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Topical Role of Ozonated *Aloe vera* Oil in Radiation Dermatitis: Expression of TGF-*β* and Collagen Density

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Abstract

BACKGROUND: The effect of ozonated *Aloe vera* in the treatment of radiation dermatitis has not been studied, while long-term use of topical steroids can cause some side effects.

AIM: The aim of the study was to analyze the effect of topical administration of ozonated *Aloe vera* oil on the expression of TGF-β and collagen density in the treatment of radiation dermatitis.

METHODS: 36 Sprague Dawley rats were randomized into six groups, namely, K1 (negative control-without therapy), K2 (positive control-hydrocortisone cream 2.5%), P1 (*Aloe vera* oil), P2, P3, and P4 (ozonized *Aloe vera* oil 300/600/1200 mg/ml). Termination and immunohistopathological analysis of TGF-β expression and collagen density were performed after 7 days of treatment.

RESULTS: Measurement of TGF-β expression by ANNOVA test showed a significant difference between groups p = 0.001. The *Post Hoc* LSD test showed significant differences between groups K1 and P1, P2, P3, and P4 also between groups K2 and P2, P3, and P4. Measurement of collagen density by Kruskal–Wallis test showed a significant difference between the treatment groups p < 0.001. *Post hoc* Mann–Whitney test of collagen density found a significant difference between groups K1 and P1, P2, P3, and P4 also between groups K2 and P2, P3, and P4. Spearman's rho correlation test showed a strong and unidirectional relationship between TGF-β and collagen (p < 0.001 and r = 0.722).

CONCLUSION: Topical ozonated *Aloe vera* oil increased TGF-β expression and collagen density in radiation dermatitis.

Introduction

Dermatitis is an inflammatory skin reaction that is sensitive to various endogenous and/or exogenous stimuli, causing clinical abnormalities in the form of polymorphic fluorescence (erythema, edema, papules, vesicles, scales, and lichenification) and itching complaints. Polymorphic signals do not always occur simultaneously, maybe even only a few (oligomorphic) [1].

Radiation is one of the causes of dermatitis, often found in patients undergoing radiotherapy. Acute and chronic damage from radiation is a collection of damage to germ cells [2]. Acute side effects (in hours) that appear are in the form of itching, erythema, dryness, cracking, and pain ranging from mild to severe [3]. Radiation reduces neovascularization, fibroblast proliferation, collagen deposition, and level of growth factors for wound healing. The inhibited growth factors are transforming growth factor β-1 (TGFβ-1) and basic fibroblast growth factor (bFGF) in the acute phase, where the inhibition of TGFβ-1 will also inhibit other controlled components, namely, fibroblast proliferation, transformation into myofibroblasts, stimulation of extracellular matrix production, and collagen density [4].

Wound healing is complex processes with cellular and molecular mechanisms. The success of wound healing depends on the inflammatory process, cell proliferation, angiogenesis, wound contraction, epithelialization, and matrix remodeling. These processes require the continuous function of neutrophils, macrophages, fibroblasts, and endothelial cells to regulate interactions between cells, extracellular matrix proteins, and growth factors. Disruption of any of these healing processes will result in abnormal wound healing [4]. Collagen contributes a lot to wound healing. Collagen affects the mechanical strength and elasticity of tissues and acts as a natural substrate for cellular attachment, proliferation and differentiation. Collagen is a key component of the extracellular matrix that plays an important role in the regulation of the wound healing

phase whether in its native, fibrillar conformation, or as a soluble component in the wound environment [4].

Topical steroids have long been used for the prevention and treatment of radiation-induced toxic dermatitis. There are several studies stating that the use of topical steroids can reduce burning, itching and erythema and slow the progression of the skin. However, long-term use of steroids can cause several local and systemic side effects, thus many studies have been carried out with herbal plants as replacement therapy for topical steroids [5].

Oral and topical administration of *Aloe vera* gel was reported to be effective in wound healing. In a recent study, *Aloe vera* gel or its extract content, namely, *acemannan*, *β sitosterol* and others, has been reported to cause faster wound healing by stimulating fibroblast proliferation, collagen deposition, angiogenesis, and growth factor production [4]. In addition, *Aloe vera* also increases TGF-β gene expression which will accelerate the wound healing process [6].

Ozone (O3) is a much stronger oxidant than oxygen and has the ability to oxidize many substances that are inert to oxygen under normal conditions [7]. Ozone is widely recognized as one of the best bactericidal, antiviral, and antifungal agents, which currently are used as therapeutic mediators in wounds to reduce bacterial infection, heal skin damage, and increase oxygen tension on exposure [8].

Until now, the effect of ozonated *Aloe vera* in the treatment of radiation dermatitis has not been studied, while long-term use of topical steroids can cause some side effects. This study aimed to compare the expression of TGF-β and collagen density between the topical administration of ozonated *Aloe vera* oil in various doses groups and the control group on the healing of radiation dermatitis in Sprague–Dawley rats on day 7 and analyze the effect of topical administration of ozonated *Aloe vera* oil on the expression of TGF-β and collagen density on the healing of radiation dermatitis in Sprague–Dawley rats on day 7.

Materials and Methods

Experimental animals

The samples used were 36 adult male Sprague–Dawley rats weighing 150 ± 50 g and 2–3 months old; and there were three rats that died after receiving radiation, so the number of samples became 33 adult male Sprague–Dawley rats. Rats were fed and given water *ad libitum* and acclimatized for 1 weeks before the start of the experiments. Samples were randomly divided into six groups:

K1 (Negative control): radiation with no treatment
K2 (Positive control): radiation + hydrocortisone cream 2.5%
P1: Radiation + non ozonated *Aloe vera* oil
P2: Radiation + ozonated *Aloe vera* oil 300 mg/ml
P3: Radiation + ozonated *Aloe vera* oil 600 mg/ml
P4: Radiation + ozonated *Aloe vera* oil 1200 mg/ml.

The K1, K2, P1, P2, P3, and P4 groups were terminated on day 7 to be performed with wound tissue taking (sampling), preparations making, as well as histopathological, and immunohistochemical staining.

Establishment of the radiation-induced dermatitis rat model

The anesthetic was administered to the rats by an intraperitoneal injection of ketamine-xylazine (ketamine 80 mg/kg and xylazine 10 mg/kg), each rat was placed in a prone position to expose the back area of 4 × 4 cm before being radiated with a dosage of 7 Gy from a linear accelerator (LINAC). The radiation was done by the accredited radiation oncology facility. The subject was returned to a clean tray/container after radiotherapy, and then it was left until a spontaneous consciousness occurred before topical intervention was applied on the following day. The radiation wound was marked by macular erythema and dry desquamation, Grade I dermatitis, based on RTOG/EORTC [9].

Topical treatment applications

Non-ozonated *Aloe vera* oil and ozonated *Aloe vera* oil were obtained from the Center for Plasma Research, Universitas Diponegoro, produced using the method that had been explained in a study conducted by Vahlepi *et al*. [10]. Topical intervention was given 24 hours after radiotherapy, based on the treatment group. The intervention was conducted for 7 consecutive days, given twice a day.

Expression of TGF-β and collagen density analysis

TGF- β immunohistochemistry staining was conducted in the Department of Anatomical Pathology, Faculty of Medicine, *Universitas Sebelas Maret,* Solo, and the reading was carried out in the Department of Anatomy, Faculty of Medicine, *Universitas Diponegoro*, Semarang. Euthanasia and tissue excision were performed after giving topical intervention. The tissue excision was carried out on the tissue that was exposed to radiation beams along with less normal skin along the side edge. The tissue was fixated using 10% formalin buffer and cut into 3 mm pieces that were then prepared to be paraffin blocks. The paraffin block tissues were cut into 3-μm pieces follows by the TGF β antibody staining.

The evaluation of TGF- β expression on the wounds of radiation dermatitis was measured in a semi-quantitative method using the Allred score: proportion score + intensity. The proportion score (A), 0 = 0% stained cells; 1 = <1% stained cells; 2 = 1–10% stained cells; 3 = 11–33% stained cells; 4 = 34–66% stained cells, and 5: ≥ 67% stained cells. The intensity score (B), 0: non-stained, 1: poor intensity, 2: moderate intensity, and 3: strong intensity. The proportion score was added by the intensity score and the final score was 0–8.

The collagen density was evaluated and scored by seeing the percentage of collagen distribution that filled the vision of field in 1 vision of field and was scored, the observation was calculated over 5 visions of field using a binocular microscope with 400x magnification using hematoxylin and eosin (HE) staining. The collagen density score: 1 = collagen density on 10% wound area; 2 collagen density on 20% wound area; 3 = collagen density on 30% wound area; 4 = collagen density on 40% wound area; 5 = collagen density on 50% wound area; 6 = collagen density on 60% wound area; 7 = collagen density on 70% wound area; 8 = collagen density on 80% wound area; 9 = collagen density on 90% wound area; 10 = collagen density on 100% wound area, so the final score was 1–10.

The qualitative evaluation of both TGF- β and collagen density was taken from five visions of field using a binocular microscope with 400× magnification for each sample to obtain the average score. The preparation and the immunohistochemistry analysis were conducted by two accredited pathologists.

Statistical analysis

The statistical analysis used the SPSS version 22 (IBM corporation, AS). The testing for the normality of data was conducted using the Shapiro–Wilk test. The TGF-β expression of data was normally distributed, so the One-Way ANOVA testing was conducted and followed by the *post hoc* LSD test after finding a significant difference. The collagen density of data was not normally distributed, so the test was done using the Kruskal–Wallis test and was followed by the Mann–Whitney test. The correlation analysis was performed using the Spearman test. p < 0.05 was considered important statistically.

Ethical approval

All the research subjects used in this study were located and conducted in a certified facility appropriate to the guideline for medical and animal research. The ethical clearance for experimental research using animals was obtained from the ethical committee that regulates the subject (No. 66/EC/H/FK-UNDIP/VII/2021) (Figure 1).

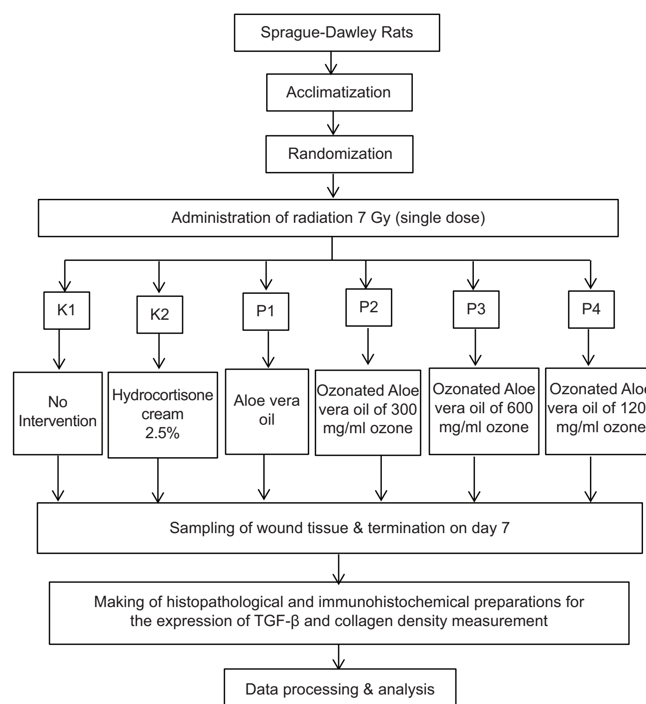

Figure 1: Research flow

Results

Expression of TGF-β

Statistical test of expression of TGF-β

The results of the expression of TGF-β measurement for all study groups are shown in Table 1. The average results are also depicted by a boxplot graph as shown in Figure 2. In this study, the normality test was carried out using the Shapiro–Wilk test, while the homogeneity test was carried out using the Levene's test. The Shapiro–Wilk test is intended to show that the sample come from a normally distributed population, but if the significance obtained is < 0.05 then the sample data do not come from a normally distributed population. The results of the Shapiro–Wilk test for the expression of TGF-β showed a significance of p > 0.05 in all groups as shown in Table 1. This shows that the data were normally distributed.

Table 1: Descriptive and normality test results of the Expression of TGF-β using the Shapiro–Wilk

Group	Mean ± SD	Median (min–max)	p	Note
Control	3.60 ± 0.40	3.6 (3.2–4.0)	0.119	N
Hydrocortisone	4.20 ± 1.02	4.2 (3.0–5.4)	0,692	N
Aloe vera	5.00 ± 0.81	4.8 (4.2–6.2)	0.627	N
Ozonated AV 300 mg	5.33 ± 0.63	5.0 (4.8–6.4)	0.054	N
Ozonated AV 600 mg	5.47 ± 0.78	5.5 (4.2–6.4)	0.741	N
Ozonated AV 1200 mg	5.93 ± 1.17	5.5 (5.0–8.0)	0.124	N

Description: N: Normal, Normal (p>0.05).

After the normality test was carried out, it was continued with the Levene's test to determine the homogeneity of the sample variations. The Levene's test results showed a significance of p = 0.280 (p > 0.05) indicating that the population variance was homogeneous and the assumptions were met, thus fulfilling the requirements for the ANOVA test (Table 2).

Table 2: One-way ANOVA test results of the expression of TGF-β

Group	Mean ± SD	p	Levene
Control	3.60 ± 0.40	0.001	0.280
HA	4.20 ± 1.02		
AV	5.00 ± 0.81		
Ozonated AV 300 mg	5.33 ± 0.63		
Ozonated AV 600 mg	5.47 ± 0.78		
Ozonated AV 1200 mg	5.93 ± 1.17		

Description: *Significant ($p < 0.05$); **Homogeneous ($p > 0.05$)

The results of the ANOVA test showed a significance of $p = 0.001$ ($p < 0.05$), meaning that that there was a significant difference in the treatment between groups. To find out which groups were significantly different, a follow-up LSD test was carried out. The results of the LSD test are shown in Table 3.

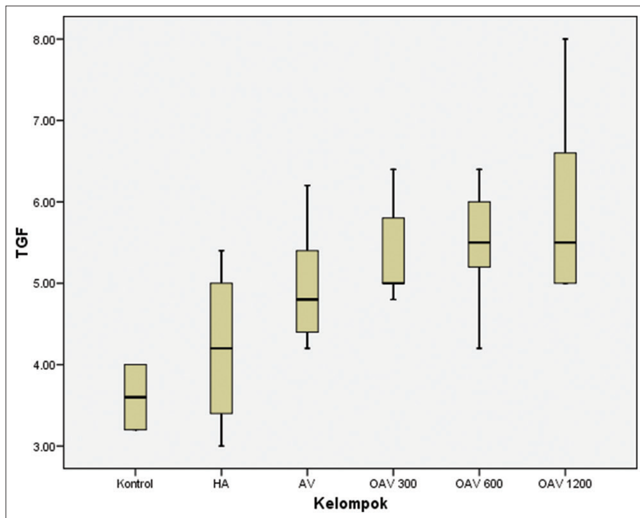

Figure 2: Boxplot graph of the expression of TGF-β of research samples

In the *post hoc* LSD test, there was a significant difference between the control group and the AV, Ozonated AV 300 mg, Ozonated AV 600 mg, and Ozonated 1200 groups; also there was a significant difference between the hydrocortisone group and the Ozonated AV 300 mg, Ozonated AV 600 mg, and Ozonated AV 1200 mg groups.

Overview of TGF-β immunohistochemical examination

Based on immunohistochemical examination of TGF-β from wound tissue cuts in experimental animals in this study, the results are shown in Figure 3.

Collagen density

Statistical test of collagen density

The results of collagen density measurement for all study groups are shown in Table 4 and 4a. The average results are also depicted by a boxplot graph as shown in Figure 4. The results of the Shapiro–Wilk test for collagen density showed a significance of $p>0.05$ in all groups except the P4 group ($p = 0.003$) as shown

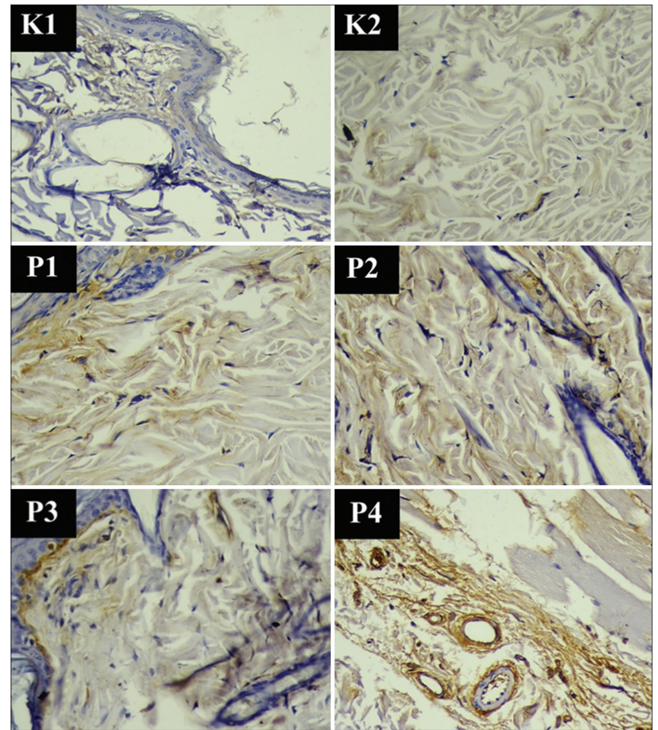

Figure 3: Immunohistochemical description of TGF-β wound tissue. (K1) Without intervention. (K2) Hydrocortisone cream 2.5%. (P1) Aloe vera oil. (P2) Ozonated Aloe vera oil of 300 mg/ml. (P3) Ozonated Aloe vera oil of 600 mg/ml. (P4). Ozonated Aloe vera oil of 1200 mg/ml

in the following table. This shows that the data were not normally distributed and the ANOVA requirements were not met, so the analysis was continued with the Kruskal–Wallis test.

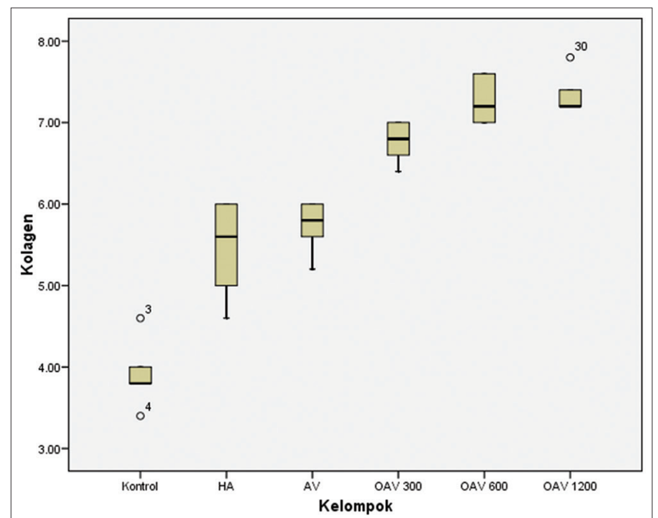

Figure 4: Boxplot graph of collagen density of research samples

The results of the Kruskal–Wallis test showed a significance of $p = <0.001$ ($p < 0.05$), meaning that there was a significant difference in the treatment between groups. To find out which group was significantly different, a follow-up Mann–Whitney test was carried out. The results of the Mann–Whitney test are shown in Table 5.

Table 3: *Post hoc* LSD test results of the expression of TGF-β

Group I	Group II	p	Information
Control	Hydrocortisone	0.271	Not significant
	AV	0.014	Significant
	Ozonated AV 300 mg	0.002	Significant
	Ozonated AV 600 mg	0.001	Significant
	Ozonated AV 1200 mg	< 0.001	Significant
Hydrocortisone	AV	0.146	Not significant
	Ozonated AV 300 mg	0.035	Significant
	Ozonated AV 600 mg	0.020	Significant
	Ozonated AV 1200 mg	0.002	Significant
AV	Ozonated AV 300 mg	0.520	Not significant
	Ozonated AV 600 mg	0.370	Not significant
	Ozonated AV 1200 mg	0.079	Not significant
Ozonated AV 300 mg	Ozonated AV 600 mg	0.787	Not significant
	Ozonated AV 1200 mg	0.229	Not significant
Ozonated AV 600 mg	Ozonated AV 1200 mg	0.347	Not significant

In the *post hoc* Mann–Whitney test, here was a significant difference between the control group and the hydrocortisone, AV, Ozonated AV 300 mg, Ozonated AV 600 mg, and Ozonated 1200 groups; between the hydrocortisone group and the Ozonated AV 300 mg, Ozonated AV 600 mg and Ozonated AV 1200 mg groups; between the AV group and the Ozonated AV 300 mg, Ozonated AV 600 mg and Ozonated AV 1200 mg. Furthermore, the Ozonated AV 300 mg group was significant to the Ozonated AV 600 mg and Ozonated AV 1200 mg groups.

Table 4: Normality test results of collagen density using the Shapiro–Wilk

Group	N	Mean ± SD	Median (min–max)	p	Note
Control	5	3.92 ± 0.44	3.8 (3.4–4.6)	0.607	N
HA	5	5.44 ± 0.62	5.6 (4.6–6.0)	0.332	N
AV	5	5.72 ± 0.33	5.8 (5.2–6.0)	0.314	N
Ozonated AV 300 mg	6	6.77 ± 0.27	6.8 (6.4–7.0)	0.065	N
Ozonated AV 600 mg	6	7.27 ± 0.27	7.2 (7.0–7.6)	0.093	N
Ozonated AV 1200 mg	6	7.33 ± 0.24	7.2 (7.2–7.8)	0.003	TN

Description: N = Normal, TN = Abnormal, Normal (p > 0.05)

Table 4a: Kruskal–Wallis test results of collagen density

Group	Mean ± SD	p
Control	3.92 ± 0.44	< 0.001
HA	5.44 ± 0.62	
AV	5.72 ± 0.33	
Ozonated AV 300 mg	6.77 ± 0.27	
Ozonated AV 600 mg	7.27 ± 0.27	
Ozonated AV 1200 mg	7.33 ± 0.24	

Overview of collagen density examination

Based on histological examination using H&E staining to assess the collagen density from wound tissue cuts in experimental animals in this study, the results are shown in Figure 5.

Table 5. Post hoc Mann–Whitney test results of collagen density

Group I	Group II	p	Information
Control	HA	0.011	Significant
	AV	0.009	Significant
	Ozonated AV 300 mg	0.005	Significant
	Ozonated AV 600 mg	0.006	Significant
	Ozonated AV 1200 mg	0.005	Significant
HA	AV	0.517	Not significant
	Ozonated AV 300 mg	0.005	Significant
	Ozonated AV 600 mg	0.006	Significant
	Ozonated AV 1200 mg	0.005	Significant
AV	Ozonated AV 300 mg	0.005	Significant
	Ozonated AV 600 mg	0.006	Significant
	Ozonated AV 1200 mg	0.005	Significant
Ozonated AV 300 mg	Ozonated AV 600 mg	0.012	Significant
	Ozonated AV 1200 mg	0.003	Significant
Ozonated AV 600 mg	Ozonated AV 1200 mg	0.492	Not significant

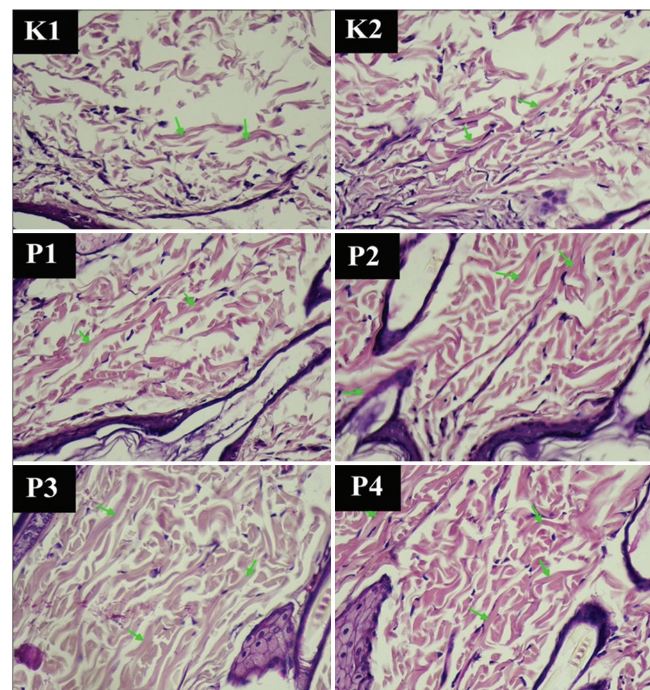

Figure 5: Collagen density using H&E staining of wound tissue. (K1) Without intervention. (K2) Hydrocortisone cream 2.5%. (P1) Aloe vera oil (P2). Ozonated Aloe vera oil of 300 mg/ml. (P3) Ozonated Aloe vera oil of 600 mg/ml. (P4) Ozonated Aloe vera oil of 1,200 mg/ml

Correlation between expression of TGF-β and collagen density

Correlation test was conducted to determine whether there was a correlation between the expression of TGF-β and collagen density variables in radiation dermatitis wound healing.

The results of the data normality test using Shapiro–Wilk showed that the expression of TGF-β data were normally distributed, namely, p = 0.534 (p > 0.05), while the collagen density data were not normally distributed p = 0.005 (p < 0.05), so the correlation test used was the Spearman correlation test (Table 6).

Table 6: Spearman correlation test results of TGF-β and collagen density

Variable	p	r
TGF-β Collagen	< 0.001	0.722

The results of the Spearman's rho correlation test showed a value of p = <0.001 and r = 0.722, from these results, it can be concluded that there was a strong and unidirectional significant correlation between TGF-β to collagen.

Discussion

In this study, the expression of TGF-β was expressed by cells in wound tissue that were seen on immunohistochemical staining and was measured semiquantitatively using the Allred score to see the

proportion per 100 cells stained; and the intensity of staining was calculated per 100 cells/field of view in five fields of view with a 400× magnification. After that, the average was calculated. Meanwhile, for collagen density in the dermatitis wound tissue, examination was calculated in five fields of view with a 400× magnification binocular microscope and Hematoxylin eosin staining.

Ozone is widely recognized as one of the best bactericidal, antiviral, and antifungal agents. Ozone's antiseptic properties can help reduce potential pathogens in wounds, thereby suppressing neutrophil counts, stopping inflammation, and accelerating wound repair [11], [12], [13]. Ozone also causes an increase in the expression of TGF-β, PDGF, and VEGF which are known to play a role in the wound healing process; this has been reported in clinical and experimental studies with ozone therapy. TGF-β is one of the growth factors that stimulate angiogenesis, fibroblast proliferation, and myofibroblast differentiation, which, in turn, will increase collagen production thereby accelerating the wound healing process [13], [14], [15].

In this study, it was proven that the administration of *Aloe vera* oil with or without ozonation resulted in better healing than the group without therapy and was equivalent to or better than the hydrocortisone group.

Radiation dermatitis

Radiation dermatitis occurs as a result of cutaneous or subcutaneous lesions from exposure to external beam radiation. Radiation reduces neovascularization, fibroblast proliferation, collagen deposition, and level of growth factors for wound healing. The inhibited growth factors are transforming growth factor β-1 (TGFβ-1) and basic fibroblast growth factor (bFGF) in the acute phase. In the chronic phase of wound healing in radiation scars, TGFβ-1 levels increase [4].

In this study, external radiation of 7 Gy gamma rays on the backs of rats of 4 × 4 cm showed a significant effect in causing radiation dermatitis. This is consistent with the results of study by Ran *et al*., where external gamma ray radiation of 1–8 Gy causes wounds accompanied by exudate, necrotic tissue, or inflammation [16]. The results of this study are supported by a previous study by Andrade *et al*., who used animal models to study radiation-induced skin lesions. In this study, skin lesions were induced in Wistar rats by irradiating an electron beam of energy 4 MeV, using a dose rate of 240 cGy/min, for 3 different doses (10 Gy, 40 Gy, and 60 Gy). The skin was observed on days 5, 10, and 25 after exposure to ionizing radiation. The inflammatory infiltrate process was observed on days 5 and 10 for the 40 Gy and 60 Gy groups by assessing the progressive increase in TGFβ-1 and the characterization of collagen fibers in the high dose group. The study showed that lesions caused by ionizing radiation in Rats were very similar to radiodermatitis in patients undergoing radiotherapy as was the case with the results of this study [17].

Expression of TGF-β

From the results of the study, it was found that giving *Aloe vera* to dermatitis wounds with or without ozonation showed an effect, where the average expression of TGF-β in radiation dermatitis wounds tended to increase significantly compared to the group that did not receive *Aloe Vera*.

This study has proven that the administration of ozonated *Aloe vera* oil can increase the expression of TGF-β higher than the positive and negative control groups. The results of this study are supported by previous studies conducted by Atiba *et al*. and Zhang *et al*. This is because during the wound healing process, platelets will produce various growth factors and inflammatory cytokines such as TGF-β, PDGF, EGF, and FGF, all of which will appear in the inflammatory phase and some of their functions are chemoactants [4], [14], [18]. *Aloe vera* in this study is useful in the wound healing process because of its extract content, namely *acemannan, β sitosterol* which activates macrophages in wounds. Besides, the role of ozone is to stimulate TGF-β and other growth factors to the wound area. TGF- β and other growth factors induce the fibroblasts proliferation where the process is affected by oxygen supply [15]. In radiation dermatitis there are pathological changes such as cellular depletion, matrix changes and microvascular damage that cause hypoxia in the wound tissue so that the wound healing process as mentioned above is disrupted [19].

In a previous study by Travagli *et al*., ozone has the ability to heal wounds, due to its "oxidative killing" effect on organisms. The use of ozone as a therapy provides many benefits because ozone will eliminate pathogens and activate the fibroblasts proliferation by releasing O2. Furthermore, the formation of intercellular matrix will occur with the keratin fibroblasts proliferation and so, wound healing begins [13].

Collagen density

The average collagen density in the ozonated or non-ozonated group compared to the negative control and hydrocortisone cream 2.5% groups was higher. This study is supported by a previous study conducted by Taqwim Hidayat *et al*., which showed that the administration of ozonated *Aloe vera* oil had an effect on collagen thickness [16].

This study has proven that the collagen density in the group that was given ozonated *Aloe vera* oil was higher than that of the control group. This is because

ozone therapy is an alternative therapy that can be used as a disinfectant and can induce strong oxidative stress, thereby stimulating cell and organ protection mechanisms [20]. In ozone therapy, various anti-oxidant systems are activated to defend the body against oxidation and the occurrence of ROS (Reactive Oxygen Species) in the form (O3, H2O2, and OH+), which leads to the production of antioxidant enzymes. These ROS act as secondary messengers for various immunocytic and non-lymphoid cells involved in the wound repair process and also play important roles in repairing inadequate tissue and coordinating the recruitment of lymphoid cells to the site of injury [8]. Ozonated *Aloe vera* oil increases reactive oxygen species (ROS) and reactive nitrogen species (RNS) around the wound site, such as platelets, macrophages, fibroblasts, endothelial cells, and keratinocytes that act as wound healing radicals. Fibroblasts around the wound site will produce collagen so that the wound healing process occurs [19], [21], [22]. With the increasing number of fibroblasts, it will increase the production, metabolism of collagen and alters the composition of collagen (more in type III) and increase the degree of collagen cross-linking so that the wound healing process is not disrupted and becomes faster [19], [21].

Correlation of TGF-β and collagen density on wound healing

The results of this study indicate that there was a strong and unidirectional significant correlation between the expression of TGF-β and collagen density variables. The content of *acemannan, β sitosterol,* and glycoproteins in *Aloe vera* will activate macrophages in wounds and the role of ozone will stimulate TGF-β and other growth factors to the wound area. This allows TGF-β and other growth factors to induce the fibroblasts proliferation, which in turn will significantly increase collagen synthesis [15]. *Aloe vera* and ozone do not only cause an increase in the collagen content of the wound but also change the composition of collagen (more in type III) and increase the degree of collagen cross-linking. This can accelerate wound contraction and increase the breaking strength of the resulting scar tissue [19], [21].

TGF-β will stimulate angiogenesis, fibroblast proliferation, myofibroblast differentiation which, in turn, will increase collagen production thus accelerating the wound healing process [13], [14], [15]. This shows that the occurrence of the collagen production process can increase or decrease depending on the number of growth factors, so that the higher the expression of TGF-β the more collagen is produced and the wound healing process is also faster.

The limitations of this study are related to expensive equipment and costs, so that radiation is only given once. Besides, the immunohistochemical examination is also relatively expensive.

Conclusion

1. There was a significant difference in the expression of TGF- β between the group treated with 300, 600, and 1200 mg/ml ozonated *Aloe vera* and the group that did not receive any therapy (negative control) and the group that received hydrocortisone therapy (positive control).
2. There was a significant difference in the collagen density between the group treated with 300, 600. and 1200 mg/ml ozonated *Aloe vera* and the group that did not receive any therapy (negative control) and the group that received hydrocortisone therapy (positive control).
3. There is a significant correlation between the expression of TGF-β and collagen density in healing radiation dermatitis.

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