Literature Review

The Role of Propolis in Pulp Pain by Inhibiting Cyclooxygenase-2 Expression

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ABSTRACT

Background: *Inflammation of the pulp can lead to elicit pain. Pain in inflammation is induced by the cyclooxygenase-2 enzyme (COX-2) which induces prostaglandin E2 (PGE2) resulting in pain. Pain in the pulp can be relieved by eugenol. In its application, eugenol is toxic to pulp fibroblasts. Due to the side effect, it is worth considering other biocompatible materials with minimal side effects, such as propolis. Flavonoids and phenolic acids that contained in propolis can inhibit COX-2. Therefore, an analysis outlined in the literature review is needed to examine the results of research related to the role of propolis as pulp pain relief by inhibiting COX-2 expression.* ***Purpose:*** *To analyze the role of propolis in pulp pain by inhibiting COX-2 expression.* ***Reviews****: Propolis extract that extracted by ethanol, water, and hydroalcohol has pain relief properties in the pulp by inhibiting COX-2 by directly binding to the COX-2 receptors and by reducing the production of proinflammatory cytokines which are COX-2 inducers, proven through in vivo, in vitro, and in silico studies in various target cell organs.* ***Conclusion:*** *Propolis extract has high prospect as inflammatory pain inhibitor in the pulp by inhibit COX-2 expression.*

Keywords: *propolis extract; pain relief; pulp; COX-2*

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INTRODUCTION

Pain is a sensory and emotional experience related to tissue damage that can interfere our daily activities. Treatment of odontogenic pain requires a better approach because this condition is still a major health problem in worldwide.[1] Odontogenic pain can be sourced from the pulpodentinal or periapical tissue.[2] Pain in the pulp is mostly caused by bacteria, but can also be caused by trauma, heat, and chemistry.[3] In 2011, based on Indonesian Health Profile, pulp and periapical diseases ranked fourth out of ten diseases that occurred in outpatients in hospitals in Indonesia with total 209,637 patients. It shows that attention is needed related to oral health problems, especially dental diseases that have reached the pulp and periapical level.

45% of pain in the orofacial region is stimulated by acute pulpitis.[4] Pain in the pulp can be relieved by compounds that have ability to relief pain. One of the compounds that have pain relief properties is eugenol .[5] Eugenol is a phenolic component that has allyl, phenol, and ether functional groups.[6,7] Eugenol is able to relieve pain by inhibiting COX-2.[8] In its application, eugenol has several weakness. According to the research of Escobar-García *et al.,* (2016)[9] eugenol is toxic when applied directly to human dental pulp fibroblasts. The toxic properties of eugenol can irritate periapical tissue and necrotize the bones and cementum. Excessive use of eugenol can cause side effects such as seizures, nausea, fast heartbeat, and dizziness as a result of toxicity to the immune system. Eugenol toxicity can cause mutations in the body's DNA, which can lead to cancer.[10] Safe and effective pain management is an important goal for all dentists in treating patients, so it can be concluded that new therapeutic strategies are needed in medicine to relieve pain with fewer side effects.

One of the alternative materials that can be used in dentistry for various applications is propolis.[11] Propolis is a bee product that consists of plant resin and beeswax.[12] The chemical substance and bioactivity of each type of propolis depends on the species of bee, the type of plant as a food source, geographic area, and variations in resin composition.[13] Propolis contains polyphenolic compounds, including flavonoids (such as pinocembrin, chrysin, and quercetin) and phenolic acids and esters (such as caffeic acid and phenethyl caffeic acid phenethyl ester (CAPE)) which play an active role as anti-inflammatory, anti-bacterial, antioxidant, immunomodulatory, and tumor inhibitor.[11,14,15] Inflammatory pain is induced by the cyclooxygenase-2 enzyme (COX-2) which induces prostaglandin E2 (PGE2) resulting in pain.[16]Flavonoids and phenolic acids that contain in propolis are able to inhibit cyclooxygenase (COX) and lipoxygenase.[17] This proves that propolis can act as a pain relief through its anti-inflammatory effect. According to

Sabir (2019)[18] propolis can provide effective results in relieving tooth pain because it has good antibacterial and anti-inflammatory effects. Therefore, an analysis outlined in the literature review is needed to examine the results of research related to the role of propolis as an pulp pain reliever by inhibiting COX-2.

REVIEW(S)

Pain

Odontogenic pain is the result of dangerous physical stimulation because it mechanism is through via inflammatory mediators that stimulate receptors that located at the terminal ends of nociceptive C and A δ afferent fibers. Physical stimuli give an effect on dentinal fluid flow, it can activate nociceptors that innervate the dentinal tubules, resulting in the perception of odontogenic pain.[3] An inflamed tooth can be very painful, it may cause throbbing, spontaneous and intense pain that will get worse because of hot and cold stimuli. Pain in the pulp can occur in conditions as dentin hypersensitivity, reversible pulpitis, or irreversible pulpitis. If the pain is stimulated and does not go away after the stimulus is gone, the pulpitis is called irreversible pulpitis. If the pain quickly disappears, it can be referred as reversible pulpitis.[5] Pain in non-vital teeth is usually spontaneous and exacerbates if the apex is inflamed, it does not react to cold or heat.[19]

The complex neurophysiological process that known as nociceptive consists of four components: transduction, transmission, modulation and perception, starting from the occurrence of strong stimuli in the periphery to the central nervous system (cortex cerebri). Transduction is the process which a noxious stimulus (heat, cold, mechanical distortion) becomes an electrical impulse at the end of sensory or peripheral nerves. There are three types of nerve fibers that involved in this process: A-beta, A-delta, and C fibers. A-delta and C fibers are fibers that respond optimally to non-noxious stimulation which are classified as pain-conducting fibers. Transmission is the conduction of electrical impulses to the CNS through A-delta and C fibers from the periphery to the spinal cord along the sensory tract to the brain. In modulation, there is a process of change in pain transmission (inhibition and excitability), especially in the dorsal horn of the spinal cord. The result of the transduction, transmission, and modulation processes which will ultimately produce a subjective process known as pain perception that mediated through the thalamus with the cortex as a sensory commodity. The organs of the body that receive pain are pain receptors, also called nociceptors.[20,21]

The pathophysiology of odontogenic pain originates from mechanical, thermal, or chemical stimulus that stimulates mechanoreceptors in the dentinal tubules through fluid flow with the velocity about 2-4 mm / sec. Mechanoreceptor stimulation initiates neurological impulses in the subodontoblastic Raschkov plexus and Bradlow's interodontoblastic plexus on pulp that perceives pain. In the process of pain perception, the pain stimulus is carried through the branches of the trigemina nerves: ophthalmic nerve, maxillary nerve, or mandibular nerve which will enter the trigeminal ganglion. Pain stimulus is transmitted to the caudal subnucleus and is continued by second order neurons that called Nociceptive Specific (NS) and Wide Dynamic Range (WDR). If the pain stimulus is in the hot or pinch category it will be continued by the NS nerve, if it is included in the tactile stimulus category it will be continued by the WDR nerve. Furthermore, the stimulus enters the thalamus, which contains third order neurons to perceive pain, which involves the limbic system, hypothalamus, and cortical brain .[22,23] Tissue damage induced by bacterial toxins, mechanical, chemical or thermal stimulation cause the formation and release of chemical substances due to cell disruption and activation of various specialized cells including macrophages.[24] Macrophage cells will secrete pro-inflammatory cytokines that have algesic properties, such as TNF α, IL-1β (Interleukin) and IL-6 .[25] The NF-κβ transcription pathway has an important role in the inflammatory response by regulating transcription of cytokine genes such as interleukin IL-1β, IL-6, and tumor necrosis factor-alpha / TNF-α.[26] TNF-α simultaneously releases IL-6, which stimulates IL-1β production. IL-1β increases the regulation of hyperalgesic prostaglandin production, by mobilizing arachidoic acid and and inducing expression of the cyclooxygenase-2 (COX-2) gene.[27]

High level of ROS will activate cytosol phospholipase A2 (cPLA) that can damage phospholipid membrane, so it will produce arachidonic acid compounds.[28,29] COX-2 (PGH synthase) performs the cyclization process and donates two oxygen molecules for arachidonic acid to synthesize Prostaglandin G2 (PGG2). Peroxidase process is the reduction stage of PGG2 to an unstable endoperoxide compound called Prostaglandin H2 (PGH2). PGH2 is an intermediate compound in the biosynthesis of active protanoids such as PGE2. PGE2 plays some roles in inflammatory processes such as vasodilation of blood vessels, edema and pain. In peripheral tissue, PGE2 works synergistically with several inflammatory and pain mediators, including bradykinin, serotonin and histamine to activate nociceptive neurons that can trigger pain in the pulp.[17,30]

On the surface of the myelinated A-δ nerve cell membrane there are TRPV1 receptors. PGE2 activates TRPV1 receptor, causing membrane depolarization so that ion channels open, K + ions will leave the cell, while Ca ++ and Na + ions enter the cell. If the membrane potential increases, the channels will also open wider , Ca ++ and Na + ions will also enter much more, so that peptides such as SP and CGRP will be released, where SP and CGRP have the function of causing pain impulse conduction along the sensory nerve membrane.[31,32] SP can also trigger the release of further inflammatory mediators from platelets, mast cells, and activated immune cells.[24,33] Conduction of pain impulses along the sensory nerve membrane that called transmission process will undergo modulation which occurs in the dorsal

horn of the spinal cord which then causes the perception of pain that mediated through the thalamus.[20,21]

Cyclooxygenase-2 enzyme

Cyclooxygenase (COX) enzyme is an enzyme that produces prostaglandins, which is inflammatory mediator that is a product of arachidonic acid metabolism. COX consists of 2 isoenzymes: COX-1 and COX-2. Although all isoforms of this enzyme converts arachidonate to prostaglandins, both of them have significant differences in their distribution and roles in the body. COX-1 is expressed constitutively and plays a role in catalyzing prostaglandins which are responsible for carrying out physiological regulatory functions such as hemostasis, digestion and kidney functions, while COX-2 is induced if there is a trigger and plays a role in catalyzing the prostaglandins that cause inflammation, pain and fever.[34,35] Arachidonic acid is released from membrane phospholipids by phospholipase A2 when stimulated. When the catalytic domain binds to arachidonic acid, COX-2 starts the cycles and adds two molecules of O2 to arachidonic acid to form cyclic hydroperoxide PGG2 in the first cyclooxygenation step, then reduces PGG2 become PGH2 in the second peroxidation step. PGH2 is a highly unstable endoperoxide, which function is an intermediate substrate for biosynthesis of PGs E2, F2, D2 and I2 and TXA2 series. PGE2 that released from inflamed tissue can sensitizes afferent nerve fibers that work either on the sensory peripheral nerves or at central locations in the spinal cord and brain to generate pain.[30,35]

Propolis

Propolis is a bee product that consists of plant resin and beeswax.[37] Bees collect and use resin as propolis on purposes, such as gluing cracks in the hive, creating a smooth surface for attachment of young bees, as protection by eliminating parasites, and reducing microbes in beehives.[38] In general, raw propolis consists of about 50% resin, 30% wax, 10% essential oil, 5% pollen, and 5% other organic compounds.[39] Propolis contains several useful ingredients. Components that are often used in medicine or dentistry are flavonoids, phenolic acids and esters which play an active role as anti-inflammatory, anti-bacterial, antioxidant, immunomodulatory and anti-tumor.[11,15] Several classes of flavonoids are flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols, anthocyanidins, chalcones, and aurones.[40] Each class has examples of different compounds such as flavones (for example, chrysin, apigenin, and luteolin), flavonols (for example, quercetin, kaempferol, myricetin, and fisetin), flavanones (for example, flavanones, hesperetin, and naringenin), and others.[41] Phenolic acid, which can also be referred to as phenolkarboxylic acid is a type of aromatic acid compound.[42]

DISCUSSION

Pulp and periapical pain are the main causes of patients went to the dentists. Toothache begins from pulp inflammation and stimulation of the tooth pulp nerve fibers. Acute pulpitis is an acute inflammatory response that cause severe pains. In acute pulpitis, the pulp is still vital and the percussion is negative.[30] Patients with acute reversible pulpitis have a brief pain, and disappears after the stimulation is removed. There are no spontaneous pain and the patient know exactly where the pain is. Teeth that diagnosed with acute irreversible pulpitis are very responsive to cold stimuli, the pain occurs within minutes to hours, sometimes appears spontaneously, disturbs the quality of sleep, and occurs when bending over. Pain in non-vital teeth is usually spontaneous and exacerbates if the apex is inflamed, it does not react to cold or heat.[19] Tissue damage induced by bacterial toxins, mechanical, chemical or thermal stimulates leukocytes to release cytokines such as IL-1 and TNF (endogenous pyrogen) which can increase the COX-2 enzyme, which can convert arachidonic acid to PGE2.[43] The amount of PGE2 was found in the inflamed pulp tissue.[44] Increase of TRPV1 in patients with acute pulpitis will peak within 24 hours after stimulation. PGE2 activates TRPV1 at the sensory nerve endings of the tooth pulp, which releases SP and CGRP one hour after the injury that cause conduction of pain impulses along the sensory nerve membrane.[45-47] Management of acute pulpitis emergency that still used until now is topical eugenol. According to the research of Escobar-García *et al.,* (2016)[9] eugenol is toxic when applied directly to human dental pulp fibroblasts. The toxic properties of eugenol can irritates the periapical tissue and occur necrosis of bones and cementum. According to Sabir (2018)[18] propolis can provide effective results in relieving tooth pain because it has good antibacterial and anti-inflammatory effects.

The effectiveness of propolis as a pain relief in dentistry has been investigated. According to the research of Shabbir *et al.,* (2020)[48] giving propolis as an intracanal medicament after endodontic surgery using the VAS method shows that most patients (> 78%) do not experience or only experience mild inter-appointment pain. It shows that propolis is able to act as an anti-microorganism and anti-inflammatory through COX-2 inhibition which can prevent or suppress flare up, namely acute exacerbation pain without any side effects. This study was supported by the research of Sabir (2019)[49] which the ethanol extract of propolis was able to significantly inhibit COX-2 expression in the pulp ($p < 0.05$) than the control and was better than Ca (OH) 2 in pulp capping treatment. Reduction of pain through COX-2 inhibition in the pulp using propolis has also been studied by Madan et al., (2020)[50] that 15% of propolis tincture is able to relieve pulp pain through pulpotomy treatment, its effectiveness is comparable to MTA, supported by Abd-El Moneim's research, Bayoumy, & Barakat, (2017)[51] stated that propolis is able to relieve pulp pain through pulpotomy treatment and its effectiveness is comparable to formocresol. Ethanol extract 70% -propolis can inhibit TNF-α expression in Odontoblast like cells so it can reduce COX-2 expression in the pulp.[52] Research by Iswanto, Kuswandari & Mahendra (2016)[53] proved that giving topical application of propolis after persistent tooth extraction can relief wound pain on days 1 and 3 (0%), whereas in the group without propolis

application there was pain in the wound (100%) showed that propolis was able to suppress post-extraction inflammatory pain. Research by Askari *et al.,* (2017)[54] proved that a solution of hydroalcohol-propolis extract was able to reduce inflammatory pain after crown-lengthening treatment.

In order to study pain transmission, identify new pain targets, and characterize a profile of new compounds for pain relief, various experimental animal pain models have been developed especially in rodents.[55] A number of different irritants are injected into the skin, feet, muscles, joints, and visceral organs to produce tissue damage and hyperalgesia which is an acute inflammatory pain associated with neutrophil infiltration as well as a more sustained pain response associated with macrophage infiltration.[56] Acetic acid-induced constriction test or writhing test was described as a typical model of inflammatory pain. The nociceptive response generated in this test was due to the participation of several mediators such as prostaglandins, proinflammatory cytokines such as IL-1b, IL-8, TNF-a, sympathomimetic amines, acetylcholine, and substance P.[57] In rodents, acetic acid contributes to release the arachidonic acid from phospholipid membranes and synthesizes PGE2 via the cyclooxygenase (COX) pathway.[58] Compounds that can reduce stretching of the rodents are able to cause inhibition of prostaglandin synthesis from COX-2, which is a peripheral pain mechanism involving local peritoneal receptors on the surface of cells lining the peritoneal cavity.[59] Based on the research of Sun, liao & Wang (2018)[60] the ethanol extract of Chinese propolis from *Apis mellifera* honey bees is able to overcome inflammatory pain. This study used 40%, 70%, and 95% ethanol extracts and 40% water extract. The highest total flavonoids in propolis are in ethanol extract 40% (40E) that is 142.7 ± 0.6 mg QE / g, it is higher than ethanol extract 70% (70E), 95% (95E) and extract with 40% water (40W). The highest total phenolic levels were also found in the 40% ethanol extract of 515.8 ± 4.0 mg GAE / g, higher than 70%, 95% ethanol extract and 40% water extract. The writhing test results showed that 40E showed a significant reduction ($p < 0.05$), compared to the control group and was the most effective compared to other extracts, it directly suits to the 40E content which contained the highest total flavonoid, phenolic and CAPE compounds compared to other extracts. This research was supported by the research of Tanvir (2018)[58] which 100% ethanol extract - Bangladesh propolis which contained total polyphenol that is 80.16 ± 0.79 mg gallic acid equivalent (GAE) / g and total flavonoids 10.95 ± 0.74 mg catechin equivalent (CE) / g showed inhibition in the writhing test with statistical value ($p < 0.05$). A difference was found in the research of Franchin *et al.,* (2012)[57] the 100% Brazilian geopropolis ethanol extract produced by *Melipona scutellaris* bees had a total phenolic 127.7 ± 1.9 mgGAE / g and no total flavonoids were found which could be caused by low or no flavonoid content in propolis. Differences of chemical composition of propolis can be caused by geographic location and different vegetation. Despite of the absence of total flavonoids, their ability in inhibiting writhing test (geopropolis extract 3, 10 and 30mg / kg), resulted that it able to effectively inhibit abdominal pain 31, 56 and 75% with statistical values ($P < 0.05$) compared to the control group. Brodkiewicz *et al.,* (2018)[59] also examined the ethanol extract of geopropolis from 2 different bees, 70% - ethanol extract geopropolis Argentine *S jujuyensis* (ESP) and ethanol extract 70% - geopropolis Argentine *T. fiebrigi* (ETP). Both extracts had high levels of total phenolic and low levels of flavonoids. Both were able to inhibit stomach constriction with statistical value ($p < 0.05$) compared to the control group. The ethanol extract of 95% -propolis Iran has been studied by Parandin (2019)[61] to inhibit stomach constriction with statistical values at doses of 100 ($p < 0.05$), 200 ($p < 0.001$), & 400 mg / kg ($p < 0.001$). Water-propolis extract has been studied.[62-63] Water-black morrocan propolis extract was able to inhibit stomach constriction significantly with statistical values at doses of 2.5% ($p < 0.05$) and 5% ($p < 0.01$). Iraqi propolis water extract was able to significantly inhibit stomach constriction at a dose of 100mg / kg (54.64% inhibition), 200mg / kg (68.56% inhibition) 300 mg / kg (75.77% inhibition) with a statistical value of $p < 0.0001$. Research using hydro alcohol extract - red propolis was able to significantly inhibit stomach constriction at doses of 10 mg / kg and 30 mg / kg with a statistical value of $p < 0.0001$ compared to controls.[64] The alcohol-propolis extract has been studied.[65] It significantly inhibit stomach constriction at doses of 50 mg / kg and 100 mg / kg with a statistical value of $p < 0.05$ compared to the control group. Based on the experiments above, both propolis extracted using ethanol, water, hydroalcohol, and alcohol can reduce writhing test with inhibits prostaglandin synthesis from cyclooxygenase-2, indicating that propolis extract is able to suppress peripheral inflammatory pain mechanisms involving peritoneal receptors that localized on the surface of cells that layering the peritoneal cavity.

The effectiveness of propolis extract against inflammatory pain due to tissue damage in the skin of the hind legs has been investigated using the formalin induced pain test. Formalin injection produces a biphasic behavioral response which the first phase (0-5 minutes) is characterized by the occurrence of neurogenic pain with direct stimulation of the nociceptive afferent tip. Formaldehyde stimulating sensory C-fibers then release substance P. The second phase (15-30 minutes) is characterized by peripheral inflammation and involves a period of pain sensitization during inflammatory process that associated with elevated of PG levels, COX stimulation, and nitric oxide (NO) release, suggesting that this test indicates the effectiveness of the compound in central and peripheral antinociceptive action.[57,66] In the formalin test, COX-1 that synthesized prostaglandins were not involved in nociceptive transmission, but COX-2 played an important role in this test. Ethanol extract of geopropolis showed antinociceptive activity in both the neurogenic and inflammatory phases with inhibition values of 51 and 50% for doses of 10 and 30 mg / kg ($p < 0.05$) and the results were not significant for doses 1 and 3 mg / kg in the first phase, and the inhibition values were 68, 52, 51 and 56% for the doses of 1, 3, 10 and 30 mg / kg, respectively ($p < 0.05$) in the second phase.[57] The results of this study were

supported by research of Brodkiewicz *et al* (2018)[59] the ethanol-propolis extract of *T. fiebrigi* (ETP) and *S. jujuyensis* (ESP) propolis showed significant antinociceptive activity ($p < 0.05$) compared to negative controls in both test phases. Research by Parandin & Daroogari (2019) [61] showed Iranian ethanol-propolis extract had an antinociceptive effect on both phases of formalin-induced nociception which was significantly inhibited in mice treated intraperitoneally with morphine and Iranian propolis extract. In the first phase (0 - 5 minutes), the inhibition was 88.97% ($p < 0.001$), 14.11% ($p < 0.01$), 26.66% ($p < 0.001$), and 62.31% ($p < 0.001$) for morphine & ethanol extract of propolis 100, 200, and 400 mg / kg. In the second phase (20-30 minutes), the inhibition was 89.85% ($p < 0.001$), 25.73% ($p < 0.001$), 46.40% ($p < 0.001$), and 56.32% ($p < 0.001$) on morphine & ethanol extract of propolis 100, 200, and 400 mg / kg. The water-propolis extract effective in inhibiting pain in the skin of the back legs. It was tested by Mountassir (2014)[62] with the results that the extract with dose of 5% was the most effective at inhibiting pain in the formalin induced pain test in both phases ($P < 0.05$) with phase 1 inhibition by 35% and phase 2 inhibition by 71%. Hydro alcohol extract -propolis red Brazilian HERP at 10 and 30 mg / kg resulted in marked inhibition of the formalin-induced neurogenic phase (36.2 and 66.9%, respectively, $p < 0.05$) and inflammation (76, 4 and 82.3%, respectively, $p < 0.001$). Research by Hariri & Abualait (2020)[65] revealed that both propolis groups (P50 and P100) showed a significant reduction ($p < 0.05$) in formalin-induced nociceptive behavior in both phases. This indicates that propolis extracted using ethanol, water, and hydroalcohol is able to overcome pain due to tissue damage in the skin of hind legs in the neurogenic and inflammatory phases through COX-2 inhibition.

Research on the effectiveness of propolis in inhibiting pain through COX-2 inhibition on various target cells has been investigated by in vivo and in vitro experiments. 500 mg Iranian propolis extract capsules are able to reduce pain in primary dysmenorrhea, which is caused by excess production of prostaglandins which is the result of arachnoid acid biosynthesis through the COX-2 system in the uterine muscles during the ovulation cycle, which cause uterine contractions and ischemia. Significant changes were found in the mean pain scores during the first ($P < 0.001$) and second ($P < 0.001$) months in the propolis group versus the placebo group.[67] Post-tonsillectomy pain was significantly lower in the topical propolis group compared to the control group on days 3 and 7-10 postoperatively ($p < 0.05$). Pain and edema mostly occur after tonsillectomy as a result of thermal effects and the expression of inflammatory mediators that stimulate pharyngeal nociceptors.[68] Hydroalcohol red propolis extract can be used to control post ovariohysterectomy (OH) abdominal inflammation pain.[69] Propolis lotion has been studied to reduce the pain intensity of shingles sufferers due to skin inflammation which causes intense itching accompanied by severe pain within 14 ± 2 days.[70] Propolis is able to inhibit pain due to inflammatory mediators in mucosal epithelial cells. Propolis-sesame oil paste and propolis-olive oil paste were effective in reducing the pain intensity of recurrent aphthous ulcers significantly ($p < 0.05$) with 90 and 95% success compared to the placebo formula, only 35%.[71] Propolis was able to significantly reduce the intensity of recurrent aphthous stomatitis pain which is an ulcerative inflammation ($p < 0.001$) compared to the placebo group.[72] Water -Anatolian propolis extract was able to provide anti-inflammatory effects on mice tails through the tail flick test.[73] In RAW 264.7 cells, the ethanol extract of propolis was able to reduce the expression of TNF α, COX-2, IL-6, IL-1β and iNOS, ROS.[74,75] This study was supported by Shahinozzaman *et al.,* (2018)[76] that the flavonoid compounds in prenylated Okinawa propolis ethanol extract had the ability to reduce COX-2 activity in the LPS-stimulated RAW 264.7 cell model. The results of the docking test of propolis extract on COX-1 and COX-2 had a fairly equivalent inhibitory behavior, indicating that the samples tested did not have selective inhibitory activity for COX-2. [77] However some compounds from the phenolic are more selective than Celecoxib. The results of the in silico molecular docking test by Flamandita *et al.,* (2019)[78] stated that the Xanthoxyletin propolis Lawang compound was able to act as the strongest COX-2 inhibitor with a binding affinity of -8.9 kcal / mol. Artepilin C phenolic compounds in propolis extract were able to reduce COX-2 activity in RAW 264.7 cells through inhibition of IL-1β and TNF-α .[79] Arachic Acid Ethyl Ester (PEN4) in the *Tala-Mokolo* propolis-ethanol extract which is a class of alkylphenol compounds effective for acute and chronic inflammatory pain and central analgesic properties in the hotplate test.[80] CAPE on propolis is able to relieve neuropathic pain through the CCI test on mice & on BV-2 cells by inhibiting COX-2 through reducing TNF-α, IL-1β and IL-6 activity.[81]

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