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Gallus domesticus egg-white gel accelerate wound healing after tooth extraction

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ABSTRACT

Background: Tooth extraction is an action taken to prevent or treat the onset of a disease. However, there are still many complications after tooth extraction, such as a wound healing process that runs slowly and even causes excessive bleeding which can cause death. Gallus domesticus egg-white gel is a medicament that has growth factors that can help the wound healing process run smoothly. Purpose: To determine the number of inflammatory cells involved in the wound healing process after tooth extraction by the application Gallus domesticus egg-white gel. Methods: This research is an experimental laboratory in vivo involving 32 male Sprague dawley rats divided into 4 groups: treatment group (egg-white gel), positive control group (iodine glycerin), negative control group (gel base and saline). Each group will have the left mandibular central incisor extracted under ketamine. All samples were sacrificed on day 3 and 7 for histopathological examination. Results: The Kruskal-Wallis test showed a significance value of 0.00 ($p < 0.05$) meaning that there was a significant difference in the number of inflammatory cells on the 3rd and 7th day. The significance value between treatment groups was 0.320 ($p > 0.05$), which means there was no significant difference between groups. Conclusion: There was a decrease in the number of inflammatory cells in the wound healing process after tooth extraction by application of Gallus domesticus egg-white gel histologically to iodine glycerin on the 3rd and 7th day, but statistically there was no significant difference ($p > 0.05$).

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INTRODUCTION

The most frequent surgical treatment carried out in the field of dentistry is tooth extraction (Pranskunas et.al., 2019). Tooth extraction is the removal of a tooth, either the whole tooth or its root without pain with minimal injury (Mehrota, 2020). However, post-extraction trauma will inevitably occur, both to the bone tissue and surrounding soft tissue, which can lead to

complications such as bleeding, dry socket, infection, alveolus fracture, mandibular fracture, soft tissue injury, and others (Alhasyimi, 2018).

Tooth extraction will inevitably cause soft tissue wounds that interfere with the normal function and structure of the body (Alhasyimi, 2018). Therefore, handling after tooth extraction needs to be done properly. The wound healing process is a dynamic process involving many factors, such as nutrition, age, immunology, drugs, local regeneration, and systemic factors (Purnama et.al., 2017).

In general, the wound healing process is divided into four phases: first one is hemostasis phase, second one is inflammation phase, third phase is proliferation, and the last one is remodeling phase. The hemostasis phase is the first stage of wound healing, with the goal of preventing excessive blood loss and providing a matrix for the following stage (Delpachitra et.al., 2021). In general, the hemostasis phase involves vascular vasoconstriction, platelet plug formation, and activation of the coagulation cascade that takes place shortly after the wound is inflicted until a few minutes later. The second phase is the inflammatory phase, which occurs after the hemostatic state is achieved until the fifth day after the wound. The inflammatory phase involves important cells such as leukocytes including neutrophils, lymphocytes, basophils, eosinophils, and monocytes, as well as macrophages (Landén dan Ståhle, 2016).

The inflammatory phase is a very important phase because it is the front line in fighting all infections (Landén and Ståhle, 2016; Primadina and Perdanakusuma, 2019). The third phase that occurs is the proliferation phase, which occurs from the third to the fourteenth day and includes three main stages: angiogenesis, fibroplasm, and re-epithelialization (Primadina and Perdanakusuma, 2019). The proliferation phase is characterized by provisional matrix turnover and epithelial proliferation. The remodeling phase, which starts during the second or third week and lasts for approximately a year or more, is the final stage of wound healing (Gonzalez et.al, 2016). The remodeling phase's objective is to maximize tensile strength by rearrangement, resynthesis of extracellular matrix, and degradation (Guo, 2010).

The wound healing process post-tooth extraction can be accelerated by using medicated materials that help prevent infection from worsening. Some medicaments used for wound healing after tooth extraction are povidone iodine and iodine glycerin (da Silveira et.al, 2019; Ferdina, 2022; Ningsih et.al., 2019). Both povidone iodine and iodine glycerin contain iodine which is useful as an antiseptic and anti-inflammatory agent (Gupta et.al., 2022). Iodine glycerin has the ability to induce re-epithelialization and prevent wound infection from causing serious complications (Alhasyimi, 2018). However, iodine glycerin has the disadvantage of causing local erythema, hypersensitivity reactions, and pain when used in the long term (Sa'diyah et.al., 2020).

An alternative material that can be used to overcome the weaknesses of the chemical medicaments above is the egg-white of *Gallus domesticus*. Egg-white is a rich source of pure protein in the form of amino acids and vitamins needed by the body (Jahani et.al., 2019). The protein contained in egg-white can be absorbed by the muscles easily and almost no fat content is found in it (King, 2012). Albumin protein contained in *Gallus domesticus* consists of ovalbumin, ovomucin, ovomucoid, ovotransferrin, ovomacroglobulin, ovoinhibitor, globulin G2 & G3, avidin, and cystatin (Kartiwa et.al., 2016).

Albumin protein is useful for accelerating the wound healing process because it can stimulate the arrival of macrophage cells so that the phagocytosis process occurs (Sakerebau, 2020). Egg-whites of *Gallus domesticus* are able to stimulate the recovery of damaged body cells and are able to form tissues, due to the content of albumin protein and can form tissue, due to the amino acid content that can spur growth factors (Hendriati et.al., 2018).

Egg-white of *Gallus domesticus* are made into gel preparations to accelerate the wound healing process and easy penetration of drugs into the injured tissue (Hendriati et.al., 2018). In addition, gel preparations are able to minimize the unpleasant odor obtained from egg-white of *Gallus domesticus*. Gel preparations are obtained by mixing gelling agents (such as HPMC and

CMC), solvents, humectants, and preservatives (Elrefay et.al., 2022 and Ardana et.al., 2015 and Astuti et.al, 2021). This study aims to determine the number of inflammatory cells involved in the wound healing process after tooth extraction by application of iodine glycerin and *Gallus domesticus* egg-white gel on days 3 and 7.

RESEARCH METHOD

This study used an in vivo laboratory experimental design that was approved by the Ethics Committee of the Faculty of Medicine and Health Sciences University of Muhammadiyah Yogyakarta with registration number No. 037/EC-HC-KEPK FKIK UMY/VII/2023. The subject of the study is male *Sprague dawley* rats within total sample are 32 rats which will be divided into 4 groups, namely *Gallus domesticus* egg-white gel as the treatment group, iodine glycerin as the positive control group, gel base as negative control A, and saline (NaCl 0,9%) as the control group B.

The manufacturing process *Gallus domesticus* egg-white gel is obtained by separating the egg-white and yolk *Gallus domesticus* then weighed by 20 grams. One gram of HPMC and was moistened using 4 grams of glycerin and aquadest then stirred evenly. The mixture was allowed to stand for 1 hour until a consistency of in the form of a gel. After that, 20 grams of free-range *Gallus domesticus* egg-white. The prepared egg-white gel was then preserved by adding nipagin 0.09 gram, and nipasol 0.01 gram, after which it was dissolved in 1gram propylene glycol.

Sprague dawley rat samples were extracted from the central incisor of the left mandible under anesthesia solution of ketamine 50mg/kg intraperitoneally. After tooth extraction, each rat sample was given an intervention according to their respective groups until days 3 and 7. All samples were decapitated on days 3rd and 7th day and then be made histology preparations were then cut longitudinally. Preparations that have been prepared were stained using Hematoxylin-eosin (HE) staining.

Observation of the number of inflammatory cells was performed under a light microscope magnification of 400x using optilab software. Data was analyzed using IBM SPSS version 20 software with the Kruskal-Wallis tests.

RESULTS AND DISCUSSIONS

This study obtained the results of the number of inflammatory cells in the socket after tooth extraction of *Sprague dawley* rats observed in a 400x magnification light microscope and taken using Optilab viewer software. The calculation of the number of inflammatory cells was carried out using the direct method, namely researcher 1 identifying inflammatory cells (PMN, MN, and macrophages) then the identification made by researcher 1 was corrected by researcher 2, with this calculation method it is expected that there will be a small error in calculating the number of inflammatory cells. The number of inflammatory cells in histology preparations stained in Hematoxylin-eosin (HE) and viewed under a light microscope can be seen in figures 1 and the number of inflammatory cells after application of medicament can be shown in the figure 1:

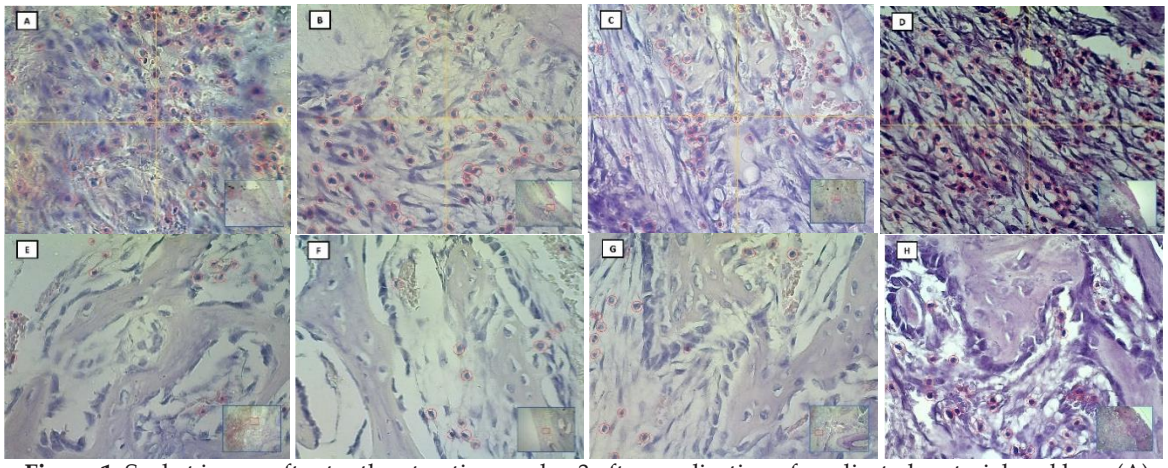

Figure 1. Socket image after tooth extraction on day 3 after application of medicated materials gel base (A), *Gallus domesticus* egg-white gel (B), iodine glycerin (C), saline (D). And gel base (E), *Gallus domesticus* egg-white gel (F), iodine glycerin (G), saline (H). Note: (o) is the inflammatory cells

Table 1. The number of inflammatory cells after application of edicament materials on days 3 and 7

Day	Material	Sample code of Sprague dawley	The number of inflammatory cells
3	Gel base	1-3-1	83.83
		1-3-2	46.89
		1-3-3	234.83
		1-3-4	51.69
	Egg-white gel	2-3-1	28.5
		2-3-2	100.5
		2-3-3	44.67
		2-3-4	150.56
	Glycerin iodine	3-3-1	51.89
		3-3-2	77.61
		3-3-3	109.95
		3-3-4	89
	Saline Steril	4-3-1	87.72
		4-3-2	110.28
		4-3-3	148.83
		4-3-4	127.89
7	Gel base	1-7-1	13.83
		1-7-2	19.11
		1-7-3	11.94
		1-7-4	11.22
	Egg-white gel	2-7-1	9.611
		2-7-2	13.22
		2-7-3	13.44
		2-7-4	12.33
	Glycerin iodine	3-7-1	15.17
		3-7-2	19.06
		3-7-3	12.11
		3-7-4	13.72
	Saline	4-7-1	38.83
		4-7-2	47.78
		4-7-3	31.5
		4-7-4	30

Table 2. The kruskal-wallis test results of inflammatory cell after application of medicament materials

Groups	Kruskal-Wallis		
	Chi-Square	df.	Asymp. Sig.
Treatment	3.503	3	0.320
Day	21.841	1	0.000*

Notes: (*) = $p < 0.05$, meaning there is a significant difference between groups

Based on table 2, the statistical Kruskal-Wallis test results is there is a significant difference for the day group ($p = 0.000$). However, there is no significant difference ($p = 0.320$) between the treatment group (egg wthie gel, gel base, saline and glycerine iodine.

The second stage of wound healing is the inflammatory phase, which is the body's defense against newly developing infections. The inflammatory reaction appears shortly after the wound and begins to decline after the fifth day. The inflammatory phase involves various PMN cells (including neutrophils, eosinophils, and basophils), MNs (monocytes and lymphocytes), and macrophages or monocytes that differentiated around the wound. According to Landén et al. (2016) neutrophils are cells that are first recruited to the inflamed area and will survive for about 2-5 days before finally performing phagocytosis to remove cell debris and pathogens. Around 3rd day after tooth extraction, monocytes which differentiate into macrophages will be recruited to the inflamed area to support the healing process the wound healing process. Macrophages are cells that role as facilitators of the phase transition from phase transition from inflammation to proliferation (Landen et al., 2016).

The results shown in Figure 1 show that in the gel base group, PMN cells dominated over MNs and macrophages on day 3, while the results that can be read from Figure, on the 7th day, inflammatory cells that dominated were MN cells and macrophages. The group given iodine glycerin treated group showed a balanced distribution of PMN, MN, and macrophages both on days 3 and 7. Whereas in the *Gallus domesticus* egg-white gel group and sterile saline, MN cells and macrophage cells dominated both on 3rd and 7th day. All treatment groups had macrophages present, allowing the proliferation phase has begun. Macrophages that play a role at the beginning of the proliferation phase proliferation phase are M2 macrophages for anti-inflammatory and proangiogenesis (Gonzales et.al., 2016).

Based on the observation histological examination shown in Figure 1 and 2, it was found that the number of inflammatory cells is most visible on day 3 and decreased on day 7. On day 3 post tooth extraction, the picture of inflammatory cells and red blood cells dominated the socket area. However, in some sockets, elongated cells with oval nuclei referred to as fibroblast cells already appeared in the socket area on day 3. The presence of fibroblast cells is a sign of the proliferation phase. This means that on day 3, the inflammatory phase and proliferation phase were overlapping. This is consistent with research by Roma et al. (2021), which found that the inflammation and proliferation phases of the burn wound healing process overlapped in white Wistar males that received ointment (Siregar and Hariaji, 2021). According to the research of Landen et al. (2016), which argues the presence of M2 macrophages is an indication of the beginning of the proliferation phase, the inflammatory phase and the proliferation phase overlap (Landen et.al., 2019). In contrast to day 7, the presence of inflammatory cells in the socket area dropped dramatically. The picture of the socket after tooth extraction on day 7 showed the formation of bone spicules, indicating the beginning of the remodeling phase.

Statistical test using Kruskal-Wallis Table 2 shows a significant difference in the day group. It shows that the wound healing process develops significantly based on the interval of days after tooth extraction. This study demonstrates that inflammatory cells that first emerge peak on day 3 and sharply decline on day 7 (Siregar et.al., 2021). Table 1 shows disparities in the average number of inflammatory cells across all treatment groups, with the iodine glycerin group sample number 3 having the highest number on day 3 (234.83) and the sterile saline group sample number 2 having the highest number on day 7 (47.78). While the lowest average number of inflammatory cells both

day 3 and day 7 was found in the egg-white gel group *Gallus domesticus* sample number 1 (28.5) & (9.61). When viewed from the average of all samples in each treatment group, the sample preparation in the sterile saline group showed the highest average score both on day 3 (118.681) and day 7 (36.0278). While the lowest sample average was found in the *Gallus domesticus* egg-white gel group both on days 3 (81.0556) and 7 (12.1528).

Statistically using the Kruskal-Wallis test in Table 2, there was not any difference between the treatment groups that was significant ($p > 0.05$), (Gallus domesticus egg-white gel), positive control (iodine glycerin), negative control A (gel base), and negative control B (sterile saline). These findings support the notion that Gallus domesticus egg-white gel can coordinate wound healing activities on a par with other medications.

The egg-white gel from Gallus domesticus is nutrient-rich, effective as an anti-inflammatory, and promotes wound healing. According to studies by A'ziza et al. (2023), the ovalbumin protein, the biggest structure in egg white, serves as a source of amino acids that can stimulate the growth factor of inflammatory cells. This discovery is consistent with their findings (A'ziza et.al., 2023). This ability will help the initial process of inflammation, which triggers the presence of neutrophil cells (PMN) and macrophages to the wound area after extraction and eliminates various kinds of intrusive pathogens, so as to prevent prolonged infection. Research by Kusumaningrum et al. (2019) mentioned that the active protein in *Gallus domesticus* egg-white is able to inhibit bacterial growth which results in membrane rupture (Kusumaningrum et al,. 2019).

Other ingredients found in egg-white such as ovotransferrin, ovomucoid, ovomucin, ovomacroglobulin, globulin G2 & G3, and lysozyme have the ability as antimicrobials viruses and bacteria (Jahani et.al., 2019 and Widyaningsih et.al., 2021). These proteins have the ability to lower the amount of inflammatory cells needed for wound healing. The mechanism of *Gallus domesticus* egg-white gel in the wound healing process, especially in the inflammatory phase, is to keep the osmotic pressure inside and outside the cell balanced so that the swelling that occurs is not severe (Putri, 2016). The histopathological picture of the sample in the *Gallus domesticus* egg-white gel group both on day 3 and 7 Figures 1 & 2 shows the least number of inflammatory cells, so it can be said that inflammatory symptoms such as swelling, heat, pain, and loss of function are also lower than the positive and negative control groups. Egg white albumin protein *Gallus domesticus* also functions as a tissue regenerator and promoter of injured body cell recovery (Sakerebau et.al., 2020 and Andritoiu et.al., 2021).

The medical ingredients of the hen egg-white gel *Gallus domesticus*, iodine glycerin, and gel base both have glycerin content. Research conducted by Bialik-Was et al. (2021) suggested that glycerin is a humectant material that is able to absorb water quite well, low toxicity, and can hold moisture where it is applied (Bialik-Was et al., 2021). Other findings conducted by Stuot et al. (2012) suggested that glycerin is able to slow down the activity of disease causing pathogenic bacteria with minimal side effects, but has cytotoxic properties if used in high concentrations and prolonged exposure (Stuot et al., 2012). Therefore, both *Gallus domesticus* egg-white gel, gel base, and iodine glycerin have almost equal abilities in the healing phase of a wound following tooth extraction.

After a tooth extraction, the saline family of medications can aid in the wound healing process. This is due to the fact that sterile saline is an isotonic physiological solution and does not induce irritation. According to research by Evangeline et al. (2015), sterile saline can speed up the healing of wounds since it has anti-inflammatory qualities, which reduce erythema and pain when a wound arises (Evangeline et al., 2015). However, when observed further in the histological examination, the number of inflammatory cells present during post-tooth extraction tends to be large. This can be seen in the sample on day 7 that inflammatory cells still dominate, even though the inflammatory phase should have begun to be replaced by the proliferation phase. Previous research conducted by Huynh et al., (2016) showed the role of saline administration in the wound healing process in human gingival fibroblast (hGF) is able to stimulate cell migration but not

proliferation throughout the wound healing process. These findings support that the histology preparation of sterile saline treatment on day 7 is still dominated by inflammatory cells and not much fibroblast proliferation has occurred (Huynh et al., 2016).

Numerous factors, including age, nutrition, hormone changes, and others, have an impact on the wound healing process. The reason for choosing male *Sprague dawley* rats is because they are easy to control, calm, and it is expected that the wound healing process is not affected by hormonal factors, such as menstruation and pregnancy.[34] In addition, the biological condition of male *Sprague dawley* rats is more stable and drug metabolism can take place quickly.[34] In this study, the individual conditions of the rats were optimal because the room temperature was well controlled, the food was sterile, and the living quarters were maintained so that the wound healing process and the decrease in inflammatory cells in all groups on day 7 could run well.

In addition to the systemic factors of individual rats, local conditions have an impact on the wound healing process as well. Since saliva is present in the oral cavity, this study looked at how the wounds healed there. Because saliva contains antiviral and antibacterial substances including lysozyme, lactoperoxidase, immunoglobulin A, and growth factors like Nerve Growth Factor (NGF) and Epidermal Growth Factor (EGF), it aids in the healing of wounds (Eggermont, 2012). Macrophages present in the inflammatory process play a role in the secretion of EGF in animal saliva and facilitate the proliferation of fibroblasts that will form collagen to accelerate the wound healing process, this concurs with the study conducted by Wahyudi et al. (2013). Physiologically, the presence of salivary protein as an ideal local factor can accelerate the wound healing process compared to other parts of the body (Wahyudi et al., 2013).

According to the discussion that has been had, using Gallus domesticus egg-white gel to iod glycerin histologically revealed changes in the number of inflammatory cells in the wound healing process following tooth extraction. However, applying Gallus domesticus egg-white gel to iod glycerin following tooth extraction did not statistically significantly reduce the amount of inflammatory cells.

CONCLUSION

There was a decrease in the number of inflammatory cells in the wound healing process after tooth extraction by application of Gallus domesticus egg-white gel histologically to iodine glycerin on the 3rd and 7th day, but statistically there was no significant difference ($p>0.05$).

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