MECHANISM OF PURPLE LEAVES EXTRACT IN REDUCE THE NUMBER OF LYMPHOCYTES IN TRAUMATIC ULCER

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ABSTRACT

Background: Purple leaves are one of the medicinal plants that have various benefits, one of which is as an anti-inflammatory. Flavonoids in purple leaves work as an anti-inflammatory, one of which is in conditions of traumatic ulcers which in the process show symptoms of inflammation such as redness and pain. In chronic inflammation that occurs in traumatic ulcers, inflammatory cells, one of which are lymphocytes, will migrate to the trauma area and cause symptoms of inflammation. Inflammation as much as possible is limited because if it continues it can cause the wound to undergo an abnormal healing process so that it continues to become pathological inflammation. This study aims to determine the effect of giving purple leaves extract on the number of lymphocytes in traumatic ulcers of wistar rats.
Method: There were five treatment groups namely normal group, control group, treatment group one (extract 0.05 g/kg BW), treatment group two (extract 0.15 gr/kg BW), and treatment group three (extract 0,45 g/kg BW). Rats were euthanized on the 7th day for histopathological preparations. The data in this study were tested using the One-way Anova parametric statistical test.
Result: All treatment groups significantly reduce the number of lymphocytes. The highest decrease occurred in the group with the highest dose, presumably because the higher the dose, the more active substances were able to reduce the number of lymphocytes.
Conclusion: At a dose of 0.05 g/kg BW rats could significantly reduce the number of lymphocytes in tissues that had traumatic ulcers.

Key words : Purple leaves, lymphocytes, traumatic ulcer

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PRELIMINARY

Traumatic ulcers were ulcers or lesions caused by trauma due to being bitten, scratched by dentures, trauma due to heat, and so on (Khairiati, 2014). The number of cases of traumatic ulcer were quite high compared to other oral cavity lesions. In 2016 at the dental and oral hospital in Indonesia, a study was conducted on the number of patients treated at the Oral Medicine Clinic, namely the Dental and Oral Hospital, University of Jember. The results of the study stated that of 766 Oral Medicine Clinic patients, the prevalence of traumatic ulcers was 6.5% of 766 patients (Setyowati, et al, 2017). The healing process in traumatic ulcers consists of four stages or phases, namely hemostasis, inflammation, proliferation, and tissue remodeling phases (Suhartono et al, 2018). In the hemostasis phase, platelet aggregation and capillary vasoconstriction occur. Furthermore, in the inflammatory phase, platelets release inflammatory factors and migration of inflammatory cells, namely lymphocytes, PMNs, and macrophages to the trauma area plays a role in the phagocytosis of antigens in wounds (Dai et al., 2011). At the end of the inflammatory phase, the number of inflammatory cells begins to decrease, followed by a proliferation phase characterized by an increase in the number of fibroblasts, followed by a maturation or tissue remodeling phase characterized by the formation of scar tissue and collagen deposition (Robin, 2008).

One of the agents that play a role in the inflammatory process was lymphocytes. On the fifth to seventh post-traumatic day, lymphocytes began to appear significantly. Lymphocytes had an important role, namely influencing wound healing agents, one of which was fibroblasts by releasing cytokines, including fibroblast activating factor and IL-2. With the release of cytokines, inflammatory symptoms such as pain, redness, swelling, and so on also arise (Suryadi et al., 2013).

Currently, treatment using herbal plants was becoming popular with the public because it has lower side effects compared to non-natural medicines. One of the plants that has the potential to be used as an alternative medicine in the therapy of traumatic ulcers was the purple leves (Graptophyllum pictum). This plant was one of thirteen plant commodities developed by the Directorate General of POM as a superior medicinal plant (Wahyu, 2021). Purple leaf (Graptophyllum pictum) was one of the plants that is widely used by the community as a medicinal plant, including as a medicine for wounds, swelling, and so on. This was because purple leaves contain anti-inflammatory properties, namely flavonoids, alkaloids, and tannins. This was in accordance with the statement of the Ministry of Health of the Republic of Indonesia (2012) which stated that purple leaves had active ingredients that were included in the flavonoid group. Flavonoids were known to be able to inhibit inflammatory mediators, one of which is IL-1 which affects the decreased expression of chemokines and adhesion molecules that induce lymphocyte migration from lymphoid glands to tissues (Miftah, 2020). The effect of inhibiting lymphocyte migration was that it could reduce inflammation symptoms such as pain, swelling, and redness, this is because lymphocytes were able to stimulate inflammatory agents that cause pain, swelling, and redness (Sumarny et al, 2013).

Purple leaves are included in herbal plants that had low side effects so that they could be used as an alternative treatment for traumatic ulcers. Some people with traumatic ulcers had a history of allergies to non-natural medicines that contain chemicals in them, one of whichwais characterized by ongoing inflammation. Given its low side effects, purple leaves could be used as an alternative therapy for traumatic ulcers, but its effectiveness has not been proven.

Hilmarni, et al, in 2016 conducted a study on the toxicity test of purple leaf extract on the hematological profile of white mice using a dose of 0.05 g/kg BW; 0.15 g/kg BW; and 0.45 g/kg BW. The results of the study on the hematological profile stated that purple leaves extract was not toxic to the hematological profile of experimental animals. In the treatment group, the average number of leukocytes was lower than the control group, which means that purple leaves extract was thought to had the potential to reduce the number of leukocytes. From this study, the authors used purple leaves extract doses of 0.05 g/kg rat BW, 0.15 gr/kg BW rat, and 0.45 gr/kg BW rat to be applied to experimental animals with traumatic ulcer model because the dose range was has the potential to suppress the number of leukocytes, besides that the three doses have been proven not to be toxic to the hematological profile of experimental animals, considering that in this study the authors examined one of the blood cells, namely lymphocytes. Based on the description of the background, the author intends to conduct experimental laboratory research and in vivo to determine the effect of Purple Leaves Extract (EDU) on lymphocytes infiltration in the mucosa of experimental animals with traumatic ulcers.

RESEARCH METHODS

Research Place

This research was conducted at the Bioscience Laboratory of RSGM Jember University and the Physiology Laboratory and Anatomical Pathology Laboratory of the Biomedical Section of the Faculty of Dentistry, Jember University.

Tools and materials

The tools used are digital scales, amalgam stopper, binocular microscope, disposable syringe, rats dental chair, measuring cup, porcelain cup, mortal and pastel, tweezers, plastic filling instrument, microtome, object glass and deck glass, hotplate, paraffin printing equipment, and water baths. The materials used were Wistar rats, Purple Leaves Extract (EDU), ketamine, alcohol, Haematoxillin-eosin stain, sterile distilled water, ether, water, immersion oil, and buffered formalin.

Research Stages

The initial stage of the research was to make an EDU using the maceration method. The purple leaf powder was weighed as much as 500 grams. then put into a maceration vessel, then given 2500 ml of 96% ethanol (1:5) at room temperature for 1x24 hours. The vessel is closed and then stored in a place that was not exposed to sunlight, while stirring repeatedly for one hour. After 1x24 hours, the purple leaves sample was filtered, the residue obtained from the filtering was macerated again twice, so that the total length of the maceration process was three days. The obtained macerate results were collected together and evaporated to separate the solvent. Evaporation was carried out using a rotary evaporator at a temperature of 50ºC to separate the ethanol solvent to obtain a concentrated extract of purple leaves.

Furthermore, before treating animals model, the researchers asked for ethical clearance approval from the Health Research Ethics Commission, Faculty of Dentistry, University of Jember with number 1098/UN25.8/KEPK/DL/2021. The animals used were 25 male Wistar rats which were divided into five treatment groups. The first group was the normal group (N), i.e. experimental animals were not given any treatment, either in traumatic ulcer conditions or with Purple Leaves Extract (EDU). The second group was the control group (K), the experimental animals were treated with traumatic ulcers and left without EDU. Furthermore, the third group, namely treatment group I (P1) where experimental animals that had traumatic ulcers were given EDU at a dose of 0.05gr/kg BW, the fourth group, treatment group II (P2) with an EDU dose of 0.15gr/kg BW, and the fifth group, treatment group III (P3) with a dose of 0.45gr/kg BW. The three doses in previous studies were proven to have no toxic effects on experimental animals and have the potential to reduce the number of leukocytes.

In making a model of traumatic ulcer, Wistar rats were anesthetized using 10 ml of ketamine with a dose of 0.2 ml/kg of body weight of rats intramuscularly in the thighs of rats. The end of the amalgam stopper with a diameter of 2 mm was heated with Bunsen flamed for approximately 25 seconds until the end of the amalgam stopper glowed reddish, then using tweezers, the rat's mouth was retracted to open, then the end of the amalgam stopper was touched for 1 second on the buccal mucosa of the rats (which had previously been marked with OHP marker). Observations began 24 hours after the procedure for making the traumatic ulcer.

After 24 hours after the procedure for making traumatic ulcers, Wistar rats that had experienced traumatic ulcers were given EDU therapy according to the group division. EDU administration was done using a plastic filling instrument topically on traumatic ulcer lesions. EDU administration was

given once a day for seven days after the creation of the traumatic ulcer model, starting on the first day of treatment until the seventh day when the rats lymphocyte count peaked. On day 7, all samples were euthanized using ether. This step aims to obtain samples of the buccal mucosa of rats. The rats were placed in a closed box, then given liquid ether which would evaporate and waited until the rats died.

Furthermore, the samples of the buccal mucosa will be made histopathological preparations with HE staining. Then the preparations of the buccal mucosal tissue were counted using a binocular microscope with a magnification of 400x, seen from 3 fields of view with the letter V pattern, namely on the left, middle and right. Counting was carried out on the 4 tissue preparations, then tabulated the number of lymphocytes and then the average was taken.

RESEARCH RESULT

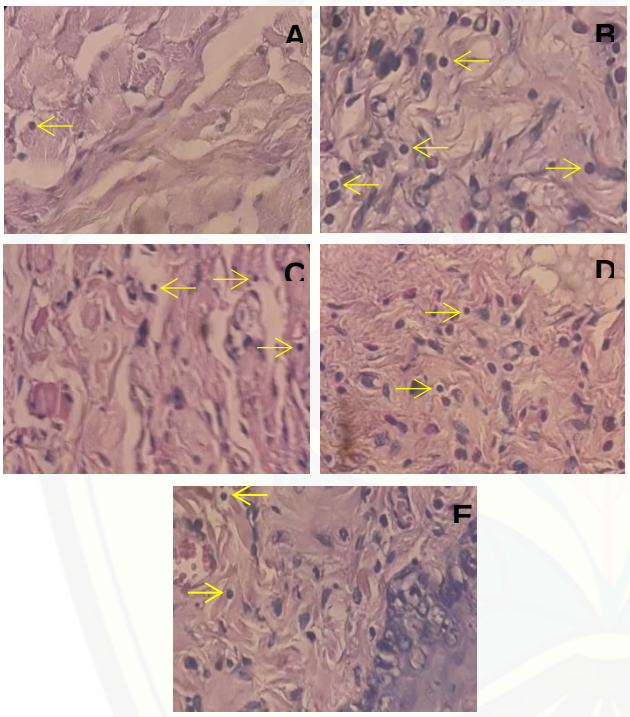

Figure 1. Histological description of lymphocyte cells in the mucosa of male Wistar rats (yellow arrows) observed using a binocular microscope at 400x magnification. (A) Group N, (B) Group K, (C) Group P1, (D) Group P2, and (E) Group P3

The following mean and standard deviation of lymphocytes count were listed in the table:

Kelompok N (*Mean*±SD)	Kelompok K (*Mean*±SD)	Kelompok P1 (*Mean*±SD)	Kelompok P2 (*Mean*±SD)	Kelompok P3 (*Mean*±SD)
6,08 ± 0,995	15,91 ± 0,569	13,91 ± 0,630	11,66 ± 1,305	9,41 ± 0,687

X ± SD : Mean standard deviation

N: No traumatic ulcer-no EDU

K: With traumatic ulcer-without EDU

P1: With traumatic ulcer-EDU 0.05 g/kg

P2: With traumatic ulcer-EDU 0.15 g/kg

P3: With traumatic ulcer-EDU 0.45 g/kg

Tables and diagrams containing data on the average number of lymphocytes showed that the control group had the highest average number of lymphocytes compared to other groups, followed by treatment group 1, treatment group 2, treatment group 3, and the group with an average lymphocytes count. the lowest was the normal group.

Based on the Saphiro Wilk normality test, a significance of $p>0.05$ was obtained which indicates that the data was normally distributed, as well as the Levene homogeneity test results obtained a significance of $p=0.330$ ($p>0.05$), which means the data was homogeneous. Furthermore, to find out whether or not there was a difference in the average in all groups, the data was carried out by using the One-way Anova parametric statistical test. The test results obtained were significance $p=0.00$ ($p<0.05$), which means that there was a significant difference in the number of lymphocytes between groups, so it was continued with the post hoc Least Significant Difference (LSD) test. From the LSD test, it was found that the significance result was $p<0.05$ in all groups which indicated that significant data differences occurred in all groups.

DISCUSSION

Based on the data obtained, EDU had the ability to suppress the number of lymphocytes. This could be seen in the average number of lymphocytes in the treatment groups, both P1, P2, and P3, all three of which had a lower average than group K, which meant that there has been decreasing in the number of lymphocytes. In the results of the LSD test, the three doses were found to be able to reduce the number of lymphocytes significantly, from the lowest to the highest dose. The P1 group which was the group with the lowest dose of 0.05 g/kg BW rats had the highest number of lymphocytes. This was because the dose used low enough so that the amount of active substance contained in the dose was quite low, resulting in decreasing the number of lymphocytes in this group not as large as the other treatment groups. Meanwhile, the treatment group with the lowest number of lymphocytes was the P3 group which had the highest dose (0.45 g/kg BW rats) which meant that the number of lymphocytes decreased as the EDU dose increased. The results of this study which stated that there was a decrease in the number of lymphocytes as the dose increased were supported by research conducted by Ayu in 2014 on the effect of *Centella asiatica* leaves extract on the percentage of lymphocytes. In this study, it was concluded that the group with the lowest percentage of lymphocytes was in the group with the highest dose of extract, namely a dose of 0.50 g/kg BW. This could happen because a high enough dose contained an active compound in sufficient amount to suppress the number of lymphocytes. One of the active compounds in EDU was flavonoid which acts as an anti-inflammatory by inhibiting the release of inflammatory mediators, one of which was IL-1 and TNF (Miftah, 2020). IL-

1 played a role in increasing the secretion of chemokines and other cytokines such as IL-6, as well as increasing the expression of adhesion molecules (Hamidy, 2017).

If IL-1wais inhibited, the secretion of chemokines and expression of adhesion molecules could also be inhibited. Chemokines and adhesion molecules were two important factors that play a role in the induction of lymphocyte migration from lymphoid glands to traumatized tissues in chronic inflammation (Lukacs, 2000). If the chemokines and adhesion molecules do not work optimally, the migration of lymphocytes to the wound tissue could decrease so that the inflammatory reaction could be suppressed.

Group K had the highest number of lymphocytes because the extract was not given. Group K and group N had a fairly large difference in the average number of lymphocytes. This was because in group N there was no inflammation so there was no spike in the number of inflammatory cells including lymphocytes. On the other hand, group K was treated with traumatic ulcers for up to seven days when the lymphocytes count peaked on that day (Jiyanto 2012). The reason for holding group N was to see if EDU was able to suppress the number of lymphocytes to close to normal conditions. When compared with group N, the number of lymphocytes in group P3 (the group with the least number of lymphocytes) still exceeds the number of group N, so it could not be concluded that EDU was able to suppress the number of lymphocytes to return to normal conditions, however, if observed the average number of lymphocytes in group P3 not much different from group N. In addition, in the Ifandari's study in 2014 the average number of lymphocytes in the normal group was 7.53. The data was not much different from the P3 data's group in this study, which was 9.41 so it was suspected that EDU had the potential to suppress the number of lymphocytes to close to normal conditions.

In this study, it was found that EDU was able to reduce the number of lymphocytes in the buccal mucosa of Wistar rats with traumatic ulcers and had the potential to reduce the number of lymphocytes to near normal in healthy conditions. The dose of EDU which showed the highest decrease in the number of lymphocytes was 0.45 g/kg BW rats. The results of this study were in accordance with other similar studied because the extract used contains compounds that were anti-inflammatory, one of which was flavonoids, so that similar results were obtained, namely a decrease in the number of inflammatory cells, especially in this study lymphocytes. In addition, the results obtained from this study were in accordance with another study conducted by Ayu in 2014 which concluded that the dose that was able to reduce the percentage of lymphocytes to the lowest was a dose of 0.50 g/kg BW, the dose was not much different from the dose in this study. namely the dose of 0.45 g / kg BW.

However, considering that this study discusses an inflammatory response in which the response should not be suppressed excessively because it was feared that the healing process could take too long due to the inflammatory cells not working against the antigen optimally, the dose of 0.05 g/kg BW rats was the most dose significantly, because even though the dose was not too high, it was able to reduce lymphocyte infiltration significantly as evidenced by the results of the LSD test between group K and group P1 at a dose of 0.05 g/kg BW rats which showed a significance of $p<0.05$. It is hoped that a sufficiently low dose could not result in excessive suppression of lymphocytic infiltration and also lower side effects.

CONCLUSION

The Purple Leaf Extract (EDU) could reduce lymphocytes infiltration at mucosa's traumatic ulcers in Wistar rats (Rattus norvegicus) at a dose of 0.05 g/kg BW significantly.

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