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ORIGINAL ARTICLE

POTENCY OF OCIMUM SANCTUM L EXTRACT OINTMENT ON THE MANAGEMENT PARTIAL-THICKNESS PALPEBRAL LACERATION, IS IT A NOVELTY TO BE CONSIDERED AS AN ALTERNATIVE THERAPY?:REVIEW ON HISTOPATHOLOGICAL CHANGES IN EXPERIMENTAL MODEL

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ABSTRACT

Introduction and Objective: *Ocimum sanctum extract (OSE) has been shown to have antioxidant, anti-inflammatory, and antibacterial properties which are responsible for wound healing. This study aims to analyze the effect of 5%, 10%, and 15% OSE ointment on changes in collagen density and fibroblast count in Wistar rats with partial-thickness palpebral laceration.*

Methods: *Laboratory experimental study with randomized post-test only control group design. The subjects were 30 rats divided into 5 groups: The placebo; Gentamicin 0.3%; OSE 5%; OSE 10% and OSE 15% group. The intervention was administered 2 times/day for 4 days. On the fifth day, the palpebral tissue was obtained to make histopathological preparations and to observe the collagen density and fibroblast count. Data were analyzed using Mann-Whitney, One-Way ANOVA, Kruskal Wallis, and Tamhane test. A p-value <0.05 was assigned to the statistically significant.*

Results: *The placebo group revealed loose collagen tissue and OSE 15% and gentamicin group revealed dense collagen tissue. The low fibroblast count was in the gentamicin and OSE 15% group and the highest fibroblast count was in the placebo group with a mean of 19.77±3.29; 22,67±0,73 and 60.9±10.66 cells, respectively. There was a significant difference (p<0.001) in the collagen density and the fibroblast count based on the treatment groups. Posthoc tests revealed differences in the collagen density level and the fibroblast count between the placebo and the whole group.*

Conclusion: *There was a significant difference between placebo and all groups of collagen density changes and fibroblast count. There was no difference between OSE 15% and gentamicin both microscopically and statistically.*

Keywords*: Ocimum sanctum L, Extract of Ocimum sanctum L, partial-thickness palpebral laceration, fibroblast cell count, collagen density*

INTRODUCTION

In today's industrialization and high-speed traffic era, the incidence of trauma is increasing in general. Like other parts of the body, the eye is also a part that often experiences trauma. [1]–[3] The incidence of ocular involvement in craniofacial trauma ranges from 15% to 60%. [4] According to the World Health Organization, approximately 55 million ocular traumas occur annually, and 750,000 cases require hospitalization, of which 200,000 are open injuries.

In addition, about 1.6 million became blind, 2.3 million experienced bilateral visual impairment and 19 million had unilateral visual impairment due to ocular trauma. The prevalence of ocular trauma in North Sumatra accounts for as much as 0.7% and is the third highest rate on the island of Sumatra.[5]

Palpebral laceration is one of the ocular trauma that may occur due to blunt trauma, sharp objects, animal bites, fights, and other trauma mechanisms. These various mechanisms can cause partial or total palpebral thickness defects. [6]–[8] Palberal laceration treatment is a surgical procedure, but medical ones are still used and remain viable if indicated. Superficial or partial lacerations that are horizontal and follow the skin line can be treated effectively without surgery by applying an antibiotic ointment along the laceration. [9], [10]

The last few years have attracted much interest from researchers in developing herbal remedies based on medicinal plants. It has been reported that these herbal treatments are relatively safe and have minimal side effects compared to synthetic drugs. [11], [12] One of the medicinal plants widely used by the community is Basil or *Ocimum sanctum* L which possesses pharmacological properties such as antimicrobial, analgesic, antioxidant, anti-inflammatory, anticarcinogenic, and immunostimulator and has been used in the Ayurveda system for the treatment of infectious diseases. *Ocimum sanctum* L has antioxidant, anti-inflammatory, and antibacterial properties which may be responsible and beneficial in wound healing. The Naibaho study in 2013 found that *Ocimum sanctum* L has antibacterial effects related to the content of alkaloids, terpenoids, saponins, and flavonoids. [2], [11], [13]

In the study of Gautam et al. in 2010, rats with an excision wound model gave better healing results in the group given *Ocimum sanctum* L extract with 10% ointment compared to the control group. A similar study was also conducted by Gautam and Goel in 2013 on diabetic rats with incisional wound models, and the results revealed better wound healing in rats given *Ocimum sanctum* L extract treatment than in controls. This effect was due to a decrease in tissue damage produced by radicals as an antioxidant effect and accelerating the deposition of collagen and other connective tissue.[11], [14]

Based on this description, the research problem can be formulated: Does Ocimum sanctum L extract ointment have potential effects as management of partial-thickness palpebral laceration, review on histopathological changes in experimental model?

METHOD

This research has been approved by the Health Research Ethics Committee of the Universitas Sumatera Utara and was conducted from April-May 2022 in the Laboratory of

Herbarium Medanese (MEDA) North Sumatra for plant identification, Department of Pharmaceutical Biology, Faculty of Pharmacy Universitas Sumatera Utara for manufacturing process of Ocimum sanctum extract ointment (OSE) 5%, 10% and 15% preparations on a hydrocarbon basis, Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara for the experimental animal's interventions, and the Department of Histology, Faculty of Medicine, Universitas Sumatera Utara for the palpebral histopathological examination. The research method uses a randomized post-test-only control group laboratory experimental design with a sample of 30 Rattus norvegicus Wistar strains (based on Federer's formula) obtained from the Department of Pharmacology and Therapeutics Faculty of Medicine, Universitas Sumatera Utara.

The rats were then divided into five groups, and then acclimatization was carried out for one week, namely 1. placebo group, 2. OSE 5% group, 3. OSE 10% group, 4. OSE 15% group, and gentamicin 0.3% group. With the specified inclusion criteria such as white male Wistar rats, 2-3 months old, body weight 250-350 grams, and in healthy condition characterized by active movement, no injuries, and nobody defects, and experimental animals will be excluded if met the criteria for being sick during the adaptation and research process. Such exclusion is possessing anatomical abnormalities in one or both eyes, 10% weight loss from initial body weight, full thickness laceration, death during the treatment period, histopathological preparations were unsuccessful and could not be read. All treatment groups were given the intervention of topical application of ointment in the laceration region two times a day for four days. On the fifth day, all Wistar rats were euthanized using a lethal dose of ketamine injection, which was three times the anesthetic dose. If there was no respiratory activity, a cut of the eyelid tissue with a size of 1.5 cm was obtained. The wound healing parameters observed were the density of collagen and the fibroblasts count. The data were analyzed using the SPSS version 25, univariate analysis to see the distribution of collagen density in each group; the difference in collagen density was carried out by the Kruskal Wallis test and followed by the Posthoc using the Mann Whitney test. Differences in fibroblast count were performed using the One Way ANOVA test and continued with the Posthoc using the Tamhane test. All statistical tests used the degree of significance of $p<0.05$.

Creating the Model of Partial Thickness Palpebral Laceration Procedure

The Wistar rats were clinically examined physically and precisely for the palpebral condition. Examination of the palpebral includes whether there were abnormalities, color, and surface texture. Then, the Wistar rats were anesthetized using the anesthetic agent ketamine and

xylazine intraperitoneally. After the anesthetic effect worked, the palpebral region was shaved, and then an asepsis procedure was performed using povidone-iodine. An incision was made in the superior palpebral region with a depth of ± 0.2 mm (up to the dermis tissue, marked by the appearance of a bleeding spot) using a marked blade with a length of 5 mm from medial to lateral.

Figure 1. The process of creating a Palpebral Laceration Wound Model Partial Thickness

RESULT

Collagen Density

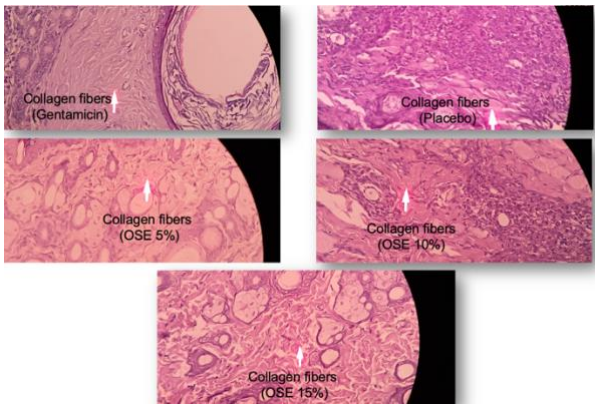

Figure 2. Histology of collagen fibers in all group

Table 1 shows the collagen density examination results in all treatment groups after treatment. In the group of Wistar rats that were given a placebo, all of them revealed loose-density collagen tissue. In the Wistar rats given OSE 5% , two rats (33.3%) showed loose-density, moderate-density, and dense-density collagen tissue, respectively. In the Wistar rats given OSE 10%, four rats (66.7%) showed dense-density collagen tissue and two (33.3%) moderate-density collagen tissue. The rats were given OSE 15% and gentamicin 0.3% showed dense-density collagen tissue for all Wistar rats.

Table 1. Collagen Density DistributionSemarang

Group	n	Collagen Density		
		Loose	Moderate	Dense
Placebo	6	6 (100)	0	0
OSE 5%	6	2 (33.3)	2 (33.3)	2 (33.3)
OSE 10%	6	0	2 (33.3)	4 (66.7)
OSE 15%	6	0	0	6 (100)
Gentamicin	6	0	0	6 (100)

Tables 2.1 and 2.2 show the results of the differences in collagen density analysis

Table 2.1. Differences in Collagen based on Treatment Group

Group	Collagen Density		P[a]
	Average (SD)	Median (Min-Max)	
Placebo	1		
OSE 5%	2 (0.89)	2 (1-3)	
OSE 10%	2.67 (0.52)	3 (2-3)	<0.001
OSE 15%	3		
Gentamicin	3		

Table 2.2. Posthoc analysis from Differences in Collagen Density based on Treatment Group

Group	Posthoc[b]			
	OSE 5%	OSE 10%	OSE 15%	Gentamicin
Placebo	0.022	0.002	0.001	0.001
OSE 5%		0.162	0.022	0.022
OSE 10%			0.138	0.138
OSE 15%				1.000
Gentamicin				

[a]Kruskal Wallis, [b]Mann Whitney

The Kruskal Wallis test showed a significant difference ($p<0.001$) in the level of collagen density in Wistar rats based on the treatment group. The results of the Posthoc test analysis revealed a difference in the level of collagen tissue density between the group of placebo and

all other groups given OSE and gentamicin. There was no significant difference in the collagen density level between the groups given OSE 5% and OSE 10%, but, there was a significant difference among the groups given OSE 5% with OSE 15%, and gentamicin. There was no significant difference in collagen density between the group given OSE 10% with OSE 15%, and gentamicin. Likewise, no difference in collagen density was found between the group given OSE 15% and those given gentamicin.

Fibroblast Cell Count

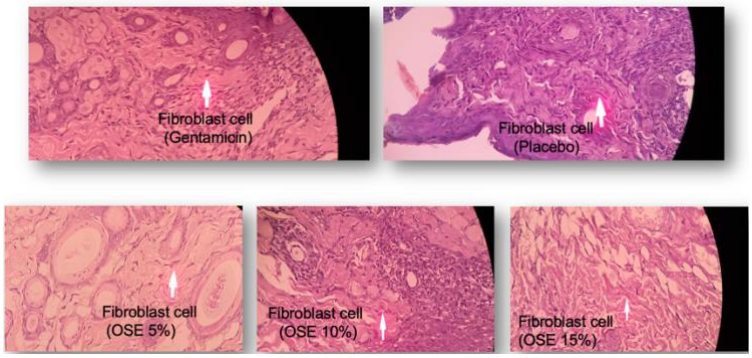

Figure 3. Histology of fibroblast cells in all group

Tables 3.1 and 3.2 show the results of fibroblast cell count examination after being given treatment in all treatment groups.

Table 3.1 Differences in Fibroblast Cell Count from the Average of Five Fields of View on Incision Wounds based on the Treatment Group

Group	Fibroblast Cell Count		p[a]
	Average (SD)	Median (Min-Max)	
Placebo	60.9 (10.66)	62.7 (43.8-73.2)	<0.001
OSE 5%	23.3 (1.88)	23.4 (20.8-25.8)	
OSE 10%	22.87 (1.22)	23 (21.2-24.6)	
OSE 15%	22.67 (0.73)	22.7 (21.6-23.6)	
Gentamicin	19.77 (3.29)	19.9 (14.2-23.2)	

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Table 3.2 Posthoc analysis from Differences in Fibroblast Cell Count from the Average of Five Fields of View on Incision Wounds based on the Treatment Group

Group	Posthoc[b]			
	OSE 5%	OSE 10%	OSE 15%	Gentamicin
Placebo	0.003	0.003	0.003	0.001
OSE 5%		1,000	0.998	0.414
OSE 10%			1,000	0.523
OSE 15%				0.584
Gentamicin				

[a]*Oneway Anova*, [b]*Tamhane*

The lowest fibroblast cell count was seen in the group given gentamicin, with a mean of 19.77 cells (SD = 3.29), and the highest number of fibroblast cell count was found in the group given a placebo with a mean of 60.9 cells (SD = 10.66). Based on the fibroblast cell count among the OSE group, the lowest fibroblast cell count was in the group given OSE 15% with a mean of 22.67 cells (SD=0.73). Using the Oneway Anova test, it was revealed that there were significant differences in the fibroblast cell count ($p<0.001$) from the five treatment groups.

The posthoc test showed a difference in the fibroblast cell count between the group given a placebo and the whole group given the OSE and gentamicin ($p<0.05$). However, there was no difference in the fibroblast cell count between all groups given OSE and the group given gentamicin ($p>0.05$).

DISCUSSION

The goal of treating palpebral lacerations is to restore the anatomical and physiological structures. In the process of restoring structure and function, basic aesthetic principles must be taken into consideration. Treatment for palpebral lacerations is surgical, but medical therapy is still used if indicated. Superficial or partial lacerations that are horizontal and follow the skin line can be treated effectively without surgery by applying an antibiotic ointment along the linear length of the laceration. [6], [15], [16]

Ocimum sanctum L is an herbal plant with pharmacological effects such as antimicrobial activity, immunomodulator, antioxidant, and anti-inflammatory, and all of these activities play a role in wound healing. The therapeutic effect is associated with the content of eugenol, which is a phenolic compound and a principal constituent in the essential oil of *Ocimum sanctum* L leaves. Additionally, it is also associated with the content of ursolic acid, which is also a principal constituent in the *Ocimum sanctum* L. [17]–[20]

The results of the collagen density examinations showed that there were significant

differences in each treatment group ($p<0.001$). In the posthoc analysis, it was found that there were substantial differences between the placebo group and the whole group, both groups treated with OSE and gentamicin. Significant differences were also found in the group given OSE 5% with OSE 15% and gentamicin. This study follows a previous study conducted by Goel, who explained that OSE 10% ointment has the effect of accelerating wound healing by inducing tumor necrosis factor-α (TNF-α) in the early stages of wound healing, where TNF-α is the main cytokine secreted by macrophages and neutrophils during the inflammatory phase. In this study, it was reported that there was an increase in TNF-α at 24 hours post-wound treatment, and the levels decreased and remained stable at 48 hours. Furthermore, another study reported that TNF-α inhibited collagen formation, but not if the levels were low. This finding is supported by a study conducted by Gautam and Goel, who stated that administration of 50% ethanol extract of *Ocimum sanctum* L in diabetic rats accelerated wound healing with antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and antiulcer effects, thereby accelerating the synthesis and deposition of collagen. [21], [22]

This study showed that the optimal effect was obtained in the OSE treatment group with the highest concentration of 15% compared to concentrations of 5% and 10% and had the same effect as gentamicin 0.3% in terms of collagen density parameters. This may be caused by differences in treatment in each group with different concentrations, such as the chemical content of eugenol, ursolic acid, saponins, flavonoids, terpenoids, and alkaloids contained in the Ocimum sanctum L leaf extract. Those substances perform a role in wound healing in experimental animals. This study is in line with the research report conducted by Irwandi, who observed the effectiveness of the ethanol extract of *Durio zibethinus* L (Durian) in animal excision wound models with collagen density parameters and concluded that the optimal concentration of the extract was the highest concentration of 15%. However, this study does not align with the research conducted by Sucita et al., who examined the effectiveness of the ethanol extract of *Caesalpinia sappan* L at concentrations of 6.5%, 15%, and 30%. This study reported that the optimal treatment effectiveness regarding collagen density parameters was at the lowest dose of 6.5%. This was associated with the concentration of the extract and resulted in a decrease in its antioxidant activity. It is also described that high concentrations can inhibit the active substances from penetrating the mucous membrane. [18], [23]

A lower dispersion rate caused the diffusion coefficient to become smaller, decreasing drug diffusion. However, in this study, the OSE ointment was manufactured with a hydrocarbon base with a greater dispersion rate than other bases (absorption base, water washable, and water-soluble). OSE ointment with a hydrocarbon base can increase the hydration (moisture) on the

skin (fatty carrier is an occlusive layer so that it can hydrate the skin). This, in turn, can increase the absorption of drug-active substances in the skin layer. Low humidity levels can cause oxygen pressure in the wound tissue to decrease, inhibiting collagen formation. On the other hand, high humidity levels due to adequate hydration can increase the oxygen pressure in the wound tissue and accelerate the process of collagen formation. All the active ingredients in OSE ointment with a hydrocarbon base can be better distributed on the wound and provide an excellent therapeutic effect. This could be the evidence that a high concentration of ointment in this study has an optimum effect on wound healing regarding collagen density. [24]–[26]

This study also assessed the difference in fibroblast cell count in all treatment groups. The results showed that the lowest mean of fibroblast cells count was observed in the group given gentamicin 0.3% with a mean of 19.77 cells (SD = 3.29), and the mean of the highest fibroblast cell count was found in the group given a placebo with a mean of 60.9 cells (SD=10.66). It was also found that there was a significant difference in the mean fibroblast cell count from the five treatment groups with a p-value of <0.001. This means that the treatment significantly affected changes in the mean of fibroblast cell count statistically. This aligns with the theory that fibroblasts are cells that synthesize collagen and several other ECM components. The proliferation of fibroblasts determines the outcome of wound healing. Fibroblasts will produce collagen that binds the wound. Under normal circumstances, fibroblast cleavage activity is very rarely seen. However, when the injury occurs, these cells will migrate toward the wound, proliferate and produce a collagen matrix to repair damaged tissue. This aligns with this study that the highest fibroblast cell count is in the placebo group, and the lowest is in the gentamicin group. The OSE 5-15% group gave almost the same mean of fibroblast cell count. Theoretically, it is known that TGF-β stimulates fibroblast migration in the wound area. The active substance in OSE (saponins) activates the TGF-β signaling pathway. With more activated TGF-β, the greater the number of migrating fibroblasts. Therefore, the collagen produced is also denser. This increased collagen density is followed by fibroblast cleavage activity which is rarely seen due to the wound's tendency to improve. Because in normal tissue, fibroblast cells are rare and usually hidden in the tissue matrix. In addition, the saponin content in OSE can increase monocyte proliferation and elevate the number of macrophages. These macrophages will secrete growth factors such as FGF, PDGF, TGF-β, and EGF, which can attract more fibroblasts to the wound area, synthesizing collagen and increasing capillary blood vessel proliferation. High fibroblast count in the placebo group indicated that fibroplasia was occurring. On the other hand, both the OSE and gentamicin 0.3% groups revealed a decrease in fibroblast cell count. It is theorized that fibroblast was already replaced by a collagen matrix

that fills the wound cavity. [25], [27]

This aligns with a study by Palumpun, which assessed the effect of applying Piper beetle extract on the fibroblast cell count. It was reported that topical Piper beetle extract with a concentration of 10% increased fibroblast cell count and collagen density in the wounds of Wistar rats compared to the control group. Dwita et al. also assessed the effect of Hemigraphis colorata W. Bull extract on burn wound healing. This study reported an increase fibroblast cell count in the placebo group on days 3, 7, and 14 compared to Hemigraphis colorata W. Bull extract 5%, 10%, and 20% ointment group, whose number fluctuated and began to decrease on day 14. It was concluded in the study that the topical application of Hemigraphis colorata W. Bull 20% can accelerate the healing of burn wounds based on the fibroblast cell count and collagen density. Another similar study was conducted by Amita, which assessed the effect of Anredere cordifolia Steenis extract 5%, 10%, and 15% on fibroblast proliferation in wound healing. Fibroblast proliferation was found to decrease when collagen production is sufficient for growth. Fibroblast proliferation will continue to occur if collagen production is less than needed. Based on this study, applying *Anredere cordifolia Steenis* 15% revealed the densest collagen. [28]–[30]

CONCLUSION

In this current study, the application of OSE 15% ointment produces the same results as the application of gentamicin 0.3% eye ointment on the collagen density, microscopically and statistically. In the fibroblast cell count, the average fibroblast cell count given OSE and gentamicin revealed a fibroblast cell count lower than the placebo group, which had a higher mean fibroblast cell count. This decrease in fibroblast is due to complete collagen fiber formation, and the process was in the late proliferative phase, while the placebo group was still in the fibroblastic phase. In the OSE groups, OSE 15% had the least mean number of fibroblasts compared to OSE 5% and 10%. This result showed that the greater the OSE concentration, the lesser the mean of remaining fibroblast. This phenomenon suggested that more than 15% ointment concentration may have an effect relatively close to gentamicin. Therefore, further research is necessary to prove these hypotheses.

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