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Effect of Purple Leaf Extract (Graptophyllum Pictum (L.) Griff) on the Number of Macrophage Cells in Pulp Perforation

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Abstract

Background: Purple leaf (Graptophyllum pictum (L.) Griff) is one of Indonesia's traditional medicinal plants, which has anti-inflammatory properties which is expected to stop the inflammation of the pulp accompanied by perforation. **Purpose:** To determine the effect of giving purple leaves (Graptophyllum pictum (L.) Griff) to the number of macrophage cells in pulp perforation. **Method**: The study used 24 Wistar rats divided into four groups, namely control (K), and 3 treatment groups P1, P2, P3. Each group consisted of 6 rats prepared, then P1 was given 10% purple leaf extract, P2 was given 15% purple leaf extract, P3 was given 20% purple leaf extract. On the 3rd day, the Wistar rat was sacrificed and continued with the preparation of HPA. Calculation of the number of macrophages was obtained from the One-way ANOVA test. **Results:** There were significant differences between the control and treatment groups (K with P1, P2, P3), on the results of the One-way ANOVA difference-result test ($p < 0.05$) **Conclusion:** Purple leaf extract (Graptophyllum pictum (L.) Griff) affected increasing the number of macrophage cells in pulp perforation after the 3rd day of treatment.

Keywords: *Purple leaf (Graptophyllum pictum (L.) Griff); macrophages; pulp perforation*

Introduction

Mechanical trauma to the pulp treatment procedure cannot be avoided especially if caries expand to deep depths. The action of cavity preparation or removal of carious tissue in deep cavities can cause pulp perforation[1]. Pulp perforation can be caused by the use of burs or other dental tools. In the condition of an open pulp, the direct pulp treatment can be performed[2,3].

The surface of macrophage cells have receptors and are immune cells[4,5] that first recognize the host cell debris as an antigen[6,7]. Various efforts continue to be made to find a drug that can cure pulp inflammation. Lately, there has been a lot of research into various types of plants that are suspected of having medicinal activities for human health purposes[8,9]. One of them is the purple leaf plant (Graptophyllum pictum (L.) Griff). Purple leaf (Graptophyllum pictum (L.) Griff) is one of Indonesia's traditional medicinal plants, which has anti-inflammatory properties so it is expected to stop inflammation of the pulp. The chemical content of purple leaves includes flavonoids, tannins, non-toxic alkaloids, steroids, saponins, and glycosides[10].

The research conducted aims to determine the effect of purple leaf extract (Graptophyllum pictum (L.) Griff) on the number of macrophage cells contributing to the healing process of pulp inflammation caused by mechanical injury. This research is an experimental laboratory study and the variable analyzed is the number of macrophages on the 3rd day after the treatment of the mechanical injury.

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Materials and Method

Materials

This research was an experimental laboratory study, using male Wistar strain rats, aged 2-3 months, with an

average body weight of 250-300 grams, obtained from the Experimental Animal Laboratory of the Department of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya. The animal experiments were 24, had been adapted for 1 week, then divided into 4 treatment groups randomly, namely control groups (K), P1, P2, and P3 each of 6 mice.

The herbs tested were purple leaves (Graptophyllum pictum L.Griff) varieties of lurid-sanguineum Sims which were obtained at the same time through plant determination tests at the Indonesian Institute of Plant Conservation Institute, Purwodadi Botanical Gardens, through Identification Certificate No. 445 / IPIL06 / HM / IV / 2019. The preparation of purple leaf extract gel based on polyethylene glycol (PEG) (Sigma-Aldrich, 25322-68-3) with a concentration of 10%, 15%, 20%, was carried out at the Prescription Formulation Laboratory of the Faculty of Pharmacy, Airlangga University, Surabaya.

Method

Extraction

Dry Simplicia was taken as much as 300 grams. The extraction was carried out by the maceration method using ethanol 96% as much as 3000 ml. Maceration was done by soaking 300 grams of Simplicia in 75 parts of 96% ethanol (2250 mL) for 5 days by shaking it periodically. After 5 days of waiting, it was squeezed so that the filtrate was obtained. Added the remaining solvent (750 mL) through the pulp until it reached 3000 mL of filtrate, and after that, it was filtered. The filtrate obtained was concentrated using a rotary evaporator at a temperature of 700 0C with a speed of 70 rpm[11].

Total Flavonoid Level Test

A total of 100 mg of sample was dissolved with 5 mL of ethanol, then diluted 10 times. The mixture was added 300 µL NaNO2, shaken for 10 seconds and left at room temperature for 10 seconds. 300 mL of AlCl 3, 2 mL of 1 M NaOH and 1.9 mL of distilled water were added to the reaction mixture, then shaken for 10 seconds and measured at the wavelength of 415 nm. Quercetin with concentrations of 10, 20, 40, 60 and 80 ppm was used as a standard solution. Purple leaf ethanol extract sample was dissolved with p.a. ethanol (2% -5%), added 0.10 ml of 10% AlCl3, and 0.10 ml of 1M sodium acetate and 2.80 ml of distilled water. The mixture was shaken homogeneously then left for 30 minutes. Then the absorption is measured using an ultraviolet-visible (UV-vis) spectrophotometer at a maximum wavelength of 4.

The test was carried out in triplo. The levels of flavonoids can be calculated using the formula:

$$F = \frac{c \times V \times f \times 10{-}6 \times 100\%}{m}$$

Information :

F: number of flavonoids AlCl3 method

c: Quercetin equality (µm / ml)

V: total extract volume

f: dilution factor

m: sample weight (g)

Animal Experiment Treatment

Before cavity preparation was performed, pain management was firstly performed on experimental animals in the form of intramuscular anesthesia with a combination of 0.2 ccs of ketamine (Ketalar ®, PT.Pfizer, Indonesia) and 0.5 cc xylazine (Xyla®, PT.Tekad Mandiri Citra, Indonesia). The maxillary left molar was prepared on an occlusal surface using a *diamond round bur low-speed* ISO 008, diameter of 0.84 mm (Intensiv, Switzerland) forming a Class I Black cavity with a depth of 1.5 mm and a diameter of 0.84 mm to reach the pulp roof, marked with a reddish color on the base of the cavity, making pulp perforation using the tip of a straight sonde (Martin, Germany), the tip diameter of a 0.35 mm sonde was visually formed a bleeding point and was confirmed with a sterile paper point tip. Furthermore, the topical application procedure of purple leaf extract at the bottom of the cavity, the extract was measured using microsyringe as much as 0.5 µl, then applied to the base of the cavity using a straight sonde tip, and toppled with the restoration of Glass Ionomer Cement (GIC) type II (Fuji IX, GC Corp, Tokyo, Japan).

On the 3rd day, the animal was terminated and the procedure was done to take the left maxilla. Maxillary tissue was fixed with 10% buffered formalin and decalcified using EDTA 10% pH 7.4. The making of histopathological preparations using Hematoxylin-Eosin (HE) staining was carried out at the Research Center of the Faculty of Dental Medicine, Airlangga

University, Surabaya. The preparation was observed under a microscope with 400x magnification.

Data Analysis

Test data normality using Kolmogorov Smirnov. Then the results of the treatment group research data were carried out homogeneity tests using Levene's test followed by the One-way ANOVA test to determine the data distribution. Data that were normally distributed continued with the Tukey HSD test to determine differences between different treated groups.

Results

The purple leaf ethanol extract used contained a total flavonoid of 1.49% (SD = 0.04) (Tabel 1). Data obtained from histopathological readings (HPA) indicated a predominance of macrophage cell numbers.

Based on Table 2, there were differences in the mean macrophage cells in the control group and the treatment group. In the treatment group with the given of purple leaves, it affected the concentration of 10%, 15%, 20%, therefore increasing the number of macrophage cells. The number of macrophage cells in each group could be seen in graph 1.

Figure (K) was the distribution of macrophage cells (arrows) in the control group treated without treatment on the 3rd day; (PGP10) was a distribution of macrophage cells in the treatment group given lesion + 10% purple leaf extract on the 3rd day; (PGP15) treatment group was given 15% purple leaf + seras + extract on the 3rd day; (PGP20) treatment group was given lesions + 20% purple leaf extract on the 3rd day.

Kolmogorov Smirnov test results on the number of macrophage cells showed a significant value between the control and treatment groups that is equal to 0.894. This value was greater than the significance level (α) = 0.05, meaning that the data on the number of macrophage cells in each treatment was normally distributed. The homogeneity test continued using Levene's test obtained p> 0.05 referring to the homogeneous data. To see the differences between treatment groups, the One-Way ANOVA test was obtained, the value of p = 0,000 (p <0.05) showing that there were significant differences between the concentration groups. Followed by the Tukey HSD Post-Hoc test to find out each sample group.

Tabel 1. Determination of total flavonoid levels of purple leaf ethanol extract by the colorimetric-AlCl3 method

Extract Weight (g)	Absorbance	%
0,24	0,35	1,49
0,22	0,30	1,46
0,20	0,29	1,48
0,21	0,34	1,53
0,21	0,30	1,48
0,21	0,31	1,53

Table. 2 Mean results and standard deviations in the number of macrophage cells in the control and treatment groups

No.	Group	n	Mean	Standard Deviation
1.	K	7	5,28	0,81
2.	P1	7	7,71	1,21
3.	P2	7	11,28	1,47
4.	P3	7	11,71	1,16

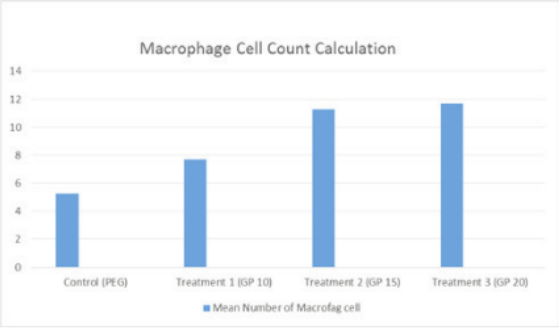

Figure 1. Graph of the mean number of macrophage cells in each group

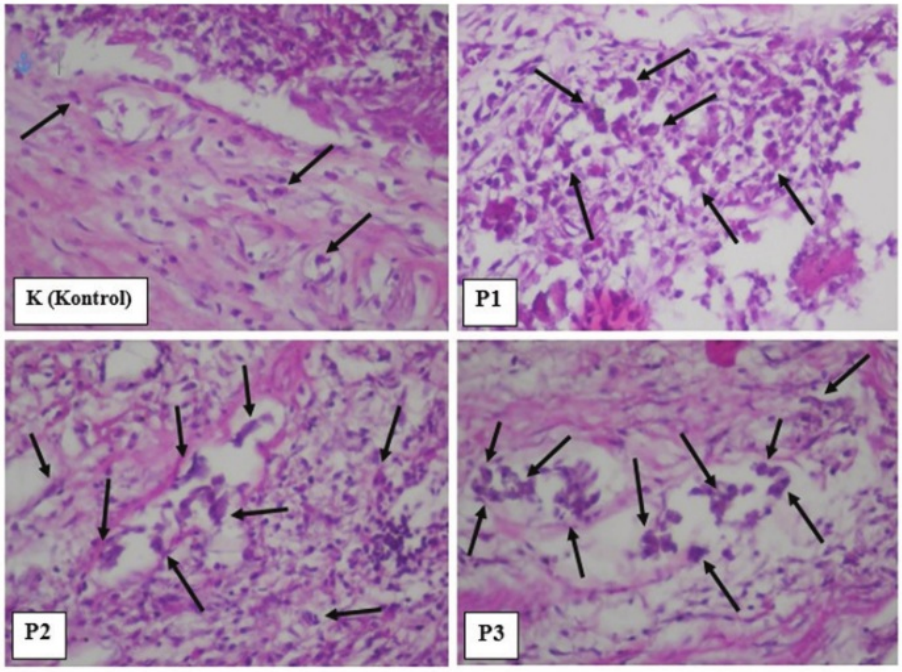

Figure 2. Picture of macrophage cells with histopathological examination of 400x magnification with 4 visual fields.

Table 3. Results of Post Hoc Tukey HSD statistical analysis of macrophage cell counts

	K	P1	P2	P3
K	-	0,040*	0,000*	0,000*
P1		-	0,000*	0,000*
P2			-	0,768
P3				-

Keterangan: (*) : terdapat perbedaan bermakna

Discussion

Pulmonary perforation caused by mechanical trauma caused the formation of debris cells from odontoblast damage that the host responded to as Damage-Associated Molecular Patterns (DAMPs), and later to be able activating pro-inflammatory macrophages, with the "classically activated" nitrite oxide (NO) phenotype that played a role in neutrophil migration to the affected tissue. When inflammation occurred, macrophage infiltration increased especially on days 3 to 5[12].

Macrophages had many roles especially in injured tissue including host defense, promotion and resolution of inflammation, apoptotic cell removal, and initiation of cell proliferation and tissue recovery after injury[13]. In the inflammatory response, there were 2 types of macrophage cells, namely M1 and M2. M1 (Classically activated) was a cell producing pro-inflammatory cytokines, recognizing, and killing pathogens, and regulating the inflammatory response. M2 (Alternatively activated) was a cell producing anti-inflammatory cytokines and activating tissue repair[14].

The herbal plants used as a treatment for inflammation of the pulp in this study were purple leaves (Graptophyllum pictum L.Griff) varieties of lurid-sanguineum Sims, because these varieties had the potential as herbal medicines in the fields of medicine and dentistry, compared to the other two varieties that were generally used for ornamental plant needs. Purple leaves with lurid-sanguineum Sims varieties were reported to have anti-inflammatory, antioxidant and analgesic activity[15]. The making of purple leaf extract in this study was done by the maceration method because the technique was simpler, safer and did not risk the damage of active substances because it was a cold extraction method without heating[16].

One of the active compounds of purple leaf ethanol extract which functioned as an anti-inflammatory mediator in response to mechanical injury that caused dental pulp perforation, one of which was flavonoids. Following the previous studies, in this study a UV-visible spectrophotometer test was observed with ultraviolet light with a wavelength of 366 nm, an intensive yellow fluorescent solution appeared, indicating the presence of flavonoids[17]. The mechanism of flavonoids as anti-inflammatory through inhibition of cyclooxygenase (COX). Cyclooxygenase (COX) functioned to trigger the formation of prostaglandins, and prostaglandins caused inflammation. If there was a decrease in the inflammatory process, the process will accelerate the initiation of the proliferation phase and the healing process and tissue repair. One of the cells that played a significant role in tissue repair was macrophages[18].

An important role of macrophages in the process of tissue healing was the production of growth factors needed to initiate the proliferation of fibroblast cells that play a role in the healing process and tissue repair[13]. The presence of macrophages in the injured area reached peak concentrations around the third and fourth days and remained in the tissue until the healing process was completed[19].

Application of polyethylene glycol (PEG) was used as a control group because PEG gave no influence in triggering the host immune response, PEG was considered a placebo in this study[20]. The results of the study proved that the administration of purple leaf extract affected in increasing the number of macrophage cells on the third day after treatment, which on the 3rd day was the end of the inflammatory phase leading to the initial fibroblastic phase, and this was when macrophages actively produced the growth factors[21].

Conclusion

This study concludes that the purple leaf extract significantly affects the number of macrophage cells in Wistar rats that are preceding given a mechanical perforation of the pulp, where between purple leaves extract concentrations of 15% and 20% have the same potential in increasing the number of macrophage cells.

Conflict of Interest : None

Source of Funding : Self-Funding

Ethical Clearance : The use of experimental animals had received approval from the Ethics Commission of the Faculty of Dental Medicine, Airlangga University (Number: 008 / HRECC.FODM / 1/2020).

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