AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 63, Issue 12, December, 2023

The Effect Of Herbal Extracts Added To Calcium Hydroxyde As A Potential Direct Pulp Capping Material From A Biological Marker Perspective : A Systematic Review

Dewi Puspitasari[1,2], Kurnia Fatwati[3], Erni Marlina[4], Maharani Laillyza Apriasari[5], Stang[6], Maria Tanumihardja[7*]

Doctoral Program, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia[1]
Department of Dental Material, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia[2]
Department of Dental Material, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia[3]
Department of Oral Medicine, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia[4]
Department of Oral Medicine, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia[5]
Department of Biostatistics, Faculty of Public Health, Hasanuddin University, Makassar, Indonesia[6]
Department of Conservative Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia[7]

Corresponding Author: 7*

Keywords:
Biomarkers, Direct Pulp Capping, Herbal extract, Reparative Dentin.

ABSTRACT

This study was aimed to evaluate the biomarkers used to mark the formation of reparative dentin on an exposed pulp following application of combination of herbal extracts with $Ca(OH)_2$ as a potential pulp capping material. Databases are obtained from PUBMED, Wiley Online Library, Google Scholar and ScienceDirect for the last 10 years from 2013 to 2023. The keywords used to search for electronic articles are: (herbal extract OR natural product OR natural medicine) AND (direct pulp capping) AND (reparative dentin). Screening of full-text articles included in the systematic review based on inclusion and exclusion criteria. A structured Population-Intervention-Comparison-Outcome of the review was as follows: Population: formation of reparative dentin; Intervention: combination of herbal extracts and $Ca(OH)_2$; Comparison: $Ca(OH)_2$; Main Outcome: biomarkers. The study selection process was included in the data using PRISMA-2020. We assessed the risk of bias using SYRCLE's Rob tool. The search yielded a total of 8 articles that fulfilled the eligibility criteria. We found the biomarkers used to evaluate the herbal extract (cocoa pod husk, green tea, propolis, mauli banana stem) combined with $Ca(OH)_2$ on the reparative dentin formation process, namely macrophage, NF-kβ, TNF-α IL-1, IL-8, IL-12, IL-6, IFN δ; IL-10, p38 MAPK, VEGF, TGF-β, DMP-1, ALP and type 1 collagen. Studies showed that limited type of biomarker used to evaluate the effect the herbal extract combined with $Ca(OH)_2$ as the potential pulp capping material on the process of reparative dentin formation. Further studies should be directed to evaluate other biomarkers to asses

reparative dentin formation.

1. Introduction

Biomarker or biological marker is defined as a characteristic that is objectively measured and assessed as an indicator of a normal biological process, a pathogenic process, or a pharmacological response to a treatment intervention. Basically, biomarkers can be considered and utilized in clinical settings as responses that are functional, psychological, biochemical at the cellular level or molecular interactions. Biomarkers are widely used in research and have been proven in the medical field to established the diagnosis and treatment, for example molecules expressed throughout the dental pulp inflammatory cascade have the potential to be employed as (diagnostic) biomarkers for the presence of inflammation in the dental pulp [1- 3].

Dental pulp is soft connective tissue, consist of cells, extracellular matrix, blood vessels and nerves. Odontoblasts are the principal cells of the dentin-pulp complex [4]. Odontoblasts form a single layer and synthesize extracellular matrix subsequently forms dentin. Dentin matrix formation through odontoblast differentiation during the mineralization process can be identified and quantified at the protein or gene expression level [5]. Several proteins secreted by odontoblasts and frequently used for biomarkers to analyze odontoblast activity include Dentin matrix protein-1 (DMP-1) and osteocalcin [6], [7].

When the pulp is exposed to injury due to mechanical trauma, chemicals and bacteria, the pulp will produce a defense reaction in the form of an inflammatory response and an immune response, which can be reversible or irreversible. In reversible pulp inflammation, treatment can be carried out to maintain the vitality of the dental pulp with a purpose to form reparative dentin or a dentinal bridge [2].

Reparative dentin is a tertiary dentin matrix secreted by new odontoblast-like cells, following the death of the original postmitotic odontoblasts cells that responsible for the secretion of primary or secondary dentin [8]. In some cases, formation of reparative dentin is not dense enough due to dentinal bridge discontinuity that creates areas of necrosis called “tunnel defect” that has been one of the weaknesses of calcium hydroxide. This is one reason that clinicians search for alternative materials, including natural herbal extracts that can replace or to be combined with calcium hydroxide [9].

Previous studies suggested that caffeic acid phenethyl ester (CAPE), an active component of propolis, can increase the production of TGF-β1 to mark an initial stage in the process of odontoblast-like cells proliferation. Moreover, CAPE can inhibit the Nuclear Factor-κB (NF-kB) pathway resulted in down regulation of proinflammatory cytokine genes, such as IL-1, IL-6, IL-8, and TNF-α. The NF-kB is a transcription factors and considered to be a regulator of innate and adaptive immunological processes, as well as a key mediator of the inflammatory response [9], [10].

Several studies reported that cocoa polyphenols can reduce the effects of inflammation by significantly preventing tumor necrosis factor α (TNF-α) that induced NF-kB translocation. These events are partially mediated by members (isoforms) of Mitogen-activated protein kinases (MAPK) family, especially p38. Other isoforms are ERK1/2, and JNK. MAPK are found in the protein cascade, which regulates gene

ISSN: 0005-2523
Volume 63, Issue 12, December, 2023

expression, cell proliferation, differentiation, motility, and apoptosis. The p38 MAPK signaling pathway is important in the migration and dentinogenesis of human dental pulp cells, especially in the repair response. Cocoa polyphenols may activate and phosphorylate ERK1/2 and p38 MAPK but substantially inhibit JNK activation [11], [12].

Green tea, other herbal extract is considered as a rich-food source of antioxidants that contain rich in polyphenols. The largest group of polyphenols in green tea that provide strong antioxidant effects are epigallocationchin gallate (EGCG). EGCG is able to reduce the expression of pro-inflammatory cytokines and reduce TLR4 resulting in down regulation of TNF-α expression and decrease free radicals that can oxidize tissue, therefore preventing destruction of cell membranes [12], [13].

Reparative dentinogenesis involves more complex biological events than reactionary dentinogenesis, due to involvement of progenitor cells and induction of differentiation of odontoblast-like cells before the secretion of reparative dentin matrix occurs. Growth factors play an important role in reparative dentinogenesis, as regulators of several cell functions such as proliferation, differentiation and matrix synthesis [8]. Various types of growth factors are involved in the regeneration of the pulp complex, including Transforming Growth Factor-β (TGFS-β), Bone Morphogenetic Protein (BMPs), Platelet Derived Growth Factor (PDGF), Insuline-like Growth Factor (IGF) and Fibroblast Growth Factor (FGFs) [8]. Basically, growth factors will be released in the dentin matrix as compensation for tissue injury and restorative procedures [14]. Studying growth factors will provide a basis for understanding the biological mechanisms of tissue regeneration in the dentin-pulp complex and the effects of endodontic treatment. The purpose of writing this systematic review is to discuss the effect of herbal extract combined with calcium hydroxide, as potential direct pulp capping material via biological markers that involved in the process of reparative dentin formation.

2. Methods

This review is a systematic analysis of literature reviews obtained through article data sources from electronic scientific journal databases PUBMED, Wiley Online Library, Google Scholar and ScienceDirect with search and data processing techniques that follow the principles of the Preferred Reporting Items for Systematic Reviews and Meta-analyses strategy (PRISMA 2020) and the results of data synthesis are described quantitatively.

2.1 Eligibility Criteria

Inclusion and exclusion criteria in the review were detailed using PICOS (Population; Intervention; Comparator; O: Outcome(s); Study design). The subject of this article is literature data obtained through the electronic scientific journal databases PUBMED, Wiley Online Library, Google Scholar and ScienceDirect. Studies were eligible for inclusion in the review with rat animal samples focusing on cases of reversible pulpitis with direct pulp capping therapy using herbal extracts, either pure or in combination with other vital pulp treatment materials such as Calcium Hydroxide. The comparator in this study is direct pulp capping using gold standard material in the form of Calcium Hydroxide. The articles used are full text and in English for the period 2013 to 2023. The studies used in this review were experimental on rat. This article presents an overview of biological markers used to evaluate the proccess of reparative dentin formation using hematoxylin-eosin (HE) and immunohistochemical (IHC) staining methods.

2.2 Search Methods for Identification of Studies

Searches for electronic articles are carried out in electronic scientific journal databases using keywords that follow the rules of "Boolean Operators", namely an article search method that combines the words "OR",

"AND", and "NOT". The databases used in this research are PUBMED, Wiley Online Library, Google Scholar, and ScienceDirect. The keywords used to search for electronic articles are: (herbal extract OR natural product OR natural medicine) AND (direct pulp capping) AND (reparative dentin).

2.3 Data Collection and Analysis

Title and abstract searches were conducted and assessed to obtain the full text of relevant articles for review. Search for electronic articles using keywords that have been determined in the search strategy in the journal database, namely (herbal extract OR natural product OR natural medicine) AND (direct pulp capping) AND (reparative dentin) with the journal search year set 2013 - 2023. Several articles are available, those identified through the search strategy will then be filtered by the author based on the title. Articles that are potentially relevant based on the title will be selected; and will be excluded the irrelevant. If the abstract is unclear or not available but the title seems relevant, then the article will be selected to be read in full-text.

Full-text articles will be searched for and read in detail by the author to screen for full-text eligibility aspects by looking at the suitability of the title and content based on predetermined inclusion criteria. Articles are then extracted in depth to see if they fall within the predetermined inclusion criteria, and if it does not meet the inclusion criteria, the article is deleted. Data were collected from the articles that had been obtained, which included Author's name, year of publication, sample, number of samples, research treatment, biomarkers of reparative dentin formation, time of formation, significance, and analysis methods. Data processing follows the principles of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA 2020) strategy, and the results of data synthesis are described quantitatively. The study selection process is summarized in the following figure.

3. Results

The search was carried out using a combination of keywords: herbal extract, natural product, natural medicine, direct pulp capping, and reparative dentin. A total of 120 articles were identified through database searches (PubMed = 8 articles, Wiley Online Library = 22 articles, Google Scholar = 1020 articles, Science Direct = 160 articles). A total of 1042 duplicates articles were excluded and finally a total of 168 articles were obtained. In the initial stage, the title was screened, then the abstract of the identified articles was screened. A total of 116 articles were excluded because they were irrelevant based on the title and abstract, while 52 other articles were included for full-text evaluation. A total of 17 articles were then checked for eligibility using the PICO method so that 8 articles met the eligibility criteria and were included in the review. The flow diagram of search results and study selection in this review is outlined in Figure 1.

ISSN: 0005-2523
Volume 63, Issue 12, December, 2023

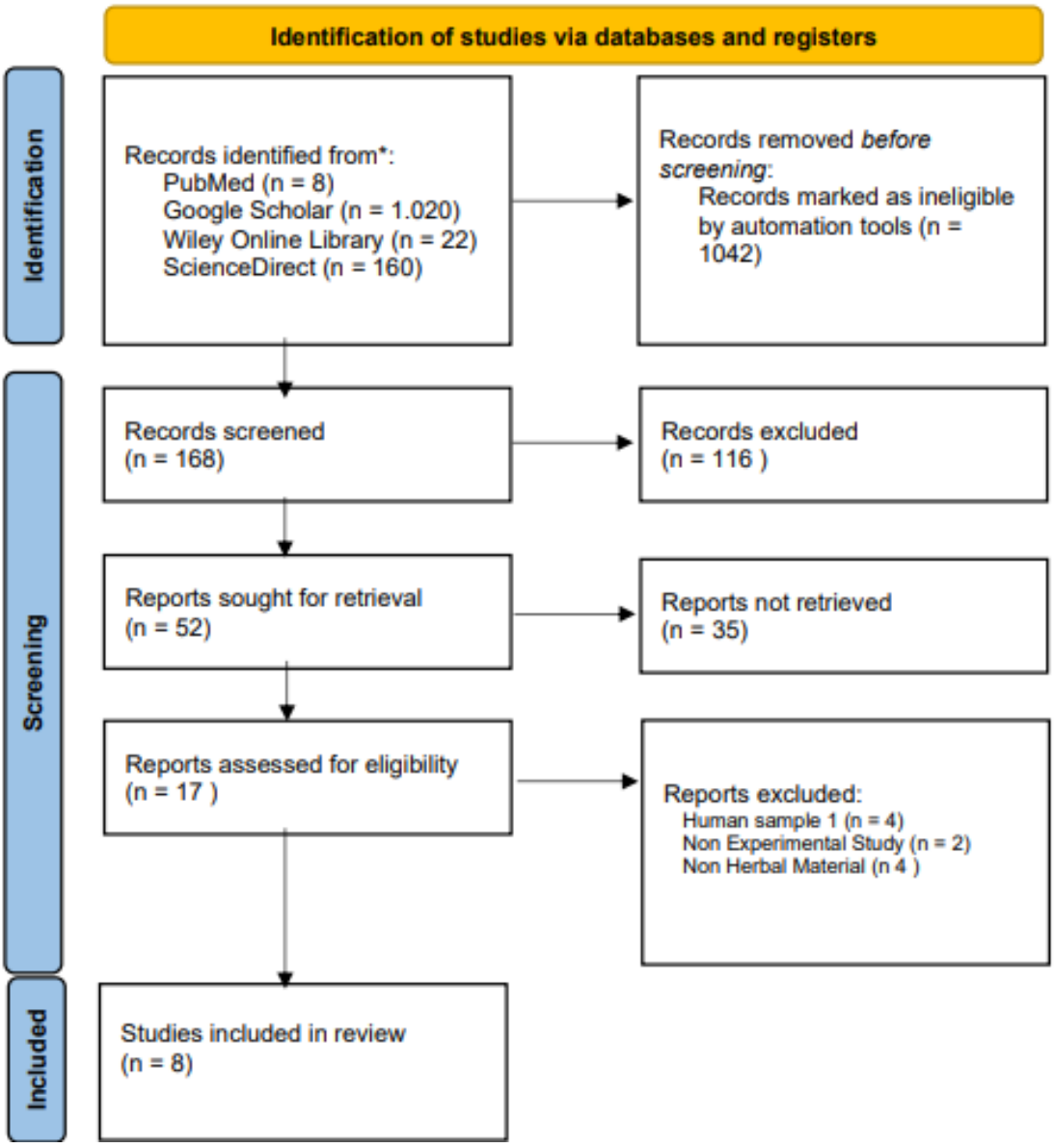

Figure 1. Flowchart of the systematic review

Table 1. Biomarkers of the included animal studies

No	Writer	Sample	Intervention	Biomarker	terms of time	Result	Sig	Analysis	Conclusion
1	[12]	36 wistar rats	cocoa pod husk extract and green tea extract	p38 MAPK and reparative dentin	Day 28	significant difference between the positive control group and the test groups for the average p38 MAPK activation value on day 7 and 28.	yes	IHC	The use of combination calcium hydroxide with green tea extract and combination calcium hydroxide with cocoa pod husk extract have significant effect on p38 MAPK activation and wide area of reparative dentin in wistar rats
2	[15]	30 wistar rats	Propolis extract	MMP-1 dan Collagen type 1	Day 7	the majority of cells in the Calcium Hydroxide and Propolis extract group showed	yes	IHC	Compared to calcium hydroxide to a combination of Ca (OH)2 and Propolis extract, MMP1 expression was lower and collagen type 1 density was higher in the rat's

						Collagen type 1 density, whereas the least number of cells showed MMP-1.			pulp.
3	[16]	80 Sprague Dawley rats	Propolis extract	Collagen type 1	Day 7	No statistically significant differences of collagen type I density among the groups for each time period were found	yes	IHC	Propolis and flavonoid propolis may increase collagen density on inflamed rat dental pulp.
4	[17]	80 Sprague Dawley rats	Propolis extract	IL-6	6^{th} hour. Day 2	All agent showed IL-6 expression in inflamed rat dental pulp tissue, and this expression was decreased with the longer of observation time periods. EEP more stronger to decreased IL-6 expression on inflamed rat dental pulp tissue than other agent.	yes	IHC	Trigona sp. propolis from south Sulawesi, Indonesia could suppressed the expression of IL-6 on inflamed rat dental pulp tissue.
5	[18]	60 Wistar rats	cocoa pod husk extract and green tea extract	C-Fos and DMP-1	Day 21	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	yes	IHC	Both cocoa and green tea mixed with calcium hydroxide have relatively the same anti- inflammatory and antioxidant properties so their active substances have the same effect to increase the number of c-FOS and DMP- 1 expressions in mice of dental pulp perforation.
6	[19]	30 wistar rats	Propolis extract	Fibroblast	-	a significant difference between the calcium hydroxide group and the calcium hydroxide-propolis group. Increased numbers of fibroblasts and angiogenesis indicate a faster inflammatory process that affects the acceleration of wound healing	yes	IHC	More number of fibroblasts and angiognesis cells found in the pulp after the application of a combination of Calcium hydroxide-propolis compared to the application of Calcium hydroxide
7	[10]	27 Wistar rats	Propolis extract	IL-10, IL-8, TGF-ß, VEGF dan TLR-2	Day 7 and 14	Day 7 : combination of propolis and $Ca(OH)_2$ showed significantly increased expression of IL-10, VEGF, IL-8,	yes	IHC	Combination of propolis and Ca(OH)2 can repair pulp tissue damage through the formation of reparative dentine. On the 14th day with propolis and Ca(OH)2 combination, VEGF

ISSN: 0005-2523
Volume 63, Issue 12, December, 2023

						TGF-β, and TLR-2 compared with the negative control. Day 14: combination of propolis and calcium hydroxide increases VEGF			expression was high so that it can help the process of angiogenesis in the dental pulp to be faster, while the group with only $Ca(OH)_2$ has anti-inflammatory properties and high IL-10 expression so as to reduce inflammation which occurs in the pulp chamber.
8	[20]	18 Wistar rats	mauli banana stem (Musa acuminata) extract gel 37.5%	Macrophage	Day 3	significant difference between mauli banana extract gel concentration 37.5% with calcium hydroxide group with p value = 0,001 and with HPMC gel group	yes	HE	Provision of mauli banana extract gel 37.5% concentration can increase the number of macrophage cells on pulp inflammation on the 3^{rd} day.

3.1 Bias Of Risk

Each animal study's methodology was assessed using SYRCLE's Rob tool (Systematic Review Centre for Laboratory animal Experimentation), an adapted version of the Cochrane RoB tool with ten items to identify sources of bias. The following domains (10 items) were examined in accordance with the guidelines: sequence generation, baseline characteristics, allocation-concealment, random housing, blinding personnel, random outcome assessment, blinding outcome assessors, incomplete outcome data, selective outcome reporting and other sources of bias. Each domain was evaluated based on signaling questions with three distinct answers (judgements) that characterised the risk of bias: a yes, no or unclear judgement coincided with a low, high or unclear risk of bias. Each domain received a yes judgement if all or most of the details provided for the signaling questions were addressed. The SYRCLE evaluation is presented in figure 2. A study scored low risk across all domains, four studies some concern, and three studies scored high risk. However, the overall risk for all studies was considered to be in "some concerns" due to susceptibility to bias was resulted from no explicit report of the domain and no differences was observed in baseline result between intervention and comparator groups.

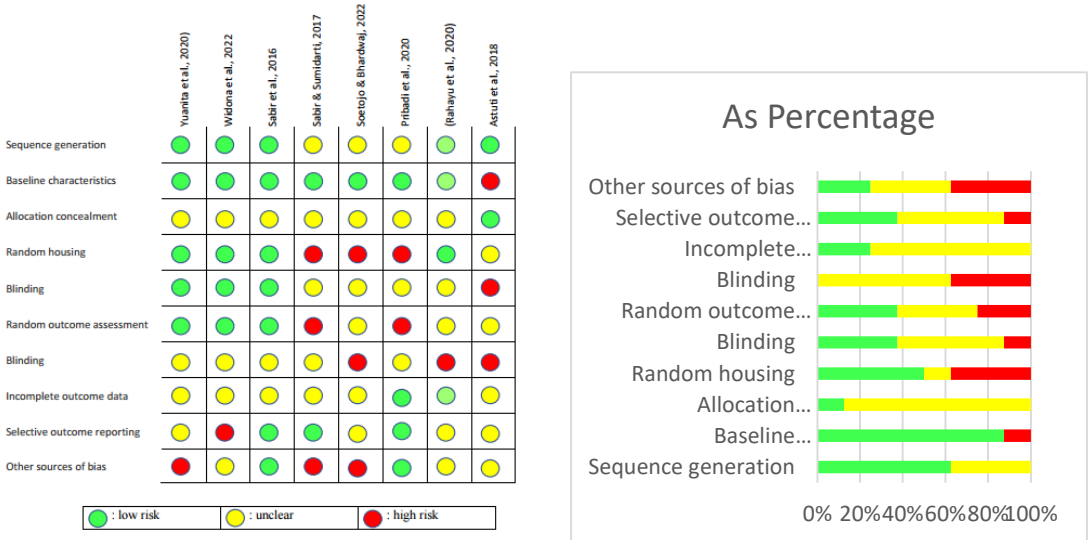

Figure 2. SYRCLE risk of bias tool for included studies.

4. Discussion

Preserving the vitality of pulp is aimed to the formation of reparative dentin in response to those pathological external stimuli and part of the defensive reaction of the dentin-pulp complex [21]. The dentin-pulp complex is capable of repair following dental injuries or exposed pulp, caused by caries removal (caries exposure), attrition, and dental procedures (cavity preparation) [21], [22].

Reparative dentin formation involves three consecutive phases, namely the haemostasis and blood clot formation phase; the inflammatory phase occurs on the 1st to the 5th days; the haemostasis phase in some literature is contained within the inflammatory phases; the proliferation phase on the 3rd to the 7th days after treatment, continued with the formation of collagen as well as non-collagen proteins on the 5th to 14th days following treatment, and the remodeling phase until dentinal bridge as a reparative dentine is formed more than 14 days following the treatment [8], [23], [24]. When the inflammatory process is continuing, it may proceed to pulp necrosis [23].

All articles showed the herbal extracts added to calcium hydroxide applied to the exposed dental pulp of rats for direct pulp capping have been assessed in terms of condition of the pulp (inflammatory state of pulp tissue) and dentin bridge development. Until now, the gold standard for assessing the development is still histological investigation [3], [21]. In the result, we found that the research of herbal extracts more conducted in rodent model due to their teeth's cellular structure and functions are identical to those of human teeth. Furthermore, animal models are necessitated for validating biological effects or safety before clinical trial [25]. Biomarker for direct pulp capping from the inflammatory phase until the formation of reparative dentin can be seen from histological assessment.

4.1 Effect of herbal extracts on the inflammatory phase and biological marker used

Inflammatory response begins with a hemostatic phase characterized by a vasodilatation, increased vascular permeability and the release of inflammatory cells (monocytes, macrophages, dendritic cells, mast cells and neutrophils) [23].

Many dental pulp cells, such as fibroblasts, odontoblasts, neurons, macrophages, endothelial cells, stem cells, and polymorphonuclear cells (PMN) express intracellular and extracellular receptors that may recognize component of bacteria. Toll-like receptors (TLRs) are the most well-characterized receptor family in pulp. They are capable of detecting a wide variety of Pathogen associated molecular patterns (PAMP), including cell wall-derived lipopolysaccharides and bacterial DNA [23]. It has been reported that there are changes in the expression of TLR-2 and TLR-4 mRNA within 3 to 9 hours after inflammatory insults [26]. TLR-2 and TLR-4 were also detected on reversible pulpitis tissues. The consequences of TLR activation include the release of various cytokines that mediate the innate immune response by regulating phagocytosis and triggering antimicrobial activity [27].

TLR involvement activation recruits several adaptor molecules, such as Myeloid differentiation primary response 88 (MyD88), then activates MAPK p38 and NF-kß to produce proinflammatory cytokines [4]. There are three well-known MAPK groups containing ERK, p38, and JNK [28]. Furthermore, NF-kß is a transcription factor that produces pro-inflammatory cytokines that are the primary target of pulp inflammation [4].

Activation of NF-kβ will release various pro-inflammatory cytokines including TNF-α, IL-1, IL-8, IL-12, IL-6, and IFN δ [23] The cytokines IL-8, IL-1, TNF-α are expressed by odontoblasts, functioning to eliminate bacterial components (for example, LPS via the TLR signaling mechanism). IL-8 is essential in

ISSN: 0005-2523
Volume 63, Issue 12, December, 2023

neutrophil recruitment and activation. The cytokine TNF-α will stimulate macrophage cells to phagocytose microbes and necrotic cells in areas of inflammation [29].

Macrophages play an indispensable role in the inflammatory response and regeneration of many tissues. Macrophages are found in the wound area from the inflammatory phase to the healing phase. Macrophages are divided into three phenotypes: unpolarized M0, M1 and M2 macrophages. M0 macrophages polarize towards M1 macrophages in response to interferon gamma (IFN-γ) produced by type 1 helper T lymphocytes, and bacterial lipopolysaccharide (LPS) [30]. M1 macrophages show an inflammatory response to pathogens and foreign bodies by secreting various proinflammatory cytokines or toxic effector molecules, such as interleukin 1β (1L-1β), tumor necrosis factor alpha (TNF-α), IL-6, reactive oxygen species, and nitric oxide [31]. M2 macrophages produce anti-inflammatory factors, such as transforming growth factor beta (TGF-β) [32], IL-10, and angiogenic vascular endothelial growth factor [33].

Interleukin-6 (IL-6) is a multifunctional cytokine synthesized in response to stimuli such as inflammation and trauma. The activity of interleukin-6 in inflammation has a dual effect, namely as anti-inflammatory, but also as pro-inflammatory in chronic diseases [16], [34]. Interleukin-6 displays a wide range of biological effects and acts as a key mediator of host responses following tissue injury and infection and inflammation. The anti-inflammatory and immunomodulatory properties of propolis and its constituents have been studied by a number of researchers. The results showed that 6 hours after application of EEP (Ethanolic Extract Propolis) and $Ca(OH)_2$, only weak IL-6 expression occurred in the inflamed dental pulp of mice. In contrast, flavonoids and non-flavonoids derived from propolis stimulated moderate and strong IL-6 expression in the same time period, but this expression decreased with the longer observation period. In addition, EEP appeared to be stronger than other test materials in inhibiting IL-6 expression in inflamed rat dental pulp tissue [16]. Additionally, IL-6 levels may be associated with severe inflammation as well as clinical symptoms [16]. The result of this systematic reviews not only show the propolis extract combined with Ca(OH)2 but also show the activity of propolis extract only that compared to $Ca(OH)_2$. The study of Sabir (2016) and Sabir (2017) show that propolis extract alone may increase collagen density and downregulate expression of IL-6 on inflamed rat dental pulp [16], [17]. Another study also proved that 37.5% mauli banana stem extract showed a higher number of macrophage cells on pulp inflammation on the 3^{rd} day compared to $Ca(OH)_2$ [20].

The study by Elsalhy (2013) showed that IL-8 levels and the IL-6/IL-10 and IL-8/IL-10 ratios have the potential to be indicators of dental pulp inflammation in cases of caries exposure. IL-8 is constantly expressed by odontoblast cells. In situations of pulp inflammation, IL-8 promotes neutrophil recruitment to aid in the healing process [35]. In the study of Rahayu (2020) showed IL-8 levels were lower in the propolis and $Ca(OH)_2$ combination group compared to the $Ca(OH)_2$ alone group [10].

The inflammatory process is complete when the number of macrophages has decreased, and entered the initial stage of proliferation, fibroblast cells and blood vessels will form granulation tissue and collagen which then mineralizes to form reparative dentin [36].

4.2 Effect of herbal extracts on the proliferation phase and biological marker used

The proliferative phase often coexists with the inflammatory phase. Later in the healing process and transition from the inflammatory to the proliferative phase, macrophages switch from a proinflammatory M1 phenotype to a reparative M2 phenotype, expressing anti-inflammatory mediators such as IL-10 and TGF-β, resulting in matrix deposition and tissue remodeling. The M1–M2 transition is critical for the resolution of inflammation and provides balance to tissue repair [36] [33].

In the formation of reparative dentin, TGF-β has an important role in regulating cell proliferation, differentiation and reparative dentinogenesis [10]. The TGF-β release from the dentinal matrix requires diffusion which generally passes through the dentin tubules to the pulp cells and stimulates the migration of dental pulp cells toward the odontoblastic layer. In order to replace odontoblasts that have been irreversibly damaged, dental pulp stem cells are drawn to the site of injury and differentiate into a second generation of odontoblasts or odontoblast-like cells [29]. This is in accordance with research results which show that by combining propolis with $Ca(OH)_2$, the expression of TGF-β is higher than the treatment group that only received $Ca(OH)_2$ [10], [29], [37].

TGF-β is a growth factor that also influences angiogenesis activity and is essential for vascular maturation and remodeling. TGF-β stimulates not only fibrous tissue deposition and fibroblast proliferation, but also collagen production. The angiogenesis process is stimulated by local factors from the microenvironment including low oxygen tension because in the inflammatory phase there is an increase in vascular permeability and reactive oxygen species (ROS) which causes the accumulation of vasoactive molecules. The increase in the number of neovasculature on day 7 aims to provide an adequate supply of oxygen and nutrients in the tissue repair process [19]. Oxygen levels in tissues directly regulate angiogenesis through interactions between proteins that regulate transcription of angiogenic and antiangiogenic genes. Synthesis of the angiogenic factor Vascular endothelial growth factor (VEGF) by capillary endothelial cells is directly increased by hypoxia through activation of the transcription factor hypoxia-inducible factor (HIF), which binds oxygen when oxygen levels around capillary endothelial cells fall. HIF-1 binds to specific DNA sequences and stimulates the transcription of specific genes such as VEGF that promote angiogenesis. When oxygen levels in the wound tissue increase, oxygen binds to HIF causing the destruction of HIF molecules in cells and a decrease in the synthesis of angiogenic factors. Binding of angiogenic factors causes capillary endothelial cells adjacent to the devascularization site to begin migrating into the matrix and forming new cells [38].

Proliferation and migration of stem/progenitor cells continue to complete the healing process by the differentiation of new pulp tissue and odontoblast-like cells that produce reparative dentine during the remodeling phase [23].

4.3 Effect of herbal extracts on the remodeling phase and biological marker used

The final phase of the repair process involves tissue formation and maturation. In the case of repair of the mineralized dentin barrier in pulpitis, fibroblast and progenitor cells activated in the proliferation phase may differentiate into odontoblast-like cells [23], [39]. However, the signals required to activate this differentiation process are complex and have not been fully elucidated [23].

Regardless of their origin and nature, these cells form a collagen matrix which then undergoes mineralization to form reparative dentin. Cells undergo a mitotic process to differentiate into odontoblast like cells which are characterized by type I collagen and dentin matrix protein (DMP-1), as well as increased alkaline phosphatase (ALP) activity. Increased ALP activity stimulates the release of minerals from cells to initiate mineralization, then the process of mineral formation occurs. The hydroxyapatite deposits combine into a collagen matrix in the mineralization process. DMP-1 synthesis increases, interacts with type 1 collagen to form sequences and undergoes mineralization, and the formation of reparative dentin occurs [12], [15].

The structure of reparative dentin generally differs from primary dentin and reactionary dentin produced by native odontoblasts of developmental origin. In general, reparative dentin is less organized and does not

ISSN: 0005-2523
Volume 63, Issue 12, December, 2023

have a continuous tubular structure like primary, secondary, and reactionary dentin; however, this lack of permeability may protect the pulp from further external insults. Several studies have shown that the wound healing response of the pulp often results in premature formation of fibrodentine with osteotypic features and this structure may not provide an effective barrier to protect the pulp from exogenous irritants [23].

However, various biomarkers generated by the dental pulp's cellular components can give an understanding of the molecular processes that lead this injured pulp tissue toward healing or necrosis. A biomarker is an objectively measurable substance and its presence is expected to be suitable and valid. It may be evaluated as a sign of a normal physiological or pathological process. For direct pulp capping intervention, the process of formation of reparative dentin through several phases including the inflammatory phase is macrophage, NF-kβ, TNF-α IL-1, IL-8, IL-12, IL-6, and IFN δ; the proliferation phase is IL-10, p38 MAPK, VEGF, TGF-β and the remodelling phase is DMP-1, ALP and type 1 collagen. may be utilised as a diagnostic or prognostic tool. Finding a way to make these biomarkers readily and easily measureable in a therapeutic context or clinical setting is still a challenge [3].

5. Conclusion

Studies showed that limited type of biological marker used to evaluate the effect the herbal extract combined with Ca(OH)2 as the potential pulp capping material on the process of reparative dentin formation. Further studies should be directed to evaluate other biological markers to asses reparative dentin formation.

6. References

[1] Califf RM. Biomarker definitions and their applications. Exp Biol Med 2018;243:213–21. https://doi.org/10.1177/1535370217750088.

[2] Emilia E, Neelakantan /. Biomarkers in the Dentin-Pulp Complex: Role in Health and Disease. vol. 39. 2015.

[3] Rechenberg DK, Galicia JC, Peters OA. Biological markers for pulpal inflammation: A systematic review. PLoS One 2016;11. https://doi.org/10.1371/journal.pone.0167289.

[4] Yumoto H, Hirao K, Hosokawa Y, Kuramoto H, Takegawa D, Nakanishi T, et al. The roles of odontoblasts in dental pulp innate immunity. Japanese Dental Science Review 2018;54:105–17. https://doi.org/10.1016/j.jdsr.2018.03.001.

[5] Ozeki N, Mogi M, Kawai R, Yamaguchi H, Hiyama T, Nakata K, et al. Mouse-induced pluripotent stem cells differentiate into odontoblast-like cells with induction of altered adhesive and migratory phenotype of integrin. PLoS One 2013;8:1–14. https://doi.org/10.1371/journal.pone.0080026.

[6] Nijakowski K, Ortarzewska M, Jankowski J, Lehmann A, Surdacka A. The Role of Cellular Metabolism in Maintaining the Function of the Dentine-Pulp Complex: A Narrative Review. Metabolites 2023;13. https://doi.org/10.3390/metabo13040520.

[7] Shah D, Lynd T, Ho D, Chen J, Vines J, Jung H-D, et al. Pulp–Dentin Tissue Healing Response: A Discussion of Current Biomedical Approaches. J Clin Med 2020;9:434. https://doi.org/10.3390/jcm9020434.

[8] Octiara E. Dentin Reparatif dan Growth Factor yang Berperan Dalam Dentinogenesis Reparatif (Reparative Dentin and The Role Of Growth Factor In Reparative Dentinogenesis). Dentika Dental Journal 2015;18:294–9.

[9] 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 2020;53:1–5. https://doi.org/10.20473/j.djmkg.v53.i1.p1-5.

[10] Rahayu RP, Pribadi N, Widjiastuti I, Nugrahani NA. Combinations of propolis and Ca(OH)2 in dental pulp capping treatment for the stimulation of reparative dentin formation in a rat model. F1000Res 2020;9:1–13. https://doi.org/10.12688/f1000research.22409.1.

[11] Rodríguez-Ramiro I, Ramos S, López-Oliva E, Agis-Torres A, Bravo L, Goya L, et al. Cocoa polyphenols prevent inflammation in the colon of azoxymethane-treated rats and in TNF-α-stimulated Caco-2 cells. British Journal of Nutrition 2013;110:206–15. https://doi.org/10.1017/S0007114512004862.

[12] 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;21:1238–44. https://doi.org/10.5005/jp-journals-10024-2950.

[13] Parolia A, Kundabala M, Rao NN, Acharya SR, Agrawal P, Mohan M, et al. A comparative histological analysis of human pulp following direct pulp capping with Propolis, mineral trioxide aggregate and Dycal. Aust Dent J 2010;55:59–64. https://doi.org/10.1111/j.1834-7819.2009.01179.x.

[14] Tomson PL, Lumley PJ, Smith AJ, Cooper PR. Growth factor release from dentine matrix by pulp-capping agents promotes pulp tissue repair-associated events. Int Endod J 2017;50:281–92. https://doi.org/10.1111/iej.12624.

[15] Widona SN, Ramadhinta Y, Kurniawan MG, Zubaidah N. Application of Combination Propolis Extract and Calcium Hydroxide as a Direct Pulp Capping Agent on MMP-1 Expression and Collagen Type 1 Density in Rat ' s Pulp Tissue 2022;18:17–23.

[16] Sabir A, Mooduto L, Kaelan C, Horax S. Impact of the use of ethanolic extract of propolis, flavonoid and non-flavonoid propolis for direct pulp capping in collagen type i density. Braz J Oral Sci 2016;15:264–8. https://doi.org/10.20396/bjos.v15i4.8650037.

[17] Sabir A, Sumidarti A. Interleukin-6 expression on inflamed rat dental pulp tissue after capped with Trigona sp. propolis from south Sulawesi, Indonesia. Saudi J Biol Sci 2017;24:1034–7. https://doi.org/10.1016/J.SJBS.2016.12.019.

[18] Soetojo A, Bhardwaj A. Journal of International Dental and Medical Research ISSN 1309-100X http://www.jidmr.com Effect of Calcium Hydroxide Combination Tamara Yuanita and et al 2022:1516–21.

[19] Pribadi N, Widjiastuti I, Nadia A. Effect of calcium hydroxide-propolis combination on the number of fibroblast cells and angiogenesis in Wistar rats pulp 2020;10:14–8.

[20] Astuti ND, Apriasari ML, Nahzi MYI. The Effect of Mauli Banana (Musa acuminata) Stem Extract

ISSN: 0005-2523
Volume 63, Issue 12, December, 2023

on Macrophage Cell Number in Pulp Inflamation (In Vivo Study In Male Wistar Rat (Rattusnovergicus) Teeth). Dentino J Kedokt Gigi 2018;3:3742.

[21] Nie E, Yu J, Jiang R, Liu X, Li X, Islam R, et al. Effectiveness of direct pulp capping bioactive materials in dentin regeneration: A review. Materials 2021;14. https://doi.org/10.3390/ma14226811.

[22] Zhao Y, Yuan X, Liu B, Tulu US, Helms JA. Wnt-Responsive Odontoblasts Secrete New Dentin after Superficial Tooth Injury. J Dent Res 2018;97:1047–54. https://doi.org/10.1177/0022034518763151.

[23] Karim IAE, Cooper PR, About I, Tomson PL, Lundy FT, Duncan HF. Deciphering Reparative Processes in the Inflamed Dental Pulp. Frontiers in Dental Medicine 2021;2. https://doi.org/10.3389/fdmed.2021.651219.

[24] Goldberg M. Central Indirect and Direct Pulp Capping: Reactionary vs. Reparative Dentins. JSM Dent 2020;8:1119.

[25] Huang H, Okamoto M, Watanabe M, Matsumoto S, Moriyama K, Komichi S, et al. Development of Rat Caries-Induced Pulpitis Model for Vital Pulp Therapy. J Dent Res 2023;102:574–82. https://doi.org/10.1177/00220345221150383.

[26] Dias ML, O'Connor KM, Dempsey EM, O'Halloran KD, McDonald FB. Targeting the Toll-like receptor pathway as a therapeutic strategy for neonatal infection. Am J Physiol Regul Integr Comp Physiol 2021;321:R879–902. https://doi.org/10.1152/ajpregu.00307.2020.

[27] Herb M, Schramm M. Functions of ros in macrophages and antimicrobial immunity. Antioxidants 2021;10:1–39. https://doi.org/10.3390/antiox10020313.

[28] Cargnello M, Roux PP. Activation and Function of the MAPKs and Their Substrates, the MAPK-Activated Protein Kinases. Microbiology and Molecular Biology Reviews 2011;75:50–83. https://doi.org/10.1128/mmbr.00031-10.

[29] Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ, et al. Dental Pulp Defence and Repair Mechanisms in Dental Caries. Mediators Inflamm 2015;2015:1–16. https://doi.org/10.1155/2015/230251.

[30] Kadowaki M, Yoshida S, Itoyama T, Tomokiyo A, Hamano S, Hasegawa D, et al. Involvement of M1/M2 Macrophage Polarization in Reparative Dentin Formation. Life 2022;12. https://doi.org/10.3390/life12111812.

[31] Sica A, Erreni M, Allavena P, Porta C. Macrophage polarization in pathology. Cellular and Molecular Life Sciences 2015;72:4111–26. https://doi.org/10.1007/s00018-015-1995-y.

[32] Befani, Christina; Liakos P. Research Article Cell Biology International 10.1002/cbin.10307 2017;41:769–78.

[33] Roszer T. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediators Inflamm 2015;2015:16–8. https://doi.org/10.1155/2015/816460.

[34] Hargreaves KM, Berman LH. Cohen's Pathway of The Pulp. 11th ed. St. Louis, Missouri: elsevier Inc; 2016.

[35] Elsalhy M, Azizieh F, Raghupathy R. Cytokines as diagnostic markers of pulpal inflammation. Int Endod J 2013;46:573–80. https://doi.org/10.1111/iej.12030.

[36] Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: a critical step during wound healing. Cellular and Molecular Life Sciences 2016;73:3861–85. https://doi.org/10.1007/s00018-016-2268-0.

[37] Więckiewicz W, Miernik M, Więckiewicz M, Morawiec T. Does propolis help to maintain oral health? Evidence-Based Complementary and Alternative Medicine 2013;2013. https://doi.org/10.1155/2013/351062.

[38] Quintero-Fabián S, Arreola R, Becerril-Villanueva E, Torres-Romero JC, Arana-Argáez V, Lara-Riegos J, et al. Role of Matrix Metalloproteinases in Angiogenesis and Cancer. Front Oncol 2019;9:1–21. https://doi.org/10.3389/fonc.2019.01370.

[39] Sangwan P, Sangwan A, Duhan J, Rohilla A. Tertiary dentinogenesis with calcium hydroxide: A review of proposed mechanisms. Int Endod J 2013;46:3–19. https://doi.org/10.1111/J.1365-2591.2012.02101.X.