Conservative Dentistry Journal Vol. 12 No. 2 July-December 2022; 86-89

Research Report

Effective dose of propolis extract against pain response in mice (*Mus musculus*) using writhing test method

Chonny Salsabilla Zamrutizahra[1], Ira Widjiastuti[2], Sri Kunarti[2]
[1]Undergraduate Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Conservative Dentistry Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: *Odontogenic pain has a high prevalence. One of the alternative materials used as medicine in dentistry is propolis. Propolis is a mixture of natural resins containing flavonoid and phenolic acids that play a role in suppressing the pain response through COX and NF-kB inhibition mechanisms. The content of active propolis compounds is influenced by the type of bee, region, geographical conditions, climate change, seasons, and botanical sources causing different effectiveness of propolis. This is supported by several studies that show a variety of effective doses that produce an optimal analgesic effect, thus a research plan was developed to discover the effective dose of propolis extract on suppressing pain response in mice using the writhing test method.* ***Purpose:*** *To determine the effective dose of propolis extract towards pain response in mice.* ***Methods:*** *This study was conducted using the writhing test method to see the analgesic effect of propolis extract. The research sample consisted of 30 mice which were divided into five treatment groups, namely the piroxicam control group, and the propolis extract group at doses of 35 mg/kgBW, 70 mg/kgBW, 105 mg/kgBW, 140 mg/kgBW.* ***Results:*** *The results showed that the propolis extract dose of 70 mg/kgBW had no significant difference with the piroxicam control group, while between the doses of 35 mg/kgBW, 105 mg/kgBW, 140 mg/kgBW there was a significant difference towards the piroxicam control group.* ***Conclusion:*** *Propolis extract 70 mg/kgBW is the effective dose in suppressing pain response in mice.*

Keywords: *propolis extract; effective dose; pain response; writhing test; medicine*

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

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.[1] Odontogenic pain, also known as toothache, originates from damaged tooth structure, pulp or periodontium. Odontogenic pain is a major health problem because it is a form of orofacial pain with a significant prevalence in the global realm, so an appropriate approach to the treatment of odontogenic pain is needed.[2] According to Riskesdas in 2018, 45.3% of the total population of Indonesia experienced dental caries or pain and the percentage was greater than the prevalence of caries in 2013 which was 25.9%.[3,4]

Propolis is a natural resinous mixture produced by honey bees from substances collected from parts of plants, buds, and exudates.[5] Propolis have a wide variety of therapeutic advantages, i.e. being cost-effective and biocompatible with the human cell, with no toxicity, limited allergic reaction and ready availability.[6] Propolis contains phytochemical compounds which include flavonoids, phenolic acids and their esters (such as caffeic acid phenetyl ester or CAPE) that have active roles as anti-inflammatory and antioxidant.[7] According to Shehata *et al.*[8], the active compounds in propolis are influenced by the type of bee, region, geographical conditions, climate change, seasons, and botanical sources thus the effectiveness produced by various types of propolis is different.

Propolis extract can induce analgesic and anti-inflammatory action by suppressing the inflammatory response induced by lipopolysaccharide.[9] Pain in inflammation is induced by prostaglandin E2 (PGE2) resulting from the conversion of arachidonic acid (AA) by cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2).[10] The flavonoid content in propolis extract mainly inhibits COX-2 so that it can suppress the activity of prostaglandin endoperoxide synthase which plays a role in causing pain.[11] Caffeic acid phenetyl esther (CAPE), a phenolic ester derivative, is a potent modulator that plays a role in inhibiting the activity of COX-1 and COX-2 as well as suppressing the activation of genes responsible for COX-2 expression that play a role in pain and inflammation.[5,12]

Several research studies show propolis extract is able to subdue pain. According to a study conducted by Sokeng

et al.[13], arachic acid ethyl ester isolated from Cameroonian propolis at a dose of 50 mg/kgBW resulted in an increase in the average latency for the hot plate test in mice. Based on the findings of Shabbir *et al*.[9] Chinese propolis paste with a concentration of 95% at a dose of 200 mg was shown to have a similar effect to the group given Ca(OH)2 for endodontic postoperative pain without side effects. Research conducted by Al-Hariri & Abualait[14] proved that an alcoholic extract of green Brazilian propolis at a dose of 50 mg/kgBW caused significant inhibition of pain by acetic acid induction with rats as a model of nociceptive pain. Research on the potential of propolis from Indonesia is still limited, so further research is needed to determine the effective dose of propolis extract as an analgesic agent.

MATERIALS AND METHODS

Propolis extract was obtained by maceration method. 250 grams of Lawang propolis were cut into thin slices with a size of approximately 1 mm, then propolis was placed in a container together with 600 mL of 70% ethanol for 48 hours. Then remaceration was carried out by adding the obtained maceration results with 600 mL of 70% ethanol in a container for 24 hours. Then the macerate was filtered through filter paper and distilled under low pressure in a rotary evaporator (BIO-RAD, USA) at a temperature of 80–90°C, a pressure of 650 mmHg and a speed of 80 rpm to remove the solvent. The EEP is then placed in a container and left for approximately three days for the remaining solvent to completely evaporate. Then the extract obtained was stored at a temperature of 20-25°C.[15] The propolis extract was then dissolved in 20 ml of saline and Tween 80.

Experimental animal as many as 30 mice were grouped into 5 groups into a control group which was given piroxicam at a dose of 10 mg/kgBW and the treatment groups was given propolis extract at a dose of 35 mg/kgBW, 70 mg/kgBW, 105 mg/kgBW, and 140 mg/kgBW. Before the experiment, the mice were acclimatized for 1 week to get used to their environment. Mice were fed with regular feed and drinking water which was given regularly during the acclimatization period.

Experiments on mice were carried out using the writing test method. Mice were given propolis extract intraperitoneally. Administration of 0.6% acetic acid as much as 10 ml/kg was injected intraperitoneally 30 minutes after being given the extract. Then observation was done on the writhing of the mice which was marked by the response of the mice by abdomen contraction, arching the back, and extending the hind legs. The writhing that is performed usually appears after 5 minutes post-injection and lasts for 30 minutes. The number of writhes is counted for 30 minutes. The results of the number of writhings of mice were calculated to obtain the mean numbers.[16,17]

RESULTS

The results of the observation of the frequency of writhing in each study group are shown in Figure 1 and Table 1 and it can be seen that the piroxicam group has the lowest average number of writhing of the mice. In addition, it was seen that the 70 mg/kgBW propolis extract group had the lowest amount of writhing than the doses of 35 mg/kgBW, 105 mg/kgBW, and 140 mg/kgBW. This shows that the propolis extract dose of 70 mg/kgBW is the most effective in inhibiting pain.

Statistical analysis was conducted using SPSS version 26. The results of the one way ANOVA test showed the value of $p = 0.000$ ($p < 0.05$), which means that there were significant differences between each treatment group. The results of the post-hoc test in each treatment group can be seen in Table 2. The results of the Tukey HSD test showed that there was no significant difference between the piroxicam and propolis 70 mg/kgBW group ($p = 0.863$). Meanwhile, there was a significant difference between the control group piroxicam with propolis 35 mg/kgBW ($p = 0.002$), piroxicam with propolis 105 mg/kgBW ($p = 0.014$), piroxicam with propolis 140 mg/kgBW ($p = 0.000$).

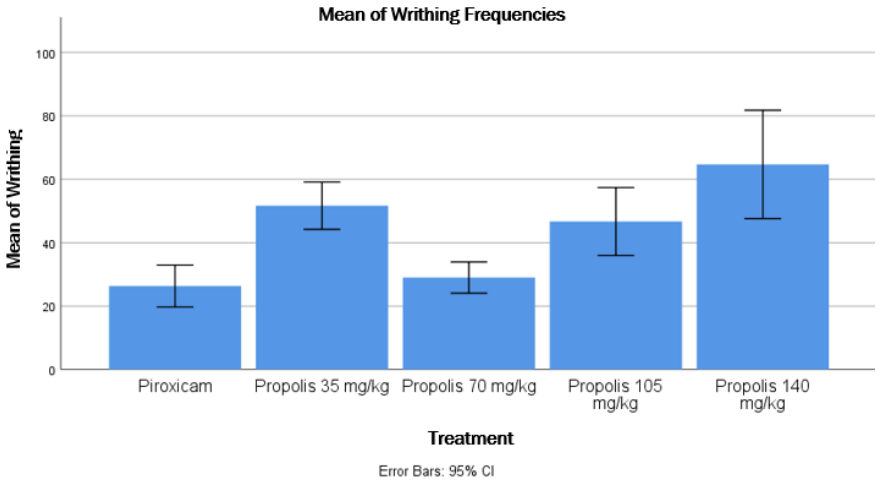

Figure 1. Bar chart of writhing frequency for each research group.

Table 1. The mean and standard deviation of the frequency of stretching for each treatment group

Treatment Groups	N	Mean	SD
Piroxicam	6	25.83	7.333
Propolis 35 mg/kgBB	6	51.67	7.090
Propolis 70 mg/kgBB	6	31.67	7.916
Propolis 105 mg/kgBB	6	46.67	10.211
Propolis 140 mg/kgBB	6	64.67	16.256

Table 2. Post-Hoc Tukey HSD test results

Treatment Group	Piroxicam	Propolis 35 mg/kgBW	Propolis 70 mg/kgBW	Propolis 105 mg/kgBW	Propolis 140 mg/kgBW
Piroxicam	-	0.002*	0.863	0.014*	0.000*
Propolis 35 mg/kgBW	-	-	0.020*	0.916	0.221
Propolis 70 mg/kgBW	-	-	-	0.120	0.000*
Propolis 105 mg/kgBW	-	-	-	-	0.042*
Propolis 140 mg/kgBW	-	-	-	-	-

DISCUSSION

The results showed that the lowest number of writhes was found in the positive control group with the average number of writhes being 25.83. Meanwhile, in the treatment group with 35 mg/kgBW (17.5 mg/20g) propolis extract, the average number of writhes was 51,67; a dose of 70 mg/kgBB (35 mg/20g) as much as 31.67; a dose of 105 mg/kgBW (52.5 mg/20g) as much as 46,67; and a dose of 140 mg/kgBW (70 mg/20g) as much as 64.67. Based on the results of the study, the dose of 70 mg/kgBW had the lowest amount of writhing. This is in accordance with research conducted by Al-Hariri & Abualait[14], which is propolis extract at a dose of 50 mg/kgBW is the most effective dose in reducing the number of writhes performed on mice. Research conducted by Sokeng *et al.*[13] showed that propolis extract at a dose of 50 mg/kgBW was effective as an analgesic agent in suppressing pain.

At doses of 35 mg/kgBW and 70 mg/kgBW, there was a decrease in the number of writhings, so it can be concluded at these doses that propolis extract was effective in suppressing the pain response. According to Robertson[18], giving the right dose will produce an optimal therapeutic effect. The propolis extract group at a dose of 35 mg/kgBW had a slightly higher average amount of writhing than the 70 mg/kgBW dose. This indicates that the propolis extract with this dose is able to suppress the pain response, but the pharmacological effect produced is less than optimal because the dose used is lower so that the concentration of drug in the systemic circulation needed to inhibit the pain mechanism is insufficient.

The amount of writhings that did not decrease at doses of 105 mg/kgBW and 140 mg/kgBW could be caused by the ratio of the solvent used being less than the solvent at doses of 35 mg/kgBW and 70 mg/kgBW. This can lead to higher suspension viscosities and large particle sizes. The viscosity of the suspension can affect drug absorption. The higher the viscosity, the lower the efficacy and absorption of the drug. The physicochemical properties of the drug, the dissolution rate of the suspension and the particle size greatly affect the absorption rate of a given drug. Slow dissolution rate causes longer drug absorption, and large particle size can prolong the release of pharmacological agents into the systemic circulation, and often leads to decreased total elimination of drug levels from the body.[18]

Statistical analysis of the data using one way ANOVA and post-hoc Tukey HSD tests showed that there was no significant difference between the piroxicam and 70 mg/kgBW propolis extract groups. This indicated that the propolis extract 70 mg/kgBW had the same analgesic effect as the positive control group. In this study, the effective dose of propolis extract was 70 mg/kgBW because it had the lowest average amount of writhing compared to other doses.

Flavonoids and phenolic acids are the most abundant active compounds in propolis and play a major role in the mechanism of inhibiting the pain response.[10] The results of the phytochemical test of propolis extract showed high concentrations of active compounds in the form of flavonoids (3.62%) and phenolic acids (4.01%).

The flavonoid content in propolis extract mainly plays a role in inhibiting COX-2 so that it can suppress the activity of prostaglandin endoperoxide synthase which plays a role in causing pai.[11] Consistent inhibition of cyclooxygenase activity will cause PGE2 production to decrease, where PGE2 is a prostanoid that plays a role in stimulating pain responses.[20]

CAPE which is an ester of phenolic acid has lipophilic properties so it can easily enter cells. CAPE can inhibit the activity of COX enzymes involved in arachidonic acid metabolism. Therefore, with the cessation of arachidonic acid metabolism, no prostaglandins are produced so that the pain can be reduced. In addition to its role in inhibiting COX activity, CAPE is also known as a specific inhibitor

for NF-kB activation. Inhibited NF-kB will block the release of IL-1β which is a proinflammatory cytokine that causes pain induction.[10]

It should be noted that factors such as bee species, region, geographical conditions, climate change, seasons, and botanical sources can affect the content of active compounds in propolis so that the effectiveness produced by each propolis variant may vary.[7] Therefore, the effectiveness of propolis in suppressing the pain response depends on the quantity of active compounds contained, in which the quantity of active compounds possessed in various types of propolis is influenced by several factors that have been mentioned. Thus, it can be concluded that propolis extract 70 mg/kgBW is the effective dose for pain response. However, further research is needed on the pharmacokinetic and pharmacodynamic effects of propolis extract and research using nanopropolis as an analgesic in pulp tissue.

REFERENCES

1. International Association for the Study of Pain. IASP Terminology - IASP. Washington: International Association for the Study of Pain; 2017 [cited 2021 May 20]. Available from: https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698#Pain
2. FDI World Dental Federation. Odontogenic Pain Management. International Dental Journal. 2018; 68(1): 20-21.
3. Balitbang Kemenkes RI. Riset Kesehatan Dasar. Jakarta: Balitbang Kemenkes RI. 2013. p.110-8
4. Riset Kesehatan Dasar. Jakarta: Badan Penelitian dan Pengembangan Kesehatan. 2018.
5. Wagh V. Propolis: A wonder bees product and its pharmacological potentials. Advances in Pharmacological Sciences. 2013; 2013: 1-11
6. Khurshid Z, Naseem M, Zafar M, Najeeb S, Zohaib S. Propolis: A natural biomaterial for dental and oral healthcare. Journal of dental research, dental clinics, dental prospects. 2017; 11(4): 265-274
7. Huang S, Zhang CP, Wang K, Li GQ, Hu FL. Recent advances in the chemical composition of propolis. Molecules. 2014; 19(12): 19610-19632
8. Shehata MG, Ahmad FT, Badr AN, Masry SH, El-Sohaimy SA. Chemical analysis, antioxidant, cytotoxic and antimicrobial properties of propolis from different geographic regions. Annals of Agricultural Sciences. 2020; 65(2): 209-217
9. Shabbir J, Qazi F, Faroqui W, Ahmed S., Zehra T, Khurshid Z.. Effect of Chinese propolis as an intracanal medicament on post-operative endodontic pain: A double-blind randomized controlled trial. International Journal of Environmental Research and Public Health. 2020; 17(2): 1-10
10. Sabir A. Does propolis can relief toothache???. Journal of Dentomaxillofacial Science. 2019; 4(2): 63-66
11. Rajoo M, Parolia A, Pau A, Amalraj FD. The Role of Propolis in Inflammation and Orofacial Pain: A Review. Annual Research & Review in Biology. 2013; 4(4): 651-664
12. Murtaza G, Karim S, Kha, S, Azhar S, Mumtaz A, Bin Asad, M. Caffeic acid phenethyl ester and therapeutic potentials. BioMed Research International. 2014; 1-9
13. Sokeng S, Talla E, Sakava P, Tagne MAF, Henoumont C, Sophie L, Mbafor J, Fohouo, FNT. Anti-Inflammatory and Analgesic Effect of Arachic Acid Ethyl Ester Isolated from Propolis. BioMed Research International. 2020; 2020: 1-8
14. Al-Hariri MT, Abualait TS. Effects of Green Brazilian Propolis Alcohol Extract on Nociceptive Pain Models in Rats. Plants. 2020; 9(9): 1-9
15. de Mendonca ICG, Porto ICCM, Nascimento TG. Brazilian red propolis: phytochemical screening, antioxidant activity and effect against cancer cells BMC Complement Altern Med. 2015: 15: 1-12
16. Yam FM, Loh YC, Tan CS, Adam SK, Manan NA, Basir R. General pathways of pain sensation and the major neurotransmitters involved in pain regulation. International Journal of Molecular Sciences. 2018; 19(8): 1-23
17. Altaee RAMK. Evaluation the analgesic and anti-inflammatory activity of aqueous extracts of Iraqi propolis in male Albino mice. Journal of Kerbala University. 2014; 12(2): 1-6
18. Robertson D. Drug action: The therapeutic effect. Nurse Prescribing. 2017; 15(5): 253-255
19. Al Shoyaib A, Archie SR, Karamyan VT. Intraperitoneal Route of Drug Administration: Should it Be Used in Experimental Animal Studies?. Pharmaceutical research. 2019; 37(1): 1-30
20. Verri WA, Vicentini FTMC, Baracat M. Flavonoids as Anti-Inflammatory and Analgesic Drugs: Mechanisms of Action and Perspectives in the Development of Pharmaceutical Forms. Studies in Natural Products Chemistry. 2012; 36: 297–330