Oral Presentation (PCS-17)

The Potency Of Sernai Leaves (*Wedelia biflora*) to Increase Healing of contusion in Rats (*Rattus norvegicus)*

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INTRODUCTION

Contusion is a type of injury caused by a collision of the body with a blunt object followed by damage to the soft parts of the body, bone damage, bleeding and swelling. The tissue Injuries cause an inflammatory or inflammatory reaction, in which the inflammatory reaction is followed by pain (dolor), heat (kolor), red (rubor), swelling (tumor), and impaired function in the area around the wound (fungsiolesa). The Inflammatory reactions are the result of increased capillary permeability and migration of leukocytes to areas of inflamed tissue. The body's defense process against injury involves cells that infiltrate the wounded areas including neutrophils, macrophages, and lymphocytes. The body's response to inflammation is characterized by the release of neutrophils from the blood vessels which function as the first defense cells that play a role in phagocytosis of microorganisms and inflammatory cells in the wound. Contusion can be cured by giving anti-inflammatory drugs that aim to relieve pain, slow and prevent the spread of the tissue destruction process.the Anti-inflammatory drugs are classified into two, namely Non-Steroid Anti-inflammatory (NSAIDs) and Anti-inflammatory Steroids (AIS), a class of anti-inflammatory drugs that have the ability to suppress signs and symptoms of inflammation. The use of anti-inflammatory drugs in humans can cause side effects that are harmful to the body such as ulcers, cardiovascular diseases and osteoporosis, while fot the animals cause the difficulties due to the large variety of species. Therefore, herbal ingredients are developed, one of which is sernai (*Wedelia biflora*). Sernai has empirical benefits as a medicine for wounds and ulcers , sernai leaf water extracts as antipyretics, antitripanosoma, antimicrobials, antifungal, and anti-inflammatory agents. Sernai contains alkaloids, terpenoids, triterpenoids and flavonoids. The Flavonoid compounds can inhibit the release of arachidonic acid, secretion of lysozyme enzymes from neutrophil cells and endothelial cells and inhibit the proliferation phase and exudation phase of the inflammatory process. If the prostaglandin pathway is inhibited, the the occurrence of local blood vessel vasodilatation will reduces and so a leukocyte infiltration decreases, then it is replaced by macrophages that function to help endothelial cell formation and new blood vessels so that wound healing can take place quickly .

MATERIALS & METHODS

Sernai leaves were taken in Lamteube Mon Ara, Kuta Baro District, Aceh Besar. the making of EEDS was done with maceration method. The rats were anesthetized with local EMLA anesthesia before treated. After the anesthesia reacted, the contusion was made by pressing the base of the rat's tail using 23 cm *doyen intestinal forceps* with a 1 degree squeeze ratchet on the handle for 1 minute 30 seconds, then released. Each group that has been bruised is treated as follows: P0 treatment: negative control, P1 treatment: contusion with EEDS 10%,P2 treatment: contusion with EEDS 20%,P3 treatment: contusion with EEDS 40%. The extract was given by applying evenly in the contusion area once a day according to dosage and the contusion area before being given treatment was measured. Then the observation was done for 8 days to see the extent of the wound by measuring the area of the contusion (length x width) using a caliper. The rat blood was collected through orbital sinus to calculate neutrophils. The Blood collection was carried out on day 0 before treatment, day 3 and day 6 after treatment. The data was tested using the One Way Analysis of Variance (ANOVA) method and continued with the Regression test.

RESULTS AND DISCUSSION

Tabel 1. Average ± SD number of neutrophils (%) of rats before and after administration of sernai leaf ethanol extract (EEDS)

Treatment	Day 0	Day 3	Day 6
P0 (no trearment)	24,66 ± 4,17	27,16 ± 2,71	25,33 ± 3,98
P1 (EEDS 10%)	25,00 ± 4,85	28,16 ± 3,12	27,66 ± 3,77
P2 (EEDS 20%)	24,83 ± 1,83	27,66 ± 3,61	27,33 ± 4,13
P3 (EEDS 40%)	25,33 ± 3,77	30,00 ± 2,96	28,00 ± 3,03

The 0th day before being given contusion and EEDS treatment, the average percentage of rat neutrophils was between 24-25%. This result is in the normal range. The average normal percentage of rat neutrophils ranged from 6.2-26.7%. Therefore, this data becomes a base line to see whether there is an increasing or decreasing in neutrophils if the animal is induced by contusion and given EEDS. The results of the statistical, there was no significant differences (P> 0.05) between treatment groups with 0, 3 and 6 days. But, there was a tendency for increasing on day 3 with a higher percentage of neutrophils compared to the base line (number of neutrophils on day 0 that had not been given the contusion induction), namely, P0 (27, 16%), P1 (28.16%), P2 (27.66%), P3 (30.00%). Furthermore, the percentage of neutrophils on day 6 was lower than day 3 and higher than day 0, namely, P0 (25.33%), P1 (27.66%), P2 (27.33%) and P3 (28.00%). When compared to the base line showed an increase in the number of neutrophils starting on day 3 along with the inflammatory process due to bruising, then the number of neutrophils decreases on day 6 compared to day 3. The Increased number of neutrophils on day 3 is affected by increased blood flow and blood capillary permeability followed by vasodilation during injury, so phagocytic cells such as neutrophils, macrophages, platelets and T lymphocytes migrate to the wound area. The number of inflammatory cells around the wound area is marked by an increased number of neutrophils, causing symptoms of swelling or edema. The wound healing process begins with increased in the number of neutrophils in the inflammatory phase, followed by the proliferation phase, which is the process of repairing the skin epithelial cells so that the wound become normal again. Remodeling phase aimed for maximizing and integrity of the wound. When the number of neutrophils decreases, the area of the contusion becomes smaller. In the EEDS with treatment groups, i.e P0 (without treatment), P1 (Piroxicam 0.5%), P2 (EEDS 10%), P3 (EEDS 20%) and P4 (EEDS 40%). On the third day the area of contusion in the group P0 (52.52 mm), P1 (30.86 mm), P2 (23.12 mm), P3 (22.15 mm) and P4 (18.57 mm), whereas on the 6th day the area of contusion was in groups P0 (18.5 mm), P1 (5.15 mm), P2 (4.48 mm), P3 (4.47 mm) and P4 (1.34 mm). EEDS with various concentrations can accelerate the inflammatory process for healing bruises. There is a response by shrinking the size of contusion with EEDS 10%, 20% and 40%, then the percentage of neutrophils in the 6th day will also decrease and be replaced immediately by macrophages to help the formation of new cells.The neutrophils increase in the inflammatory phase 0-5 days and decrease in the proliferation phase 3-14 days, the number of neutrophils in the proliferation phase is less because neutrophils have been replaced by macrophages. Macrophages support the formation of angiogenesis . However, the number of neutrophils has not approached the base line value because the measurement of neutrophil counts was only carried out until the 6th day, while the healing process averaged the reduction of the area of injury until the 8th day with the area of contusioan in the P0 group (7.14 mm), P1 (1.95 mm), P2 (0.88 mm), P3 (0.79 mm) and P4 (0.15 mm), and in accordance with the conditions of contusion in the wound healing process is not perfect. The speed of wound healing can be influenced by substances contained in the drug given, such as the drug has the ability to improve healing by stimulating faster growth of new cells.

Flavonoid content in EEDS is believed to be able to inhibit the release of arachidonic acid and the exudation phase of the inflammatory phase and also accelerate wound contraction and re-epithelialization.. The Flavonoids also function as antimicrobials that function to kill bacteria. Increased prostaglandins will stimulate the pain nerves and increase the inflammatory response, if the inflammatory response persists and lasts longer will cause tissue damage, therefore inhibition of the cyclooxygenase pathway must be done to reduce the symptoms of inflammation. The role of saponins in EEDS as antibacterial by increasing the permeability of bacterial cell membranes so that hemolysis of bacterial, tannin and terpenoid cells has antioxidant activity that plays a role in capturing free radicals that can damage cell membranes and reduce inflammation of cells .

The prostaglandin biosynthesis starting from stimulation of cells which causes damage to cell membranes, so that the enzyme phospholipase is activated to convert phospholipids to arachidonic acid and help the enzyme cyclooxygenase to produce prostaglandins. Prostaglandins are pain mediators that are specific for long-lasting pain. The active compounds contained in EEDS work to inhibit the release of serotonin and histamine which are chemical mediators to the site of inflammation, and work on the main mediator in inflammation which inhibits prostaglandin synthesis through inhibition of the synthesis of cyclooxygenase.The Stunted prostaglandin pathways will reduce vasodilation of blood vessels so that leukocyte infiltration decreases. With decreasing leukocyte infiltration, the inflammatory phase progresses rapidly so that the cell regeneration process can be formed immediately and the skin structure returns to normal. EEDS can accelerate wound healing by preventing inflammatory reactions, stimulating collagen synthesis, and vasodilation of blood vessels, and increasing the rate of angiogenesis. Thus this study shows that EEDS has the potential

as anti-inflammatory by accelerating the inflammatory process as well as analgesics by inhibiting prostaglandin synthesis

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