on day 14th compared to group 2 and III on day 3rd with *p* value >0,05. Insignificant difference were also found between group 2 and group 3 on day 3rd, 7th, and 14th with each *p* value > 0.05 (Table 3)

Table 3. *Tukey HSD Test* for Smad3 expression on day 3rd, 7th, and 14th.

Groups	$Ca(OH)_2$ + aquadest D3	$Ca(OH)_2$ + aquadest D7	$Ca(OH)_2$ + aquadest D14	$Ca(OH)_2$ + Cocoa D3	$Ca(OH)_2$ + Cocoa D7	$Ca(OH)_2$ + Cocoa D14	$Ca(OH)_2$ + Green tea D3	$Ca(OH)_2$ + Green tea D7	$Ca(OH)_2$ + Green tea D14
$Ca(OH)_2$ + aquadest D3	-								
$Ca(OH)_2$ + aquadest D7	0,075	-							
$Ca(OH)_2$ + aquadest D14	0,006*	0,989	-						
$Ca(OH)_2$ + Cocoa D3	0,000*	0,019*	0,182	-					
$Ca(OH)_2$ + Cocoa D7	0,000*	0,001*	0,019*	0,989	-				
$Ca(OH)_2$ + Cocoa D14	0,000*	0,000*	0,002*	0,716	0,996	-			
$Ca(OH)_2$ + Green tea D3	0,000*	0,004*	0,054	1,000	1,000	0,950	-		
$Ca(OH)_2$ + Green tea D7	0,000*	0,000*	0,000*	0,374	0,913	1,000	0,716	-	
$Ca(OH)_2$ + Green tea D14	0,000*	0,000*	0,000*	0,236	0,794	0,996	0,542	1,000	-

*) There are significant differences (*p* value < 0,05).
D: day

Table 4. *Tukey HSD Test* for FGF2 expression on day 3rd, 7th, and 14th.

Groups	$Ca(OH)_2$ + aquadest D3	$Ca(OH)_2$ + aquadest D7	$Ca(OH)_2$ + aquadest D14	$Ca(OH)_2$ + Cocoa D3	$Ca(OH)_2$ + Cocoa D7	$Ca(OH)_2$ + Cocoa D14	$Ca(OH)_2$ + Green tea D3	$Ca(OH)_2$ + Green tea D7	$Ca(OH)_2$ + Green tea D14
$Ca(OH)_2$ + aquadest D3	-								
$Ca(OH)_2$ + aquadest D7	0,026*	-							
$Ca(OH)_2$ + aquadest D14	0,008*	1,000	-						
$Ca(OH)_2$ + Cocoa D3	0,000*	0,052	0,132	-					
$Ca(OH)_2$ + Cocoa D7	0,000*	0,002*	0,008*	0,974	-				
$Ca(OH)_2$ + Cocoa D14	0,000*	0,000*	0,001*	0,622	0,996	-			
$Ca(OH)_2$ + Green tea D3	0,000*	0,026*	0,072	1,000	0,996	0,788	-		
$Ca(OH)_2$ + Green tea D7	0,000*	0,001*	0,004*	0,909	1,000	1,000	0,974	-	
$Ca(OH)_2$ + Green tea D14	0,000*	0,000*	0,000*	0,533	0,989	1,000	0,708	0,999	-

*) There are significant differences (p value < 0,05).
D: day

In the activation of FGF2 on day 3rd, the sample of group 1 has significant difference compared to group 2 and III on day 3rd, 7th, and 14th with p value <0,05. The significant difference were also found between group 1 on day 7th, and 14th compared to group 2 and III on day 7th, and 14th with significance level p<0,05. But there was no significant difference between sample of group 1 on day 7th, and 14th compared to group 2 and III on day 3rd with p value >0,05. Insignificant differences were also found between group 2 and group 3 on days 3rd, 7th, and 14th with p value >0.05 (Table 4).

DISCUSSION

The study result on the activation of Smad3 and FGF2 on days 3, 7, and 14 showed that the effect of the combination of $Ca(OH)_2$ and distilled water showed the lowest mean value compared to the other two groups. The highest mean value was shown by the combination of

$Ca(OH)_2$ and green tea extract, then followed by the combination group of $Ca(OH)_2$ and cocoa pod husk extract with an insignificant difference.

This condition indicates that the use of cocoa pod husk and green tea extract as a mixing agent for calcium hydroxide has been shown to increase Smad3 and FGF2 activation which is indicated by the higher average of Smad3 and FGF2 activation on each day in both groups compared to the group of combination $Ca(OH)_2$ with distilled water. The results of this study also shown the increase of Smad3 activation which is in line with the increase of FGF2 activation on days 3, 7, and 14. These findings are in line with research conducted by Strand et al which found that Smad3 plays a role in regulating the expression of FGF2 which induced by TGFβ1.[6]

The increase in FGF2 expression on day 14th obtained in this study was not in line with the results of the study by Sagomonyants and Mina which saw the stage of the specific effect of FGF2 on tooth pulp cell differentiation from day 3rd to day 21th.[25] In this study, over all the peak expression of FGF2 was on the 7th day then will decrease on the 14th day and will still be expressed in a fixed amount until the 21st day.

However, in this study it was found that the increase of FGF2 expression continued occur until day 14 in all treatment groups. This is probably due to the different types of cells studied, Sagomonyants and Mina saw the expression of FGF2 in odontoblast cells, whereas in this study the expression of FGF2 was seen in fibroblast cells, where in the pulp exposed condition, the proliferation of fibroblast cell still increase until day 21.[25] In addition, the use of calcium hydroxide as the base material for pulp capping will cause an increase in extracellular Ca^{2+} levels which trigger an increase in FGF2 expression. According to Kanaya et al., the use of calcium-based materials such as calcium hydroxide for direct and indirect pulp capping treatments will cause an increase in extracellular Ca^{2+} levels which can cause an increase in the expression of the FGF2 gene in hDP (human dental papilla) and mDP (mouse dental papilla) through PKA and ERK 1/2.[26]

The highest average value of Smad3 and FGF2 activation on the group combination of $Ca(OH)_2$ and green tea extract was probably due to the high content of polyphenols in green tea extract which was dominated by EGCG.[21] Epigallocatechin-3-gallate has anti-inflammatory effects that can inhibit the translation of NF-kB into the cell nucleus. According to previous studies, epigallocatechin-3- gallate (EGCG) will inhibit the IKK / NF-kB signal transduction pathway because it inhibits IKK phosphorylation, resulting in IkB inhibition, resulting in decreased NF-kB activity and inhibition of TNF-a expression that cause limiting the number of inflammatory cells that migrate to the wound area.[27] This condition results a shorter inflammatory reaction.

In addition, EGCG also has antioxidant properties that can inhibit iNOS production and

reduce the NO levels,[22] as well as inhibit TNF-induced ROS and cell death by supporting complex dissociation. The released of Nuclear factor E2-related factor2 (Nrf2) will translocate to the nucleus and together with other transcription factors, activate the transcription of genes containing the Antioxidant Response Element (ARE) in the promoter region which produces an inhibitory effect on NF-κB activity. On the other hand, the Kelch-like ECH-associated protein 1 (Keap1) is separated from the Nrf2-Keap1 complex and directly interacts with IKKβ and suppresses NF-κB function. In addition, Nrf2 which translocates to the nucleus and binds to ARE elements will encourage the activation of various enzymes / antioxidant proteins which will reduce oxidative stress.[28,29]

The antioxidant properties of EGCG mainly originate from the presence of hydroxyl groups or -OH groups which are easily oxidized in ring B and cause the opening of oxygen atoms so that they increase reactivity to biological polymeric bonds, bonds with heavy metals, catalyze electron transport, and capture free radicals including nitric oxide (NO) which is a free radicals produced by the enzyme nitric oxide synthase (NOS) due to friction and heat generated by the use of burs when making preparations in deep cavities. This results in vasoconstriction of blood vessels and decreased capillary permeability. As a result, there is a decrease in neutrophil cell migration into the injury area so that the acute phase of inflammation is more quickly completed. This stimulates macrophage activity as a second defense by increasing the number of macrophages to carry out the phagocytosis process, cleaning debris tissue, then releasing and activating some growth factors such as PDGF, TGFβ and FGF which stimulates the proliferation and migration of fibroblasts cells.[30]

The insignificant difference in Smad3 and FGF2 expression between the combination group of $Ca(OH)_2$ with cocoa pod husk extract and the combination of $Ca(OH)_2$ with green tea extract on days 3, 7, and 14, possibly because the cocoa pod husk extract also contained high polyphenols which were dominated by proanthocyanidin which has anti-inflammatory and antioxidant effects.[31] In addition, proanthocyanidin works as an antioxidant by interfering with one of Fenton's reactions thereby inhibiting oxidation. Cocoa pod husk also has benefits as an antioxidant because it contains the alkaloid purine class, namely theobromin. Similar to proanthocyanidin, theobromin works by intervening in one of the fenton reactions so as to inhibit the oxidation reaction.[18,32]

The ability of cocoa flavonoids as antioxidants is related to their structure. Orthodihydroxy 3 ', 4' (catechol) structure on the B ring which provides stability to the penoxyl radicals through hydrogen bonding or electron delocalization. The catechol structure in ring B increases the inhibition of fat peroxide and plays a role in capturing peroxyl, superoxide and peroxynitrite

radicals. This structureless flavonoid compound has low antioxidant activity. The C2, C3 double bonds and the conjugation with the 4-oxo group on the C ring are responsible for radical stabilization through electron delocalisation in all three rings. The presence of hydroxyl groups 3 and 5 can increase antioxidant activity. Compounds that do not have an orthodihydroxy structure in ring B but have a catechol structure in ring A show greater antioxidant activity.[33]

CONCLUSION

Cocoa pod husk and green tea extract have the same ability to increase the Smad3 and FGF2 expression in exposed dental pulp.

REFERENCES

1. Grosman, LI. Grossman's Endodontic Practice. 12th ed. Editors: B. Suresh Chandra & V. Gopi Krishna. New Delhi: Wolters Kluwer (India) Pvt., Ltd; 2010. ISBN-13:978-81- 8473-2801
2. da Rosa WLO, Piva E, da Silva AF. Disclosing the physiology of pulp tissue for vital pulp therapy. International Endodontic Journal; 2018. 51, 829–846.
3. Ariesdyanata C., Lunardhi cecilia GJ., Subiwahjudi A. Perbedaan angiogenesis pada pulpa setelah aplikasi ekstrak propolis dan kalsium hidroksida. 2019. Conservative Dentistry Journal (9): 1: 48-53.
4. Kim SG., Solomon C., Zheng Y., Suzuki T., Mo Chen, Song S., Jiang N., Cho S., Zou J., Mao J.J. Effects of Growth Factors on Dental Stem/ProgenitorCells. Dent Clin North Am; 2012. 56(3): 563–575. doi: 10.1016/j.cden.2012.05.001
5. Lucchini, M., Romeas, A., Couble, M-L., Bleicher, F., Magloire, H., Farges, JC. TGFβ1 Signaling and Stimulation of Osteoadherin in Human Odontoblasts In Vitro. Connective Tissue Research; 2009. 43. 345-353. doi: 10.1080/03008200290000790.
6. Strand DW., Liang YY., Yang F., Barron DA., Ressler SJ., Schauer IG., Feng X-H., Rowley DR. TGF-β induction of FGF-2 expression in stromal cells requires integrated smad3 and MAPK pathways. American journal of clinical and experimental urology; 2014. 2(3), 239–248.
7. Komabayashi T., Zhu Q., Eberhart R., Imai Y. Current status of direct pulp-capping materials for permanent teeth. Dental Material Journal; 2016. 35(1):1-12. doi:10.4012/dmj.2015-013
8. Brand R.W., Isselhard D.E., Satin E. Anatomy of Orofacial Structures: A Comprehensive Approach, American Speech. 2014. https://doi.org/10.2307/486972
9. Dwiandhono I., Effendy R., Kunarti S. The thickness of odontoblast-like cell layer after induced by propolis extract and calcium hydroxide. Dental Journal; 2016. 49(1):17- 21
10. Ariesdyanata C., Lunardhi CGJ., Subiwahjudi A. Perbedaan angiogenesis pada pulpa setelah aplikasi ekstrak propolis dan kalsium hidroksida. Conservative Dentistry Journal; 2019. (9): 1: 48-53.
11. Song M. Yu B., Kim S., Hayashi M. Smith C., Sohn S., Kim E., Lim J., Stevenson R.G., Kim R.H. Clinical and molecular perspectives of reparative dentin formation: Lessons learned from pulp-capping materials and the emerging roles of calcium. Dent Clin North Am; 2017. 61(1): 93–110. doi: 10.1016/j.cden.2016.08.008
12. Izzuddin AFA. and Nurkesuma A., The Potential of Cocoa (Theobroma Cacao L.) Pods Extract in Periodontal. 2015.
13. Yuanita T, Oktavianti RA, Suryani DF, Rukmo M, Kunarti S, Kusuma AH. The Inhibitory Ability of Cocoa Pod Husk Extract on Enterococcus faecalis Glucosyltransferase Enzyme

Activity. J Contemp Dent Pract; 2020. 21 (3):271-276.
14. Abeysinghe D.C. and Kumari I.P.N.P. Antioxidant Activity and Phenolic Content of Different Pod Tissues of Five Selected Cocoa Hybrid Lines. Journal of Food and Agriculture; 2012. 5(1-2), pp.5–12. DOI: http://doi.org/10.4038/jfa.v5i1-2.5177
15. Andújar I., Recio, Maria, Giner, Rosa, Rios, Jose-Luis. Cocoa Polyphenols and Their Potential Benefits for Human Health. Oxidative medicine and cellular longevity. 2012. 906252. doi:10.1155/2012/906252.
16. Marika M., Scoditti E., Carluccio M.A., Kaltsatou A., Cicchella A. Effect of Cocoa Products and Its Polyphenolic Constituents on Exercise Performance and Exercise- Induced Muscle Damage and Inflammation: A Review of Clinical Trials. Nutrients; 2019. 11, 1471; doi:10.3390/nu11071471
17. Yuwono B, Elga S, Merry D. The Potential of Cacao (Theobroma Cacao L.) Pod Husk Extract on The Number of Fibroblast Cells of Wistar Rats Wound Post-Tooth Extraction. Int J App Pharm; 2019. 11(4). pp 30-32.
18. Scapagnini G., Davinelli S., Di Renzo L., De Lorenzo A., Olarte H., Micali G., Cicero A. and Gonzalez S., Cocoa bioactive compounds: significance and potential for the maintenance of skin health. Nutrients, 2014. 6(8), pp.3202-3213.
19. Santos A., Pacheco J., Silva P., Bedran-Russo A., Rezende T., P.N.R. Pereira, Ribeiro AP. Direct and transdentinal biostimulatory effects of grape seed extract rich in proanthocyanidin on pulp cells. International Endodontic Journal; 2018. 52. 10.1111/iej.13019.
20. Yahfoufi N., Alsadi N., Jambi M., Matar C. The Immunomodulatory and Anti- Inflammatory Role of Polyphenols. Nutrients, 2018. 10(11), 1618; https://doi.org/10.3390/nu10111618
21. Kar S., Saloni S., Sindhu. Green tea - it's chemical constituents and health benefits. International journal of engineering research & technology (IJERT); 2016. Vol. 5 Issue 2. ISSN: 2278-0181
22. Ismiyatin, K., Juniarti D., Purwanto D., Primazafira A. Effect Of Epigallocatechin-3- Gallate (EGCG) On the Number of Macrophage Cells in Inflammation of Pulp with Mechanical Injury. Conservative Dentistry Journal; 2020. Vol.10(1).31-35. DOI: 10.20473/cdj.v10i1.2020.31-35.
23. Leopoldini M., Russo N. and Toscano, M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chemistry; 2011. 125(2), pp.288-306.
24. Yuanita T, Drismayanti I, Dinari D, Tedja L. Effect of Calcium Hydroxide Combinations with Green Tea Extract and Cocoa Pod Husk Extract on p38 MAPK and Reparative Dentine. J Contemp Dent Pract; 2020. 21 (11):1238-1244.
25. Sagomonyants K., and Mina M. Stage-spesific effects of FGF2 on the differentiation of dental pulp cells. Cells Tissues Organs; 2014. 199(0):311-328. doi:10.1159/000371343.
26. Kanaya S, Xiao B, Sakisaka Y, Suto M, Maruyama K, Saito M, et al. Extracellular calcium increases fibroblast growth factor 2 gene expression via extracellular signal- regulated kinase 1/2 and protein kinase A signaling in mouse dental papilla cells. J. Appl. Oral Sci.; 2018. 26: e20170231. https://doi.org/10.1590/1678-7757-2017-0231.)
27. Singh BN., Shankar S. and Srivastava RK. Green tea catechin, epigallocatechin-3- gallate (EGCG): mechanisms, perspectives and clinical applications. Biochemical pharmacology; 2011. 82(12), pp.1807-1821.
28. Jiang J., Mo Z-C. Yin K., Zhao G-J., Yun-Cheng LV., Ouyang X-P., Jiang Z-H., Fu, Y., Tang C-KE. Epigallocatechin-3-gallate prevents TNF-α-induced NF-κB activation thereby upregulating ABCA1 via the Nrf2/Keap1 pathway in macrophage foam cells. International Journal of Molecular Medicine; 2012. 29: 946-956.
29. Luyao X., Benson OAB., Songou Z., Jingying Z., Xuehong L. Inhibition of NF-κB Signaling Pathway by Resveratrol Improves Spinal Cord Injury. Frontiers in Neuroscience; 2018.12. https://doi.org/10.3389/fnins.2018.00690
30. Widjiastuti I., Setyabudi, Ismiyatin K., Purwanto DA., Sukmawati T. Effect of hydrogel

Epigallocatechin-3-gallate (EGCG) to the number of fibroblast cell proliferation in the perforation of Wistar rat tooth pulp. Conservative Dentistry Journal; 2019. Vol. 9 No. 2 July-December; 93-96

31. Laličić-Petronijević, J., Komes, D., Gorjanović, S., Belščak-Cvitanović, A., Pezo, L., Pastor, F., Ostojić, S., Popov-Raljić, J., & Sužnjević, D. Content of Total Phenolics, Flavan-3-Ols and Proanthocyanidins, Oxidative Stability and Antioxidant Capacity of Chocolate During Storage. Food technology and biotechnology; 2016. 54(1), 13–20. https://doi.org/10.17113/ftb.54.01.16.4014
32. Amarowicz, R. Tannins: the new natural antioxidants? European Journal of Lipid Science and Technology; 2007. 109(6), pp.549-551.
33. Utami, RR. Antioksidan biji kakao: Pengaruh fermentasi dan penyangraian terhadap perubahannya. Jurnal Industri Hasil Perkebunan; 2018. Vol. 13 No. 2 Desember: 75-85

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