Comparison of the Effect of Calcium Hydroxide Combination with Cocoa Pod Husk Extract and Green Tea Extract On C-Fos and Dmp-1 Expression in Exposed Dental Pulp

Tamara Yuanita[1]*, I Dewa Ayu Listiana[1], Irfan Prasetyo[1], Priskila Naomi Widodo[1], Lailatun Tedja[1], Adioro Soetojo[1], Anuj Bhardwaj[2]

1.Dept. of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga
2.Dept. of Conservative Dentistry and Endodontics, College of Dental Sciences, Rau, Indore, MP, India.

Abstract

Dental caries is still a worldwide problem including in Indonesia. Untreated caries may progress into deep lesion cause abnormalities in the pulp that can be reversible or irreversible. Direct pulp capping is one of the treatments of an exposed vital pulp due to caries, caries removal, and trauma. Calcium hydroxide has been the gold standard as pulp capping materials in pulp protection but it does have some disadvantages. To overcome these disadvantages the research was carried out with other alternative materials for direct pulp capping using natural material. To analyze the effect of calcium hydroxide mixed with cocoa pod husk extract and calcium hydroxide mixed with green tea extract on c-FOS and DMP-1 expression in mice perforation dental pulp.

Sixty upper molars in Wistar rats were perforated mechanically and applied the combination material of pulp capping then divided into three groups. The control group were treated with calcium hydroxide and distilled water The treatment groups were treated with calcium hydroxide with cocoa pod husk extract and calcium hydroxide mixed with green tea extract.

The treatment group of calcium hydroxide with green tea extract increased the expression of c-FOS and DMP-1 more than the treatment group of calcium hydroxide with cocoa pod husk extract, there were no statistically significant differences between groups.

Both cocoa and green tea mixed with calcium hydroxide have relatively the same anti-inflammatory and antioxidant properties so their active substances have the effect same effect to increase the number of c-FOS dan DMP- 1 expressions in mice perforation dental pulp.

Experimental article (J Int Dent Med Res 2022; 15(4): 1516-1521)

Keywords: Perforation, calcium hydroxide, cocoa bark extract, green tea extract, expression c-FOS expression DMP-1, immunology.

Received date: 22 June 2022 **Accept date:** 19 July 2022

Introduction

Dental caries is still a worldwide problem including in Indonesia. Basic Health Research conducted by the Ministry of Health of the Republic of Indonesia in 2018 showed the D score of the DMFT Index for the Indonesian population is 4.4 with the average index of DMFT in permanent teeth is 7,1. The prevalence of dental caries in Indonesia is also showed a high percentage (88,8%).[1]

According to Chandra et al., (2014) stimuli on the pulp can cause changes in the pulp, both reversible and irreversible depending on the intensity, duration and severity of tissue damage and the body's defense system. Mild stimuli cause reversible inflammation (Reversible Pulpitis).[2] Indirect or direct pulp capping procedures are performed to treat reversible pulpitis to keep the pulp vital.[3]

Direct pulp capping is is a technique consist placing a biocompatible material into the pulp tissue that is accidentally exposed as a result of trauma or iatrogenic causes. The main characteristics of pulp capping materials are their biocompatibility.[4] The aim of this treatment is to stimulate the pulp tissue to form reparative dentin and keep the pulp vital.[5]

Calcium hydroxide has been the gold standard in the use of pulp capping materials. However, the use of calcium hydroxide still has physical limitations such as not adhering the material to dentin and dissolving the material in tissue fluid. According to Song et al., (2018) the

Corresponding author:
Tamara Yuanita
Faculty of Dental Medicine, Universitas Airlangga
St. Mayjen. Prof. Dr. Moestopo No. 47, Surabaya, Indonesia.
E-mail: tamara-y@fkg.unair.ac.id

dentinal bridge that is formed under calcium hydroxide has many imperfections, and there is a tunnel defect that allows bacterial penetration.[6] According to Andreasen et al., (2018), a study conducted by Cox reported that there were tunnel defects in 89% of the dentinal bridges that were formed under calcium hydroxide.[7] In addition, Ca (OH) 2 can also irritate vital pulp tissue and even cause necrosis due to its pH that is too high (12.5 - 12.8). Based on these limitations, recently various materials have been developed as an alternative to Direct Pulp Capping treatment to get the maximum effect and produce the most beneficial tissue response.[8]

c-FOS is one of the component transcriptional factor AP-1 (Acivator Protein-1) which regulates gene expression in response to stimuli. AP-1 plays a role in regulating DMP-1 transcription. AP-1 began to appear on the first day of mineralization and continued to increase until the 7th day [9]. Dentin 1 matrix protein (DMP-1) is an acid extracellular matrix protein that make a major contribution in the biomineralization process of calcium phosphate in bone and dentin.[10]

Cocoa (Theobroma cacao L.) is one of the most important agricultural commodities of Indonesia which has the opportunity to be developed towards product diversification with a fairly high selling value. Cocoa pods consist of cacao pods and cocoa beans. Cocoa beans are processed into chocolate products. In processing cocoa beans into chocolate products, it produces quite a lot of cocoa shell waste. Cocoa contains polyphenols which are potential natural antioxidants that can modulate the immune system and provide a chemopreventive effect and ward off free radicals. The total phenolic content reaches 611 mg GAE per serving, while the flavonoids reach 564 mg ECE per serving. [12]

Green tea is proven to be a high source of antioxidants and polyphenols. The content of chemical compounds such as catechins and other polyphenol compounds is also quite high in green tea and is very beneficial for health. The total phenolic content reaches 165 mg GAE and flavonoids up to 47 mg ECE. EGCG is the largest group of polyphenols in green tea that provide a strong antioxidant effect.[12]

Polyphenols play a role in immune and inflammatory responses through inhibition of the NFkB signaling pathway, inhibition of the MAPK signaling pathway and the arachidonic acid signaling pathway.[13] The purpose of this study was to analyze the effect of calcium hydroxide mixed with cocoa pod husk extract and calcium hydroxide mixed with green tea extract on c-FOS and DMP-1 expression in mice perforation dental pulp.

Materials and methods

Research Samples

Before conducting the research, ethical approval has been obtained from the Research Ethics Committee of the Faculty of Dentistry Faculty, Universitas Airlangga with the certificate number 213/HRECC.FODM/IV/2020. The total sample size of this study was 54 males Rattus novergicus aged 12-16 weeks with a body weight of 250-300 grams. The samples were collapsed into 3 groups, each group consisted of 18 rats. The concentration of cocoa pod husk extract used of this study was 3.125% and green tea extract was 0.8%.

Research Methods

The tools used were first disinfected with 95% alcohol. All rats were anesthetized with 100 mg ketamine (Ketalar, Warner Lambert, Ireland) (65 mg / kg body weight) and xylazine HCl (Rompun, Bayer, Leverkusen, Germany) dissolved in sterile phosphate buffered saline (PBS), and then all rats were placed on a container. Cleaning and disinfection were carried out on the occlusal tooth surface to be prepared using a cotton pellet which was previously immersed in a 95% alcohol. Preparation of Class I-like cavity was made on the occlusal surface of the right maxillary first molar until it reached the pulp roof using a low speed handpiece with a round diamond bur (SS White Dental, Lakewood, NJ. Pulp exposure was performed using k-file no. 10. The perforated teeth were rinsed with a saline solution and then dried with a cotton pellet.

In control group, Ca(OH)2 mixed with distilled water in a ratio of 1:1; in group treatment I, Ca(OH)2 mixed with cocoa pod husk extract in a ratio of 1:1; and in group treatment II, Ca(OH)2 mixed with green tea extract in a ratio of 1:1. The material was placed in contact of the pulp exposure and then the cavity was restored with Cention N (Ivoclar Vivadent, Schaan, Liechtenstein). The mice were returned to the cages after being tagged.

Rats from control and treatment group were eutenized after being treated depend to a

predetermined time by peritoneal injection to see the expression of cFOS and DMP-1. Eighteen rats were eutenized on day 7, 14 and 21. Each day consisted of 6 rats for each

Immunohistochemical (IHC) observations on cFOS and DMP-1 expression were performed using primary antibodies (monoclonal mouse) cFOS and DMP-1. (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 examinations were conducted blinded and separately by two examiners.The Shapiro-Wilk and Levene test were used to find out the distribution the homogeneity of data. Anova was used to test the difference with a significance level of α 0.05 followed by Tukey's test.

Results

Immunohistochemical (IHC) detection of c-FOS on day 7th, 14th and 21st can be seen on figure 1-3. The arrows show activated c-FOS on fibroblast cells in all groups A. Group control; B. Group Treatment I; C. Group Treatment II. While The average of c-FOS expression in each treatment group is shown in figure 4. The highest mean value of c-FOS expression in each group was on day 21st. The average was 8.83 for group control; 15,17 for group treatment I; and 15,33 for group treatment II

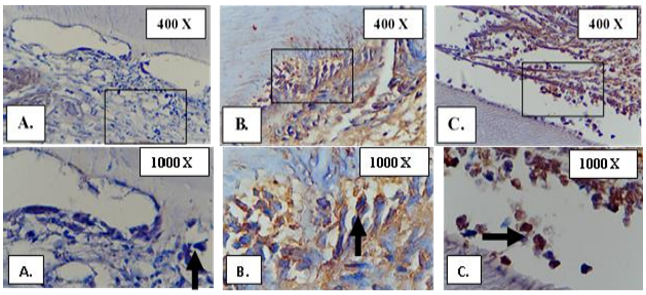

Figure 1. IHC detection of c-FOS on day 7 with a magnification of 400 times and 1000 times. The arrows show activated c-FOS on fibroblast cells in each group.

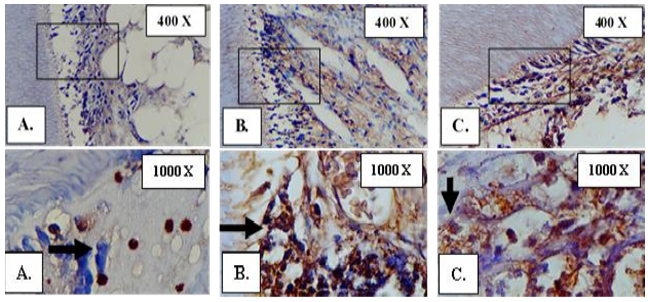

Figure 2. IHC detection of c-FOS on day 14 with a magnification of 400 times and 1000 times. The arrows show activated c-FOS on fibroblast cells in each group.

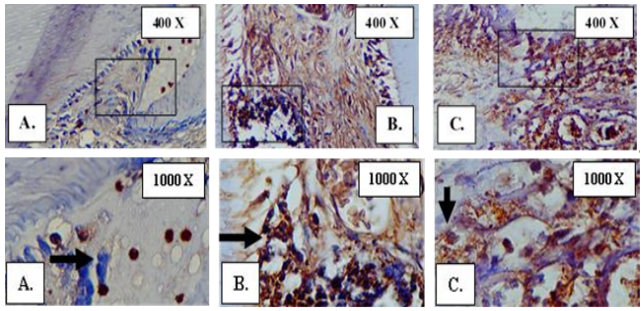

Figure 3. IHC detection of c-FOS on day 21 with a magnification of 400 times and 1000 times. The arrows show activated c-FOS on fibroblast cells in each group.

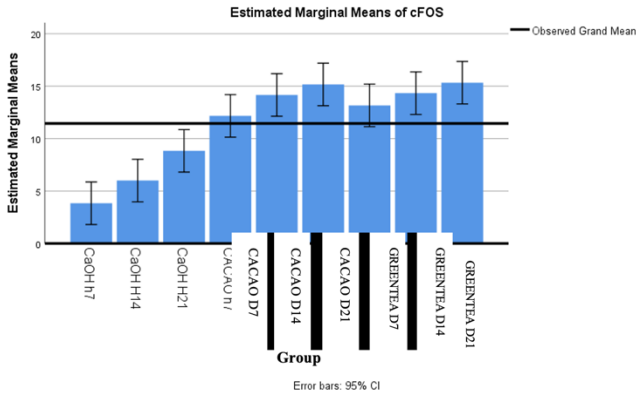

Figure 4. The mean value of c-FOS activation on day 7, 14, and 21.

IHC detection of DMP-1 on day 7, 14, and 21 can be seen on figure 5-7. The arrows show activated DMP-1 on fibroblast cells in each group. A. Group control; B. Group Treatment I; C. Group Treatment II. While The average of DMP-1 expression in each treatment group is shown in figure 8. The highest mean value of DMP-1 expression in each group was on day 21. The average was 8,00 for group control; 13,67 for group treatment I; and 14,33 for group treatment II.

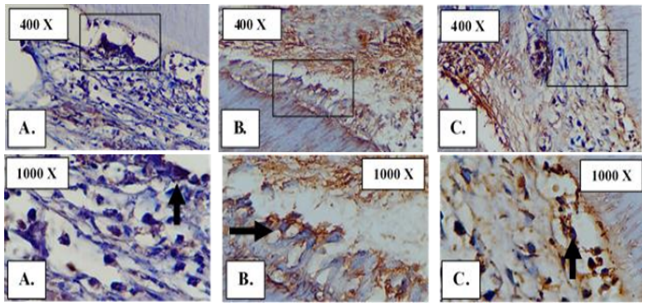

Figure 5. IHC detection of DMP-1 on day 7 with a magnification of 400 times and 1000 times. The arrows show activated c-FOS on fibroblast cells in each group.

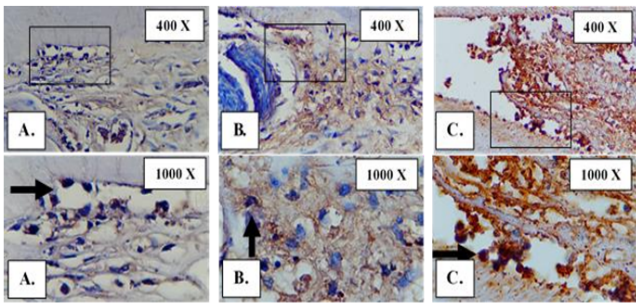

Figure 6. IHC detection of DMP-1 on day 14 with a magnification of 400 times and 1000 times. The arrows show activated c-FOS on fibroblast cells in each group.

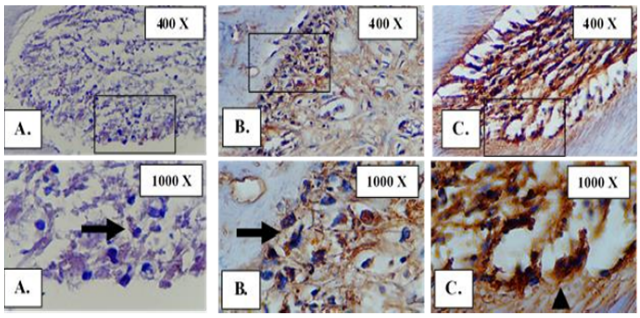

Figure 7. IHC detection of DMP-1 on day 21 with a magnification of 400 times and 1000 times. The arrows show activated c-FOS on fibroblast cells in each group.

Based on normality and homogenety test of c-FOS and DMP-1 activation, data were obtained with normal and homogeneous distributions. The results of the Anova test showed that the value of p = 0.000 (p <0.05). This shows that there are differences in c-FOS and DMP- 1 expression between treatment groups. To determine the different treatment groups, the Tukey HSD statistical test was carried out todetermine the effect of the treatment group on c-FOS and DMP-1.

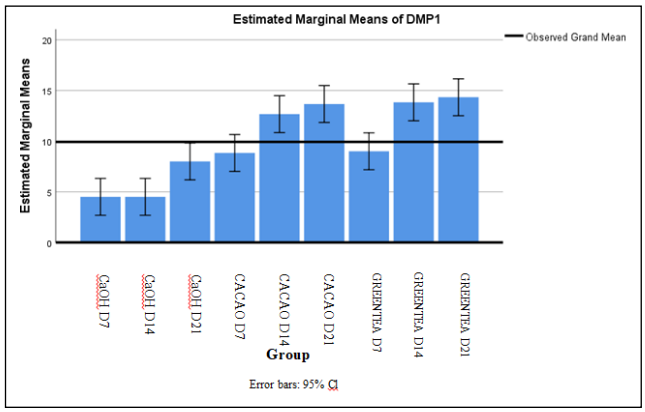

Figure 8. The mean value of DMP-1 activation on day 7, 14 and 21.

Group	Ca(OH)2 D7	Ca(OH)2 D14	Ca(OH)2 D21	Cacao D7	Cacao D14	Cacao D21	Green Tea D7	Green Tea D14	Green Tea D21
Ca(OH)2 D7				0.000 *			0.000 *		
Ca(OH)2 D14					0.000 *			0.000 *	
Ca(OH)2 D21						0.002 *			0.001 *
Cacao D7							0.999		
Cacao D14								1.000	
Cacao D21									1.000
Green Tea D7									
Green Tea D14									
Green Tea D21									

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

Table 1. The result of *Tukey HSD Test* on c-FOS activation on day 7, 14 and 21.

Table 1. Control group of Ca (OH)2 mixed with distilled water, treatment group I of calcium hydroxide mixed with cocoa pod husk extract and treatment group II of calcium hydroxide mixed with green tea extract has a p value <0.05. This shows that there is a significant difference in the expression of c-FOS between control and treatment groups. Meanwhile, the treatment I group of calcium hydroxide mixed with cocoa pod husk extract and treatment group II of calcium hydroxide mixed with green tea extract had a p value> 0.05. This shows that there is no significant difference in the expression of c-FOS between these groups.

Table 2. Control group of Ca (OH)2 mixed with distilled water, treatment group I of calcium hydroxide mixed with cocoa pod husk extract and treatment group II of calcium hydroxide mixed with green tea extract t has a p value <0.05. This

shows that there is a significant difference in the expression of DMP-1 between control and treatment groups. the treatment I group of calcium hydroxide mixed with cocoa pod husk extract and treatment group II of calcium hydroxide mixed with green tea extract had a p value> 0.05. This shows that there is no significant difference in the expression of DMP-1 between these groups.

Group	Ca(OH)2 D7	Ca(OH)2 D14	Ca(OH)2 D21	Cacao D7	Cacao D14	Cacao D21	Green Tea D7	Green Tea D14	Green Tea D21
Ca(OH)2 D7				0.036 *			.025 *		
Ca(OH)2 D14					0.000 *			.000 *	
Ca(OH)2 D21						.002*			.000 *
Cacao D7							1.000		
Cacao D14								0.991	
Cacao D21									1.000
Green Tea D7									
Green Tea D14									
Green Tea D21									

* There are signifficant differences (*p* value < 0,05). D| day

Table 2. The result of *Tukey HSD Test* on DMP-1 activation on day 7, 14 and 21.

Discussion

The results of the research on the expression of c-FOS and DMP-1 on day 7th 14th and 21st showed that the effect of the combination of Ca (OH)2 and aquades showed the lowest mean value compared to the other two groups. The highest mean value was shown by the treatment group II, Ca (OH)2 mixed with green tea extract, followed by the combination group of Ca (OH)2 and the extract of the cocoa pod with no significant difference. This condition indicates that the use of cocoa pod husk extract and green tea extract as a mixing agent for calcium hydroxide has been shown to increase the expression of c-FOS and DMP-1 as indicated by the average expression of c-FOS and DMP-1 which is higher in both groups. compared with the combination group of Ca (OH)2 with distilled water. In calculating the number of fibroblast cells, the highest mean was found in the treatment group II, Ca (OH) 2 mixed with green tea extract on the 21^{s}day, namely 15.33 for c-FOS and 14.33 for DMP-1.

This is because the high EGCG content intea extract greencan inhibit the translation of NF-kB into the cell nucleus. According to research EGCG will inhibit the signal transduction pathway by IKK / NF-kB because it inhibits IKK phosphorylation, resulting in IkB inhibition, consequently decreasing NF-kB activity and inhibiting TNF-a expression so that there is a limitation in the number of inflammatory cells that migrate to the wound area.

Green tea extract contains catechins, the types of catechins are EGCG, ECG, EGC, and EC. EGCG is the most powerful free radical scavenger compared to other catechins. The ability of EGCG to scavenge free radicals by providing stable phenol radicals is due to the presence ofgroups *galloyl* in the B and D rings that cancapture OH and Ofree radicals2-simultaneously.[14]

In addition, EGCG also has antioxidant properties that can inhibit the production of inducible nitric oxide synthase (iNOS) and reduce levels of Nitric Oxide (NO) and inhibit TNF-induced ROS and cell death. The release of nuclear factor E2-related factor2 (Nrf2) will move to the nucleus and together with other transcription factors, activate the transcription of genes containing Antioxidant Response Element (ARE) in the promoter region which produces an inhibitory effect on NF-κB activity. On the other hand, ECH-related protein 1 (Keap1) such as Kelch is separated from the Nrf2-Keap1 complex and directly interacts with IKKβ and suppresses NF-κB function. Green tea also contains carotenoids, tocopherols, ascorbic acid (vitamin C), minerals such as Cr, Mn, Se or Zn, and certain phytochemical compounds that can increase the antioxidant potential of green tea polyphenols.[15,16,17]

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 platelet-derived growth factor (PDGF), transforming growth factor β (TGFβ) and fibroblast growth factor (FGF) which stimulates the migration and proliferation of fibroblasts cells.[18]

Conclusions

There were no statistically significant differences between groups indicates that both of them have relatively the same anti-inflammatory and antioxidant properties so their active substances have the effect same effect to increase the number of c-FOS dan DMP-1 expressions in mice perforation dental pulp.

Declaration of Interest

The authors declare that there are no conflicts of interest.

References

1. Basic Health Research. Ministry of Health Research and Development in 2018. Health Research and Development Agency; 2018: 207-10.
2. Chandra BS, V Gopikrishna. Grossman's Endodontic Practice 13th Ed, Wolters Kluwer (India) Pvt. Ltd., New Delhi; 2014:32-3
3. Boutsiouki C, Frankenberger R, Krämer N. Relative effectiveness of direct and indirect pulp capping in the primary dentition. Eur Arch Paediatr Dent. 2018;19:297–309.
4. Paula AB, Laranjo M, Marto CM, et al. Evaluation of dentinogenesis inducer biomaterials: an in vivo study. Journal of Applied Oral Science. 2020; 28: e20190023.
5. Kamal EM, Nabih SM, Obeid RF, Abdelhameed MA. The reparative capacity of different bioactive dental materials for direct pulp capping. Dent Med Probl. 2018;55(2):147-152. doi: 10.17219/dmp/90257. PMID: 30152617.
6. Song M, Kim S, Kim T, et al. Development of a Direct Pulp-capping Model for the Evaluation of Pulpal Wound Healing and Reparative Dentin Formation in Mice. J Vis Exp. 2017; (119): 54973.
7. Andreasen JO, Andreasen FM, Andersson L. Textbook and Color Atlas of Traumatic Injuries to the Teeth. Stomatology Edu Journal. 2019; 6(4):279
8. Rosa WLOd, Cocco AR, Silva Md, Mesquita LC, Galarca AD, Silva AFd, Piva D. Systematic review of dental pulp capping materials. J Biomed Mater Res B Appl Biomater. 2018;106(3):1358-68 10.1016/j.dental.2016.08.185.
9. Sonoda S, Mei YF, Atsuta I, et al. Exogenous nitric oxide stimulateis the odontogenic differentiation of rat dental pulp stem cells. Sci Rep. 2018;8(1):111. doi:10.1038/s41598- 018-21183-6.
10. Porębska A, Różycka M, Hołubowicz R, Szewczuk Z, Ożyhar A, Dobryszycki P. Functional derivatives of human dentin matrix protein 1 modulate morphology of calcium carbonate crystals. The FASEB Journal. 2020; 34: 6147–65. https://doi.org/10.1096/fj.201901999R.
11. Yuanita T, Drismayant I, Dinari D, Teja L. Effect of Calcium Hydroxide Combinations with Green Tea Extract and Cocoa Pod Husk Extract on p38 MAPK and Reparative Dentine. Journal of Contemporary Dental Practice. 2020; 21(11):1238-44; https://doi.org/10.5005/jp-journals-10024-2950
12. Shahidi F, Alasalvar C. Handbook of Functional Beverages and Human Health, CRC Press. Boca Raton; 2016:690
13. Yahfoufi N, Alsadi N, Jambi M, Matar C. The Immunomodulatory and AntiInflamatory Role of Poyiphenols. Nutrients. 2018, 10(11): 1618
14. Singh V, Kuldeep, Bajaj A, Kalra M. Green tea: enhanching oral health. University J Dent Science. 2016; 2(1):61-3
15. Leopoldini M, Russo N, Toscano M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chemistry. 2011; 125(2):288-306.
16. Ismiyatin K, Subiyanto A, Suhartono M, Sari PT, Widjaja OV, Sari RP. Efficacy of topical hydrogel Epigallocatechin-3-gallate against neutrophil cells in perforated dental pulp. Dent J (Majalah Kedokt Gigi). 2020; 53(2): 1-5.
17. Cabrera C, Artacho R, Gime´nez R. Beneficial Effects of Green Tea—A Review. Journal of the American College of Nutrition. 2006; 25(2): 79–99.
18. Widjiastuti I, Subiyanto A, Ningtyas EK, Popyandra R, Kurniawan MG, Retnaningsih FD. Propolis extract as pulp capping material enhances odontoblast-like cell thickness and type 1 collagen expression (in vivo). Dent J (Majalah Kedokt Gigi). 2020; 53(1): 1– 5.