

Research Article

Aloe vera Extract Sheet and Egg Albumin (*Gallus domesticus*) Reduce the Number of Lymphocytes on Gingival Incision Wound

Aloe vera Extract Sheet dan Albumin Telur Ayam Kampung (*Gallus domesticus*) Menurunkan Jumlah Limfosit pada Luka Insisi Gingiva

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ABSTRACT

An incision in the oral cavity is a dental procedure performed in surgical procedures. Suturing is the gold standard in primary wound closure care, however, suturing can cause tissue trauma, scars, and secondary infections. Cyanoacrylate adhesive is one of the alternative tissue glue for wound closure but it has inflammatory risk as suturing, potential toxicity, and accidental adhesion risks. Aloe vera extract sheet based combined with free-range chicken egg albumin is expected to be an alternative method for wound healing of gingival incisions. This study aimed to investigate the effect of administration of Aloe vera extract wound healing sheet (WHS) and free-range chicken egg albumin on the number of lymphocytes in the gingival incision wound as the inflammatory response. Experimental laboratory research with a posttest-only control group design was conducted in 24 male rats (*Rattus norvegicus* Wistar strain) with an incised gingiva, divided into four groups, namely T1 (50% WHS concentration and chicken egg albumin), T2 (100% WHS concentration and chicken egg albumin), PC (positive control with suturing), NC (negative control without treatment). The treatment was carried out for seven days. The data obtained were analyzed statistically using One-way ANOVA and Post Hoc LSD. The use of Aloe vera extract WHS and free-range chicken egg albumin (T1 and T2) reduced the number of lymphocytes compared to control groups (PC and NC; $p < 0.05$). WHS and chicken egg albumin can shorten the inflammatory process in wound healing which is characterized by lower lymphocyte counts compared to the control groups.

Keywords: *Aloe vera*, free-range chicken albumin, tissue glue, wound healing

ABSTRAK

Insisi di rongga mulut merupakan tindakan kedokteran gigi yang sering dilakukan pada prosedur operasi sehingga menghasilkan luka sayatan. *Suturing* merupakan standar perawatan dalam penutupan luka primer, akan tetapi *suturing* dapat menyebabkan trauma pada jaringan, menimbulkan bekas luka, dan infeksi ikutan. *Cyanoacrylate adhesive* merupakan salah satu bahan lem jaringan yang terbukti efektif menutup luka, namun memiliki resiko inflamasi yang sama seperti *suturing*, bersifat toksik, serta adhesi yang tidak disengaja dapat terjadi. Lem jaringan *wound healing sheet* (WHS) berbahan dasar ekstrak *Aloe vera* dan albumin telur ayam kampung diharapkan mampu menjadi metode alternatif untuk penyembuhan luka insisi pada gingiva. Penelitian ini bertujuan untuk mengetahui respon inflamasi pada proses penyembuhan luka insisi gingiva berupa jumlah limfosit pasca pemberian WHS ekstrak *Aloe vera* dan albumin telur ayam. Penelitian eksperimental laboratoris desain riset post test-only control group design meneliti 24 ekor tikus jantan (*Rattus norvegicus* galur Wistar) dengan gingiva yang telah diinsisi dibagi menjadi empat kelompok, yaitu T1 (WHS konsentrasi 50%), T2 (WHS konsentrasi 100%), PC (kontrol positif perlakuan *suturing*), NC (kontrol negatif tanpa perlakuan). Perlakuan dilakukan selama tujuh hari. Data yang diperoleh dianalisis secara statistik menggunakan One-way ANOVA dan dilanjutkan dengan Post Hoc LSD. Pemberian WHS ekstrak *Aloe vera* dan albumin telur ayam dapat menurunkan jumlah limfosit dibandingkan dengan kelompok *suturing* maupun kelompok tanpa intervensi ($p < 0.05$). WHS ekstrak *Aloe vera* dan albumin telur ayam kampung dapat mempersingkat proses inflamasi dalam penyembuhan luka yang ditandai dengan jumlah limfosit yang lebih rendah dibandingkan dengan kelompok kontrol.

Kata Kunci: Albumin telur ayam kampung, aloe vera, lem jaringan, wound healing

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INTRODUCTION

The incision in the oral cavity is a dental procedure performed in surgical procedures, resulting in an incision wound (1). Wound closure is an important part of treatment to make the tissue work normally. Suturing is the gold standard of care in primary wound closure (2,3). Suturing can control rapid bleeding or long cuts with minimal tension, but suturing can cause tissue trauma due to needle penetration and leave scars, secondary infections, and complications. Suturing is conducted in confined spaces such as the oral cavity requires good suture skills (2-4).

Currently, alternative non-suturing wound healing methods are developed to address the shortcomings of the suturing method. Synthetic or bio-adhesive tissue glue is one of the alternative methods used (5). Wound closure with tissue glue can be conducted quickly, is relatively painless, has minimal inflammation, easy to remove, and has a lower infection rate than sutures (3). *Cyanoacrylate* adhesive is an alternative to standard suturing which is effective as a painless wound closure method and provides good esthetics (6). The disadvantages of cyanoacrylate-based tissue glue such as tissue inflammation, toxicity due to product degradation, and accidental adhesion risk to the body or equipment (7,8). Tissue glue is also known to interfere with wound healing when applied to patients with sensitivity to the components of tissue glue, patients with wounds, gangrene, or ulcer decubitus, patients with preoperative systemic disease, or patients with peripheral vascular disease, blood clotting disorders, and history of keloids (8,9).

Our previous study combined *Aloe vera* extract wound healing sheets of 50% and 100% concentrations with free-range chicken egg albumin as tissue glue for gingival incision wounds in Wistar rats. The use of *Aloe vera* sheet preparations after the topical application of albumin helps to promote wound healing as well as protect wounds in the oral cavity environment. The results of the study showed a higher number of fibroblasts compared to the natural healing process and suturing treatment after the seventh-day post-application of the preparation. Therefore, it indicates the potential of *Aloe vera* extracts wound healing sheet combination with free-range chicken egg albumin to accelerate the healing of gingival incision wounds (5).

Inflammation is the first phase in wound healing with lymphocyte infiltration occurring on the first day. The number of lymphocytes peaked on the 3rd to 6th day and decreased on the 7th day. Lymphocytes together with macrophages have a role to stimulate each other to eliminate triggering antigenic agents so fibroblasts form a strong tissue in the proliferative phase (10). *Aloe vera* contains active compounds that are potent anti-inflammatory agents and can accelerate the wound-healing process (11). The topical application of *Aloe vera* to the oral mucosa of Wistar rats is reported can accelerate the healing of incision wounds (12). Topical preparations/ ointments are less effective for use in the oral cavity because of saliva and oral microorganisms are also prone to physical trauma due to food which can trigger infection if oral hygiene is not well maintained (13,14). Hikmawati *et al.*, state that *Aloe vera*-PVA sheet preparation as wound dressing material shows low

degradation time (15). Sheet preparations will be suitable for use in the oral cavity which is prone to degradation due to the saliva.

The study on conjunctival grafts using albumin as an alternative to sutures in rabbit eyes concluded that the technique using albumin glue was better and resulted in a smaller wound gap on day one compared to the suture technique (16). Albumin glue from fresh eggs shows good shear adhesion and underwater adhesion performance, good wound healing performance during *in vivo* experiments on rats, and did not show a long-term inflammatory response (7). Free-range chicken eggs have high albumin protein compared to purebred chickens and have an affordable price (17). This study aimed to investigate inflammatory response in the gingival incision wound healing process in the form of the number of lymphocytes following treatment with wound healing sheets of *Aloe vera* extract and free-range chicken egg albumin.

METHOD

This research was a laboratory experimental study with a posttest-only control group design. The experimental animals were 24 male white rats (*Rattus norvegicus* strain Wistar), divided into four groups. The research started from August to September 2021. Ethical approval has been obtained from The Research Ethics Committee of Faculty Medicine, Jenderal Soedirman University, with registration number 143/KEPK/VIII/2021. Experimental animals were acclimatized for one week in the experimental animal unit of the Pharmacology Laboratory, Faculty of Medicine, Jenderal Sudirman University, Purwokerto. *Aloe vera* extract was made at the Laboratory of Materia Medica Batu, Malang. Sheets of *Aloe vera* extract were made at the Biomedical Laboratory, Faculty of Medicine, Jenderal Soedirman University, Purwokerto. Histology samples of rat gingiva were prepared at the Histology Laboratory, Faculty of Medicine, Brawijaya University, Malang.

Aloe vera Extract

Aloe vera extract was prepared using the maceration method. A total of 5 kg of *Aloe vera* (skin and leaf flesh) were dried and mashed. The refined *Aloe vera* was put into an Erlenmeyer tube and macerated with 1000mL 70% alcohol for 24 hours to obtain *Aloe vera* extract. The extract obtained was filtered using a white cloth. The filtering results were heated in a water bath to obtain *Aloe vera* precipitate with a concentration of 100%. *Aloe vera* extract with a concentration of 50% was obtained from dilution using distilled water.

Wound Healing Sheet *Aloe vera* Extract

The wound healing sheet was produced by dissolving 1.25 grams of sodium alginate (Na alginate), and 10 grams of polyvinyl alcohol (PVA) into 125mL of aqua DM (demineralized water) in a beaker. The PVA-Na alginate mixture was stirred using a hot plate magnetic stirrer at a speed of 1000 rpm for one hour at 25°C. The homogeneous solution of PVA-Na alginate was added with 100% concentration of *Aloe vera* extract with a ratio of 1:1 solution and extract, to make a wound healing sheet with a concentration of 100%. A wound healing sheet with 50% concentration is made with the same ratio but used 50% of *Aloe vera* extract. PVA-Na alginate solution and *Aloe vera*

extract were homogenized using a hot plate magnetic stirrer at 1000rpm for one hour at 100°C. The formed PVA-Na alginate-*Aloe vera* extract solution was poured onto a 24x24cm mold (aluminum pan) which had been coated with aluminum foil. The mixture was poured and flattened with a thickness of 0.5mm and then placed in an oven at 100°C for 1.5 hours to form a soluble sheet in the mouths of rats. The sheet formed was cut with a size of 0.75x0.5 cm to match the width of the rat's gingiva. Free-range egg albumin of 0.02mL volume as an adhesive was applied to wound-healing sheets of *Aloe vera* extract.

Treatment in Experimental Animals

The parameter observed in this research was the number of lymphocyte cells in the gingival histology preparations of experimental animals. Twenty-four rats were divided into four groups, namely T1 (50% concentration wound healing sheet and free-range chicken egg albumin group), T2 (100% concentration wound healing sheet and free-range chicken egg albumin group), PC (positive control group; wound suturing using silk thread with interrupted suturing technique), NC (negative control group; without treatment). The free-range chicken albumin used was 0.02 mL in volume. Firstly, the rats were anesthetized with zoletyl® intramuscularly on the rat's gluteus muscle, then the labial gingiva under the two mandibular incisors was incised using a surgical blade no. 11 for a length of 5 mm with a depth reaching the alveolar bone. The bleeding was stopped and cleaned using gauze and NaCl.

Application of Wound Healing Sheet

The wound healing sheet was applied by drying the rat's gingiva, then the sheet was smeared with 0,02 mL free-range chicken egg albumin using a cotton bud and then applied to the wound using tweezers. The treatment was given for seven consecutive days.

Histological Preparations

On the seventh day, rats were decapitated, gingival tissue was taken and stored in NBF solution, and histological preparations for gingival incisions were made. Histological preparations were made with Hematoxylin Eosin (HE) staining. Observations of preparations were carried out to investigate lymphocytes as markers of wound healing. Lymphocyte cells in histological preparations seen in the microscope have the characteristics of being round, single-celled, and without granules.

Observation of the Number of Lymphocytes

Observation of the number of lymphocytes was carried out by two observers. The blinding method used was a single blinding trial. Observation of the number of lymphocytes was carried out with a light microscope with a magnification of 1000x with 20 fields of view per sample and photographed using Optilab Pro to count the number of lymphocytes. The number of lymphocyte cells was calculated by the number of lymphocyte cells seen in the visual field.

Statistical Analysis

The data were tested by parametric analysis. The Shapiro-Wilk test and Levene's Test indicated that the data were normally distributed and homogeneous ($P > 0.05$). The One-way ANOVA hypothesis test indicated significant results ($p < 0.05$) and was continued with the Post Hoc LSD test.

RESULTS

Histological pictures of each group can be seen in Figure 1.

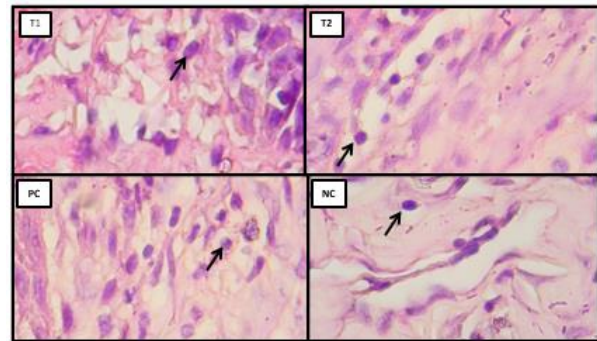


Figure 1. Histology of Lymphocyte cells on the 7th post-incision day in all treatment groups (magnification 1000x).

Note:

T1. Representative image of gingiva tissue with wound healing sheet 50% *Aloe vera* extract and free-range chicken egg albumin.
T2. Representative image of gingiva tissue with wound healing sheet 100% *Aloe vera* extract and free-range chicken egg albumin.
PC. Representative image of gingiva tissue with suturing as the positive control.
NC. Representative image of gingiva tissue without intervention as the negative control.

The results of the average number of lymphocyte cells can be seen in figure 2.

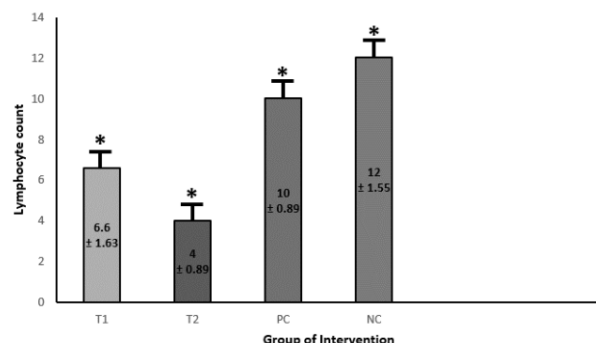


Figure 1. Lymphocyte count on the 7th post-incision day. Data were shown as mean ± S.D.

Note:

T1. Lymphocyte count in gingiva tissue with wound healing sheet 50% *Aloe vera* extract and free-range chicken egg albumin.
T2. Lymphocyte count in gingiva tissue with wound healing sheet 100% *Aloe vera* extract and free-range chicken egg albumin.
PC. Lymphocyte count in gingiva tissue with suturing as the positive control.
NC. Lymphocyte count in gingiva tissue without intervention as the negative control.

* significant difference between groups (p -value < 0.05)

The results showed that the administration of wound healing sheets with *Aloe vera* extract and free-range chicken egg albumin (T1 and T2) reduced the number of lymphocytes compared to the suturing and no-intervention groups (PC and NC, respectively). The lymphocyte count in the T1 group using 50% extract was 6.6 ± 1.63 , and the T2 group using the 100% extract was 4 ± 0.89 . The results in the T1 and T2 groups indicated that

the higher concentration of *Aloe vera* was directly proportional to the decrease in the number of lymphocytes, with the 100% concentration of *Aloe vera* wound healing sheet showing the lowest lymphocyte count compared to 50% concentration *Aloe vera* sheet as well as control groups ($p < 0.05$).

The One-Way ANOVA test results showed a significant difference in lymphocyte count between groups ($p < 0.05$). Post hoc LSD analysis showed a significant difference in the mean of lymphocyte count between all groups T1, T2, PC, with NC ($p < 0.05$). It indicated that the wound healing sheet significantly could shorten the inflammatory process in wound healing compared to the control groups.

DISCUSSION

The wound-healing process begins with an inflammatory phase when lymphocytes begin to appear and infiltrate the wound area along with neutrophils and macrophages (18). Lymphocytes migrate to the wound area on day 1, the number reaches a peak on day 3 to 6, then decreases on day 7. Lymphocytes bind to antigens, then activate and secrete lymphokines which play a role in stimulating and activating macrophages in phagocytosis and polymorphonuclear (PMN) cells that have undergone apoptosis. The activated macrophages will release cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF), which will activate lymphocytes. Macrophages and lymphocytes stimulate each other to eliminate triggering antigenic agents so that fibroblasts form a strong tissue in the proliferative phase (10). The decrease of lymphocytes on the 7th day occurs because the existing inflammatory cells will undergo apoptosis therefore their duties as phagocytic agents are replaced by fibroblasts which form new tissue in the proliferative phase (19).

The treatment groups which were treated with wound healing sheets of 50% dan 100% extract of *Aloe vera* and free-range egg albumin showed a lower lymphocyte count on day 7 compared to the negative control group without treatment. The decrease in lymphocytes as inflammatory cells indicates that the healing process has entered the next stage (19). The low number of lymphocytes compared to the control group may indicate a shorter inflammatory process and faster wound healing.

The negative control group presented the condition of the wound without treatment intervention. The negative control group produced the highest number of lymphocytes, which indicated that the wound-healing process was still in the inflammatory phase. The inflammatory process and wound healing in the negative group presumably occurred slower because in open wounds the epithelial closure was delayed and the rate of formation of granulation tissue was higher (20). The condition of the oral cavity that contains microorganisms and food debris causes the gingival wound to be prone to infection and mechanical irritation, thus making the inflammatory phase longer (21).

Aloe vera applied to wounds has the potential as an anti-inflammatory agent so that a short period of inflammation will accelerate healing to the next phase. Anthraquinones, sterols, and saponins as active ingredients of *Aloe vera* act as antibacterial and wound healing promoters. The ingredients contained in *Aloe vera* can reduce leukocyte adhesion through the cyclooxygenase and prostaglandin

pathways (22). *Aloe vera* is effective in inhibiting the inflammatory process through inhibition of IL-6 and IL-8, reduction of leukocyte adhesion, enhancement levels of IL-10, and reduction levels of TNF- α (23). Aloesin as the active compound in *Aloe vera* is also able to significantly reduce the activity of macrophages and neutrophils in the inflammatory phase, and reduce TNF- α , IL-6, and IL-1 β (24).

Wound healing is also affected by external factors (25). Wound healing in the oral cavity is prone to infection due to the condition of the oral cavity which contains microorganisms and food debris (13). The content of anthraquinone emodin, aloe-emodin, aloin, aloeresin D, isoaloeresin D, and chromones in *Aloe vera* are reported to have antimicrobial activity. Anthraquinones have antimicrobial properties by altering solute transport through membranes, cell walls, and elongation of fatty acids (26). *Aloe vera* has a high antioxidant content from anthraquinones such as barbaloin, emodin, anthranol, lophenol, and cycloartenol. Anthraquinones have strong anti-inflammatory effects acting as antioxidants and are involved in free radical-mediated reactions during the inflammatory response to inhibit free radical-mediated cytotoxicity and lipid peroxidation. Antioxidants are important as enzymatic defenses against reactive oxygen species (ROS) to reduce tissue ischemia and oxidative injury (24,27).

Albumin as a wound adhesive in this study also has a role in wound healing. Albumin contains ovalbumin which works as a cell adhesion substrate that supports attachment (15). Albumin induces epidermal growth factor (EGFR) expression by activating extracellular signal-regulated kinase $\frac{1}{2}$ (ERK1/2) and upregulating nuclear factor kappa B (NF- κ B). EGFR has a significant role in regulating the expression of transforming growth factor β (TGF- β), which plays an important role in wound healing such as inflammation, stimulates angiogenesis, fibroblast proliferation, collagen synthesis, deposition, and remodeling of the new extracellular matrix via the mothers against decapentaplegic (SMAD) pathway. Administration of albumin can reduce the expression of proinflammatory cytokines such as TNF- α , IL-1, and IL-6, as well as C-reactive protein (CRP) and matrix metalloproteinase-8 (MMP-8). The subtraction of expression of MMP-8 in tissues may be associated with decreased inflammation (28). Izzaty *et al.*, state that the content of albumin in the extract of tilapia fish could reduce the number of inflammatory phase lymphocytes in the healing process of the buccal mucosa of Wistar rats (10).

Albumin topical preparations can be absorbed by the body through transmucosal delivery. Albumin functions in regulating blood osmotic pressure and maintaining water content in blood plasma during the inflammatory phase, therefore, helps to form body tissues in surgical wounds, accelerates tissue healing, and carries away nutrients and oxygen for the formation of new tissue in the proliferative phase (29). Albumin contains natural proteins and collagen that compile the extracellular matrix which induces growth factors, cell migration and adhesion, and tissue regeneration (5). Albumin inhibits the formation of free radicals as a result of phagocytosis of PMN cells and maintains blood osmotic pressure to prevent edema (17).

The preparation of *Aloe vera* extract in the form of sheets is useful for protecting wounds from mechanical trauma and the biological environment of the oral cavity. Free-range

chicken egg albumin has properties that support cell adhesion as a tissue glue material with active compounds which also promotes wound healing. The combination of wound healing sheets of *Aloe vera* extract glued using free-range egg albumin makes this material easy to use and has the effect of protecting and accelerating the wound healing process as has been proven in our previous study (5).

The results of the inflammatory response in this study indicated that the treatment groups showed an inflammatory phase that has progressed more quickly marked by a decrease in the number of lymphocytes compared to the control groups on the seventh day. These results are in agreement with our previous study which analyzed wound healing markers at the proliferative stage. The results of the study showed that the treatment groups had the shortest wound length, the largest percentage of injuries, and the highest number of fibroblasts on the seventh day compared to the suturing positive control group and the negative group (5). This study is also in accordance with other studies examining the wound healing effects of Aloe-alginate film containing *Aloe vera* and it is stated that the ingredients contained in *Aloe vera* such as acemannan have anti-inflammatory activity by stimulating macrophages and releasing cytokines (30). Further research conducted by Koga et al. has investigated the effect of aloe-alginate film cross-linked with zinc chloride as a wound dressing on skin wounds of Wistar rats that could modulate the inflammatory phase because the ingredients contained in it can stimulate macrophage activation and promote the release of cytokines from fibroblasts (31).

Wound healing sheet of *Aloe vera* extracts and free-range egg albumin depicted lower lymphocyte count on the 7th day compared to suturing in the positive control group as the gold standard for wound care. This result can be affected by suturing only being able to facilitate wound closure and creating an optimal setting as a mechanical support for wound healing, and avoiding complications such as bleeding, infection, and irritation without being able to accelerate wound healing (32). Meanwhile the combination of wound healing sheet extracts of *Aloe vera* and free-range chicken egg albumin can help the attachment of incision wounds to the gingiva as well as its active ingredients can shorten the inflammatory process in wound healing (28,33). Needle penetration in suturing can also cause trauma to the tissue that provides

additional wounds so the inflammatory process and wound healing can also last longer (2-4).

The different concentrations of *Aloe vera* extract in the treatment groups showed different results. The wound healing sheet extract concentration of 100% *Aloe vera* showed a lower lymphocyte count than the wound healing sheet extract of 50% *Aloe vera*. These results indicate that the greater concentration of *Aloe vera* extract used shortens the inflammatory process taken in the healing of gingival incision wounds in Wistar rats. This presumably was affected by the higher concentration of *Aloe vera* extract, and the higher antibacterial and anti-inflammatory properties of the wound healing sheet, which resulted in shortened inflammatory process in the healing of gingival incision wounds.

Wound healing sheet with 100% concentration of *Aloe vera* extract and free-range egg albumin showed the most effective concentrations to shorten the inflammatory process in the healing of gingival incision wounds, so they have the potential to be used as an alternative to suturing on gingival incision wounds. Further research to develop on *Aloe vera* concentrations in the range of 50-100% to determine the most effective concentration of this wound healing sheet is needed. This research still used conventional techniques to apply wound healing sheet preparations of *Aloe vera* extract and free-range egg albumin on gingival incision wounds, therefore innovation and new technology are needed to create more practical preparations.

The results of this research indicated wound healing sheets of *Aloe vera* extract and free-range chicken egg albumin could shorten the inflammatory process in wound healing, which was characterized by lower lymphocyte counts compared to the control group. The active compounds possessed by *Aloe vera* and free-range chicken egg albumin affected the inflammatory process shorter so the healing process occurred faster. It means wound healing sheet extracts of *Aloe vera* and free-range egg albumin had the potential to be used as alternatives to suturing on gingival incision wounds.

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