Conservative Dentistry Journal Vol. 13 No. 1 January-June 2023; 7-10

Research Report

Effective dose of nano propolis as anti-pain in animal models of Mus musculus using writhing test method

Annisa Salsabila Witjaksana[1], Ira Widjiastuti[2] and Devi Eka Juniarti[2]
[1]Undergraduate Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: *Odontogenic pain has a high prevalence where this pain is the result of noxious physical stimuli or calming inflammatory mediators that stimulate receptors located at the terminal ends of nociceptive C and Aδ afferent nerve fibers. Nano propolis is an alternative material that is used as a medicine in dentistry. Nano propolis is a mixture of natural resins containing flavonoids and phenolic acids which play a role in suppressing pain response through COX and NF-kB inhibition mechanisms. Nanoparticle technology is capable of preparing active drug ingredients in nano-sized particles and can affect drug effectiveness, because particle size affects the process of solubility, absorption and distribution of drugs. Research on the potential of nano propolis as an anti-pain is still minimal, so further research is needed on the function of nano propolis as an alternative anti-pain agent.* ***Purpose:*** *To determine the effective dose of nano propolis on pain response in Mus musculus.* ***Methods:*** *This research was conducted using the writhing test method to see the analgesic effect of nano propolis. The research sample consisted of 28 Mus musculus which were divided into four treatment groups, namely the piroxicam control group, and the nano propolis treatment group of 17.5 mg/kg, 35 mg/kg, and 70 mg/kg.* ***Results:*** *The results showed that the four treatment groups did not have a significant difference, in other words, they had the same anti-pain effect.* ***Conclusion:*** *Nano propolis 35 mg/kgBB is an effective dose in suppressing pain response in Mus musculus.*

Keywords: *effective dose; nano propolis; pain response; writhing test*

Correspondence: Ira Widjiastuti, Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga. Jl. Mayjen Prof. Dr. Moestopo No. 47, Surabaya 60132, Indonesia. Email: ira- w@fkg.unair.ac.id

INTRODUCTION

Based on The Global Burden of Disease Study (2016), dental and oral health problems, especially dental caries, are a disease experienced by almost half of the world's population (3.58 billion people).[1] Dental caries is a disease that occurs in the hard tissue of the teeth which is characterized by the occurrence of demineralization of the hard tissue of the teeth which is then followed by the breakdown of the organic matter. As a result, there is bacterial invasion and death of the pulp and the spread of the infection to the periapical tissue which can cause pain.[2]

Pain is one of the reasons patients come to see a doctor. Based on the International Association for the Study of Pain (IASP), pain is defined as an unpleasant sensory and emotional experience due to tissue damage.[3] Pain is also often referred to as an alarm to minimize contact with noxious agents. One form of pain with a high prevalence is odontogenic pain. Odontogenic pain or dental pain is the result of noxious physical stimuli or relieves inflammatory mediators that stimulate receptors located at the terminal ends of nociceptive C and Aδ afferent nerve fibers.[4] There are topical analgesics such as eugenol which are often used in dentistry to relieve pain, one of which is pain from pulpitis. Besides having anti-pain properties, eugenol is also anti-inflammatory, antimicrobial, antifungal, antiviral and also antiseptic.[5] However, eugenol has several drawbacks, which can irritate the periapical tissue, cause necrosis of the bone and cementum, risk interfering with the growth and development of permanent replacement tooth seeds.[6]

Natural products such as propolis can be used in the traditional treatment of pain.[7] Propolis is a non-toxic resinous substance produced by bees which has antimicrobial, antifungal, anti-inflammatory, antioxidant, anti-pain and antitumor properties. Propolis is a mixture of plant extracts mixed with bees' own saliva, which varies according to the place of origin. The composition of propolis is vegetable resin (50%), wax (30%), essential and aromatic oils (10%) and pollen and other organic substances (10%). The chemical composition of propolis also depends on geographical location, botanical origin, and also the bee species. The main components of propolis are flavonoids and phenolic esters such as caffeic acid phenethyl ester (CAPE). The flavonoids contained

DOI: https://doi.org/10.20473/cdj.v13i1.2023.7-10

in propolis are antimicrobial, anti-inflammatory and immunomodulating, which are very useful for treating aphthous ulcers, candidiasis, gingivitis and periodontitis.[8] Meanwhile, according to research conducted by Cheng et al., (2018), phenolic esters such as caffeic acid phenethyl ester (CAPE) contained in propolis can relieve neuropathic pain induced by chronic constriction injury (CCI) through inhibition of nuclear factor-kappa B signaling (NF-κB) and expression of proinflammatory cytokines in microglia.[9]

Nanoparticle technology is a new trendsetter in the world of science. Nanotechnology is a technology capable of preparing active drug ingredients in nano-sized particles (one millionth of a meter) at speeds smaller than one micrometer. The shape and size of the particles is one of the factors that influence drug effectiveness, because particle size is very influential in the solubility, absorption and distribution of drugs. If the particle size is smaller, the surface area of the particle is greater so that the rate of solution increases and will accelerate drug absorption through the blood circulation so that the therapeutic effect is achieved more quickly.[10]

One example of the use of nanoparticle technology in herbs is nano propolis. Nano propolis is nano-sized propolis particles (1–100 nm in diameter) that are cooled together to make them more effective without changing their properties by changing the size of the propolis with different methods.[11] Nano propolis has a smaller size to make it easier for the body to absorb.[12] Research on the potential of nano propolis as an anti-pain is still minimal, so further research is needed on the function of nano propolis as an alternative anti-pain ingredient. This research was conducted in vivo using experimental animals Mus musculus. Mus musculus were used as experimental animals because the anatomical, physiological and genetic structures of Mus musculus were similar to humans.[13] The purpose of this study is to prove the effect of nano propolis on the anti-pain effect on the musculature by the writhing test method and to determine the effective dose of nano propolis as an anti-pain agent in the musculature using the writhing test method.

MATERIALS AND METHODS

Before conducting the experiment, ethical clearance was obtained from the Health Research Ethical Clearance Commission Universitas Airlangga Faculty of Dental Medicine with number 453/HRECC.FODM/VII/2022. This research uses DDY male mice aged 2-3 months weighing between 17-35 grams were obtained from Pusat Veteriner Farma, Indonesia. Prior to the research, the experimental animals were acclimatized for one week so that the experimental animals could adapt to the new environment. Mice will be placed in a cage with a length of 40 cm x width 30 cm x height 18 cm for a density of 2 mice. The animals were kept at a constant temperature of 25 ± 2°C and on a 12-hour light/dark cycle. The animals were fed conventional animal feed and given free access to water.[14,15]

For the manufacture of propolis extract, 20 g of 100% ethanol extract of propolis was put into a 250 ml Erlenmeyer and added with 120 ml of 70% ethanol. 85 g of maltodextrin coating material was dissolved in 80 ml of distilled water and added 5 g of Mg stearate and then stirred with a stirrer until thoroughly mixed for 30 minutes. Furthermore, the solution was mixed with propolis which was dissolved in 70% ethanol and the mixture was quickly stirred again with a stirrer for 30 minutes. Then, the solution was dried with a vacuum dryer at a temperature of ≤ 50°C.[16] The propolis extract in this study was obtained from Tawon Rimba Raya Farm, Lawang District, Malang Regency, East Java.

For the manufacture of nano propolis, a total of 20 g of 100% ethanol extract of propolis was put into a 250 ml Erlenmeyer and added with 120 ml of 70% ethanol. 85 grams of maltodextrin coating material was dissolved in 80 mL of distilled water and added 5 grams of Mg stearate and then stirred with a stirrer until evenly mixed, then quickly homogenized at 22,000 rpm for 30 minutes. Then after 30 minutes the coating was homogenized, the dissolved propolis with 70% ethanol was mixed and the mixture was quickly homogenized again at 22,000 rpm for 30 minutes. After that, the solution was dried with a vacuum dryer at 50°C. The powder formed was then crushed and generalized with High Energy Milling (HEM) so that nano-sized particles were formed and identification of their size was carried out using Scanning Electron Microscopy (SEM).[16] Nano propolis in this study was made by PT. Nanotech Indonesia Global, South Tangerang City, Banten.

This study used 28 samples divided into four treatment groups, namely the piroxicam control group, nano propolis group used a dose of 17,5 mg/kg BW, nano propolis group used a dose of 35 mg/kg BW, and nano propolis group used a dose of 70 mg/kg BW. The control group used piroxicam at a 10 mg/kg BW dose, which was later dissolved in a saline solution. The nano propolis group used a dose of 17.5 mg/kg BW, 35 mg/kg BW, and 70 mg/kg BW dissolved in saline solution and 0.2% tween.

To carried out the acetic acid-induced writhing test, each tested animal was placed in the test site and then injected intraperitoneally according to the treatment group. Thirty minutes later, 10 ml/kg of 0.6% acetic acid solution was injected intraperitoneally. After 5 minutes, the mice were observed and the writhing reaction counted for 30 minutes. Acetic acid-induced writhing behavior in mice is characterized by the writhing reaction such as stretching, constriction of the abdomen, and extension of the hind leg.[17] Then, the average number of writhing reactions was calculated. To eliminate bias and subjectivity, the number of writhing reactions was calculated by two observers. After obtaining the required data, the mice were sacrificed ethically using chemicals. The ether compound is used as an inhalation agent and placed in a box until the animal is unconscious and dies.[18]

The data was then processed using the Saphiro Wilk test to determine if the data collected were normally distributed. The Levene test was used to test in the variance

Table 1. Mean and standard deviation of writhing response for the tested groups

Tested Group	N	Mean	Standard Deviation
Control Group (Piroxicam)	7	18.21	6.97
Nano Propolis Group Dose of 17.5 Mg/Kg BW	7	21.50	11.79
Nano Propolis Group Dose of 35 Mg/Kg BW	7	14.86	8.56
Nano Propolis Group Dose of 70 Mg/Kg BW	7	21.07	11.75

homogeneity test to examine if the data variance was homogenous. One-way ANOVA is used to test the difference in the average mean due to the sample group tested is more than two groups.

RESULTS

This study was conducted to determine the effective dose of nano propolis on pain response in Mus musculus. The research data were obtained by calculating the number of acetic acid-induced writhing behavior using the writhing test method. The analgesic effect results can be seen through the mean and standard deviation shown in Table 1.

Table 1 shows that the lowest amount of writhing response was found in the nano propolis group dose of 35 Mg/Kg BW, with an average value of 14.86 ± 8.56. The highest amount of writhing response was found in the nano propolis group dose of 17.5 Mg/Kg BW with an average value of 21.50 ± 11.79.

The first statistical test that was carried out was the Shapiro Wilk test, whose results showed that the data were normally distributed. The homogeneity test, namely the Levene test, showed that the data variance was homogeneous. In the comparison test using one-way ANOVA, it was found that there was no significant difference between treatment groups.with a p-value > 0.05. Because there was no significant difference, it is not continued to the post hoc test.

DISCUSSION

This study used the writhing test method to determine the effective dose of nano propolis as a pulp medicament against pain response. The writhing test method is a method used to test analgesics by targeting peripheral nerves. The nerve tissue contained in the pulp is included in the peripheral nerves, so stimulation of these nerves is needed to see the pain response.[17]

The dose of nano propolis used in this study was 17.5 mg/kg, 35 mg/kg, and 70 mg/kg. This dose was taken because the effective dose of propolis after being converted to a Mus musculus dose according to Al-Hariri and Abualait is 70 mg/kgBW[19] and for nano propolis the dose is reduced to 17.5 mg/kgBW, 35 mg/kgBW and also 70 mg/kgBB because it is expected that with a smaller dose, the effectiveness of nano propolis is better than propolis.[20]

From nano propolis at a dose of 17.5 mg/kgBW to nano propolis at 35 mg/kgBW, there was a decrease in the amount of stretching, so it can be concluded that nano propolis at a dose of 35 mg/kgBW was effective in suppressing pain response. The 17.5 mg/kgBW nano propolis group had an average number of stretches which was slightly higher than the 35 mg/kgBW dose. This indicates that the dose of nano propolis is able to suppress pain response, but because the dose used is lower, the resulting pharmacological effect is less than optimal, so that the drug concentration in the systemic circulation needed to inhibit pain mechanisms is insufficient.[21]

Nano propolis at a dose of 70 mg/kgBB experienced an increase in the average amount of stretching from nano propolis at a dose of 35mg/kgBB. This could be due to the ratio of the solvent used is smaller than at a dose of 35 mg/kg, which causes a higher suspension viscosity. A higher suspension viscosity can reduce drug absorption and efficacy.[22]

The main components of nano propolis are flavonoids and phenolic esters such as caffeic acid phenethyl ester which play a role in the mechanism of inhibiting pain responses.[8] The results of the nano propolis phytochemical test conducted at BPPKI Ketintang showed active compounds with high concentrations in the form of flavonoids (6.9%) and phenolic acids (7.81%).

Flavonoids present in nano propolis play a role in inhibiting the expression of inflammatory mediator genes, including IL-13, TNF-α and IL-6. In addition, flavonoids are known to be able to inhibit the release of arachidonic acid.[19] Then the flavonoids contained in propolis are able to inhibit cyclooxygenase (COX) and lipoxygenase. Therefore it will reduce leukotriene production and affect neutrophil phagocytosis activity thereby suppressing the inflammatory process.[23]

One of the phenolic acid derivatives contained in nano propolis is caffeic acid phenethyl ester (CAPE). CAPE contained in nano propolis can attenuate neuropathic pain induced by chronic constriction injury (CCI) through inhibition of nuclear factor kappa B (NF-κB) signaling and the expression of proinflammatory cytokines in microglia.[9]

Based on Zamrutizahra's research (2022)[24], the average number of propolis doses of 70 mg/kgBW is 31.67 ± 7.916, and when compared to nano propolis doses of 17.5 mg/kgBW, 35 mg/kgBW, and 70 mg/kgBW, the number of stretches is higher. The results of the test for the size of the nano propolis particles showed that nano propolis had a diameter of 84.3 nm, while for propolis it had a diameter of 1,080 nm. Nano particle size can enhance the absorption of pharmacological agents. This can be caused because the speed of drug dissolution is directly proportional to

its surface area, meaning that the smaller the particle size the greater the contact surface area so that the better the dissolution or solubility.[25] Then if the particle size is smaller, the surface area of the particle is greater so that the rate of solution increases and will accelerate drug absorption through the blood circulation so that the therapeutic effect is achieved more quickly.[10] Based on the research findings, it can be concluded that nano propolis doses of 35 mg/kgBB is an effective dose in suppressing pain response in Mus musculus because it has the lowest average number of stretches compared to other doses.

REFERENCES

1. The Global Burden of Disease Study. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. 2016;
2. Listrianah L, Zainur RA, Hisata LS. Gambaran Karies Gigi Molar Pertama Permanen Pada Siswa – Siswi Sekolah Dasar Negeri 13 Palembang Tahun 2018. JPP (Jurnal Kesehat Poltekkes Palembang). 2019;13(2):136–49.
3. Barrett KE, Barman SM, Boitano S, Brooks H. Sequencing and identification of expressed Schistosoma mansoni genes by random selection of cDNA clones from a directional library. Vol. 90, Memórias do Instituto Oswaldo Cruz. 2012. 215–216 p.
4. Hargreaves KM, Goodis HE. Seltzer And Bender's Dental Pulp Second Editon. 2012;
5. Yanti N, Bakti FU. Efek Analgetik Ekstrak Lerak Sebagai Bahan Pereda Nyeri Gigi. Dentika Dent J. 2014;18(2):190–3.
6. Ameliana Y, Herawati H, Pradopo S. Daya antibakteri penambahan Propolis pada zinc oxide eugenol dan zinc oxide terhadap kuman campur gigi molar sulung non vital (The antibacterial effect of propolis additional to zinc oxide eugenol and zinc oxide on polybacteria of necrotic primary molar). Dent J (Majalah Kedokt Gigi). 2014;47(4) : 198.
7. Sokeng SD, Talla E, Sakava P, Fokam Tagne MA, Henoumont C, Sophie L, et al. Anti-Inflammatory and Analgesic Effect of Arachic Acid Ethyl Ester Isolated from Propolis. Biomed Res Int. 2020;2020.
8. López-Valverde N, Pardal-Peláez B, López-Valverde A, Flores-Fraile J, Herrero-Hernández S, Macedo-De-sousa B, et al. Effectiveness of propolis in the treatment of periodontal disease: Updated systematic review with meta-analysis. Antioxidants. 2021;10(2):1–14.
9. Cheng H, Zhang Y, Lu W, Gao X, Xu C, Bao H. Caffeic acid phenethyl ester attenuates neuropathic pain by suppressing the p38/NF-κB signal pathway in microglia. J Pain Res. 2018;11:2709–19.
10. Hasan Z, Artika IM, Rosaline V, Sari N. Penerapan teknologi nanopartikel untuk sediaan obat (antibiotik berbasis bahan alam, Propolis trigona spp). Chem Prog. 2012;5(1):1–6.
11. Seven PT, Seven I, Gul Baykalir B, Iflazoglu Mutlu S, Salem AZM. Nanotechnology and nano-propolis in animal production and health: an overview. Ital J Anim Sci [Internet]. 2018;17(4):921–30. Available from: https://doi.org/10.1080/1828051X.2018.1448726
12. Abdel Mageid AD, Shaheen AA, Gad NS, Zahem RM. Ameliorative effect of propolis and nanopropolis supplementation against oxidative stress induced by microcystis aeruginosa in oreochromis niloticus. Egypt J Aquat Biol Fish. 2020;24(1):573–82.
13. Mutiarahmi CN, Hartady T, Lesmana R. Use of Mice As Experimental Animals in Laboratories That Refer To the Principles of Animal Welfare: a Literature Review. Indones Med Veterinus. 2021;10(1):134–45.
14. Taher YA, Samud AM, El-Taher FE, ben-Hussin G, Elmezogi JS, Al-Mehdawi BF, et al. Experimental evaluation of anti-inflammatory, antinociceptive and antipyretic activities of clove oil inmice. Libyan J Med. 2015;10.
15. Nugroho RA. Mengenal Mencit sebagai Hewan Laboratorium. 2018.
16. Prasetyorini, Hasan AZ, Siregar R. Penerapan Teknologi Nanopartikel Propolis Trigona spp asal Bogor sebagai antibakteri Escherichia coli secara in-vitro. Ekologia. 2011;11(1):36–43.
17. Yam MF, Loh YC, Oo CW, Basir R. Overview of neurological mechanism of pain profile used for animal "pain-like" behavioral study with proposed analgesic pathways. Int J Mol Sci. 2020;21(12):1–26.
18. Siregar KS. Teknik Eutanasi Dan Nekropsi Tikus. Natl Lab Anim Cent. 2018;8–11.
19. Al-Hariri MT, Abualait TS. Effects of green Brazilian propolis alcohol extract on nociceptive pain models in rats. Plants. 2020;9(9):1–9.
20. Martien R, Adhyatmika, Irianto IDK, Farida V, Sari DP. Technology Developments Nanoparticles as Drug Delivery Systems. Maj Farm. 2012;8(1):133–44.
21. Rokiban A, Dwiauliaramdini D, Sitijuwariyah S. Analisis Drug Related Problems (Drps) Pada Pasien Rawat Jalan Diabetes Melitus Tipe 2 Di Upt Puskesmas Rawat Inap Gedong Air Bandar Lampung. JFL J Farm Lampung. 2021;9(2):134–42.
22. Al Shoyaib A, Archie SR, Karamyan VT. Intraperitoneal Route of Drug Administration: Should it Be Used in Experimental Animal Studies? Pharm Res. 2020;37(1):1–30.
23. Ernawati DS, Sari AP. Expression of vascular endothelial growth factor and matrix metalloproteinase-9 in Apis mellifera Lawang propolis extract gel-treated traumatic ulcers in diabetic rats. 2018;11:304–9.
24. Zamrutizahra CS. Dosis Efektif Ekstrak Propolis Terhadap Respons Nyeri Pada Mencit (Mus musculus) Dengan Metode Writhing Test. 2022.
25. Nila A, Halim M. Dasar-dasar farmakologi 2. Kementrian Pendidik dan Kebud. 2013;9–15.